

THE EFFECTS OF SECONDARY SEWAGE EFFLUENT ON THE WATER QUALITY,
NUTRIENT CYCLES AND MASS BALANCES, AND ACCUMULATION OF SOIL
ORGANIC MATTER IN CYPRESS DOMES

BY

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA .

1980

ACKNOWLEDGMENTS

This investigation was funded by a grant through the Center for Wetlands jointly sponsored by the RANN Division of the National Science Foundation and the Rockefeller Foundation.

I am especially grateful to the chairman of my supervisory committee, Dr. P. L. Brezonik, for his constructive suggestions on the experimental work and preparation of this manuscript.

I would also like to thank the other members of my committee for their help, especially Dr. K. C. Ewel with respect to the decomposition model and Dr. Graetz for his interest and support on various aspects of the nitrogen cycle.

I also want to express sincere appreciation to Dr. H. T. Odum for his continuous financial commitment during the course of this investigation.

It is impossible to acknowledge each individual who in some way assisted in the development of a dissertation of this nature. This especially applies to the untold scores of graduate students and technicians who performed nitrogen analyses on the AutoAnalyzer. However, some individuals deserve special recognition for their help. Appreciation is extended to Neil Carriker, who initiated me into the subtleties of sampling swamps, to Charles Fellows, for his many helpful suggestions and serving in the essential role of confidant, to Chuck Hendry and Eric Edgerton, whose assistance helped make the rain-fall/thru-fall section of higher quality, and to Pete Straub, Steve

Weeks and Steve Hall, who assisted me in both field sampling and lab analyses. Klaus Heimburg deserves special recognition for his patience and helpfulness in unraveling some of the hydrologic perplexities. Others whose efforts helped make possible this dissertation include Joan Breeze for her expeditious typing of the rough draft; Philip J. d'Almada and Dr. Ken Portier for their statistical expertise; Dr. Walter Judd and Shirley Kooijman for their botanical assistance; and Curt Pollman for his mercurial efforts in essential liaison work.

Lastly, my wife and son deserve special recognition for their sacrifices during the course of this research effort. I also wish to thank my wife for the highly professional job in typing the final manuscript. Special credit belongs to my mother, whose devotion to her children during untoward times made it possible for me to achieve this goal.

It is no understatement that without the zeal, wisdom and constant support shown by the above-named colleagues, committee members, and family, this investigation would not have been completed.

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Abstract of Dissertation Presented to the Graduate Council
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

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August 1980

Chairman: Patrick L. Brezonik

Major Department: Environmental Engineering Sciences

Considerable alteration in the nitrogen cycle of natural cypress domes occurs when they are used for treated sewage disposal. Both the rates of the major nitrogen transformation processes and the controlling environmental factors are significantly changed by input of secondary sewage effluent. Nitrogen and phosphorus are conserved in both the natural and sewage-enriched domes because nitrification is inhibited, rates of mineralization are low, and significant surface overflows do not occur. Although most sites within natural cypress domes do not fix significant amounts of nitrogen, excised, non-nodulated roots of the dominant woody vegetation of cypress swamps were found to be capable of low rates of fixation, as measured by the acetylene reduction assay. This finding opens a new area of research in the cycling of nitrogen in cypress wetlands.

The capability of cypress domes to act as efficient nutrient traps has been demonstrated for a natural and sewage-enriched cypress

dome. Mass balance models indicated that 87 percent of the nitrogen and 92 percent of the phosphorus loadings were retained within a cypress dome receiving secondary sewage effluent, removal efficiencies that are among the highest reported for any wetland ecosystem receiving treated sewage. Thus, cypress domes seem to be effective natural tertiary treatment systems. Cypress tree uptake accounted for only 1 percent of the phosphorus and 24 percent of the nitrogen inputs in the sewage-receiving dome, leaving the majority stored in the sediments. For a natural dome, 67 percent of the phosphorus and 27-58 percent of the nitrogen remaining in the dome were taken up and stored in cypress trees. Field data and laboratory investigations demonstrated that denitrification was a major nitrogen sink in both natural and sewage-enriched domes.

Bulk precipitation was the most important factor in supplying new minerals and nutrients (except nitrogen) to the natural cypress dome. Potassium, phosphate, ammonium, and nitrate plus nitrite had lower levels in the surface water than in throughfall, indicating that these elements are important in the biological and chemical processes of these domes.

Analyses of standing waters of natural and sewage-enriched cypress domes near Gainesville, Florida, over a 4.5-year period showed that discharge of secondary sewage effluent altered the soft, acid water found in natural domes to a neutral, moderately hard condition. Furthermore, the absence of dissolved oxygen, high levels of phosphorus, nitrogen, biochemical oxygen demand, and the presence of hydrogen sulfide mean that treated sewage effluent has a substantial impact on water quality. Standing water quality following the cessation of

sewage pumping did not return to the same quality as found for the natural dome within 20 months. Water samples from shallow wells, ceramic soil moisture tubes, and laboratory percolation columns indicated that the underlying organic matter and sands were serving as an effective barrier to the transport of sewage pollutants to the shallow aquifer immediately below. The largest percentage removals for all measured water quality parameters through the entire conventional treatment plant/dome system occurred between the surface and groundwater stations.

Cypress leaves decomposed more rapidly in a sewage-enriched cypress dome than in an undisturbed cypress dome. A model incorporating both labile and refractory components of cypress leaves was found to produce a better fit to decomposition data than a simple exponential decay model. Simulated rates of litterfall and decomposition under natural conditions and with sewage enrichment indicated that duckweed productivity generated by sewage addition will have minimum effect on the storage capacity of dome basins.

CHAPTER ONE INTRODUCTION

Forested wetlands have received little scientific investigation in the past because of the low recreational and economic values placed on them by man. Only recently have they been recognized as valuable resources because of their important roles in water conservation and maintenance of water quality. As the true costs of the 1983 "zero discharge" requirement of the 1972 Federal Water Pollution Control Act have become apparent in recent years (estimated \$594 billion by the National Commission on Water Quality), wetland disposal of treated sewage effluent has received increased attention as a possible low-cost alternative to conventional tertiary treatment methods. Wetland disposal may have the further advantage of simultaneous recharge of groundwater aquifers. However, the efficacy of using forested wetlands as sites for long-term treated sewage disposal has yet to be completely demonstrated and many questions involving the long term effects of treated sewage disposal on the swamp ecosystem still remain unanswered. For example, the quantitative aspects of nutrient mass balances are poorly understood in forested wetlands. Only a prolonged research effort by investigators representing many disciplines will adequately answer all these questions.

Some answers to these questions have been reached in an interdisciplinary research effort at the University of Florida dealing with the following question: Can cypress domes process

secondary-treated sewage effluent to a tertiary state in an economic and efficient manner?

The mass or materials balance approach is useful in evaluating the efficacy of using wetlands as treatment systems for nitrogen and phosphorus removal in secondary-treated effluent. The mass balance concept has been used in solving problems involving the cycling of essential elements (nitrogen, phosphorus, sulfur, and carbon) on global (e.g., SCOPE 1976; CAST 1976), regional (e.g., Messer 1978), and ecosystem (e.g., Bormann et al. 1977) levels.

For elements that have complex physico-chemical interactions and transformations, such as nitrogen, erroneous or incomplete data lead to large uncertainties in the values for some of the reservoirs or flows. Because the conclusions based on a mass balance model can only be as precise, accurate and sensitive as the data from which the model was constructed, the completeness and quality of the data are of paramount importance in producing a model that has meaningful relationships among its system components. Unfortunately, the manpower and analytical facilities required to achieve a complete and accurate mass balance model for nitrogen have been too great to be undertaken within budgetary limitations. Because of these constraints, nitrogen mass balance models in wetland ecosystems are often incomplete.

Qualitative information on the transformations, fluxes, and storages of nitrogen and phosphorus is abundant. Complete and detailed reviews have considered nitrogen and phosphorus cycling in wetland and aquatic systems (Erickson 1978; Good et al. 1978; Keeney 1973; Ponnampereuma 1972; Syers et al. 1973; Wetzel 1975).

Wetlands have long been recognized as nutrient traps. Van der Valk et al. (1978) reviewed 17 studies on various types of wetlands. Phosphorus was removed in all 16 studies that measured phosphorus, and 12 of the 14 studies involving nitrogen reported at least seasonal removal. Two studies found wetlands to act as nitrogen sources.

Studies on palustrine wetlands (i.e., nontidal wetlands that are not confined by channels and are not marginal to lakes) have indicated nitrogen and phosphorus removals during the entire year (Boyt et al. 1977; Fetter et al. 1978; Hermann 1979; Nessel 1978; Richardson et al. 1978; van der Valk et al. 1978). Removal efficiencies in northern marshes decline in the spring and fall when high hydraulic loadings wash out some of the nutrients assimilated during the previous growing season and mineralized during the previous fall (Lee et al. 1975; Spangler et al. 1977). Only one study reported that assimilative capacity of a palustrine wetland (Everglades) was exceeded by the discharge of high nutrient effluent (Steward and Ornes 1975).

The capability of wetlands to trap nutrients has resulted in recent studies aimed at exploring how they respond to the addition of wastewater (Boyt et al. 1977; Odum et al. 1975; Sloey et al. 1978; Richardson et al. 1978; Whigham and Simpson 1976; Zoltek et al. 1979). Sloey et al. (1978) point out that palustrine wetlands are more amenable to management for wastewater treatment than other major wetlands (tidal, riverine, and lacustrine) because palustrine wetlands are hydraulically isolated from open surface water and hydraulic residence times are high.

Recent reviews on nutrient dynamics in wetlands (van der Valk et al. 1978; Whigham and Bayley 1978) have stressed the absence of studies that assess the nutrient removal efficiency of wetlands on an annual, areal basis. In fact, few studies have quantified any aspect of nutrient cycling within wetlands. For example, only 8 of the 21 papers reviewed by Whigham and Bayley (1978) contain any input or output data. Prentki et al. (1978), Deghi (1977), and Zoltek et al. (1979) have provided partial mass balance models for nutrients in wetlands.

The specific questions which my research addresses are:

- 1) How efficient are cypress domes in removing the pollutant loads in secondarily-treated discharge?
- 2) How long can chemical and microbial processes effectively treat secondarily-treated sewage to a tertiary state?
- 3) What are the chemical characteristics of surface and groundwaters in a natural cypress dome and how are they altered by sewage disposal?
- 4) To what extent and how fast can the major water quality parameters revert back to natural levels once sewage disposal has been discontinued?
- 5) How does sewage disposal alter the processes involved in the nitrogen cycle of a natural dome?
- 6) What are the nitrogen and phosphorus budgets of sewage-enriched and natural cypress domes?
- 7) To what extent does treated sewage disposal increase the rate of organic accumulation on the swamp floor, thereby reducing the hydraulic capacity of the dome basin?

CHAPTER TWO
AREAS OF INVESTIGATION, SAMPLING PROCEDURES,
AND ANALYTICAL METHODS

Site Description

The main study site consists of three cypress domes located about 5 km north of Gainesville, Florida, on a large pine plantation owned by Owens-Illinois, Inc. (Figure 2-1).

A "package treatment plant" serving a 155-unit mobile home park adjacent to the site supplied secondarily-treated sewage effluent to sewage dome 1 (0.51 ha) and sewage dome 2 (1.05 ha), respectively (Figure 2-2). Pumping was initiated in March 1974 to sewage dome 1 and December 1974 to sewage dome 2. The sewage plant is an extended aeration waste treatment facility which is currently treating about 95 m³/day (25,000 GPD), 83 percent of its design capacity of 114 m³/day (30,000 GPD). The treated effluent is then discharged into an oxidation pond.

During the period from March 1974 to March 1975, the sewage pumped to the cypress domes came directly from the package treatment plant, which was not operating efficiently. Hydraulic loadings were as low as 0 cm/wk (June 1974) and as high as 14 cm/wk (August 1974). Sludge occasionally was pumped into the domes. After March 1975, more even and constant sewage applications (2.5 cm/wk with no sludge) were obtained using effluent that had been in the oxidation pond for an average detention time of 10 days. The influent pipe within the

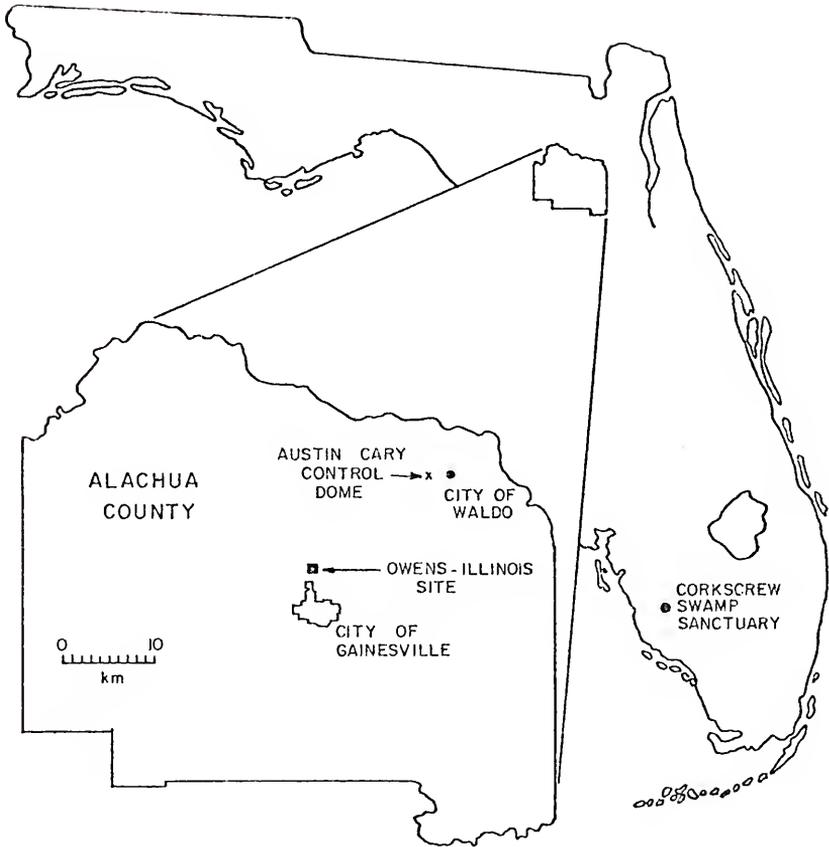


Figure 2-1. Locations of the two major study sites in Alachua County.

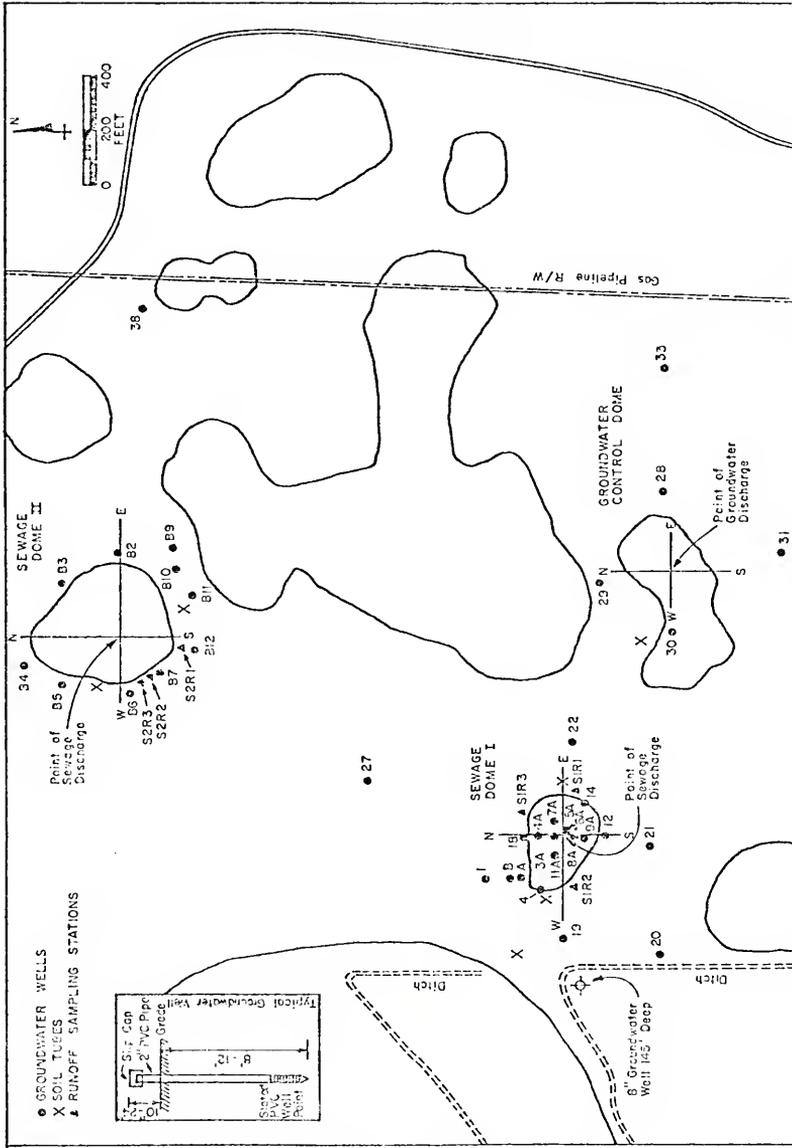


Figure 2-2. Site plan, groundwater monitoring wells, soil tube locations, and runoff sampling stations at the Owens-Illinois research site.

oxidation pond was relocated in March 1976 to a point closer to the treatment plant.

A combination of high sewage application rates, heavy rainfall, and abnormally high surface runoff during summer, 1974, caused overflow from sewage dome 1. A weir was constructed on the west side of the dome to maintain the standing water at a more constant level and to allow the overflow to be measured and sampled.

A third dome, the groundwater control dome (0.70 ha) at the Owens-Illinois site received groundwater from a deep well (~50 m) according to the same schedule and loading rate as sewage dome 1 beginning in March, 1974. It served as a hydrologic control, separating the response of acidic cypress domes to application of hard, alkaline groundwater, from the effects of sewage pollutants (nutrients and organic matter).

Two of the three domes, the groundwater control dome and sewage dome 1, were burned extensively in an unintentional forest fire on December 4, 1973, while the third dome, sewage dome 2, was burned only along the southwest edge.

A larger dome (4.2 ha), located about 17 km northeast of the Owens-Illinois site in the Austin Cary Memorial Forest (Figure 2-1), served as a "natural" control whose water levels fluctuated according to the normal hydroperiod. Activities connected with the research project (well drilling, installation of a boardwalk, tower, equipment shed, and various monitoring devices) have somewhat altered the natural character of this dome.

Sampling Procedures

Standing Waters, Sewage, and Shallow Groundwaters

The standing waters, sewage, and shallow groundwaters on the Owens-Illinois site and the standing waters of the Austin Cary dome have been routinely monitored for major cations and anions, nutrients, and other water quality parameters since March 1974 (Table 2-1). Samples were collected on a monthly basis until December 1976, followed by quarterly sampling until May 1979, from the Owens-Illinois stations listed in Table 2-2. Beginning in April 1978, monthly sampling of Austin Cary dome surface waters was resumed until July 1979.

Table 2-1. List of physical and chemical parameters for which analyses were performed.

Basic Water Chemistry and Quality	Nutrient Forms	Major Cations and Anions
Alkalinity	NH_4^+ -N	Ca^{+2}
pH	$(\text{NO}_3^- + \text{NO}_2^-)$ -N	Mg^{+2}
Color	Organic N	Na^+
Dissolved oxygen	PO_4 -P	K^+
Turbidity	Total P	Al^{+3}
Specific conductance		F^-
BOD_5		Cl^-
Inorganic Carbon		SO_4^{-2}
Organic Carbon		
Sulfide		

Table 2-2. Schedule for routine sampling

Site	No. Samples	Descriptions
Sewage	2	Treated effluent, oxidation pond effluent
Groundwater	1	Deep well supplying groundwater control dome
Wells 22, 28, 33, and 38	4	Background for shallow water table aquifer
Austin Cary natural dome	1	Standing water, Austin Cary dome center
Groundwater control dome	2	Standing water; center, 30 m west
Well surrounding groundwater control dome	1	Well 30
Sewage dome 1	3	Standing water; center, 30 m east, weir overflow
Wells inside and surrounding sewage dome 1	3	Wells 4A, 8A, and 19
Sewage dome 2	3	Standing water; center, 40 m north, 30 m south
Wells surrounding sewage dome 2	4	Wells B-3, B-5, B-7, and B-9

Beginning in March 1976, the frequency of analysis was reduced for parameters that showed constant or predictable trends. At that time, monthly hydrogen sulfide analyses on the surface waters were added and the frequency of measurements of pH, color, specific conductance, and major cations was reduced for both surface and groundwater stations from a monthly to a quarterly basis. Alkalinity and sulfate were analyzed semi-annually instead of quarterly for all

surface and groundwater stations. All other parameters were measured quarterly instead of monthly at the groundwater stations. Monthly analysis of all parameters in the standing waters of Austin Cary were resumed in April 1978 and continued to July 1979.

In addition to the regularly scheduled sampling stations listed in Table 2-2, wells 20, 27, 29, 31, 34, 35, 36, 37, and 38 near the groundwater control dome; wells 3A, 5A, 6A, 7A, 11A, and 9A inside sewage dome 1; wells B-2, B-4, B-6, B-8, B-10, B-11, and B-12 at sewage dome 2; and wells 1, 4, 12, 14, 18, and 21 surrounding sewage dome 1 were sampled occasionally, especially in the early years of the monitoring program (Figure 2-2). All wells are shallow (3-6 m deep) and they just penetrate a hardpan stratum.

In order to provide a basis for comparing the groundwater quality among the various groups of wells and to assess the removal of sewage pollutants from nearby sewage domes, the well data were composited into five groups: 1) B-1 through B-12 surrounding sewage dome 2 (B-wells); 2) wells 1, 4, 12, 14, 18, 19, 20, and 21 surrounding sewage dome 1 (S-1 wells); 3) wells 3A, 4A, 5A, 6A, 7A, 8A, 9A, and 11A located within sewage dome 1 (A wells); 4) wells 29 through 31 surrounding the groundwater control dome (GC wells); and 5) wells 22, 27, 28, 33, 34, 35, 36, 37, which are distant from any of the experimental or control cypress domes and can be considered representative of the natural groundwaters of the area. Wells 22 and 28 on the east sides of sewage dome 1 and groundwater control dome, respectively, were not included in the sewage dome 1 and groundwater control dome well composites, since the groundwater flow around these domes is in a westerly direction (Heimburg 1976).

The procedure for sampling wells involved pumping each well dry (or removing at least 2 liters) two or three days before sampling so fresh percolate could be collected. Samples were collected with a hand vacuum pump (Nalgene) by drawing water through $\frac{1}{4}$ inch i.d. Tygon tubing that was custom-fitted to each well (down to the perforated zone, 0.3-0.6 m above the bottom of the casing) (Figure 2-3). The first liter of water drawn was discarded to clean the tubing of contaminants. Samples were then collected directly in acid-washed 125-ml polyethylene bottles for major cations, specific conductance, and chloride analyses. Samples were also collected into acid-washed 500-ml polyethylene bottles for nutrient and other water quality analyses. One-half milliliter saturated HgCl_2 was added to the 500-ml polyethylene bottle, which was placed on ice in the field.

Surface water "grab" samples were taken with a 1-liter polyethylene bottle for BOD_5 analysis, and two glass 300-ml BOD bottles, one each for H_2S and dissolved oxygen determinations. The last two samples were collected using a BOD sampler (Wildco) in order to insure against the introduction of oxygen during sampling. The bottles containing samples for sulfide and BOD_5 analyses were placed on ice, while the bottles containing samples for dissolved oxygen determinations were fixed in the field by addition of MnSO_4 and alkali azide iodide. Surface water "grab" samples were also collected in 125-ml and 500-ml polyethylene bottles. The parameters measured and the methods of preservation were the same as described previously for the well samples.

Upon return to the laboratory (usually within 4 hours of collection), nutrient samples containing HgCl_2 preservative were stored at

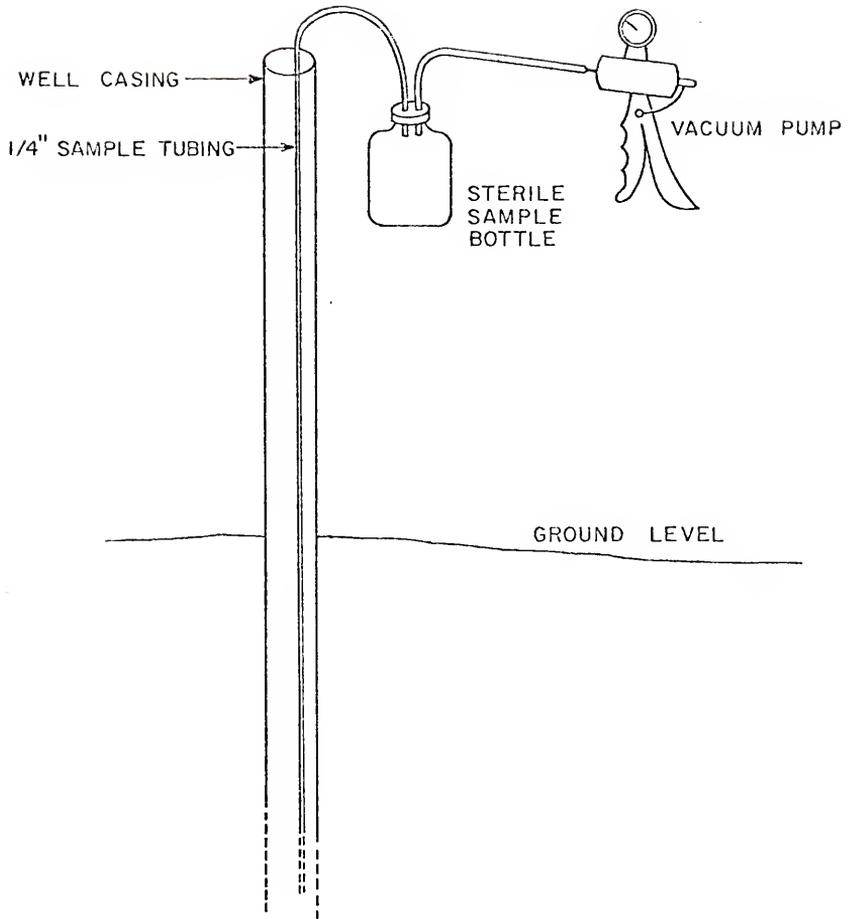


Figure 2-3. Sampling equipment and technique for sampling the shallow groundwaters.

4°C. Sulfide, BOD₅, dissolved oxygen, and pH were determined within 24 hours of sampling. All other parameters except sulfate, fluoride, chloride and the major cations were analyzed within two weeks.

Runoff

From August 2, 1976, to January 20, 1978, 10 rainfall events were sampled at stations near sewage domes 1 and 2 (Figure 2-2) to determine N and P loadings from surface runoff. Two additional rainfall events were sampled at three stations at the Austin Cary site on December 25, 1977, and January 20, 1978. Samples were collected at intervals ranging from 15 min to 1 hr, depending on the time elapsed since initial flow into the domes.

Bulk Precipitation and Throughfall

Precipitation was collected weekly from April 18, 1978, to April 19, 1979, at stations above and below tree canopy in the Austin Cary dome. The station above the dome was situated atop the tower at the center of the dome (27 m high and located above the tree crowns), while the station below the dome canopy was located near the boardwalk 20 m northeast of the dome center (Figure 2-4).

Each collector consisted of a polypropylene funnel (surface opening of 552 cm²) fitted with a teflon wool plug (Figure 2-5). A vapor barrier was provided by a loop in the tygon tubing, thus preventing ammonia loss or gain from the atmosphere and evaporation from the reservoir. An overflow tube allowed excess water to drain during large precipitation events (>7.6 cm rain).

The funnels were not replaced, in order to collect the dry fall-out deposited on them since the last rain event, but containers were replaced after collecting samples. Litter accumulated in the funnel

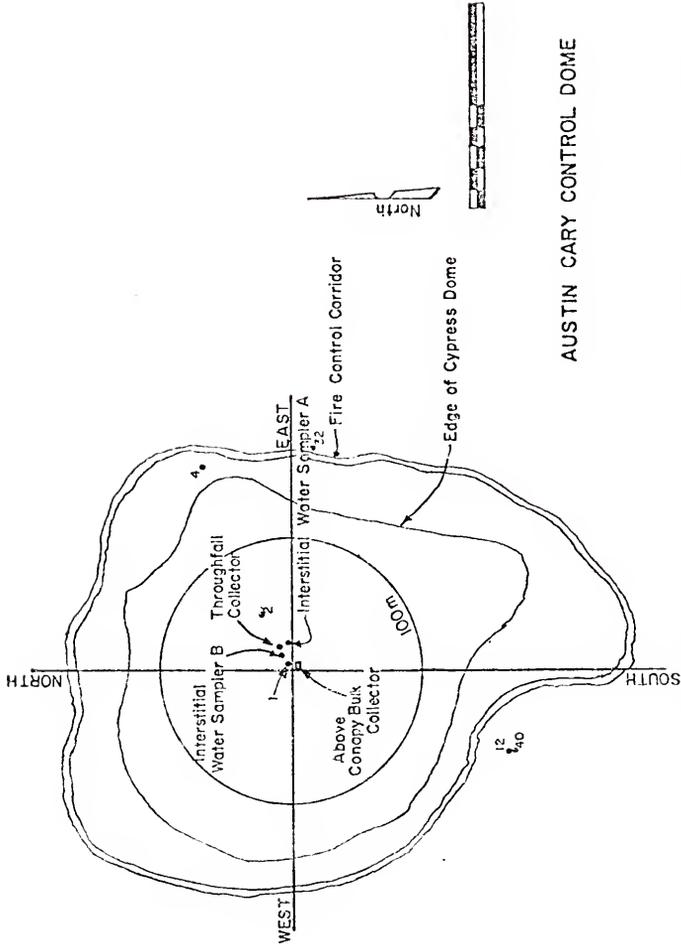


Figure 2-4. Site plan, groundwater wells (numbered dots), interstitial water sampling stations, and the locations of the bulk precipitation collectors at the Austin Cary natural dome research site.

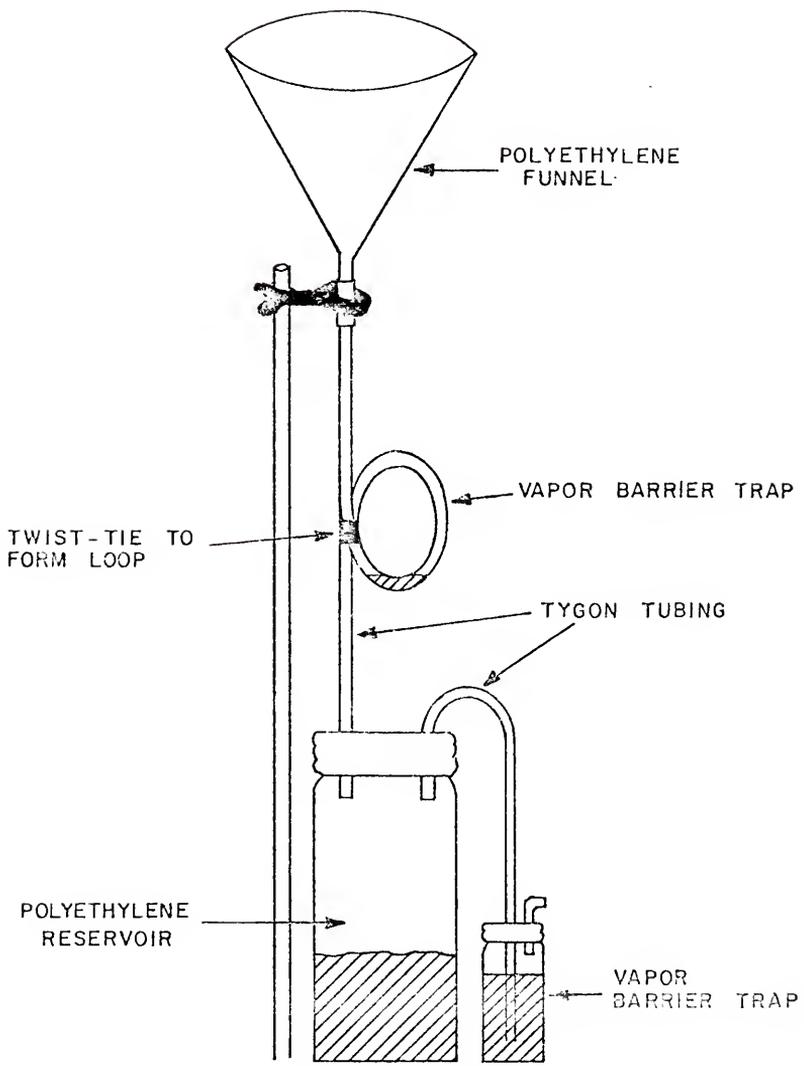


Figure 2-5. Precipitation collector.

was discarded. The volume collected during each sampling time was measured, except when precipitation events produced more water than the container could hold. Data from the University of Florida Beef Research Unit located 8 km southwest of the Austin Cary dome were used to estimate the amount of rainfall.

Plant Biomass

Yearly measurements were made of the diameter at breast height (DBH) for 100 trees in each of the three study domes at the Owens-Illinois site and 200 trees in Austin Cary dome. Diameter at breast height was measured at 1.37 m above ground surface or above severe butt swell. In addition, yearly samples of foliage, branches, and stems were taken from eight cypress trees in each dome for determination of nitrogen and phosphorus content beginning in September 1973 and ending in July 1977.

Tree cores were obtained with an increment borer. Foliage and branches were sampled with a 16-gauge shotgun (No. 2 and 4 magnum load). The samples were cut into small pieces in the field and stored in paper bags until dried at 80°C and ground in a Wiley mill to pass a No. 20 mesh screen.

Peat

A 4 cm diameter aluminum pipe with a beveled tip was used to obtain cores from the four domes during May 1977. The cores were extruded in the field and segmented at 5 cm intervals. The segments were put into polypropylene bags and frozen until the time when dry and ash weights and nitrogen content were determined.

MethodsWater Quality Parameters

Analysis of major cations was performed using a Varian Techtron Model 1200 Atomic Absorption Spectrophotometer with the instrument settings recommended by the manufacturer. Sample atomization was by an air/acetylene flame. One milliliter of a La-HCl solution (29 g La_2O_3 and 250 ml concentrated HCl diluted to 500 ml) was added to each 10 ml of a Ca and Mg sample. AutoAnalyzer procedures were used to determine nitrate plus nitrite (cadmium reduction method), ammonium (indophenol method), Kjeldahl nitrogen (semi-micro digestion followed by determination of ammonium), chloride (ferric thiocyanate method), aluminum (eriochrome cyanine R method), sulfate (methylene blue method) and silica (molybdenum blue method); procedures for each parameter were obtained from U.S. EPA (1974) or APHA (1976). Soluble reactive phosphorus and total phosphorus (persulfate digestion) were determined spectrophotometrically by the molybdenum blue method (APHA 1976). Sulfide analysis was by the method of Strickland and Parsons (1972), where Lauth's violet formed from p-phenylenediamine is measured spectrophotometrically. Fluoride was measured by ion-selective electrode (APHA 1976), and pH by glass electrode. Dissolved organic and inorganic carbon were analyzed using a Beckman Model 915 carbon analyzer. Dissolved oxygen was determined by the azide modification of the Winkler method. Five-day biochemical oxygen demand was determined by the amount of oxygen lost after incubating for 5 days in the dark at 20°C (APHA 1976). Alkalinity was measured titrimetrically with 0.02 N H_2SO_4 as the titrant and methyl red-bromocresol green as the end point indicator (APHA 1976). Total acidity (Austin Cary surface

water only) on He-purged and unpurged samples was determined by titrating a 500 ml sample (closed to the atmosphere) with 0.00907 N NaOH (standardized with potassium biphthalate) using a pH electrode (Figure 2-6). Turbidity (Hach Model 2100 nephelometer), color (spectrophotometer), and specific conductance measurements followed APHA (1976). Sulfate in samples from the Owens-Illinois site was determined turbidimetrically (APHA 1976).

Analytical problems were encountered with the organic-colored surface waters. Analyses involving spectrophotometric methods were particularly susceptible to positive interference from color present in the samples. The extent of such interference depended on the concentration of the measured parameter, wavelength used, and pH of the final reaction mixture. For the levels of color normally found in the standing waters of Austin Cary (300 to 800 CPU), blank correction procedures were instituted for the parameters (Table 2-3).

EPA reference samples were analyzed along with the samples for calcium, magnesium, sodium, potassium, sulfate, chloride, fluoride, Kjeldahl nitrogen, ammonium, nitrate plus nitrite, phosphate, total phosphorus and BOD, and were consistently within 10 percent of the stated concentration.

Nitrogen and Phosphorus Removal Studies

Soil-solution studies

Soil-solution sampling tubes consisting of porous ceramic cups attached to 3.8 cm i.d. PVC pipes were installed at three soil depths (60, 120, and 180 cm) near the edges of the groundwater control dome and sewage domes 1 and 2 (see Figure 22 for locations of soil tubes). For the stations closest to the sewage domes, duplicate tubes were

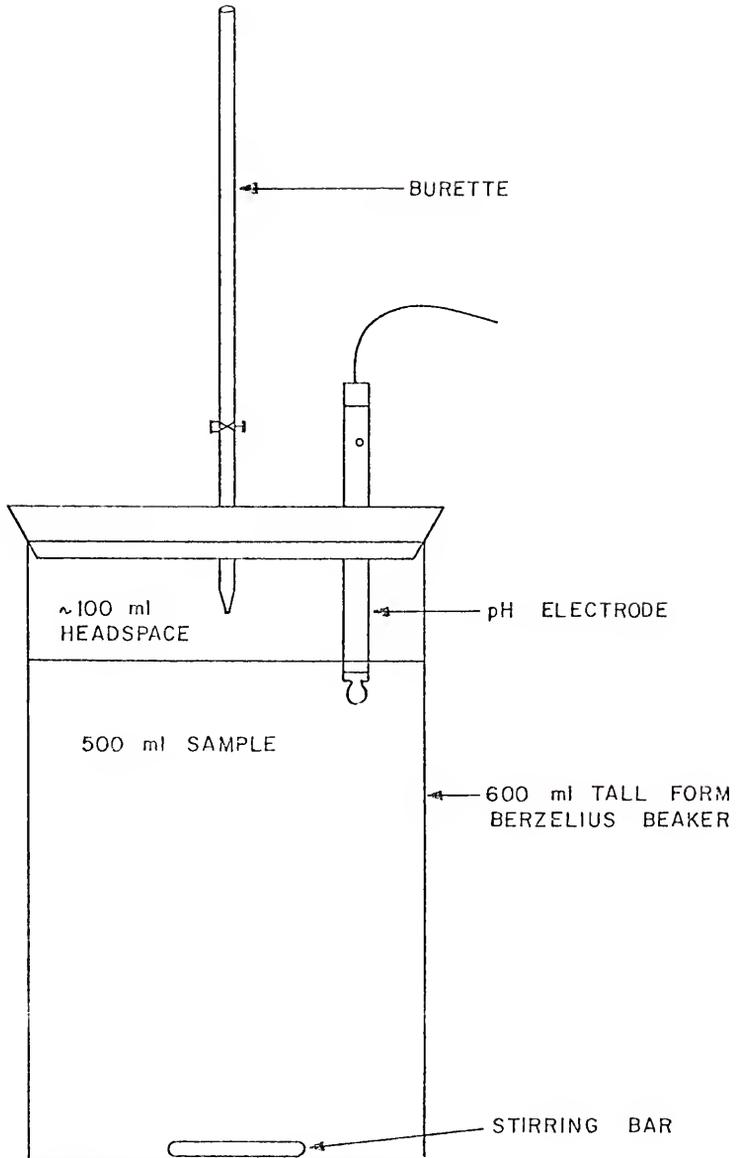


Figure 2-6. Apparatus used in total acidity titrations.

Table 2-3. Parameters influenced by organic color and the blanking procedures used to compensate for the interference.

Parameter	Blanking Procedure
Aluminum	Blank concentration obtained by adding 2 drops of a $10^{-3}M$ EDTA solution to 2 mls of sample diluted 1:10, thereby complexing the Al.
Chloride	Absorbance obtained by substituting deionized water for the mercuric thiocyanate reagent was subtracted from the sample absorbance measured with all reagents added.
Nitrate plus nitrite (only for samples with color concentrations >800 CPU) (i.e., leachate from columns)	Blank concentration obtained by deleting the sulfanilimide from the 1 percent sulfanilimide-0.1 percent N-(1-naphthyl) ethylene diaminedihydrochloride (NNED-HCl) reagent was subtracted from the measured concentration.
Phosphate	Blank concentration obtained by substituting deionized water for the ascorbic acid in the mixed reagent was subtracted from measured concentration.
Sulfate	At low levels of sulfate, high levels of organic color can interfere with the precipitation of $BaSO_4$ in the turbidimetric method (APHA 1971) and with the quantitative complexation of excess Ba^{+2} by methylthymol blue in the automated colorimetric method. Organic color for Austin Cary standing waters was oxidized by adding 1 ml of a 30 percent reagent grade H_2O_2 to 100 ml of sample and then heating. Because sulfate was an impurity in the H_2O_2 , distilled water blanks also were heated with 1 ml H_2O_2 . The sulfate level found in the blanks were then subtracted from the concentration measured in the samples. Concentrations obtained by this method may exceed the true sulfate concentration in the sample because of the oxidation of reduced organic sulfur compounds. The magnitude of this error is unknown, since reduced sulfur species in the standing waters were not analyzed.
Sulfide	Double beam spectrophotometer was used with the reference cuvette containing sample without $FeCl_3$ reagent.

placed at each depth. The other stations had only one soil tube at each depth. The ceramic cups were washed in 1:2 concentrated HNO_3 and rinsed in demineralized water before installation in the field. Soil solution was sampled by drawing a vacuum in the tubes and collecting the sample 24 hours later. Any water that had accumulated in the tubes before a vacuum was applied was discarded. Samples were obtained in November and December of 1976 and January, May, and August of 1977. Nutrient concentrations were determined by the same procedures cited above.

Laboratory leaching studies

Six columns were filled to a depth of approximately 17 cm (volume ~200 cc) with surface sediments from the groundwater control dome (Figure 2-7). The sediments consisted of 58 percent water. One pair received deep groundwater (from the Floridan Aquifer) as the eluant, another pair received treated sewage (from the oxidation pond) as the eluant, and the remaining pair received the same treated sewage spiked with either 20 mg nitrate-N/L or 30 mg nitrate-N/L plus 13.1 mg P/L as orthophosphate as the eluant. Frozen 100 ml aliquots of each of the above eluants were thawed and used whenever the eluant volume within the columns approached the surface of the sediments. All eluants were filtered through a Whatman no. 4 qualitative filter before being applied to the columns. The columns were allowed to drain freely in the dark at 24°C over a 21-month period, and 14 to 15 pore volumes of eluant were passed through the columns during this time. The flow rates approximated percolation rates measured for sewage in the field (0.3 to 0.9 cm/day) (Heimburg 1976). The filter paper (Whatman no. 4) at the bottom of each column was periodically

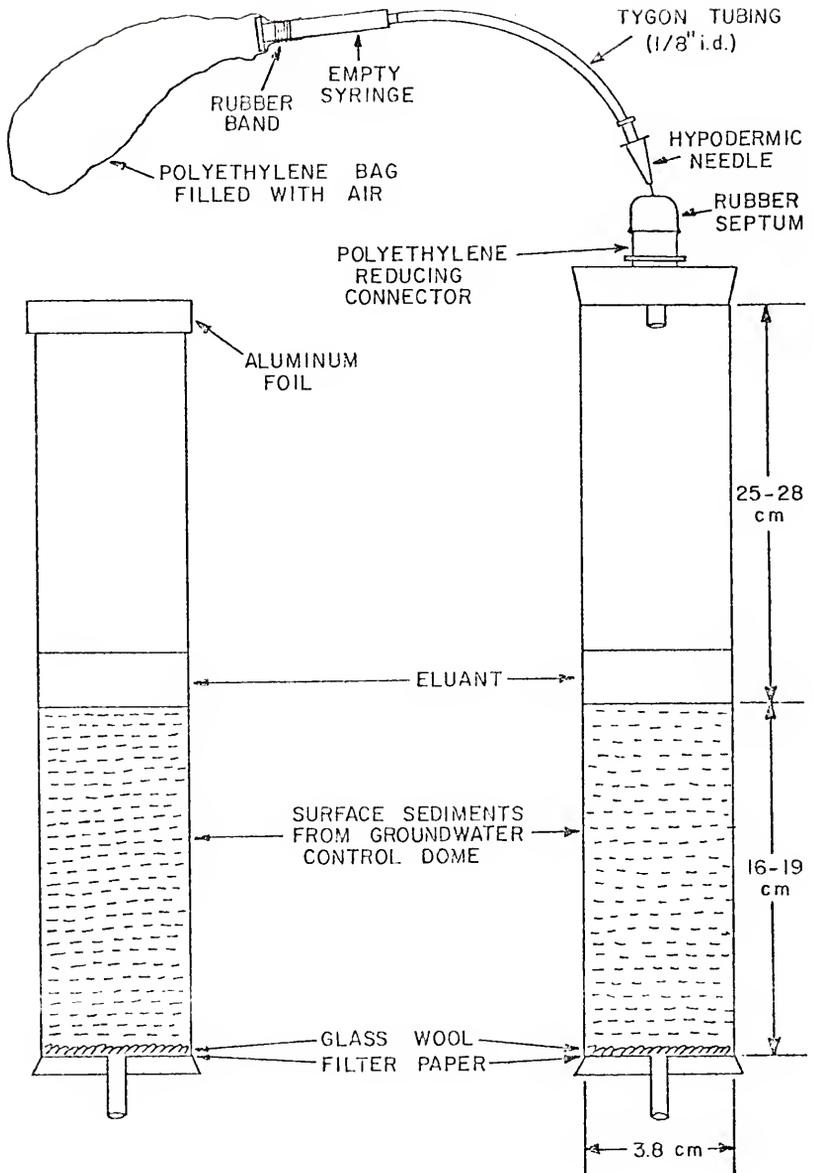


Figure 2-7. Setup for nutrient removal (left) and acetylene blockage (right) experiments.

replaced to maintain consistent flow rates. The column effluent was collected on every second or third day in 25 ml graduated cylinders, which had 1 drop of saturated HgCl_2 added prior to collecting the leachate. Successive sample collections were pooled into 50 or 100 ml aliquots and frozen. A layer of eluant was maintained over the sediments at all times.

After leaching had proceeded for 8 months, the columns (Figure 2-7) were rendered gas-tight, and the build-up of N_2O in the headspace with and without 0.1 atm of C_2H_2 was measured by injecting 500 μl of headspace gas into a Tracor 550 gas chromatograph equipped with a ^{63}Ni electron capture detector. The detector was operated at 350°C in pulsed mode with an argon-methane (95:5) carrier gas. A 1.9 m column of Poropak Q operated at 55°C was used to separate N_2O from the other gases.

Oxidation reduction potential (redox potential) was measured before and after the acetylene blockage experiment with a shiny platinum electrode positioned a few centimeters above the surface sediments. A saturated calomel electrode was used as reference electrode, and an Orion 401 ion meter served as a potentiometer. Calibration of electrodes and potentiometer was made against a $\text{Fe}^{+2}/\text{Fe}^{+3}$ standard solution (Light 1972). Adjustments were made in the measured potentials to a standard hydrogen reference electrode (Light 1972) at pH 7 (Patrick and Mahapatra 1968).

Percent dry weight and specific gravity (i.e., dry weight density) were determined by filling a 14 or 19 cm^3 vial with sediment from the 0-6 cm, 6-12 cm, and 12 cm-bottom depth intervals for each column. These were weighed and allowed to dry overnight at 105°C and

then reweighed. Percent volatile solids was also determined on these samples by ashing overnight at 550°C and then reweighing.

Total ammonium (free and exchangeable) associated with the sediments was measured by shaking 4 mg (wet wt) of sediment from the 0-1 cm, 6-7 cm, 12-13 cm, and terminal cm depths for 24 h in 100 ml 2 M KCl, followed by filtering through a 934 AH Whatman glass fiber filter. Ammonium concentration was measured on the AutoAnalyzer as previously described, except ammonium standards were made up in 2 M KCl instead of distilled water.

The mass of NH_4^+ -N (in mg) in each 6 cm interval of each column was calculated using the specific gravity of sediments, the volume occupied by a 6 cm length column, and the average mg NH_4^+ -N per gram dry wt of sediments before adding three intervals per column in order to arrive at the total amount for the column.

In addition, total nitrogen by the micro-Kjeldahl method was determined on the 0-6 cm, 6-12 cm, and 12 to terminal cm segments of the sediments after leaching and on the entire sediment before leaching. The same calculation as described above for determining the mass of NH_4^+ -N was used to obtain the mass of total N (in mg) in each 6 cm interval for each column before adding the three intervals per column in order to arrive at the total amount for the column.

Cypress Leaf Decomposition

Freshly fallen cypress leaves were collected from both sewage dome 2 and the Austin Cary natural dome from November 6-10, 1977. Fallen litter was harvested from cleared surfaces on the boardwalks, platforms, and utility shed roofs on alternate days. The leaves were stored in paper bags at room temperature until January 1, 1978.

Then, between 30 and 45 g fresh weight (nearest 0.1 g) were enclosed in 2 mm x 2 mm mesh fiberglass screen bags. The bags were stapled along the sides and the top at 5 cm intervals which left gaps large enough for most invertebrates to enter, yet prevented loss of the plant material.

On January 1, 1978, 40 bags containing litter from the natural dome were placed in the center of that dome in one of the deepest spots. Forty bags containing litter from sewage dome 2 were placed near the center of sewage dome 2 but away from the turbulence associated with the effluent. Litter bags were not placed at the edges of either dome since Deghi (1977) found no significant differences in the decomposition rates of cypress leaf litter between center and edge sites.

In addition, five bags of cypress litter from each of the two domes were carried to the field and then immediately returned for determination of dry-wet weight ratios. These ratios were subsequently used to estimate the amount of dry weight originally present in the bags.

Litter bags were collected in groups of five from each site after decomposition had proceeded for 15, 29, 58, 114, 205, 297, 390, and 570 days. Their contents were removed within 24 hrs of collection, dry weights were determined after drying at 67°C for at least five days, and percent loss of dry weight for each bag was calculated. Total nitrogen and carbon were calculated as follows:

percent total N or C remaining =

$$\frac{100 \times \text{final dry wt} \times \text{final N or C concentration}}{\text{initial dry wt} \times \text{initial N or C concentration}} \quad (1)$$

Decomposition constants characterizing cypress leaf decomposition were calculated using a linearized version of the standard exponential decay model:

$$y = e^{-kt}, \quad (2)$$

where y is the fraction remaining of the original amount of litter at time, t , k is the decomposition constant, and e is the base of the natural logarithm.

Model of Organic Matter Accumulation on the Swamp Floor

The data obtained from the cypress decomposition study described above were combined with other available data to model the effect of secondary sewage effluent on the accumulation of organic matter in cypress domes.

Two models describing organic matter accumulation were formulated: one for the natural cypress dome, in which cypress trees provide the bulk of the litter, and one for the sewage dome, in which duckweed deposition is also a major component. Cypress litter fall as well as duckweed production and decomposition rates were obtained from previous studies (Deghi 1977 and Price 1975b, respectively).

Differential equations describing the change in organic matter accumulation in each dome were written for labile and refractory components of each litter category:

$$\frac{dQ}{dt} = J - kQ, \quad (3)$$

where Q is the quantity of each litter component on the forest floor expressed as g organic matter/m² and J is the input of each litter component expressed as g organic matter/m²-yr.

The natural dome model therefore comprised two equations: fast and slow components of cypress litter. The sewage dome model contained two additional equations for the fast and slow components of the duckweed litter. These models were simulated on an EAI MiniAC analog computer after scaling and plotted directly from the analog computer with a Hewlett-Packard X-Y recorder. The amount of organic matter that had accumulated on the floor of the natural dome (20.3 kg organic matter/m²) was used to establish the initial conditions for the refractory component of the cypress leaves in both domes. Initial levels of the cypress labile component and both components of the duckweed were set at zero.

An analysis of variance by the least squares method was used to test the closeness of fit of the observed cypress leaf decomposition rates to two decomposition models: 1) the two-compartment model,

$$y = pe^{-k_1 t} + (1-p)e^{-k_2 t}, \quad (4)$$

where p is the percentage that undergoes fast decomposition, and 2) the standard exponential decay model (Equation 2). The residual sum of squares was minimized using the multivariate search procedure of Hooke and Jeeves (1961). The analysis of variance by the least squares method was also used to estimate the fast and slow decomposition rate coefficients and the percent of total litter amounts undergoing fast and slow decomposition for the two-compartment model. Only data from the first six sets of litter bags were used, since later data were confounded by subsequent litter fall.

Sediments and Peat

A portion of each 5 cm section of core was filled into either a 14 cm³ or 19 cm³ vial and dried at 105°C, weighed, ashed at 550°C overnight and then reweighed to determine the grams of organic matter per square meter.

Nitrogen concentration was determined by the micro-Kjeldahl method after the samples were ground by mortar and pestle. A 100 mg subsample (dry wt) was mixed with 3 ml concentrated H₂SO₄, 10 g K₂SO₄, and 0.3 g CuSO₄ in a 30 ml micro-Kjeldahl flask and allowed to digest. The flasks were brought up to 30 ml with distilled water, and 4 ml of 40 percent KOH were added to a 5 ml aliquot of the digestate. This was distilled into 10 ml of a 2 percent boric acid solution and then titrated with 0.01 N HCl. Duplicate samples were within 5 percent after subtracting the blank. Table 2-4 lists the means, standard deviations, relative errors, and coefficients of variation for primary standards and a peat sample. The areal concentration (g N/m²) calculated for each core was then multiplied by the area circumscribed by the core location. The amount of nitrogen (kg) calculated for each area was then summed to estimate the total nitrogen in the swamp floor of each dome.

Oxidation-reduction potentials of the waterlogged swamp floors were measured during dry conditions in July 1977 with an Orion No. 9678 combination electrode positioned 6 ± 2 cm below the sediment surface. Calibration of electrode and potentiometer was checked before each measurement with a Fe⁺²/Fe⁺³ standard solution (Light 1972), and any deviation from the standard potential of +430 mv was compensated for in the potentials measured on the sediments. Adjustments were

Table 2-4. Analysis of the precision and accuracy of the micro-Kjeldahl method used in the determination of total N in the peat and decomposing leaf litter.

Standard or Sample	%N	\bar{X}	S.D.	Rel. Error	C.V.
L-Tyrosine	7.98	7.59	0.42	1.8%	5.5%
	7.83				
	7.50				
	7.04				
Sulfanilimide	15.85	15.63	0.39	3.9%	2.5%
	16.05				
	15.29				
	15.83				
	15.14				
Peat from center of sewage dome 2	1.77	1.83	0.08	--	4.2%
	1.76				
	1.82				
	1.95				
	1.85				

made in the measured potentials to a standard hydrogen electrode reference electrode (Light 1972) at pH 7 (Patrick and Mahapatra 1968).

Denitrification

Surface peat samples were collected from the centers of the Austin Cary natural dome and sewage dome 2 on October 28, 1979, by immersing containers and displacing the surface water in the containers with the upper 3 cm of peat. Experiments were initiated in the lab within three hours of sample collection.

Portions of wet peat (10 ml; 1 g dry weight) were placed in 250-ml Erlenmeyer flasks. The flasks were closed with one-hole neoprene stoppers whose bottoms were coated with silicon vacuum grease (Dow Corning) to prevent N_2O absorption. A Nalgene quick-disconnect connector (9/16 to 3/8 in. diameter) with a serum stopper fastened at one end was inserted into each one-hole stopper. The flasks were evacuated three times and refilled with helium to 1.0 atm to render the flasks anaerobic. Blackmer and Bremner (1977) have reported that denitrification by soil microorganisms is not significantly affected by substitution of He for N_2 in soil atmosphere.

In order to remove the small amounts of endogenous nitrate (0.03 and 0.05 mg $NO_3^- + NO_2^-$ nitrogen/L for the natural and sewage domes, respectively), the flasks were preincubated 19 h at 24°C. Depending on the treatment, $NaNO_3$ (1 mg NO_3^- -N/L final concentration or 100 μ g NO_3^- -N/g peat (dry wt)) was added either by itself or in combination with glucose (20 mg/L final concentration or 0.2 percent by weight with peat (dry wt)) and/or $NaHCO_3$ (300 mg/L alkalinity as $CaCO_3$ final concentration). All the above spikes were dissolved in 1 ml deionized water and added by syringe through the stoppers. In flasks where

inhibition of the N_2O reductase was desired, enough C_2H_2 was injected to yield a final concentration of 0.094 ml C_2H_2 /ml H_2O . Excess pressure was then released by piercing the serum stopper with a hypodermic needle to attain an initial internal pressure of 1.0 atm. The flasks were swirled and incubated statically in the dark at 22°C. Controls without C_2H_2 and added NO_3^- always showed negligible amounts of N_2O production. The amount of $(NO_3^- + NO_2^-)$ -N remaining in solution (after compensating for that remaining in controls) after the incubation period was subtracted from the 1.0 ppm nitrate nitrogen spike when calculating the percentage nitrate denitrified.

In order to determine the amount of N_2O dissolved in the peat suspensions and to check for leakage and absorption of N_2O by the stoppers, duplicate flasks prepared as above each received 1 ml of a saturated $HgCl_2$ solution to stop microbial activity. Each flask then received 247 nM N_2O , and 0.1 atm C_2H_2 (0.094 ml C_2H_2 /ml H_2O). The amount of N_2O in the gas phase was determined after equilibration, and the amount dissolved in the suspension was calculated as the difference between that added and that found in the gas phase.

At appropriate intervals, 50 μ l of gas phase was removed and diluted to 500 μ l with ambient air in a 1 ml gas-tight syringe (B-D tuberculin plastic syringe). All flasks were swirled vigorously prior to gas sampling. Nitrous oxide analysis was made by injection of 500 μ l gas samples into a Tracor 222 gas chromatograph equipped with a ^{63}Ni electron capture detector. Separation of N_2O from other gases was achieved with a 4 m x 0.32 cm column of Porapak Q (100-120 mesh), using an Ar (95 percent)/methane (5 percent) carrier gas at a flow rate of 20 ml/min. The column was run at 50°C following a

conditioning period at 150°C, and the detector temperature was 370°C. Full-scale deflection at 1 mV on the recorder corresponded to 2.0 nM N₂O/ml gas phase, and minimum detectable N₂O concentration in the gas phase of the bottles was 0.01 nM/ml. Nitrous oxide retention time was just under 4 minutes. Peak height was linearly proportional to concentration in the range of 0.01 to 1.0 nM/ml.

Calibration for N₂O was carried out by preparing flasks of standard gas to appropriate concentrations. All results presented here are means of triplicate flasks, except for the control flasks poisoned with HgCl₂ and spiked with N₂O and C₂H₂. Duplicate flasks were used for this treatment. Accuracy and precision of the gas chromatograph and injection techniques employed in the acetylene blockage experiments are presented in Table 2-5.

Table 2-5. Analysis of the precision and accuracy of the Tracor 222 and Tracor 550 gas chromatographs used in the measurement of N₂O during acetylene blockage experiments.

Standard or Sample	Range (nM/ml)	\bar{x} (nM/ml)	S.D. (nM/ml)	Rel. Error	C.V.
1.0 nM/ml primary standard injected into the Tracor 222 gas chromatograph	0.875-1.025	0.969	0.055	3.1%	5.7%
Sample from nitrate-spiked leaching column injected into the Tracor 550 gas chromatograph	16.4-18.5	17.3	0.92	--	5.3%

Nitrification

Surface sediments. Surface sediments from sewage dome 2, sewage dome 1, groundwater control dome, and Austin Cary natural dome were obtained on July 2 and July 30, 1978, using two methods. The first method utilized a sterile 50 cc plastic syringe with a small tygon tube attached to the aperture. The tygon tubing was then brought into contact with the surface of the swamp floor while the syringe remained above the surface water level. The syringe plunger was then slowly raised while the Tygon tubing was moved over the surface of the sediments. After the barrel of the syringe was nearly filled with a sediment-water mixture, the Tygon tubing was removed and the plastic cap inserted into the needle aperture. The syringe was then placed in ice and returned to the laboratory. The second method of sample collection involved piston coring of the peat and sediments. The cores were stored at 4^oC until they were extruded in the lab 24 h after taking the cores in the field. Only the top 2 cm of each core was used in the MPN test for sutotrophic nitrifiers. In all cases, duplicate cores and duplicate syringes were taken at each site.

The Most Probable Number (MPN) method of Alexander and Clark (1965) was used to enumerate autotrophic nitrifiers. Samples were processed within 24 h of field sampling, and the method was slightly modified by substituting Bray's nitrate-nitrite powder (Bray 1945) for the Griess-Ilosvay reagent.

Surface waters. Surface water was collected from the Austin Cary natural dome on May 3, 1979, and immediately filtered through a Whatman glass fiber filter (grade 934 AH). A portion of the filtrate was then eluted through a DEAE cellulose (Cellex D) column

(bed volume = 50 cc) at a flow rate of 100 ml/h to remove the organic color. The column, which had been stored in a 0.02 percent NaN_3 solution to prevent bacterial contamination, was eluted with 3,000 ml (equivalent to 60 bed volumes) of .05 M NaCl (pH adjusted to 4.6) prior to color removal. Both the filtered colored and decolored water were kept at 4°C until the initiation of the experiment 72 h later.

Two-hundred milliliters of either the colored or decolored water were poured into BOD₅ bottles and spiked with NH_4^+ -N to a final concentration of 5 mg NH_4^+ -N/L. The following four treatments were performed on duplicate bottles:

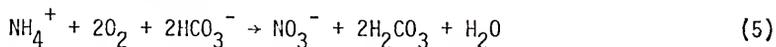
1. Colored water with pH adjusted to 8.5 with 500 mg NaHCO_3 /L
2. Decolored water with pH adjusted to 8.5 with 500 mg NaHCO_3 /L
3. Colored water with pH unadjusted but 350 mg NaCl/L added
4. Decolored water with pH unadjusted but 350 mg NaCl/L added

Table 2-6 lists the initial conditions of the experimental and control bottles.

Table 2-6. Initial conditions of the experimental and control bottles for the nitrification experiment on the surface water from Austin Cary natural dome. Each value is the mean of duplicate bottles.

Treatment	pH	Color (CPU)	$(\text{NO}_3^- + \text{NO}_2^-)\text{-N}$ (mg/L)	$\text{NH}_4^+\text{-N}$ (mg/L)	Alkalinity (mg CaCO_3 /L)
Experimental					
Colored-pH adjusted	8.5	935	0.08	5.1	300
Decolored-pH adjusted	8.5	0	0.08	5.1	300
Control					
Colored-pH unadjusted	4.5	705	0.06	5.1	0
Decolored-pH unadjusted	6.1	0	0.07	5.2	0

The pH of 8.5 is within the optimum range for autotrophic nitrification (Engel and Alexander 1958). The high initial alkalinity (300 mg CaCO₃/L) was needed to buffer the medium from the generation of acid that accompanies nitrification:



According to the stoichiometry of the above reaction, 7.1 mg of alkalinity (as CaCO₃) is lost per mg of NH₄⁺-N oxidized to NO₃⁻. An initial alkalinity of 300 mg CaCO₃/L thus could titrate the acidity produced by oxidation of 42 mg NH₄⁺-N/L. Since the initial concentration of NH₄⁺ was only 5 mg N/L, adequate alkalinity was available to insure a constant pH throughout the experiment.

The NaCl was added to the two unbuffered controls in order to maintain an ionic strength equal to that of the pH-buffered experimental bottles.

To insure that an adequate seed of nitrifying organisms would be present, each BOD bottle was inoculated with 0.2 ml of activated sludge collected immediately beforehand from the effluent end of the Gainesville-Kanapaha Sewage Treatment Plant aeration tank. The MPN method of Alexander and Clark (1965) described above indicated an autotrophic nitrifier population of 4.9 x 10⁵ cells/ml in the inoculum, yielding 4.9 x 10² cells/ml present initially in the experimental and control bottles.

The bottles were stoppered and incubated in the dark on a shaker at 22.5⁰C. A 1 ml sample was withdrawn from each bottle for analysis of ammonium and nitrate plus nitrite periodically over a 195 h incubation period, and the pH of each bottle was also measured at these times.

Nitrogen fixation

Nitrogenase activity associated with various samples from sewage dome 2 and Austin Cary natural dome was assayed by the acetylene reduction method (Stewart et al. 1967; Hardy et al. 1973). Samples collected from each dome included standing water, leaf litter, peat, lichens (Usnea sp. and Parmelia sp.), Spanish moss (Tillandsia usneoides), bladderwort (Utricularia sp.), Azolla caroliniana, duckweed (Spirodela oligorhiza), and the roots of pond cypress (Taxodium distichum var. nutans), black gum (Nyssa sylvatica var. biflora), and buttonbush (Cephalanthus occidentalis). Acetylene was generated from calcium carbide and water immediately before use. All samples were analyzed for ethylene by injecting 500 μ l of gas into a Varian-Aerograph model 600 D gas chromatograph with hydrogen flame ionization detector and a 2.7-m x 0.3-cm column packed with Poropak R. Nitrogen was the carrier gas (flow rate = 17 ml/min), and the column temperature was 56-57°C. The air and H₂ flow rates were 300 ml/min and 26 ml/min, respectively. Because of problems associated with the use of various metabolic poisons in terminating the acetylene reduction reaction (Jones 1974; Schell and Alexander 1970; Thake and Rawle 1972), injection into the gas chromatograph was performed immediately after incubation whenever possible. Amounts of ethylene were calculated from a calibration curve of peak height determined with dilutions of pure ethylene. A factor of 1.5 based on the theoretical ratio of 1.5 moles of ethylene produced per mole of ammonia fixed (Stewart et al. 1967) was used to convert ethylene production rates to equivalent nitrogen fixation rates (expressed in mg N/g (dry wt)-h).

In all assays, controls without C_2H_2 were set up; however, these almost always proved to be negligible. Ethylene contamination of the acetylene was always known and allowed for in the final calculations.

Leaf litter, peat, and standing water. Monthly surface litter and standing water samples were obtained by submerging a 22 or 70-ml serum bottle to the litter surface, where it was turned upright, allowing bottom water to displace the air inside. Organic matter composed almost entirely of cypress and black gum leaf litter was put in the serum bottles while they were still submerged. The serum bottles were then brought to the surface and immediately capped with a rubber serum stopper so that the organic matter was at no time exposed to the air. They were transported back to the lab on ice and kept refrigerated at $4^{\circ}C$ until injected with acetylene (within 5 h of field collection). A high percent aqueous phase was maintained (~80 percent) to avoid exposing the acetylene-reducing organisms to more oxygen than that present in the bottom waters and to increase the sensitivity of the acetylene-reduction method (Flett et al. 1976). Air and acetylene were injected (3 ml air followed by 10 ml acetylene) to achieve a final concentration of dissolved acetylene of 0.15 ml C_2H_2 /ml H_2O . Air and acetylene were injected by pushing a free hypodermic needle through the stopper of an upright serum bottle so that water was displaced by the gas being injected through a 10 ml glass syringe, thus maintaining a constant pressure and not exposing the inside of the serum bottle to more air than injected. The serum bottles were then vigorously shaken by hand for 15-30 s to equilibrate the vapor and aqueous phases and immediately incubated at in situ water temperatures in the dark for 3 to 25 h. At the end of the

incubation period, the serum bottles were again vigorously shaken for 1 min to equilibrate the vapor and aqueous phases, before 0.5 ml was withdrawn for measurement by gas chromatography of ethylene production. Corrections for ethylene solubility in an equilibrated closed system were made according to Henry's Law (Flett et al. 1976).

To determine vertical variation in acetylene reduction activity (ARA) in peat from the natural dome, two PVC cores were obtained with a piston-corer on March 8 and April 12, 1979. The cores (with overlying water) were sealed by rubber stoppers to prevent exposure to the air, returned to the laboratory, and extruded within 3 h of sampling. Horizontal slices of 2 cm thickness were made by pushing the sediment up inside the core tubes with a piston, and slicing off the sediment at the top. Each 2 cm sample (0-2 cm, 3-5 cm, 6-8 cm, and 8-10 cm) was divided into four subsamples, of which three were incubated under C_2H_2 and one incubated without C_2H_2 (as a control for endogenous production of C_2H_2), using the procedures already described for surface litter assays. Both control and experimental serum bottles were purged with He prior to injection with acetylene to maintain anoxic conditions during the incubation period.

Surface water was assayed regularly to determine the amount of fixation accounted for by the water used to prepare the peat and litter samples for acetylene reduction.

Floating and submerged macrophytes. Samples of macrophytes Azolla caroliniana, Spirodela oligorhiza, Lemna perpusilla, and Utricularia sp. were incubated in an aerobic atmosphere injected with acetylene to yield 12-17 percent acetylene in the gas phase. Two to three replicate samples were incubated under ambient light conditions

simultaneously with 1-2 samples incubated in the dark. Care was taken to equalize the pressure of injected materials. After acetylene addition the 70 ml glass serum bottles (57 percent aqueous phase) fitted with airtight rubber serum caps were incubated for 1 h at ambient water temperatures under natural sunlight. Duplicate sets of controls were incubated simultaneously. One set of controls consisted of a macrophyte sample with no acetylene injected; the other set was injected with 2 ml formalin prior to acetylene injection. Control ethylene peaks were always low to undetectable. Peak heights of the controls were subtracted from peak heights of the samples after corrections for ethylene solubility in an equilibrated closed system were made according to Henry's Law (Flett et al. 1976). After incubation, biological activity was terminated with 2 ml of formalin. To avoid possible leakage, serum caps were wrapped with masking tape; and to avoid possible contamination from the rubber serum stoppers, areas exposed to acetylene during incubation were coated with petroleum jelly.

For Azolla, one to three 0.25 m² plots were harvested from each dome, and the dry weight biomass determined for each assay to obtain an areal rate (g N fixed/m²-h). Daily N₂-fixation was estimated by multiplying mean hourly values of the light bottles by 14. Nitrogenase activity was assumed to be negligible at night (Brotonegoro and Abdulkadir 1976). Yearly values were estimated by integrating the daily rates associated with the period of activity. The area covered by Azolla at the time of incubation was estimated by assuming the area to be rectangular in shape and then multiplying the measured distance of two of its sides.

Rhizosphere-endorhizosphere of woody vegetation. Nitrogenase activity was measured on washed excised roots (<0.4 cm in diameter) from three major tree species (Taxodium distichum var. nutans, Nyssa sylvatica var. biflora, and Cephalanthus occidentalis) by the acetylene reduction method (Stewart et al. 1967). Whole plants or excised roots were taken from the Austin Cary natural dome between August 1978 and August 1979. In addition, pond cypress roots were taken from sewage dome 2 on March 10, 1979, and a pond cypress tree was taken from the margin of a soft lake (Newnans Lake) near Gainesville on October 23, 1978. In the pond cypress root experiments, small trees (DBH = 2-6 cm; height = 182-467 cm) with intact roots were transported in a bucket containing substrate back to the laboratory where the roots were excised. Roots of all other trees were excised in the field, and 2-4 h elapsed between sample collection and C_2H_2 addition (0.1 atm) to excised roots in assay containers. All excised roots were washed free of soil with de-ionized water before being inserted (0.7-4.0 g (fresh wt)) into assay containers (9.5-70 ml) and were incubated either aerobically (ambient air) or anaerobically (He). Incubations were carried out in the dark at room temperature (24-26°C) with de-ionized water or dome water added (filling 10 percent of the container volume) to keep the atmosphere saturated with water. Each C_2H_2 incubation consisted of 3-8 replicates.

Gas samples for analysis of C_2H_4 by gas chromatography were withdrawn on at least four occasions during the ensuing 24-120 h. Acetylene reduction activity was calculated from the linear phase of C_2H_4 production, which generally followed a lag period of 10 to 20 h.

Incubations without C_2H_2 to determine background C_2H_4 consistently yielded negligible quantities of C_2H_4 .

A surface sterilization experiment was conducted on the roots of a 366-cm tall cypress tree (3 cm DBH). The roots were excised in the lab. One-third were immersed (except the proximal cut end) in 10 percent chloramine T (Eastman; $C_7H_7ClNO_2Na \cdot 3H_2O$), another third were immersed (except the proximal cut end) in 1 percent chloramine T, and the remaining third were immersed in 0.05 M sterile phosphate buffer (pH = 7) for 30 minutes, followed by two washes in sterile distilled water. All controls and experimental assay bottles (70 ml capacity) contained 25 ml in situ water in addition to the excised roots. Pressure within the incubation containers was maintained at 1 atm by either bleeding excess pressure with a syringe after adding 5 ml (0.1 atm) C_2H_2 or replacing each 0.5 ml of gas phase withdrawn for injection into the gas chromatograph by 0.5 ml He. In situ water incubated without excised roots in the presence of C_2H_2 produced negligible amounts of C_2H_4 .

CHAPTER THREE
THE EFFECT OF SECONDARY SEWAGE EFFLUENT ON THE SURFACE AND
GROUNDWATER QUALITY OF CYPRESS DOMES

This chapter summarizes the basic water quality data over the five-year period beginning March 1974 and ending in May 1979. Its primary intent is to assess the capability of cypress domes to treat domestic sewage effluent from a water quality standpoint. Emphasis is placed on the parameters considered to be most important as polluting substances in the application of treated sewage to cypress domes.

Standing Water Quality

Surface water in pristine cypress domes (e.g., the Austin Cary dome) is typically very soft, acidic, and highly colored (Table 3-1). The dominant ions (Na and Cl) are derived from rainfall and are present in the surface waters at levels similar to those of bulk precipitation (cf. Chapter 4). In contrast, the surface water of sewage domes 1 and 2 changed considerably with the addition of sewage--to less acidic (pH 6.2-6.4), hard, and turbid. Sodium and chloride concentrations increased greatly (Table 3-1), reflecting dietary use of salt, and remained the dominant ions. Calcium and magnesium (total hardness = 62.5 mg CaCO₃/L) and alkalinity concentrations (59.4 mg CaCO₃/L) were also much higher in the sewage domes than in the natural dome, reflecting the origin of the water from the artesian Floridan Aquifer. Dissolved solids (as indicated by specific conductance) increased more than five-fold, from a mean value of 60 µmho/cm in the

Table 3-1. Summary of the mean concentrations and standard deviations of selected chemical parameters for standing waters from March 1974 to May 1979. All values as mg/L unless otherwise noted.

Parameter	Groundwater			
	Austin Cary	Control Dome center	Sewage Dome 1 center ^a	Sewage Dome 1 edge ^a
Alk (mg CaCO ₃ /L)	1.8 ± 2.9	157 ± 69	91 ± 43	46 ± 29
pH ^b	4.51± 0.36	6.91± 0.52	6.13± 0.64	5.88± 0.75
E _{h7} (mV)		+35 ^c	-185 ^c	-265 ^c
Color (CPU)	456 ±162	179 ±192	334 ±180	646 ±367
Turb (FTU)	2.8 ± 8.7	2.0 ± 1.9	20.8 ± 33.0	8.9 ± 6.9
Cond (µmho/cm)	60 ± 17	368 ±123	373 ±119	278 ±129
D.O.	2.03± 1.79	4.39± 2.90	0.17± 0.38	0.20± 0.71
BOD ₅	2.9 ± 1.5	2.3 ± 1.5	16.7 ± 9.4	11.9 ± 6.8
Si	2.0 ± 1.7	8.3 ± 4.7	---	---
Inorganic C	7.1 ± 5.7	44.2 ± 10.3	23.2 ± 6.7	16.2 ± 10.8
Organic C	39.9 ± 12.9	28.3 ± 33.7	30.5 ± 15.2	42.3 ± 13.9
HCO ₃ ⁻	2.2 ± 3.5	191.5 ± 84.0	110.5 ± 52.3	56.5 ± 35.6
Chloride	8.2 ± 4.2	11.8 ± 4.7	57.5 ± 21.9	46.3 ± 20.3
Fluoride	0.03± 0.01	0.41± 0.17	0.38± 0.20	0.22± 0.11
Sulfate	2.6 ± 2.7	14.1 ± 6.8	28.9 ± 11.9	17.6 ± 18.2
H ₂ S	<0.01± 0.00	<0.01± 0.00	2.00± 2.06	1.06± 1.16
Calcium	2.87± 0.99	45.94± 17.14	18.25± 8.23	10.22± 5.33
Magnesium	1.37± 0.59	16.75± 5.81	11.59± 7.95	6.80± 3.34
Sodium	4.94± 1.60	9.36± 1.48	39.05± 14.97	34.04± 15.19
Potassium	0.34± 0.24	0.89± 0.39	7.96± 3.52	6.48± 3.44
(NO ₃ ⁻ + NO ₂ ⁻) - N	0.08± 0.19	0.09± 0.31	0.38± 0.77	0.14± 0.19
NH ₄ ⁺ - N	0.14± 0.19	0.20± 0.59	4.2 ± 4.7	1.9 ± 2.4

Table 3-1. - extended.

Sewage Dome 1 weir ^a	Sewage Dome 2 center	Sewage Dome 2 40 m north	Sewage Dome 2 30 m south
51 ± 21	101 ± 63	43 ± 24	22 ± 29
6.20± 0.34	6.58± 0.30	5.92± 0.61	5.63± 0.78
	-255 ^b	-110 ^b	-95 ^b
464 ±211	311 ±223	766 ±301	878 ±296
7.0 ± 7.9	9.2 ± 7.8	9.2 ± 9.3	7.8 ± 9.3
266 ±105	461 ±115	323 ±104	275 ±120
0.48± 0.65	0.29± 0.70	0.11± 0.28	0.88± 1.56
8.5 ± 3.8	11.8 ± 6.0	---	7.7 ± 5.0
---	7.5 ± 4.1	6.0 ± 2.8	4.4 ± 2.3
15.7 ± 6.7	23.4 ± 6.2	14.6 ± 5.9	9.8 ± 5.6
35.3 ± 6.6	41.8 ± 35.6	42.3 ± 14.6	55.8 ± 15.3
61.9 ± 25.6	122.7 ± 77.2	52.5 ± 29.0	26.9 ± 35.8
46.4 ± 13.5	46.9 ± 18.5	46.2 ± 20.9	55.3 ± 26.1
0.34± 0.19	0.44± 0.15	0.28± 0.10	0.21± 0.13
21.6 ± 13.6	34.4 ± 12.6	26.2 ± 13.8	15.1 ± 13.9
0.46± 1.4	1.09± 1.74	2.09± 2.12	0.10± 0.27
10.25± 3.73	20.98± 7.83	10.37± 6.47	6.89± 5.16
5.82± 1.97	9.60± 3.39	6.14± 2.49	5.09± 3.21
30.78± 11.94	43.25± 11.13	36.69± 11.64	35.91± 14.86
5.34± 2.95	8.43± 3.57	6.45± 3.58	5.05± 3.14
0.21± 0.39	0.27± 0.35	0.17± 0.31	0.14± 0.16
1.9 ± 2.1	8.5 ± 11.1	3.8 ± 5.1	3.1 ± 5.4

Table 3-1. - continued.

Parameter	Austin Cary	Groundwater Control Dome center	Sewage Dome 1 center ^a	Sewage Dome 1 edge ^a
Organic N	1.4 ± 1.4	1.0 ± 1.0	5.9 ± 7.0	6.0 ± 5.2
Total N	1.6 ± 1.3	1.2 ± 1.4	11.9 ± 8.7	8.1 ± 6.4
Ortho-P	0.07± 0.11	0.09± 0.11	4.6 ± 2.9	3.3 ± 2.5
Organic P	0.11± 0.32	0.18± 0.43	2.0 ± 3.1	1.7 ± 2.3
Total P	0.18± 0.38	0.27± 0.44	6.5 ± 3.7	4.9 ± 3.7

^a Covers the period of sewage pumping only (March 1974 to September 1977).

^b H⁺ is normally distributed; therefore pH is normally distributed and the mean indicates the central tendency toward a particular value.

^c 5-8 cm below sediment surface.

Table 3-1. - extended continued.

Sewage Dome 1 weir ^a	Sewage Dome 2 center	Sewage Dome 2 40 m north	Sewage Dome 2 30 m south
4.0 ± 4.2	8.4 ± 13.6	5.0 ± 5.0	7.3 ± 10.3
6.3 ± 5.0	17.1 ± 17.7	9.0 ± 6.4	10.6 ± 10.5
3.6 ± 2.0	6.7 ± 7.8	4.1 ± 1.9	3.8 ± 1.8
2.1 ± 2.9	1.7 ± 2.3	2.0 ± 3.0	1.5 ± 1.8
5.8 ± 3.7	8.4 ± 8.2	6.1 ± 2.7	5.3 ± 2.6

natural dome to 329 $\mu\text{mho/cm}$ in the sewage-enriched domes. Sulfate and fluoride also were much higher in the sewage domes than in the natural dome, reflecting the high levels normally associated with sewage (Table 3-1).

The dominant ions in the groundwater control dome were calcium and bicarbonate, reflecting the limestone aquifer that supplies the water to the dome. Magnesium also was high, suggesting a dolomitic limestone aquifer, and total alkalinity in the groundwater control dome was the highest (119 mg CaCO_3/L) of the four domes. The monovalent/divalent cation ratio was only 0.16 (based on equivalent weight), indicating the dominance of limestone as a source of dissolved solids in the groundwater. Both sulfate and fluoride levels were relatively high in the groundwater control dome, corresponding to the levels of these ions observed in the groundwater influent. However, levels of dissolved solids were not so high as those in the sewage domes (Table 3-1).

In the natural dome the mean dissolved oxygen level was 2.0 mg/L. Higher dissolved oxygen levels (3.8 mg/L) were found in the groundwater dome, but the sewage domes were usually anoxic because of both the biological oxygen demand originating from the effluent and the secondary BOD from induced plant growth. The high levels of sulfate in the effluent, coupled with the anaerobic condition of the surface water, frequently resulted in production of sulfide. However, total organic carbon did not increase with the addition of treated sewage; for example, TOC averaged 39.9 mg/L in the Austin Cary dome and 30.5 mg/L and 41.8 mg/L in the centers of sewage dome 1 and sewage dome 2, respectively. Nevertheless, the levels of inorganic carbon accompanying

the treated sewage influent caused the total carbon at the centers of both sewage domes to increase to concentrations higher than the natural dome.

Apparent color (determined on raw samples without filtration or centrifugation) was lower at the centers of the sewage domes and groundwater control dome than at the Austin Cary dome, but water at the edges was similar to the natural dome. Addition of treated sewage resulted in higher levels of turbidity.

Ammonium, organic nitrogen, orthophosphate, and total phosphate concentrations were much higher in the surface waters of the sewage-enriched domes than in either control dome (groundwater control dome and Austin Cary). In addition, ammonium comprised a larger fraction and organic nitrogen a smaller fraction of the nitrogen in the sewage domes than in the other two. Nitrate plus nitrite rarely exceeded 10 percent of the total nitrogen concentration in any dome. The C:N:P ratio was also lowered by the addition of sewage, reflecting the large amount of phosphorus added with the sewage.

Concentrations of nitrogen and phosphorus species, pH, BOD, Ca, Mg, Na, K, SO_4 , inorganic carbon, alkalinity, F, and specific conductivity decreased from the center of the sewage domes to the edges (Table 3-1). Chloride decreased from center to edge in sewage dome 1 but not in sewage dome 2. On the other hand, concentrations of TOC and color increased near the edges of the sewage domes. Since the sewage had high levels of all the above parameters (except TOC and color) compared to natural cypress dome standing water, the decreasing concentrations toward the edges may be partially explained by simple dilution.

Dissolved oxygen levels in the sewage domes were essentially zero at the center and were very low (often zero) at the edges. This reflects both the biochemical oxygen demand of the sewage and low rates of reaeration in the quiet standing water.

The two edge stations in sewage dome 2 differ some in water quality. Chemical parameters associated with the incoming sewage (i.e., fluoride, SO_4 , H_2S , alkalinity, pH, ortho and total P) were consistently higher at the north station than at the south station of sewage dome 2.

Concentrations of total and ortho P generally increased with distance from the center in the groundwater control dome. The groundwater pumped into the center of this dome has lower levels of nutrients than are found in surface waters of natural domes (Tables 3-1 and 3-2). Organic carbon, BOD, K, Na, and color also increased at the edges. On the other hand, Ca, Mg, F, SO_4 , inorganic carbon, alkalinity, pH, dissolved oxygen, and specific conductivity were more concentrated at the center of groundwater control dome than at the edges. The variations in these parameters simply reflect the composition of the groundwater, which is pumped from a limestone aquifer. The higher concentrations of dissolved oxygen at the center can be attributed to physical aeration from the pumping action, to a large standing crop of filamentous algae (Spirogyra sp.) often present at the center, and to the oxygen demand and consequently normally depressed dissolved oxygen values of the colored waters near the edge.

Table 3-2. Summary of the mean concentrations and standard deviations for selected parameters of composited shallow groundwaters from April 1974 to May 1979. All values as mg/L unless otherwise noted.

Parameter	Deep Groundwater Well	Control Wells		Control Wells		Wells		Well 19
		Distant Groundwater Control Dome	From Groundwater Control Dome	Surrounding Groundwater Control Dome	Wells Inside Sewage Dome 1	Surrounding Sewage Dome 1 and Sewage Dome 2		
Alk (mg CaCO ₃ /L)	228 ± 5	13 ± 15	13 ± 15	5 ± 9	25 ± 40	8 ± 26	48 ± 64	
pH	7.21± 0.32	5.38± 0.66	5.38± 0.66	5.05± 0.76	4.83± 0.76	4.74± 0.77	5.74± 0.57	
Color (CPU)	20 ± 25	123 ± 126	123 ± 126	132 ± 109	53 ± 39	230 ± 224	297 ± 206	
Turb (FTU)	1.3 ± 1.5	63.9 ± 114.9	63.9 ± 114.9	39.0 ± 82.8	7.0 ± 8.3	19.8 ± 36.0	53.3 ± 67.5	
Cond (µmho/cm)	432 ± 98	90 ± 67	90 ± 67	173 ± 36	125 ± 61	126 ± 60	131 ± 37	
Si	---	3.9 ± 1.9	3.9 ± 1.9	5.1 ± 0.9	---	4.8 ± 0.3	---	
Inorganic C	53.4 ± 4.2	6.4 ± 5.3	6.4 ± 5.3	8.0 ± 7.5	18.9 ± 18.3	11.2 ± 10.3	8.6 ± 6.9	
Organic C	6.5 ± 6.2	17.6 ± 16.9	17.6 ± 16.9	23.2 ± 16.9	13.0 ± 11.2	26.2 ± 19.9	21.4 ± 35.2	
HCO ₃ ⁻	278.2 ± 6.4	16.6 ± 17.8	16.6 ± 17.8	6.5 ± 10.7	30.0 ± 48.6	10.3 ± 31.8	58.8 ± 78.0	
Chloride	7.0 ± 5.6	9.6 ± 3.5	9.6 ± 3.5	40.3 ± 6.7	20.7 ± 12.7	36.3 ± 20.5	22.0 ± 6.9	
Fluoride	0.53± 0.21	0.18± 0.18	0.18± 0.18	0.12± 0.03	0.17± 0.18	0.14± 0.20	0.72± 0.29	
Sulfate	19.4 ± 0.9	5.4 ± 5.7	5.4 ± 5.7	5.1 ± 9.1	2.6 ± 4.7	4.5 ± 7.1	9.2 ± 11.3	
H ₂ S	---	---	---	---	---	---	---	
Calcium	64.81± 7.73	9.54± 8.40	9.54± 8.40	9.23± 5.90	7.86± 7.47	6.95± 8.18	17.16± 5.32	

Magnesium	20.75±	4.91	1.50±	1.24	2.50±	0.52	4.18±	4.28	1.55±	1.13	3.42±	0.90
Sodium	9.44±	1.26	5.62±	1.84	10.14±	1.50	8.37±	2.40	10.04±	5.71	5.33±	1.28
Potassium	0.84±	0.29	0.43±	0.34	0.44±	0.18	0.35±	0.28	0.45±	0.36	0.64±	0.21
(NO ₃ ⁻ + NO ₂ ⁻) - N	0.03±	0.02	0.09±	0.18	0.11±	0.27	0.03±	0.03	0.10±	0.26	0.10±	0.18
NH ₄ ⁺ - N	0.04±	0.03	0.26±	0.94	0.15±	0.17	0.07±	0.06	0.28±	0.29	0.09±	0.11
Organic N	0.39±	0.26	0.62±	0.47	0.63±	0.56	0.71±	0.50	0.70±	0.89	1.04±	2.15
Total N	0.46±	0.27	0.86±	0.60	0.83±	0.42	0.80±	0.49	1.04±	0.82	1.23±	2.00
Orcho-P	0.06±	0.05	0.21±	0.33	0.11±	0.21	0.08±	0.12	0.32±	1.24	1.8 ±	3.0
Organic P	0.10±	0.14	0.20±	0.24	0.11±	0.14	0.09±	0.12	0.16±	0.34	0.5 ±	0.8
Total P	0.16±	0.17	0.41±	0.42	0.21±	0.27	0.17±	0.17	0.49±	1.30	2.3 ±	2.9

Surface Water Quality Following the Cessation of Sewage Pumping to
Sewage Dome 1

The treated sewage influent pumped to sewage dome 1 was discontinued in September 1977. Sampling was continued on an irregular schedule (depending on the presence of standing water) for the following 20 months in order to determine the extent of recovery in surface water quality. Wide variations were observed for most water quality parameters during this period (Table 3-3). These variations resulted from two major factors: 1) elapsed time since cessation of sewage influent; and 2) the frequency, extent, and intensity of rainfall prior to sampling.

Thus, because of an extended dry period (October-November 1977), the first sampling of the surface water during recovery (December 1977) yielded higher levels of specific conductance, minerals, nutrients, total carbon, BOD and turbidity than occurred in any other subsequent period. Higher levels of major cations and sulfate were found than occurred in sewage dome 1 standing water during sewage loading. The oxidation of reduced sulfur that had been immobilized as organic matter or ferrous sulfide under the low redox potential present during sewage pumping apparently accounted for the unusually high (237 mg/L) sulfate level after the dry period. Other sampling dates on which high concentrations were found for the water quality parameters (May 1978, and February and May 1979) were also preceded by dry periods that resulted in the disappearance of standing water from the dome, thus allowing a build-up of the products of aerobic decomposition and oxidation.

The sampling dates on which the lowest concentrations were found for the water quality parameters (February, March, and August 1978)

Table 3-3. Changes in water quality at the center station of sewage dome 1 after cessation of sewage loading on September 16, 1977. All values as mg/L unless otherwise noted.

Date	Conduc- tivity ($\mu\text{mho/cm}$)	pH	Ca	Mg	Na	K	SO ₄	HCO ₃ ^a	Cl	F
8/2/77 ^b	520	6.80	18.3 ^c	11.6 ^c	39.0 ^c	8.0 ^c	28.9 ^d	125.6	63.0	0.65
12/16/77 ^e	547	5.10	41.9	23.2	75.4	12.8	237.0	4.9	65.0	0.13
2/1/78	151	5.00	3.5	3.5	18.2	2.0	9.0	7.3	26.0	0.08
3/7/78	73	3.30	2.3	1.5	7.3	3.9	43.0	0.0	8.5	0.06
5/6/78 ^e	269	5.25	8.6	5.8	24.5	6.7	60.0	8.5	22.5	0.11
8/4/78	35	4.21	2.6	1.0	2.6	2.5	<1.0	0.0	2.5	0.04
2/22/79 ^e	119	4.60	4.8	2.3	13.0	1.3	18.0	0.0	14.2	0.08
5/26/79	100	5.43	4.2	2.0	9.0	1.2	10.0		17.0	

^a Calculated from $\text{H}^+ - \text{HCO}_3^-$ equilibrium using alkalinity.

^b Last date of sampling before cessation of treated sewage loading.

^c Mean of 17-18 surface water samples taken at dome center from April 1974 - August 1976 during sewage loading. No measurements were made from September 1976 - September 1977.

^d Mean of 5 surface water samples taken at dome center from August 1975 - March 1977 during sewage loading. No measurements were made from April 1977 - September 1977.

^e Dry period with no standing water preceded sampling date.

Table 3-3. - extended.

NO_3^- -N and NO_2^- -N	NH_4^+ -N	Org. N	TN	TP	TC	Si	BOD_5	H_2S	D.O.	Tur- bidity (NTU)	Color (CPU)
4.10	8.30	1.2	13.6	8.10	38.5		26.8	6.2	1.05	18.0	148
<0.01	4.50	3.3	7.8	2.72	98.0	15.2	18.8	<0.01	0.00	13.0	380
0.02	1.50	0.4	1.9	1.44	72.0	4.3	3.3	<0.01	4.25	4.0	620
0.04	1.85	0.4	2.3	0.69	100.5	0.5	2.8		4.10	0.0	479
<0.01	0.92	2.1	3.0	2.40		3.9	10.0	<0.01	0.35	0.0	522
0.04	0.22	2.8	3.0	0.40	57.5	1.3	2.6	<0.01	0.00		800
0.01	0.08	1.2	1.3	0.50			3.5	<0.01	6.10		683
0.03	1.18	2.0	3.2	1.20	82.0	1.0	2.9	<0.01	0.30		1200

were preceded by extensive rainfall that diluted the pollutants remaining from the period of sewage loading. On one occasion (August 1978), all water quality parameters except potassium and total phosphorus were within the ranges measured for the natural dome.

The effects of the sewage could still be discerned 20 months after sewage inputs had ceased. Specific conductance, major ions, fluoride, ammonium, and total phosphorus were still higher than the mean values reported for the natural dome (see Tables 3-1 and 3-3). Thus, the sediments and vegetation on the swamp floor continued to release these substances to the standing waters long after the end of sewage pumping. However, many important water quality parameters, including pH, nitrate, BOD, total carbon, H_2S , dissolved oxygen, turbidity, color, and silica, returned to natural background levels within 8 months after discontinuation of sewage pumping.

Removal of Major Pollutants

Conventional Treatment Plant/Dome System

The ability of cypress domes to reduce the levels of sewage pollutants in their surface and groundwaters is illustrated in Figures 3-1 to 3-4. Stations have been combined into four major groups; Tables 3-1, 3-2, and 3-4 give the mean values and standard deviations for the parameters for the surface water and composited groundwater stations. The first three (package plant and oxidation pond; sewage dome surface water; and wells) follow the route of the sewage through the treatment plant and cypress dome system, and the fourth major grouping represents a control: the surface water of the Austin Cary dome. The first bar in the treatment plant group represents the raw sewage influent; the second is treated sewage effluent; the third is

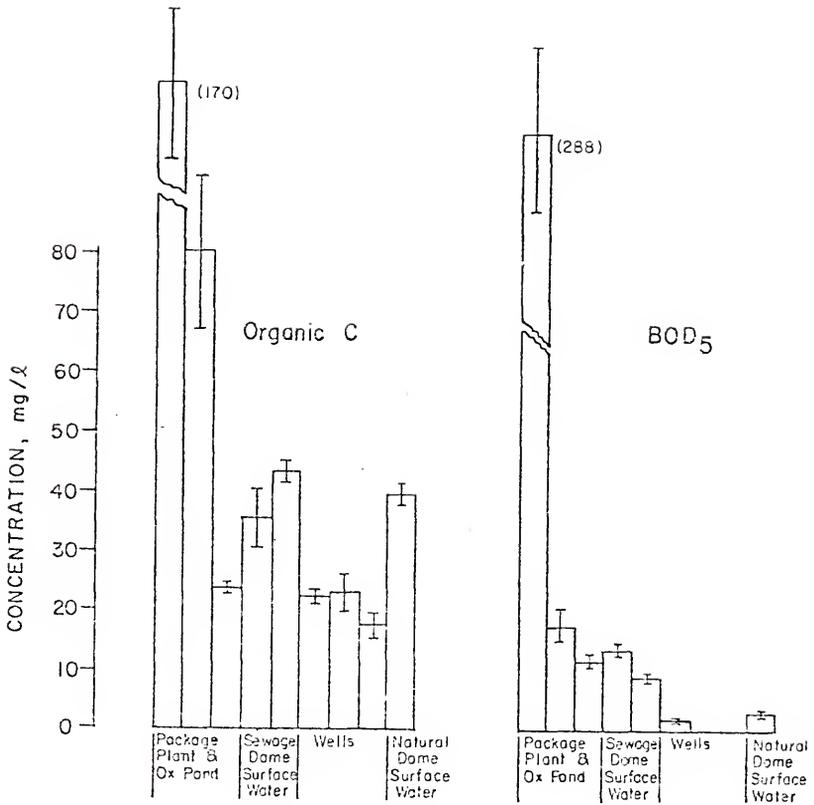


Figure 3-1. Average concentrations (± 1 S.E.) of organic carbon and biochemical oxygen demand found at various points within the conventional treatment plant/dome system and in natural waters representing control sites over the entire study period (March 1974-May 1979).

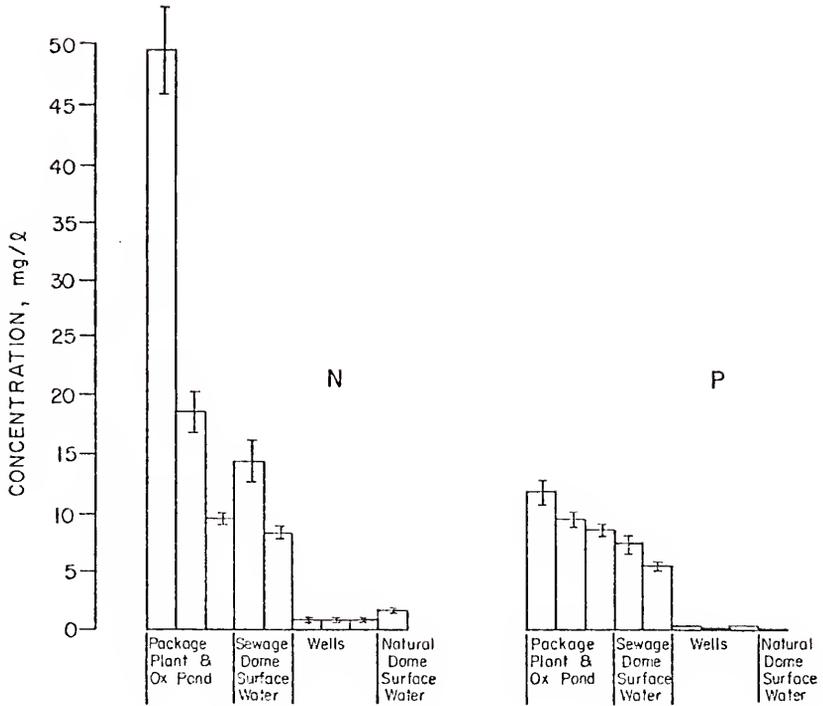


Figure 3-2. Average concentrations (± 1 S.E.) of total nitrogen and total phosphorus found at various points within the conventional treatment plant/dome system and in natural waters representing control sites over the entire study period (March 1974-May 1979).

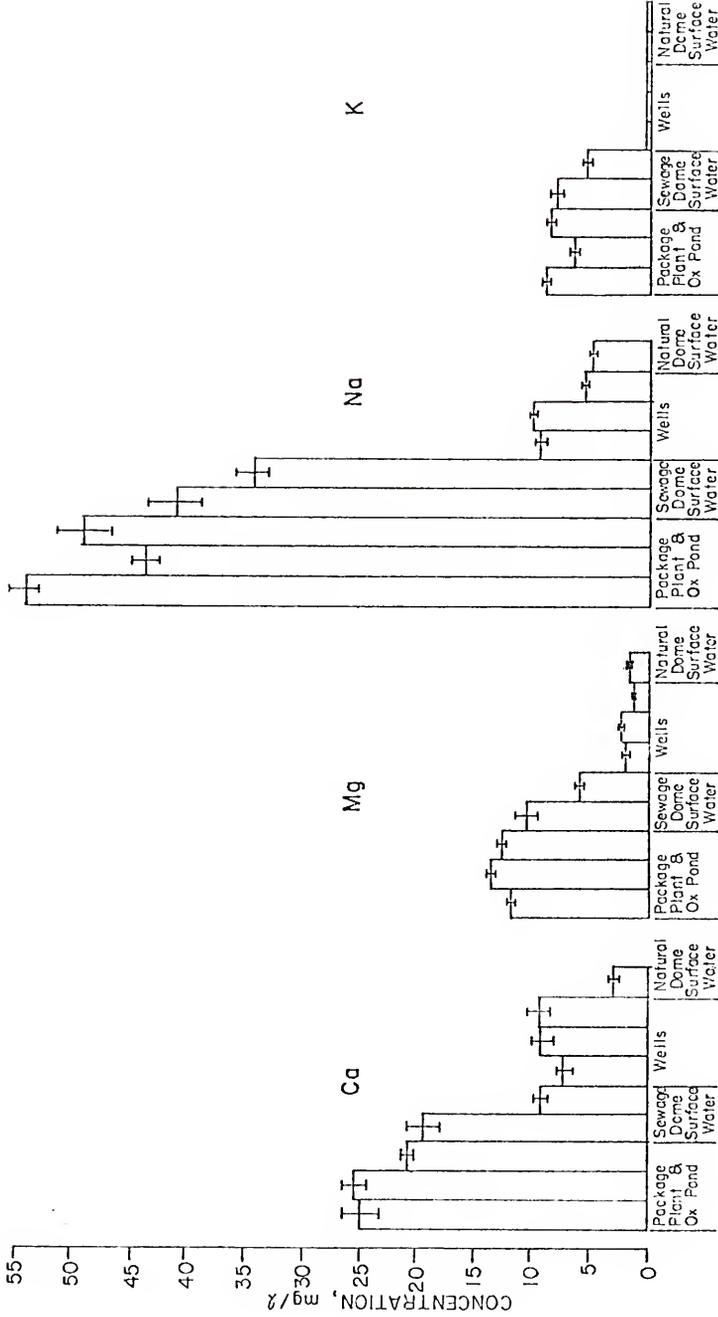


Figure 3-3. Average concentrations (± 1 S.E.) of calcium, magnesium, sodium, and potassium found at various points within the conventional treatment plant/dome system and in natural waters representing control sites over the entire study period (March 1974-May 1979).

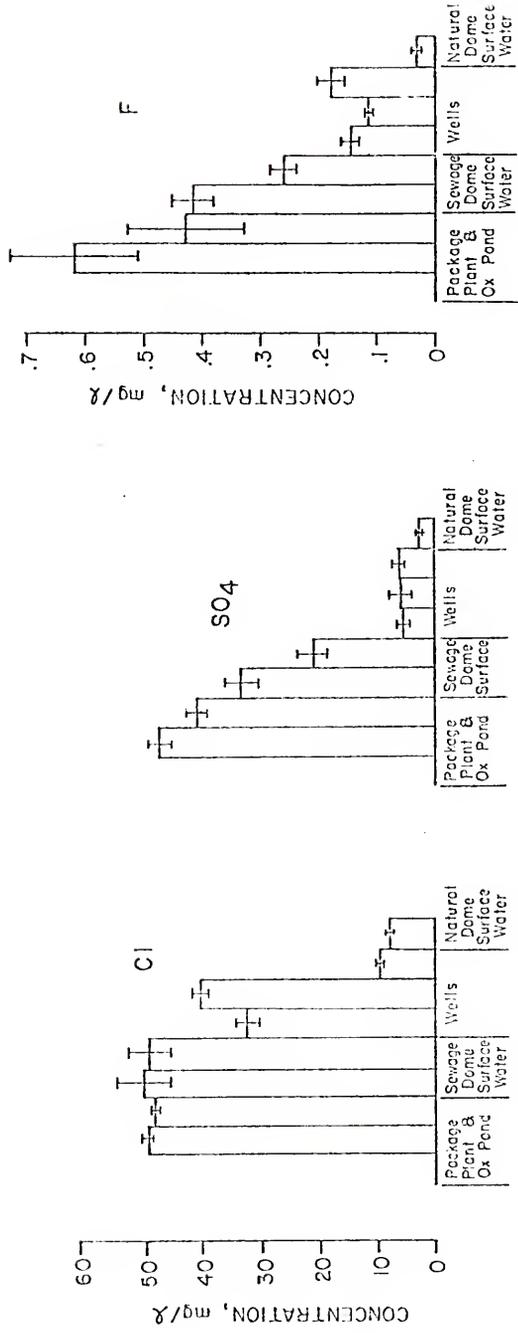


Figure 3-4. Average concentrations (± 1 S.E.) of chloride, sulfate, and fluoride found at various points within the conventional treatment plant/dome system and in natural waters representing control sites over the entire study period (March 1974-May 1979).

Table 3-4. Summary of the mean concentrations and standard deviations of selected chemical parameters throughout the treatment plant/oxidation pond treatment system. All values as mg/L unless otherwise noted.

Parameter	Raw Sewage ^a Influent	Treatment Plant ^a Effluent	Oxidation Pond ^a
Alk (mg CaCO ₃ /L)	179 ± 54	84 ± 32	115 ± 20
pH	7.42± 0.36	7.27± 0.56	7.57± 0.52
Color (CPU)	---	90 ± 60	179 ± 85
Turb (FTU)	---	7.7 ± 18.0	15.0 ± 18.1
Cond. (µmho/cm)	---	486 ± 109	444 ± 103
D.O.	---	---	---
BOD ₅	288 ± 147 ^b	18.2 ± 23.5 ^b	11.9 ± 9.2 ^b
Si	---	---	---
Inorganic C	47.7 ± 17.8	19.5 ± 15.1	22.8 ± 8.8
Organic C	170.4 ± 70.0	81.0 ± 86.4	23.8 ± 13.9
HCO ₃ ⁻	218.6 ± 65.5	102.4 ± 39.0	140.5 ± 24.0
Chloride	---	49.7 ± 5.5 ^c	48.0 ± 0.7
Fluoride	---	0.62± 0.22	0.43± 0.18
Sulfate	---	46.6± 4.2	39.6± 1.9
Calcium	25.0 ± 12.0 ^d	25.8 ± 11.4 ^e	21.9 ± 8.3 ^d
Magnesium	11.8 ± 2.1 ^d	13.4 ± 4.9 ^e	12.4 ± 2.8 ^d
Sodium	54.3 ± 7.4 ^f	43.8 ± 9.1 ^g	49.2 ± 7.6 ^f
Potassium	8.9 ± 1.7 ^f	6.5 ± 2.0 ^g	8.7 ± 1.0 ^f
(NO ₃ ⁻ + NO ₂ ⁻) - N	0.52± 0.53	5.8 ± 5.7 ^h	1.8 ± 1.3
NH ₄ ⁺ - N	27.0 ± 15.2	4.3 ± 5.0 ^h	3.7 ± 3.8
Organic N	21.8 ± 12.6	7.2 ± 7.9 ^h	4.1 ± 4.3

Table 3-4. - continued.

Parameter	Raw Sewage ^a Influent	Treatment Plant ^a Effluent	Oxidation Pond ^a
Total N	49.6 ± 20.7	18.7 ± 13.4 ^h	9.6 ± 6.3
Ortho-P	7.2 ± 3.5	7.3 ± 3.2 ^h	7.2 ± 2.3
Organic P	4.5 ± 4.1	2.0 ± 3.1 ^h	1.3 ± 1.7
Total P	11.7 ± 5.0	9.3 ± 4.4 ^h	8.5 ± 2.9

^a Alkalinity, pH, inorganic carbon, organic carbon, calcium, magnesium, nitrate plus nitrite, ammonium, organic nitrogen, orthophosphorus, and total phosphorus from Zoltek and Whittaker (1975); Zoltek (1976); and Zoltek and Neff (1977).

^b Alachua County Pollution Control (1974-78); primarily grab samples.

^c Overman (1974 and 1975).

^d Carriker unpublished data (1974-76); Zoltek and Whittaker (1975); Zoltek (1976); Zoltek and Neff (1977).

^e Carriker unpublished data (1974-76); Zoltek and Whittaker (1975); Zoltek (1976); Zoltek and Neff (1977); Overman (1974 and 1975).

^f Carriker (1974-76).

^g Carriker (1974-76); Overman (1974 and 1975).

^h Overman (1974 and 1975); Zoltek and Whittaker (1975); Zoltek (1976); Zoltek and Neff (1977).

the oxidation pond. The sewage dome surface waters are divided into center (mean of center stations of both sewage domes) and edge (mean of four stations for both sewage domes) sites. The first of the well histograms represents the pooled averages of wells within sewage dome 1 and surrounding sewage dome 1 and sewage dome 2 (primarily the regularly sampled wells B-3, B-5, B-7, B-9, 4A, 8A, and 19); the second bar is the pooled averages of three wells around groundwater control dome (wells 24, 30, and 31), and the last bar in this group is the average of wells distantly removed from the sewage domes (primarily the regularly sampled wells 22, 28, 33, and 38). (See Figure 2-2 for well locations.) Ranges and the mean levels for water quality parameters of individual wells have been previously reported (Brezonik et al. 1975; Dierberg and Brezonik 1976; Dierberg and Brezonik 1978).

Large decreases in the concentrations of the major cations and anions, organic matter, and nutrients were observed between the surface waters of both sewage domes and the wells surrounding the sewage domes. With the exception of chloride, fluoride, and calcium, the levels found associated with wells within and surrounding the sewage domes were the same as those from control wells. The package plant treatment process resulted in a larger percentage decrease in organic matter and nitrogen than in the other parameters. Only small reductions were measured in the surface waters of the sewage domes compared to the influent levels, with calcium, magnesium, and fluoride being the exception.

Total organic carbon and biochemical oxygen demand (BOD)

Biochemical oxygen demand values more closely reflect the effect of sewage effluent on the highly colored surface waters of the cypress domes than does total organic carbon (TOC) (Figure 3-1). The package treatment plant removed 94 percent of the BOD of the raw sewage, and the oxidation pond removed an additional 2 percent. The remaining BOD was then discharged into the cypress domes where small reductions (35 to 38 percent) were observed between the centers and edges of both sewage domes. The groundwater from the four wells surrounding sewage dome 2 indicated removal to levels below those found in the natural dome surface water.

The two experimental domes (Figure 3-5) had BOD levels comparable to the two control domes when the influent pipe to the sewage domes was located far from the treatment plant (October to February 1976). Prior to that and thereafter, BOD levels were significantly higher in both experimental domes, probably from a combination of lower rainfall and higher levels of organic carbon in the incoming sewage. Relocation of the influent pipe to a point closer to the discharge from the treatment plant in late March 1976 increased the input of organic carbon to the domes (Zoltek and Whittaker 1975 and Zoltek 1976). Mean TOC values were 12.3 mg/L for the 8 months prior to relocation and 30.2 mg/L for the 14 months after relocation.

The BOD levels for both control domes normally were less than 5 mg/L, whereas the BOD levels in the sewage domes usually were above 5 mg/L, (the standard of the Florida Department of Environmental Regulation (1965) for finished waters of domestic tertiary sewage treatment). It should be noted that all BOD samples were filtered

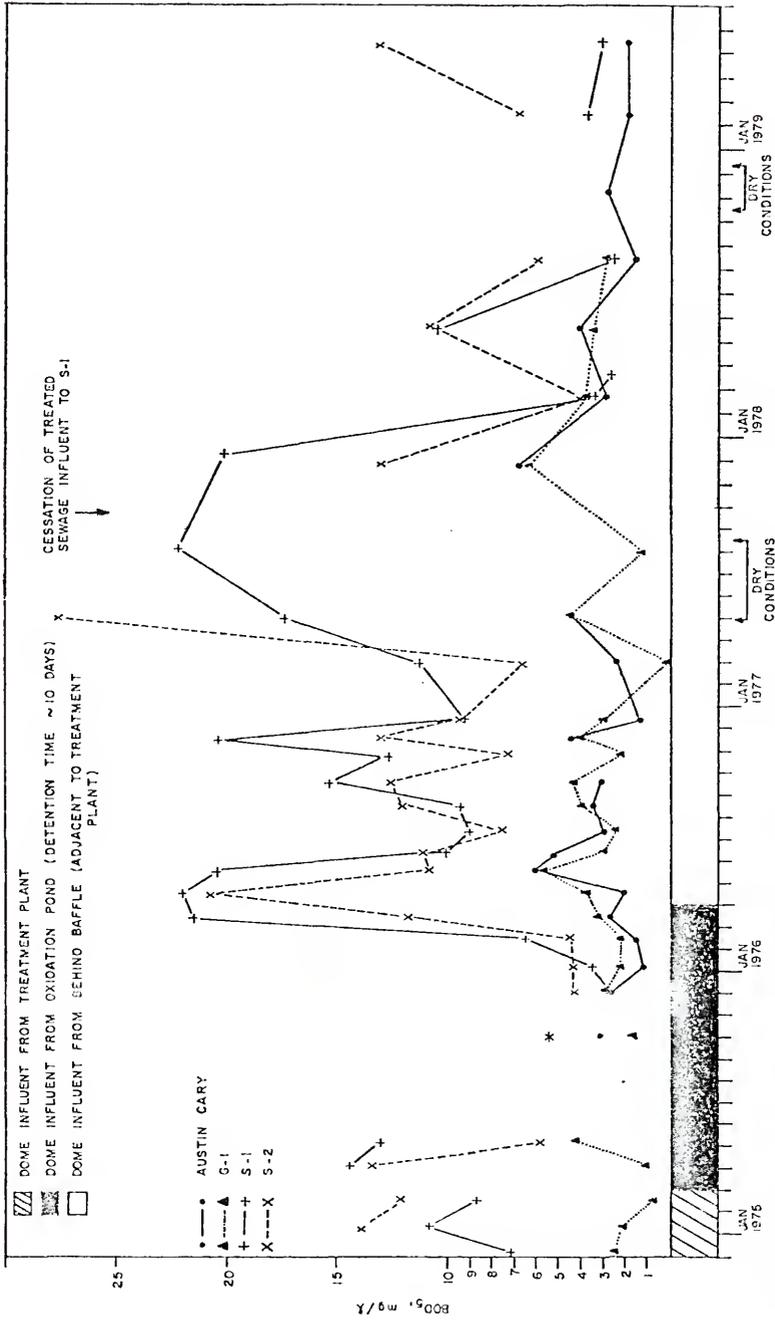


Figure 3-5. Mean BOD_5 levels in the surface waters of control and experimental domes. Each point is the mean of one center and one or two edge stations for each dome, except Austin Cary where only the center station is plotted. (G-1 is the groundwater control dome, S-1 is sewage dome 1, and S-2 is sewage dome 2.)

through Whatman No. 4 qualitative filters to remove duckweed and sediments before incubation. This procedure gave a better measurement of BOD levels attributable to the incoming sewage, but it probably resulted in an underestimate of the true BOD levels.

Nitrogen

The package treatment plant substantially reduced the raw nitrogen influent levels (from 50 to 19 mg/L at the effluent or a 62 percent removal (Figure 3-2)). The oxidation pond further reduced the average concentration by another 19 percent. Higher mean concentrations at the centers of the sewage domes than in the oxidation pond can be explained in part by the fact that secondary effluent with higher concentration was pumped to the domes directly from the treatment plant during 47 of the 60 months of the study. Nitrogen concentrations in the wells surrounding and within the sewage domes maintained the same levels as the control wells.

Except for a brief three month period (October 1975 to December 1975), total nitrogen (TN) concentrations in the surface waters of the sewage-treated domes were clearly higher than in the control domes (Figure 3-6). The mean TN concentrations for sewage dome 1, sewage dome 2, Austin Cary, and groundwater control dome over the study period were 8.6, 12.3, 1.6, and 1.5 mg/L, respectively. The low TN levels during late 1975 and the increase beginning in February of 1976 reflect changes in the nature of the incoming sewage effluent, as discussed in the section on BOD.

The center stations of the sewage domes had higher levels of TN than did the edge stations. For example, TN was 33 percent lower at one edge station (n=27) and 44 percent lower at another (n=27) than

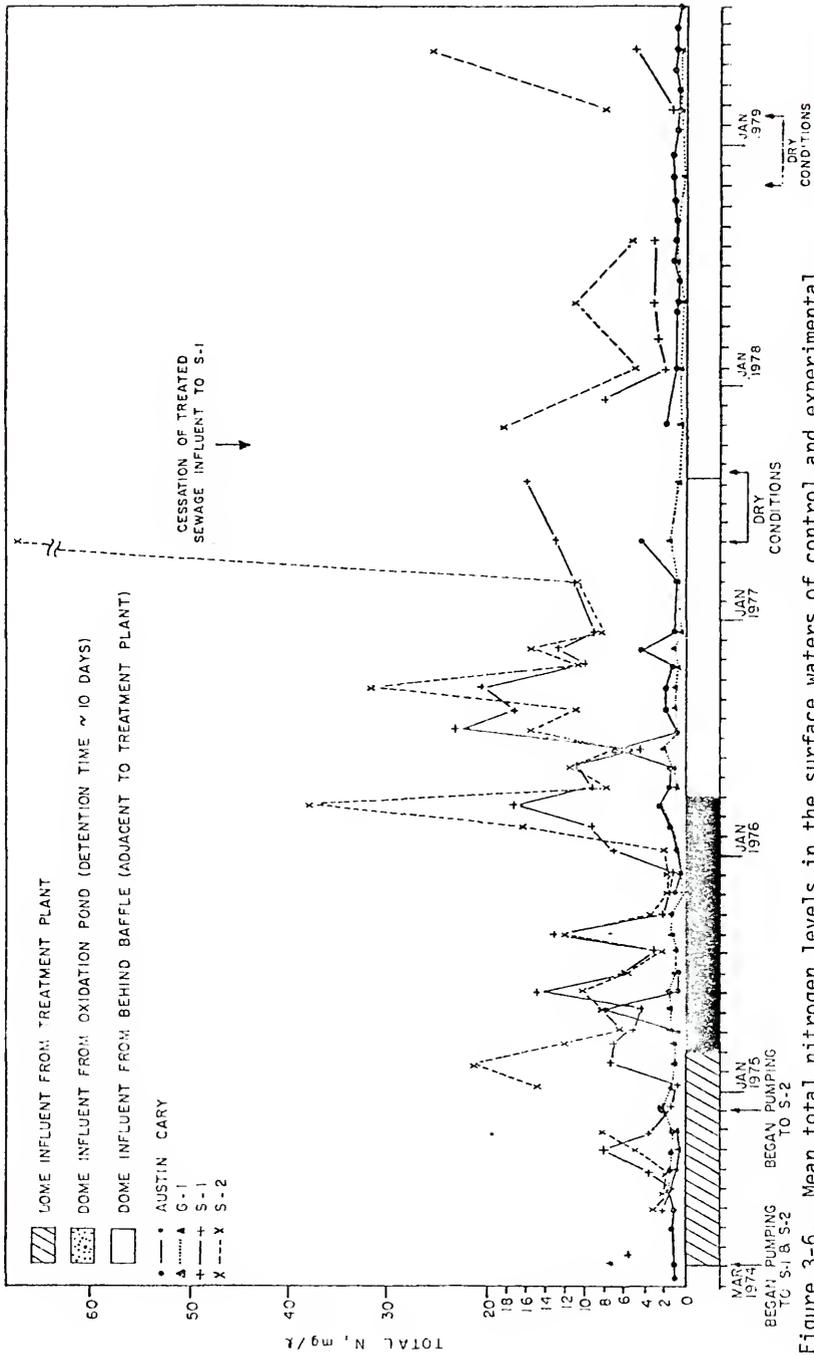


Figure 3-6. Mean total nitrogen levels in the surface waters of control and experimental domes. Each point is the mean of one center and one or two edge stations for each dome, except Austin Cary where only the center station is plotted. (G-1 is the groundwater control dome, S-1 is sewage dome 1, and S-2 is sewage dome 2.)

the average TN (12.7 mg/L) at the center of sewage dome 1. Average TN levels of the northern and southern edge stations in sewage dome 2 were 41 percent and 31 percent lower (n=31), respectively, than the center station (15.2 mg N/L). The low redox potentials in the sewage domes explain the preponderance of ammonium among the inorganic forms (Table 3-1).

Levels of TN were more variable from month to month than were the levels of total phosphorus. Perhaps this reflects the more complex biological cycle of nitrogen. The large temporal fluctuations of TN concentrations in the surface waters of the sewage domes also may be explained by several physical factors, including alterations in the placement of the sewage influent pipe within the oxidation pond, varying treatment plant efficiencies, and variations in precipitation and evapotranspiration. High levels of nitrate in the inflowing sewage tend to be associated with lower TN concentrations in the domes, because nitrate could be denitrified in the anoxic dome waters. Figure 3-7 supports this possibility; high percentages of nitrate plus nitrite in the inflowing nitrogen were associated with lower TN concentrations in the dome centers; most of the nitrate probably was dissimilated to N_2 by denitrifying bacteria (see section on Laboratory Leaching Studies). Since the sewage domes are anoxic, oxidation of ammonium to nitrate does not occur. Concentrations of nitrate plus nitrite were lower in the centers of sewage dome 1 and sewage dome 2 than in the influent (Tables 3-1 and 3-4). This was not a result of simple dilution, since the percentage nitrate plus nitrite of the TN in the centers of both experimental domes was always considerably lower than the percentage nitrate plus nitrite of the TN in the influent (Figure 3-8).

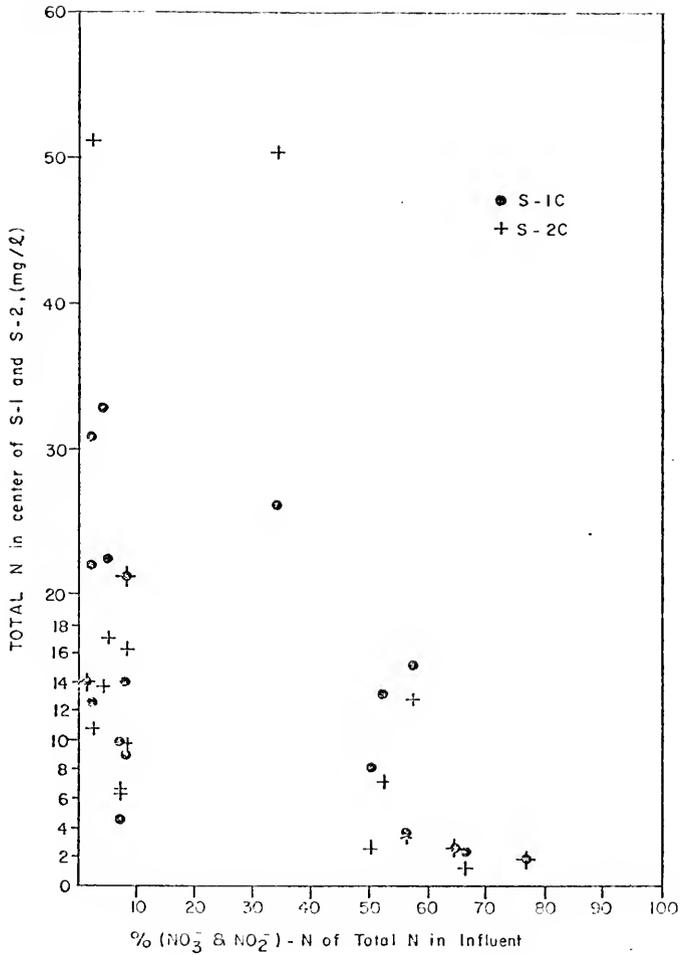


Figure 3-7. Total nitrogen in the centers of sewage dome 1 (S-1C) and sewage dome 2 (S-2C) in relation to the percent nitrate plus nitrite nitrogen of the total nitrogen in the influent.

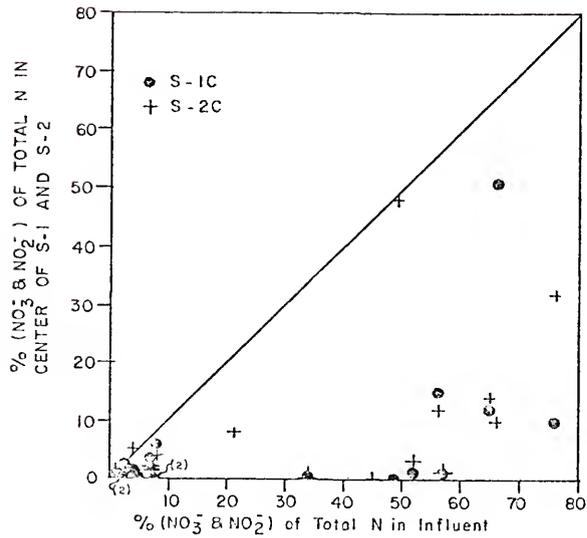


Figure 3-8. Percent nitrate plus nitrite nitrogen in the treated sewage influent vs. percent nitrate plus nitrite nitrogen in the centers of sewage dome 1 (S-1C) and sewage dome 2 (S-2C).

Total nitrogen levels in the surface waters of sewage dome 1 and sewage dome 2 generally were lower during the first two years of sewage inputs (Figure 3-6), corresponding with a period of higher levels of nitrate and lower TN levels in the sewage effluent (Overman 1974, 1975; Zoltek and Whittaker 1975; Zoltek 1976). Thus, it appears possible to maintain some control over the levels of total nitrogen in the surface waters of cypress domes receiving sewage by maintaining a nitrified effluent.

Phosphorus

The substantial reductions shown for BOD and nitrogen through the treatment plant did not occur for phosphorus (Figure 3-2). Only 20 percent of the total phosphorus (TP) was removed by the plant, and another 7 percent by the oxidation pond. As a result, the concentrations of TP in the sewage domes were nearly the same as those for TN, even though TN levels were five times higher than TP levels in the raw sewage. With only one exception, concentrations of TP in the shallow wells show complete removal of phosphorus within the domes (Table 3-1). The exception (well 19) is attributed to a naturally high background level.

Well 19 was always anomalous, yielding turbid samples and high levels of alkalinity and calcium, in addition to high levels of fluoride and phosphate (Table 3-1). Although the values for those parameters never reached the levels in the surface water of sewage dome 1 (with the exception of fluoride), they were consistently higher than any other well samples (Dierberg and Brezonik 1978). Besides the possibility of subsurface contamination by sewage percolate, it was also possible that surface overland flow could be responsible for

contamination of the well water. However, the drainage characteristics of the area suggest that such flooding would be highly unlikely. Furthermore, since the well was pumped out one day prior to sampling, overland contamination does not seem likely.

A third possibility exists: the high levels of orthophosphate, fluoride, calcium and alkalinity could be attributable to a naturally occurring geological deposit. This explanation is supported by several chemical, microbiological, mineralogical, radiological, and lithological facts.

1) If sewage were the cause of the high concentrations, other ions that have high concentrations in sewage also should have high concentrations in water from well 19. Sodium, potassium, and ammonium exhibit comparatively high concentrations in surface water of sewage dome 1 (Table 3-1), but occur only at background levels in water from well 19 (Table 3-2). Moreover, fluoride is twice as high in the well 19 water as in standing water of sewage dome 1 (Tables 3-1 and 3-2).

2) Data on total phosphate in soil solution obtained from ceramic cups placed between the edge of sewage dome 1 and well 19 indicated no elevated phosphate levels in the subsurface water down to 2 m. The original depth for well 19 was 4.5 m (Cutright 1974).

3) Fecal coliform numbers from well 19 were consistently low (Price 1975a; Allinson and Fox 1976).

4) Since fluorapatite ($\text{Ca}_5(\text{PO}_4)_3\text{F}$) is composed of the elements found in excess at well 19, and since this mineral is a common phosphate rock in the area, it seems likely that this mineral is the cause of the high levels in well 19 water. Based on the solubility equilibrium for fluorapatite, a theoretical phosphate concentration of

7.04×10^{-5} M was calculated by using the mean concentrations of Ca, F, and the mean pH measured for well 19. This compares favorably with the measured mean of 5.81×10^{-5} M.

5) Further support for this explanation was found in gamma ray logs of stratigraphic sections from wells A and B located on the north-west edge of sewage dome 1 (Figure 2-2). Zones of concentrated phosphate are indicated by areas of intense gamma radiation of the associated radon, thorium and uranium. Intense gamma radiation was logged beginning at 3 m in an interbedded sandy clay and clayey sand (Smith 1975).

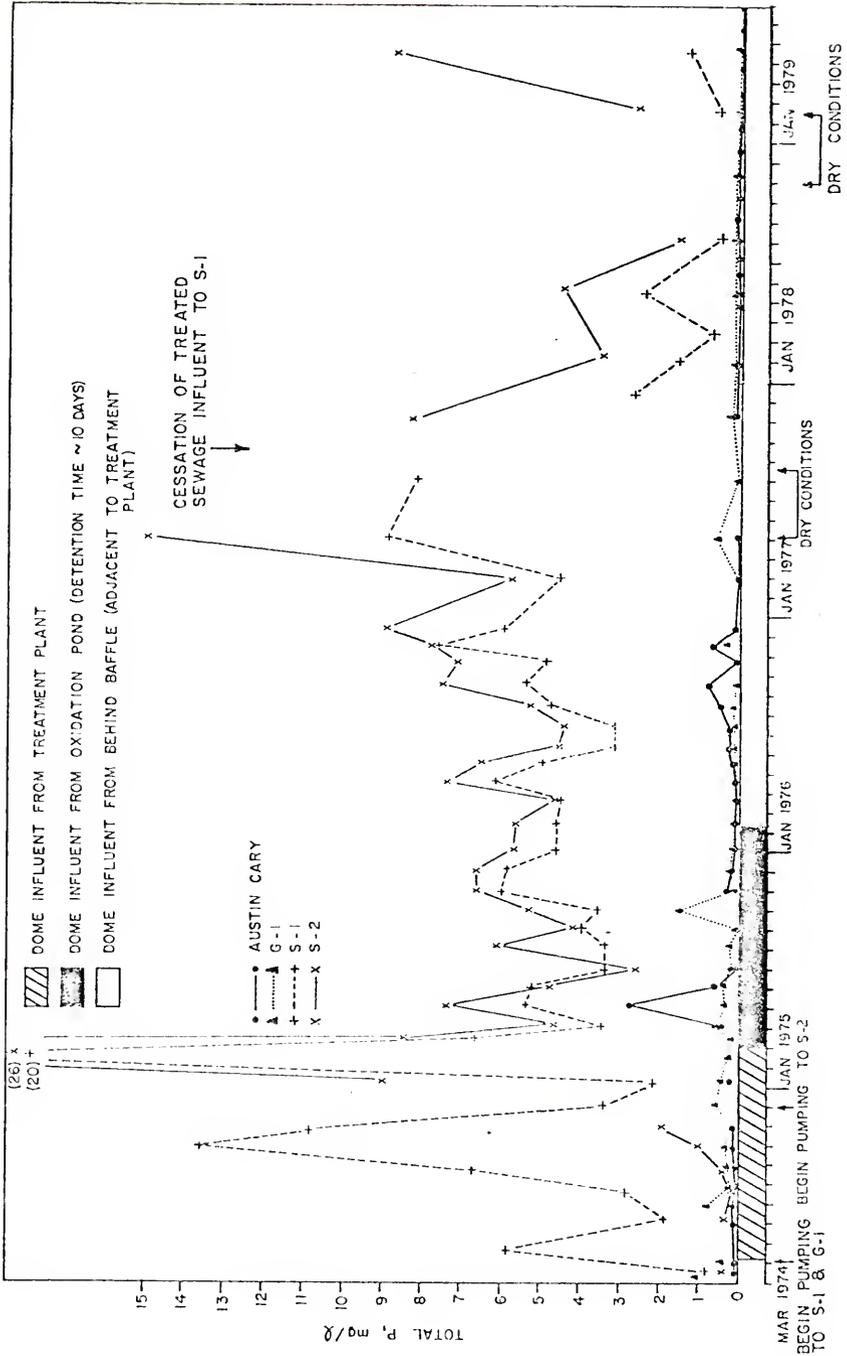
6) According to Cutright (1974), who drilled well 19, the core from 1 to 2.5 m from this well consisted of greyish white clayey sand, with clay content increasing rapidly with depth. From 2.5 to 4 m, the core consisted of blue-grey sandy-clay, and from 4 to 5 m only blue clay was found. The sand-size particles were medium to fine-grained quartz and the clay was predominantly kaolinite. The sand content decreased with depth until, between 5 to 6 m, the deposit was pure blue clay. Cutright believed this to be the top of the Hawthorn Formation, which provides the phosphorus-rich deposits that are mined commercially in Florida. Although none of the other wells on the west and north side of sewage dome 1 (wells 18, 20, 21, and 1) behaved like well 19, a deeper well (well 33, 4.3 m deep) distant from the sewage and control domes displayed similar (though lower) levels of fluoride, phosphate and calcium (Dierberg and Brezonik 1978). Cutright's (1974) well logs indicated that wells 1 and 19 were drilled 4.6 m deep, while wells 18, 20, and 21 were drilled to 3.7 m depths. The deeper nature of well 19 and control well 33 would put them closer to the Hawthorn

Formation. Thus, the high concentrations in well 19 would not seem to be the result of movement of contaminated water from sewage dome 1.

Concentrations of TP in the surface waters of sewage dome 1 and sewage dome 2 ranged from 2 to 100 times higher than TP concentrations in the two control domes (Figure 3-9), which had mean TP concentrations of 0.27 mg/L (groundwater control dome) and 0.18 mg/L (Austin Cary). Sewage dome 2 consistently had higher concentrations than did sewage dome 1, and concentrations were generally lower at the edges of both domes than at the center (Figure 3-9 and Table 3-1). However, in most instances the decrease was less than 50 percent, and (except on one occasion in sewage dome 1) concentrations were always higher than the 1 mg/L standard recommended by the Florida Department of Environmental Regulation for finished waters of tertiary sewage treatment (FDER 1965). This situation also prevailed at the weir station of sewage dome 1. Over the entire study period, the average TP at the weir was 31 percent lower than the average at the center of sewage dome 1 (7.1 mg/L), and at the site 30 m east of the center of sewage dome 1 the average was 29 percent less than that at the center. Average concentrations of TP at the edge stations in sewage dome 2 were 25 percent (north station) and 35 percent (south station) lower than the average at the center (8.2 mg/L).

The large temporal fluctuations in TP levels in sewage dome 1 and sewage dome 2 (Figure 3-9) at the beginning of the study reflect the variable nature of the sewage source. Relocation of the influent pipe within the oxidation pond in March 1976 to a point closer to the treatment plant did not result in any observable changes of TP concentrations in either the incoming sewage (Zoltek 1976) or the standing

Figure 3-9. Mean total phosphorus levels in the surface waters of control and experimental domes. Each point is the mean of the one center and one or two edge stations for each dome, except Austin Cary where only the center station is plotted. (G-1 is the groundwater control dome, S-1 is sewage dome 1, and S-2 is sewage dome 2.)



water of sewage dome 1 and sewage dome 2 (Figure 3-9). As mentioned previously, this was not the case for levels of BOD and TN. It is also noteworthy that much of the TP in the sewage domes was orthophosphate (Table 3-1), which is readily utilized as a nutrient by plants.

Sodium and potassium

The monovalent cations were similar to phosphorus in their pattern of reduction (Figure 3-3). Reductions of 77 percent for sodium and 95 percent for potassium were found in the wells compared to the levels in the surface water at the centers of the two sewage domes. During 1978 and 1979, sodium levels in the wells surrounding sewage dome 2 increased to concentrations approximately half those found in the surface water. This increase may be the result of continual transpiration from the shallow groundwater aquifer.

Calcium and magnesium

Concentrations of the divalent cations were relatively constant through the treatment plant and oxidation pond, but they were lower (52 percent for Ca and 44 percent for Mg) at the edges of the sewage domes than at the centers (Figure 3-3). In comparison, little change was noted in monovalent cations between the centers and edges. The decreased concentrations of divalent cations may reflect fixation onto exchange sites of the sediments. Concentrations of Mg in the groundwater under and surrounding the two sewage domes were equal to background levels measured in the control wells, and Ca levels were lower in the wells near the sewage domes than in the natural control wells. The proximity of the calcareous Hawthorn Formation to the control wells probably explains these differences, since these wells were drilled

deeper (ave. depth = 4.27 m) than the wells surrounding the groundwater control dome and sewage dome 2 (ave. depth = 3.66 m) (Cutright 1974).

Sulfate

A considerable decrease in the concentration of sulfate (39 percent) occurred between the center and edge stations of the sewage domes, primarily because of the reduction of sulfate to sulfide (Figure 3-4). Sulfate levels in the wells were similar to those in the control wells.

Chloride

Because of dietary use of salt, chloride concentrations were higher in domestic sewage than in most freshwaters. Because chloride is also a conservative ion (i.e., it is not adsorbed by soil components or assimilated by plants), it serves as a crude tracer of sewage. Levels of chloride in the wells thought to be influenced by sewage percolation were more than half the values found in the sewage dome centers (Figure 3-4). Average Cl concentrations were the same from the raw sewage to the dome edges, pointing to the conservative nature of chloride. The higher chloride concentrations in the wells surrounding groundwater control dome are unexplainable since the groundwater pumped into this dome has low chloride levels (~7 mg/L). However, since the phosphate mineral, apatite, can contain chloride (Hem 1970; Krauskopf 1967), it is possible that the hydroxide ions associated with the influent from the Floridan Aquifer (median pH = 7.2) may substitute for chloride ions on the mineral surfaces. During 1978 and 1979, chloride levels in the wells surrounding sewage dome 2 increased to concentrations comparable to those in the surface water

(as was found also for sodium). In some wells, chloride levels exceed surface water concentrations by threefold (Mountain 1979). The increase in chloride concentrations in the shallow wells may be the result of continual transpiration from the shallow groundwater aquifer.

Fluoride

A decrease in the concentration of fluoride (37 percent) between the center and edge stations of the sewage domes suggests that chemical precipitation may be occurring. Considering the low solubility of fluorapatite ($K_{sp} \approx 10^{-55}$) and the levels of PO_4^{-3} and Ca^{+2} , the observed concentrations of fluoride at the dome edges are within the equilibrium activity of fluorapatite, suggesting removal due to precipitation. Fluoride in the wells surrounding groundwater control dome were similar to those in the wells receiving water from the sewage domes. However, fluoride was higher in the control wells because of their depth (Figure 3-4).

Hydrogen sulfide

Concentrations of H_2S greater than 1 mg/L were found regularly at several stations in the experimental domes. Treated sewage has high concentrations of sulfate (~ 40 mg/L) compared to the natural surface and groundwaters of this area, and apparently some sulfate is reduced in the anoxic waters and sediments of the experimental domes. During infrequent periods when the surface water in the experimental domes was oxygenated, sulfide was not detectable. However, the anaerobic sediments continued to have detectable levels of H_2S (0.3 mg/L for pore water at a 30 cm depth). The amount of sulfide in the sediments during (more typical) periods when the surface water was anoxic was negligible compared to that in the surface water. For example, two

30 cm deep interstitial water samples collected in May 1979 had only 0.02 mg/L of total sulfide, whereas three surface water samples from sewage dome 2 had a mean sulfide level of 2.6 mg/L.

Hydrogen sulfide is a weak acid ($pK_1 = 7.0$). Because the median pH values for the surface waters of sewage dome 1 and sewage dome 2 are slightly lower than 7.0, the undissociated species (H_2S) predominates, and this volatile form comprises 65-92 percent of the total dissolved sulfide in the water.

Soil-Solution Studies

Chloride concentrations indicate that infiltration from sewage dome 1 occurs in a westerly direction, and the extent of infiltration increases with depth at increasing distances from the dome (Table 3-5). On the other hand, infiltration from sewage dome 2 occurs about equally to the southeast and northwest. The unusually high chloride levels (up to 1000 mg/L) at the 60 cm depth southeast of sewage dome 2 are an artifact originating from the disposal of filtrate high in $MgCl_2$ during virus sampling. Concentrations of chloride in standing water at the edges of the sewage domes ranged from 12 to 105 mg/L for the study period ($\bar{x} = 46$ mg/L for sewage dome 1 and 51 mg/L for sewage dome 2). It is evident from the chloride data that the soil tubes at the sites 7 m west of sewage dome 1 (all depths), 13 m southeast of sewage dome 2 (all depths except 180 cm), and 4 m northwest of sewage dome 2 (all depths) were in zones receiving a large amount of infiltration from the surface waters of the sewage domes. However, nutrient concentrations in these samples consistently were low, clearly indicating removal of the high concentrations of nutrients in the dome standing waters. Nutrient concentrations in soil solution samples with large

Table 3-5. Mean levels for selected chemical parameters of the interstitial water taken at three depths from soil tubes surrounding the cypress domes at the Owens-Illinois site in November, December, January, May, and August (1976-77).

Station	Depth (cm)	$(\text{NO}_3^- + \text{NO}_2^-)\text{-N}$ (mg/L)	$\text{NH}_4^+\text{-N}$ (mg/L)	Organic N (mg/L)	Total P (mg/L)	Specific Conductance ($\mu\text{mho/cm}$)	Cl (mg/L)	pH ^a	N ^b
Groundwater Control Dome 3 meters west of edge	60	0.01	0.35	0.6	0.02	54	6	4.2	5
	120	0.03	0.14	0.6	0.02	49	10	4.4	5
	180	0.02	0.25	0.7	0.01	84	10	4.5	2
Sewage Dome 1 15 meters east of edge	60	0.01	1.1	0.6	0.03	67	11	5.0	5
	120	0.01	0.05	0.5	0.01	38	7	4.6	5
180	0.02	0.02	0.3	0.02	0.02	37	7	4.8	5
Sewage Dome 1 36 meters west of edge	60	0.02	0.07	0.5	0.04	91	20	4.0	5
	120	0.02	0.13	0.8	0.02	88	21	4.5	5
	180	0.03	0.04	0.7	0.02	132	34	4.8	5
Sewage Dome 1 7 meters west of edge (replicate soil tubes)	60	0.03	0.37	0.7	0.01	144	39	4.3	5
	60	0.03	0.12	0.4	0.01	156	40	4.2	5
	120	0.03	0.05	0.7	0.01	166	45	4.4	5
	120	0.03	0.05	1.2	0.01	157	42	4.6	5
	180	0.02	0.07	0.5	0.02	169	42	4.5	5
	180	0.03	0.09	0.5	0.01	171	44	4.4	5
Sewage Dome 2 13 meters southeast of edge	60	0.03	0.28	0.9	0.01	1415	>100	4.7	5
	120	0.04	0.11	0.5	0.01	200	55	4.4	5
	180	0.06	0.06	0.6	0.02 ^c	95	26	4.4	5
Sewage Dome 2 4 meters northwest of edge (replicate soil tubes)	60	0.09	0.06	1.1	0.005	179	46	4.4	5
	60	0.02	0.05	0.5	0.01	179	48	4.3	5
	120	0.02	0.19	0.8	0.02	156	43	4.2	5
	120	0.02	0.16	0.3	0.02	150	39	4.4	5
	180	0.02	0.12	0.5	0.01	150	37	4.1	5
	180	0.03	0.09	0.8	0.005	149	39	4.2	5

^a Median pH.

^b Number of samples.

^c Based on two samples.

fractions of sewage infiltrate in the soil solutions (as indicated by chloride) did not differ significantly from the nutrient concentrations in solutions with low or negligible fractions of sewage in the soil solution (Table 3-5).

Nitrate contamination in groundwater thus is not a danger at present loading rates, and enrichment of adjacent waterways by other nitrogen and phosphorus species also does not seem a matter of concern. Apparently the spodic horizon is important in the removal of phosphorus, and the depth at which it occurs is the depth to which phosphorus can move freely (Hortenstine 1976). Hortenstine (1976) reported that phosphorus from secondary sewage effluent sprayed onto a spodosol near Orlando, Florida, moved freely in the soil solution above the spodic horizon. However, efficient removal of phosphorus by the spodic horizon was indicated by the absence of detectable phosphorus in soil solution samples below this layer. According to Coultas and Calhoun (1975), the spodic horizon at sites adjacent to the cypress domes occurs at depths of 20 to 30 cm; this fact probably explains the low levels of phosphate even in the shallow (60 cm) soil tubes.

The field data from the soil tubes corroborate the results from the monitoring wells and from laboratory column leaching studies (see following section). Efficient removal (>95 percent) of phosphorus, nitrate, ammonium and total nitrogen by the sediments and soils surrounding the cypress domes occurred throughout the 4.5 year period of sewage disposal into the domes.

Laboratory Leaching Studies

Column leaching studies were undertaken in the laboratory in order to study the nitrogen and phosphorus removal capacity of cypress

dome sediments under more controlled conditions. Concentrations of pertinent chemical constituents in each of three eluants is shown in Table 3-6.

Nitrate-nitrite

Nitrate and nitrite removal by sediments is of primary concern in evaluating the feasibility of sewage disposal into cypress swamps. A lack of nitrate or nitrite removal could lead to high concentrations in the groundwater, and this could have serious public health implications (NRC 1978). Since nitrate plus nitrite was a small fraction of the major forms of nitrogen (organic N, ammonium, and nitrate plus nitrite) in the sewage effluent (Table 3-4), the capacity of the dome to reduce high concentrations of nitrate-nitrite associated with a more efficient sewage plant could not be evaluated in the field. However, the results obtained from leaching of sewage effluent containing 20-40 mg ($\text{NO}_3^- + \text{NO}_2^-$) - N/L through sediment columns provide a partial answer to this question.

Over a 21-month period, 14-15 pore volumes of eluant passed through the columns, and almost complete removal of nitrate occurred in the two columns receiving sewage amended with 20-30 mg NO_3^- - N/L (Figure 3-10 and Table 3-7). Because of the high removal efficiency of nitrate, the total cumulative ($\text{NO}_2^- + \text{NO}_3^-$) - N in the effluent of the nitrate-spiked columns did not exceed the effluent levels of the columns leached with unamended sewage or groundwater. Similar removals were reported by Chen and Keeney (1974) for undisturbed sediment cores from two lakes. Contrary to the high removals found in this study, Struble (1977) found only a 32 percent reduction of nitrate in water containing 10 mg NO_3^- - N/L and 0.01 M CaCl_2 passed through a column

Table 3-6. Concentrations (mg/L) of pertinent chemical constituents in the groundwater, treated sewage, and treated sewage plus nitrate- and phosphate-spiked eluants used in the sediment leaching study.

Eluant	pH	PO ₄ -P	Total P	NH ₄ ⁺ -N	NO ₃ ⁻ -N	NO ₂ ⁻ -N	Org. N	Total N	Total C
Groundwater									
9/29/77 to 6/19/78	9.1	<0.005	0.01	0.07	<0.005	<0.003	0.33	0.40	--
6/19/78 to 6/14/79	9.2	0.02	0.02	0.02	0.024	<0.003	0.09	0.12	27
Treated Sewage									
9/29/77 to 6/19/78	9.2	5.3	5.8	11.02	0.77	0.041	3.6	15.3	48
6/19/78 to 6/21/79	8.8	10.5	11.0	2.15	10.1	0.005	1.35	13.6	28
Treated Sewage + Nitrate and Phosphate									
9/29/77 to 6/8/78 ^a	9.0	5.3	5.8	11.02	20.7	0.046	3.6	35.4	48
6/8/78 to 6/28/79	---	20.0	20.5	2.15	39.6	0.052	1.35	43.1	28

^a Eluant was not spiked with phosphate until the ninth month of leaching (June 1978).

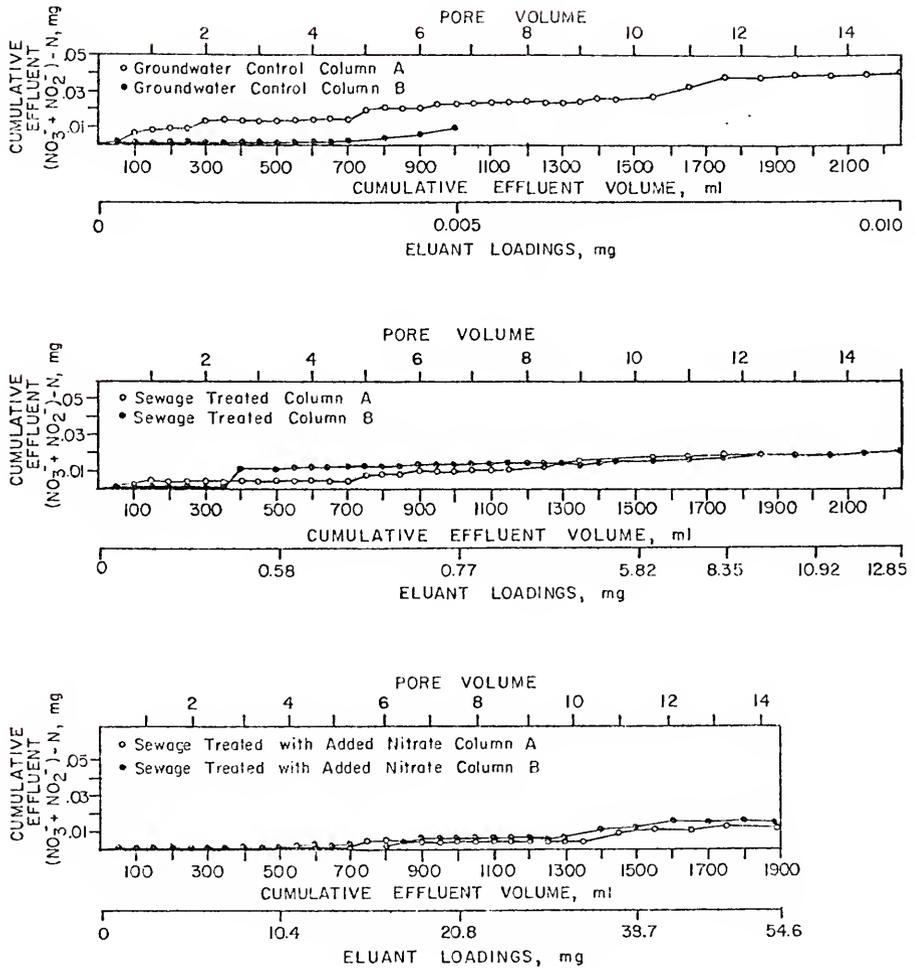


Figure 3-10. Cumulative nitrate plus nitrite nitrogen in the leachates of sediment leaching columns receiving groundwater, treated sewage, and treated sewage with added nitrate plus phosphate eluants.

Table 3-7. Average concentrations, maximum concentrations, and percent removals of nitrate plus nitrite nitrogen in the leachates from 17-cm free-draining columns of groundwater control dome sediments over a 21-month period.

Concentration	Groundwater Eluant		Treated Sewage Eluant		Treated Sewage + Nitrate- and Phosphate-Spiked Eluanta	
	A	B	A	B	A	B
	Measured mg/L in eluant					
9/77 to 6/78	<0.005	<0.005	0.77	0.77	20.8	20.8
6/78 to 6/79	0.024	0.024	10.1	10.1	39.6	39.6
Average mg/L in leachate	0.018	0.009	0.011	0.010	0.006	0.008
Maximum mg/L in leachate	0.14	0.04	0.09	0.24	0.23	0.24
Loading						
Total mg in eluant	<0.005	<0.005	8.81	11.53	54.14	53.84
Total mg in leachate	0.041	0.009	0.020	0.022	0.012	0.016
Removal (percent)	*	*	99.8	99.8	100.0	100.0

^a Eluant was not spiked with phosphate until the ninth month of leaching (June 1978).

* Denotes larger percentage leached than was added due to indigenous production of nitrate plus nitrite.

of screened sediment from the groundwater control dome mixed 1:1 with clean builder's sand. Slightly higher removals (50 percent) were found for sediments from sewage dome 1. The higher rates of removal reported here probably result from a much faster flow rate or the use of treated sewage rather than synthetic percolate. The treated sewage has a higher level of dissolved organic matter, which serves as both an energy source for denitrifying bacteria and a seed of denitrifying bacteria.

To investigate the role of denitrification in the removal of nitrate from the sediment columns, the acetylene blockage assay was used, where the final step in denitrification (i.e., reduction of N_2O to N_2) is blocked when about 1 percent acetylene is present in the gas phase (Yoshinari et al. 1977). After an incubation period of eight days, the concentration of N_2O in the head space of the two columns receiving 20 mg N/L of nitrate was 18.5 nM/cm^3 (Figure 3-11). The columns with unamended sewage and with groundwater eluants had less than 1 nM/cm^3 over the measurement period (six days). Calculation of the total amount of N_2O in the head space and dissolved in solution and comparison of this value with the amount of nitrate lost in the eluant indicated that 66 to 85 percent of the nitrate disappearance was attributable to denitrification.

Conversion of the average N_2O evolution rate ($2.3 \text{ nM/cm}^3\text{-day}$) in the nitrate-spiked columns to an annual areal rate results in a nitrate removal rate of $5.5 \text{ g N/m}^2\text{-yr}$ attributable to denitrification. This rate is lower than the rate ($15.0 \text{ g N/m}^2\text{-yr}$) that was calculated by multiplying the total nitrate nitrogen lost in the column eluant by 75 percent (the percentage of the loss attributable to denitrification).

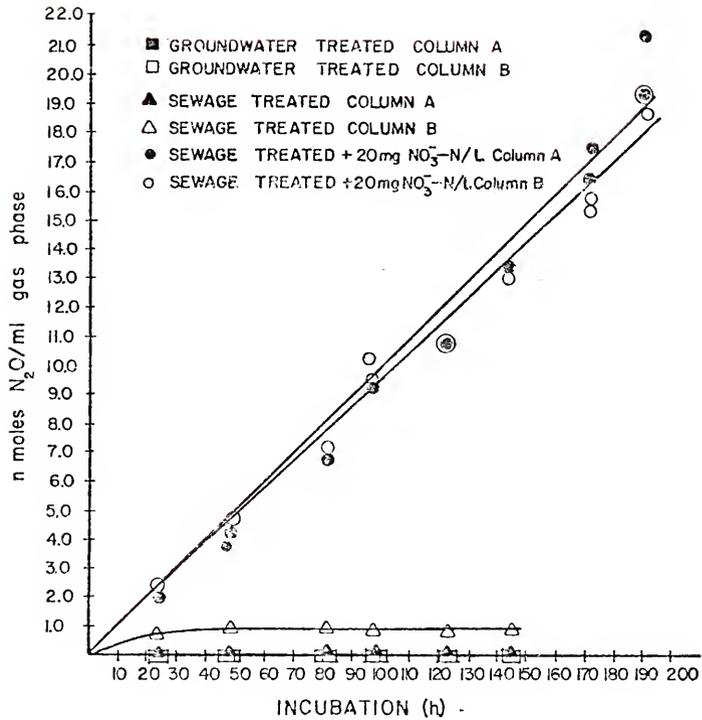


Figure 3-11. Time course of N₂O buildup following acetylene blockage in sediment leaching columns. The sediments were taken from the groundwater control dome and had received the eluants listed above for 8 months prior to the addition of 0.1 atm C₂H₂.

The lower rate probably reflects the short duration of the experiment (8 days) and suggests that steady state production of N_2O had not yet been attained. The measured rate reflects more the denitrifying capacity of the overlying water rather than the total column capacity, since C_2H_2 was injected into the headspace without shaking the sediment or overlying water to facilitate transport. Consequently, diffusion after dissolution was the only mechanism by which C_2H_2 could reach the active denitrifying sites and inhibit the reduction of N_2O to N_2 . Acetylene has a high solubility in water (Wilhelm et al. 1977), which explains the lack of a lag phase (Figure 3-11). However, the redox potential (E_{h_7}) of the eluant overlying the sediment in the columns was ~ 350 mV. This is only a moderately reduced condition (Patrick and Mahapatra 1968) and slightly greater than the redox potential (+300 mV) where denitrification begins (Meek et al. 1969; Patrick and DeLaune 1972). In summary, it appears that the low denitrification rate obtained in the 8-day acetylene blockage experiment resulted from slow transport of acetylene to the more active denitrifying sites in the sediments. Both the measured and the calculated rates are much lower than the average removal rate of nitrate by undisturbed soil cores from a freshwater swamp (69.5 g N/m^2 -yr) (Engler and Patrick 1974).

Incubation of the leaching columns in the presence and absence of acetylene provides information on the mole fraction of N_2O in the products of denitrification. Measurements of N_2O in the presence of acetylene provide an estimate of the total amount of denitrification, and incubation without acetylene measures the natural evolution rate of N_2O for the environmental conditions under which the assay was

performed. The ratio of N_2O/N_2 production by denitrification is of interest because of the role of N_2O as a sink for stratospheric ozone (Crutzen 1971; see NRC 1978). The end product of denitrification is pH dependent, with little N_2O formed above pH 6-7 (Van Cleemput et al. 1975). Because of the relatively high pH in the sediment (6.2-6.3) and eluant (6.6-7.7) of the soil tubes receiving the nitrate spikes, the mole fraction ($N_2O/N_2 + N_2O$) was found to be moderately low (0.17).

Ammonium

Elution curves for ammonium from the various columns are presented in Figures 3-12 and 3-13. The flow rates of both pairs of sewage and sewage plus nitrate columns were approximately the same, resulting in similar elution curves for each duplicate set, but the slower flow rate of groundwater Column B yielded higher ammonium levels per pore volume of column effluent than found in Column A (Table 3-8). On a time basis, however, both columns eluted approximately the same amount of ammonium, comparable to the total ammonium leached from the four sewage-treated columns.

A comparison of measurement made before and after the leaching period (Table 3-9) shows that the sediments lost from 1.16 to 3.93 mg of free and exchangeable ammonium nitrogen during the leaching period. The sediments leached by groundwater lost more than the sediments leached by sewage effluent. The net loss of the free and exchangeable ammonium during the course of the leaching period was likely the result of: 1) high levels of polyvalent cations in the eluants; 2) redox reactions favoring the production of ferrous and manganous ions; and 3) the low concentration of ammonium (2.15 mg N/L) in the sewage eluant during the last 12 months of leaching. These conditions

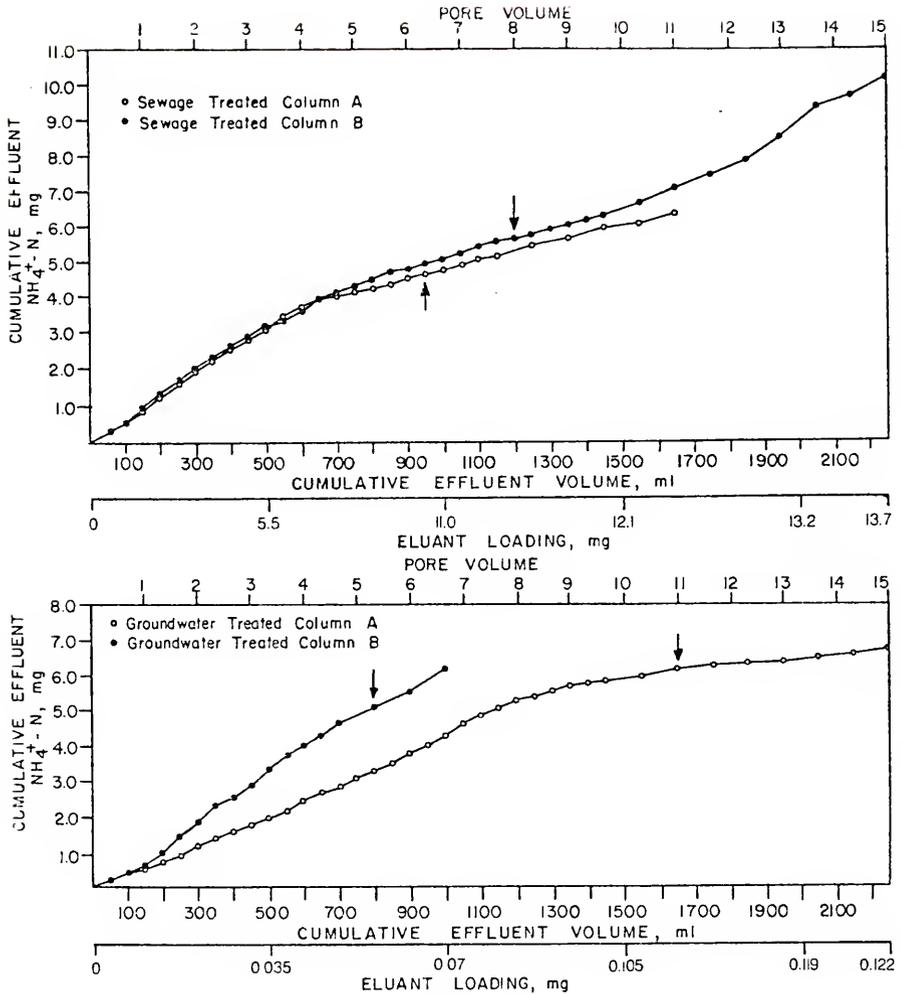


Figure 3-12. Cumulative ammonium nitrogen in the leachates of sediment leaching columns receiving treated sewage and groundwater eluants. Arrows denote when eluant concentration of ammonium decreased (in sewage treated columns) or increased (in groundwater treated columns).

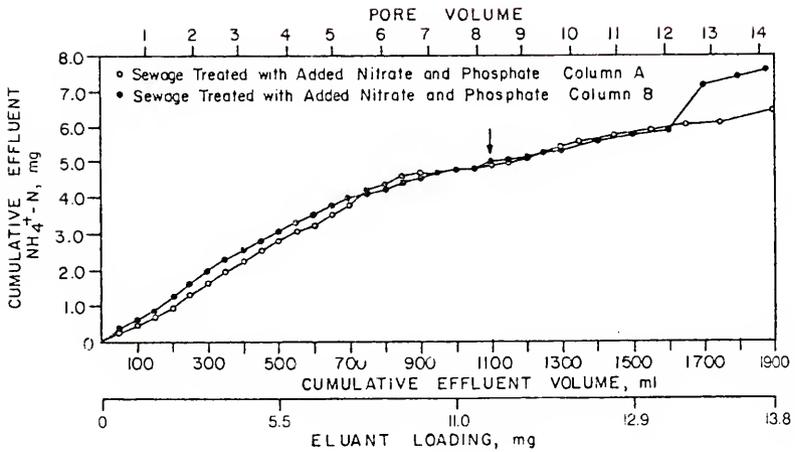


Figure 3-13. Cumulative ammonium nitrogen in the leachates of sediment leaching columns receiving treated sewage with added nitrate plus phosphate as the eluant. Arrow denotes when eluant concentration of ammonium decreased.

Table 3-8. Average concentrations, maximum concentrations, and percent removals of ammonium nitrogen in the leachates from 17-cm free-draining columns of groundwater control dome sediments over a 21-month period.

Concentration	Groundwater Eluant		Treated Sewage Eluant ^a		Treated Sewage + Nitrate- and Phosphate-Spiked Eluant ^a	
	A	B	A	B	A	B
Measured mg/L in eluant						
9/77 to 6/78	0.07	0.07	11.03	11.03	11.03	11.03
6/78 to 6/79	0.01	0.01	2.15	2.15	2.15	2.15
Average mg/L in leachate	2.97	6.18	3.74	4.49	3.36	4.02
Maximum mg/L in leachate	5.7	9.35	7.4	8.7	7.8	12.3
Loading						
Total mg in eluant	0.12	0.07	12.3	15.5	13.8	13.8
Total mg in leachate	6.69	6.18	6.73	10.11	6.39	7.56
Removal (percent)	*	*	45.3	34.6	53.7	45.2

^a Eluant was not spiked with phosphate until the ninth month of leaching (June 1978).

* Denotes larger percentage leached than was added or lost from the original amount associated with the sediments due to indigenous production.

Table 3-9. Changes in the amounts of ammonium and total nitrogen associated with the sediments in the leaching columns during the 21-month leaching period.

	Groundwater Eluant		Treated Sewage Eluant		Treated Sewage + Nitrate- and Phosphate-Spiked Eluant ^a	
	A	B	A	B	A	B
Sediment Ammonium Nitrogen (mg)^b						
Before leaching	5.82	5.38	5.00	5.07	4.95	4.93
After leaching	1.89	2.13	2.41	3.45	3.55	3.77
Amount lost	3.93	3.25	2.59	1.62	1.40	1.16
Sediment Total Nitrogen (mg)						
Before leaching	549	507	471	478	477	464
After leaching	658	601	639	555	618	529
Amount added	109	94	168	77	152	65

^a Eluant was not spiked with phosphate until the ninth month of leaching (June 1978)

^b Includes free and exchangeable ammonium.

result in the displacement of ammonium from the exchange complex (Patrick and Mahapatra 1968; Toetz 1970), even though the cation exchange capacity of the sediment was relatively high (Table 3-10).

Since the lost free and exchangeable ammonium was part of the measured ammonium in the leachate, it was subtracted from the amount measured in the leachate when determining percent retention by the columns (Table 3-11). From 45 to 66 percent of the ammonium nitrogen added to the four columns receiving sewage eluant was retained (Table 3-11), but the two columns receiving groundwater eluant leached more ammonium than was added because of the large pool of indigenous ammonium and organic nitrogen in the sediments (and the low concentration of ammonia in the input groundwater). After taking into account the loss of free and exchangeable NH_4^+ - N, I still found an excess of 2.64-2.86 mg NH_4^+ - N in the leachate from the groundwater columns (comparing inputs and outflows). The excess apparently represents the amount contributed from mineralization of organic nitrogen.

The rather high levels of ammonium found in the column leachates are at variance with the low levels of ammonium found in shallow wells and soil tubes. Lower soil horizons in the field evidently are sites of ammonium adsorption. Moreover, biota not included in the leaching columns (e.g., duckweed and cypress trees) could assimilate large quantities of ammonium in the field.

The total nitrogen content of all the sediment columns increased during the leaching period (Table 3-9). The reason for this is not certain. Only a small percentage (<12 percent) of the increase was due to the amount of ammonium in the eluants that was retained in the columns. Because of the mineralization of the organic nitrogen pool

Table 3-10. Selected chemical and physical properties of the sediments used in nutrient removal columns (after Coultas and Calhoun 1975).

Horizon	Depth cm	Bulk Density g/cc	Hydraulic Conductivity cm/hr	pH	CEC meq/100g	Total N %	Organic C %	Extractable P ppm	Fe %	Al %
01	8-0	---	---	3.6	144.49	1.748	55.10	75	0.153	0.285
All	0-18	0.35	125.0	3.7	41.61	0.495	8.82	7	0.56	0.313

Table 3-11. Ammonium and total nitrogen mass balances (mg) for the sediment leaching columns.

	Groundwater Eluant		Treated Sewage Eluant		Treated Sewage + Nitrate- and Phosphate-Spiked Eluanta	
	A	B	A	B	A	B
	Ammonium nitrogen					
Column effluent	6.69	6.18	6.73	10.11	6.39	7.56
Leached from sediments	3.93	3.25	2.59	1.62	1.40	1.16
Leached from eluant	2.76	2.93	4.14	8.49	4.99	6.40
Eluant loadings	0.12	0.07	12.3	15.5	13.8	13.8
Amount retained	-2.64	-2.86	8.16	7.01	8.81	7.40
Percent retained	*	*	66.3	45.2	63.8	53.6
Total N added to sediments	109.7	93.9	167.7	77.1	151.6	65.1

^a Eluant was not spiked with phosphate until the ninth month of leaching (June 1978).

* Denotes larger percentage leached than was added or lost from original amount associated with sediments due to indigenous production of ammonium and total nitrogen.

within the sediment column, an even smaller percentage of the total nitrogen increase was found (<6 percent) when the retention of organic nitrogen was included. The total nitrogen increases in all columns may simply reflect the occurrence of nitrogen fixation. From 0.31 to 0.79 mg N/g (dry wt) -yr would have had to have been fixed if nitrogen fixation accounted for the excess nitrogen measured in all columns. These rates are about an order of magnitude higher than the rate reported for sediments from estuarine sediments (Brooks et al. 1971). However, the calculated rates compare favorably with the rate measured for sediments from sewage dome 2 (0.21 mg N/g (dry wt) -yr) (see Chapter 6).

Phosphorus

No discernible differences were found in the cumulative effluent total phosphate among the six columns (Figure 3-14 and Table 3-12). Thus, the phosphorus added by the sewage and amended sewage was effectively removed by the sediment; the average removal rate for the four columns receiving sewage was 99 percent over leaching volumes of 12-15 pore volumes. Aluminum and/or iron in the acid soil may react with soluble phosphate to form crystalline and amorphous precipitates (Patrick and Mahapatra 1968). Table 3-10 shows high percentages of both aluminum and iron in the horizons of the sediments used in the experiment. Alternatively, phosphate may be retained by adsorption to clays via a cation bridge mechanism. Based on a loading concentration of 20.5 mg/L applied to two columns for 6 pore volumes, a column area of 11.36 cm², and a column leaching rate of 1.1 L/yr, removal of about 20 g P/m²-yr was calculated. It should be noted that although this removal was based on the maximum concentration of phosphorus

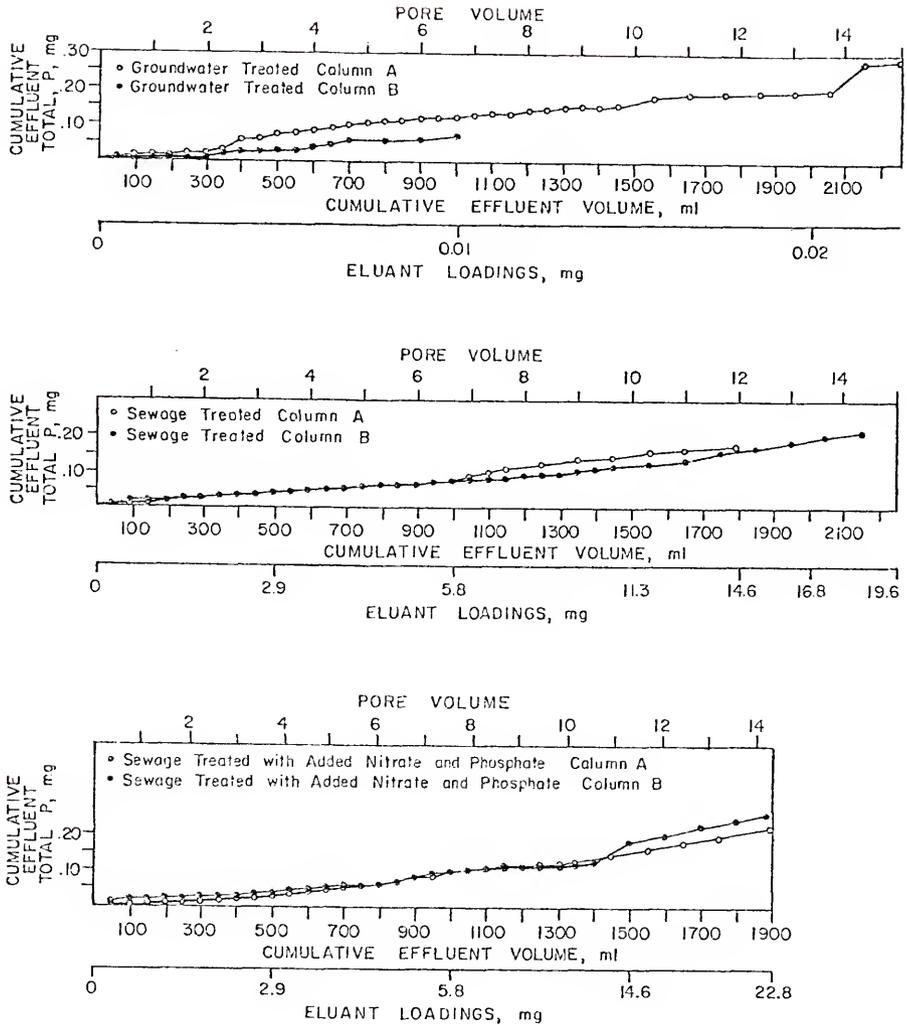


Figure 3-14. Cumulative total phosphorus in the leachates of sediment leaching columns receiving groundwater, treated sewage, and treated sewage with added nitrate plus phosphate eluants.

Table 3-12. Average concentrations, maximum concentrations, and percent removals of total phosphorus in the leachates from 17-cm free-draining columns of groundwater control dome sediments over a 21-month period.

Concentration	Groundwater Eluant		Treated Sewage Eluant		Treated Sewage + Nitrate- and Phosphate-Spiked Eluant ^a	
	A	B	A	B	A	B
Measured mg/L in eluant						
9/77 to 6/78	0.01	0.01	5.8	5.8	5.8	5.8
6/78 to 6/79	0.02	0.02	11.0	11.0	20.5	20.5
Average mg/L in eluant	0.13	0.07	0.09	0.10	0.12	0.14
Maximum mg/L in leachate	0.81	0.20	0.25	0.27	0.20	0.60
Loading						
Total mg in eluant	0.023	0.010	14.80	18.51	22.6	22.4
Total mg in leachate	0.284	0.073	0.170	0.217	0.218	0.255
Removal (percent)	*	*	98.9	98.8	99.0	98.9

^a Eluant was not spiked with phosphate until the ninth month of leaching (June 1978).

* Denotes larger percentage leached than was added due to indigenous production.

applied in the eluant, breakthrough never occurred. Therefore, it is still uncertain how much longer the nearly complete removal of total P by the sediment columns would have occurred. Based on the length of this leaching study, realistic flows and loadings, and the shortness of columns, it appears that the microbial and substrate compartments in the cypress domes can remove all the phosphorus in treated sewage for a long period of time.

CHAPTER FOUR
CHEMICAL CHARACTERISTICS OF NATURAL WATER IN
A FLORIDA CYPRESS DOME

Even though cypress domes are a dominant geomorphological landform in north-central Florida, there has been no systematic study to characterize their natural waters chemically, and to examine the biogeochemical factors that contribute to their chemical composition. In this chapter, I shall describe the chemical characteristics of water sources (i.e., rainfall, throughfall, and groundwater) to a natural cypress dome, compare the water chemistry of a cypress dome swamp to that of other swamps, and evaluate the movement of major ions through the hydrologic cycle of the swamp.

General Chemical Composition

Conductivity and Dissolved Solids

The surface waters of Austin Cary dome are characterized by low dissolved solids. Specific conductance is typically low ($\bar{X} = 51 \mu\text{mho/cm}$). This is slightly higher than bulk precipitation under the tree canopy ($\bar{X} = 32 \mu\text{mho/cm}$) for the same time period (Figure 4-1). The values illustrate both the importance of rainfall in determining the ionic composition of Austin Cary surface waters and the lack of influence of artesian groundwater in the surface water chemistry. The ionic strength of the standing water is typically about 6.2×10^{-4} (neglecting the dissociated carboxyl functional groups of dissolved organic matter). This is comparable to the ionic strength (3.6×10^{-4}) found by Beck et al. (1974) for swamp water in Georgia.

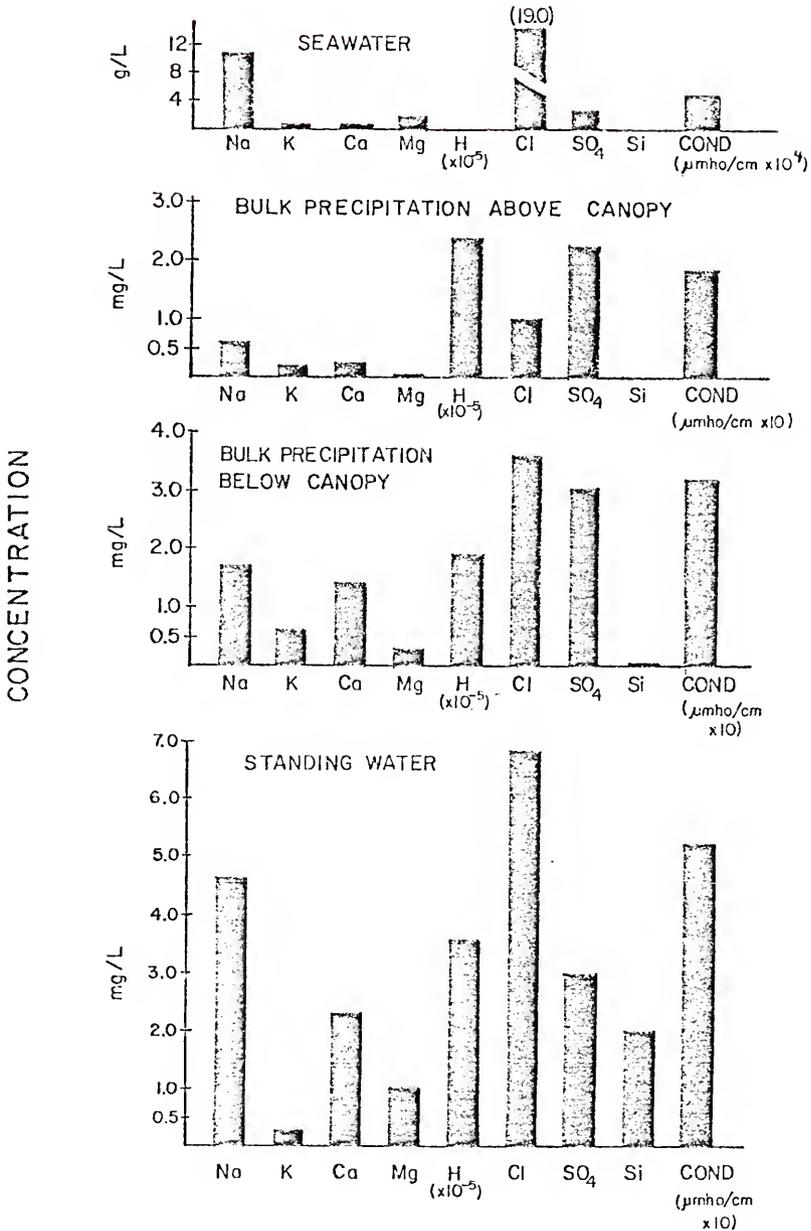


Figure 4-1. Major ions, silica and conductivity in seawater and the natural waters of Austin Cary cypress dome.

It is apparent that the water levels vary greatly over the period of a year (Figure 4-2). The oscillations in water level correspond roughly in an inverse manner to changes in dissolved solids (Figure 4-3). However, correlation of specific conductance with water level yielded a relatively low correlation coefficient ($r = -0.40$; $r^2 = 0.16$), indicating that although the correlation is statistically significant ($P < 0.05$), it explains little of the variance in conductivity.

A value of 28 mg/L was calculated for total dissolved inorganic solids in Austin Cary surface waters (from the average concentrations of major ions in Table 4-1), and the mean concentration of organic carbon was 40 mg/L. If dissolved organic matter is 50 percent carbon, then dissolved organic matter is almost 3 times greater than the dissolved inorganic matter, and the level of total dissolved solids is 108 mg/L. The high ratio of organic to inorganic matter makes the standing water of the Austin Cary dome different from other surface water systems (e.g., rivers, most lakes, and springs).

Based on the concentrations of the major inorganic cations and anions in the surface water, a theoretical value for specific conductance of 47 $\mu\text{mho/cm}$ was calculated, which is lower than the average 60 $\mu\text{mho/cm}$ found by direct measurement. The difference is probably attributable to dissolved organic matter; humic and fulvic acids contain many ionizable functional groups.

Hydrogen Ions

The mean hydrogen ion concentration (pH) of the surface waters was 4.51, and the range was 3.5 to 5.4. Comparable values were obtained for rainfall ($\bar{X} = 4.63$; range 3.99-5.72) and throughfall ($\bar{X} =$

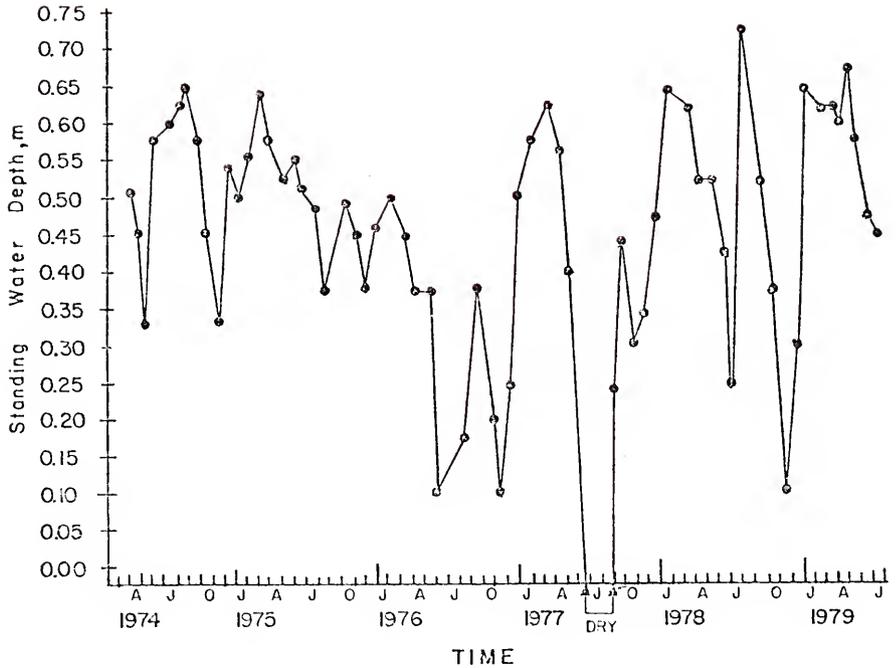


Figure 4-2. Monthly variations in the depth of the standing water at the center of Austin Cary cypress dome.

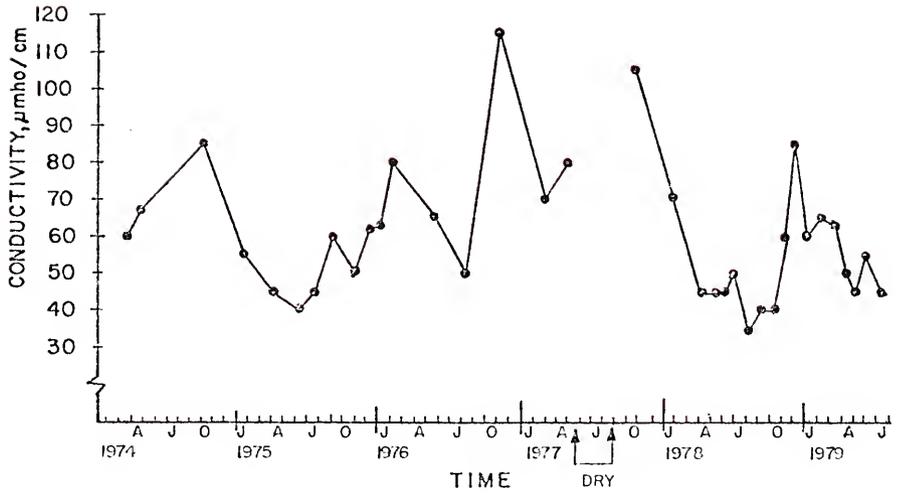


Figure 4-3. Monthly variations in the conductivity for Austin Cary cypress dome.

Table 4-1. Major ionic composition of Austin Cary cypress dome surface water during study period (January 1974 - June 1979)

	n	mg/L		meq/L	
		Range	Mean	Range	Mean
Cations					
H ⁺	51	0.004- 0.316	0.031	0.004-0.316	0.043
Na ⁺	38	2.27 - 9.82	4.94	0.099-0.427	0.215
K ⁺	38	0.08 - 1.07	0.34	0.002-0.027	0.009
Ca ²⁺	39	1.10 - 5.50	2.87	0.050-0.274	0.143
Mg ²⁺	39	0.42 - 3.08	1.37	0.035-0.253	0.113
Anions					
Cl ⁻	23	1.5 -19.5	8.19	0.042-0.550	0.231
SO ₄ ²⁻	25	<0.5 -12.0	2.6	0.000-0.250	0.054
HCO ₃ ⁻	13	0.00 - 9.75	2.16	0.000-0.160	0.035
Sp. Cond. ^a	41	34 -114	60		

^a $\mu\text{mho/cm}$

4.72; range 4.05-8.00). The acidic pH of rainfall at the Austin Cary site is typical of rainfall throughout northern Florida (Brezonik et al. 1980a,b). The pH of groundwater under and surrounding the dome was consistently higher (\bar{x} = 6.6).

Although the acidity of the rainfall undoubtedly affect the dome's surface waters, the low pH normally found in colored water is attributable mostly to the acidic nature of humic substances, which are characterized as polyhydroxy, polycarboxyl, aromatic macromolecules (Christman and Ghassemi 1966). Beck et al. (1974) reported a linear correlation between concentration of organic matter and H^+ activity in the Satilla River (Georgia), and they suggest that organic acids predominate over inorganic acids in the river. However, two swamp water samples deviated from the correlation between organic matter and H^+ found for the Satilla River, and in the present study, no correlation between H^+ and dissolved organic carbon (DOC) was discerned for Austin Cary surface waters. Furthermore, depth of standing water in the dome had a negligible effect on the observed variation in H^+ (r = -0.18).

Clymo (1967) found that the acidity of northern bog systems is much greater in communities dominated by Sphagnum, a plant that also occurs in the Austin Cary dome. Clymo indicated that Sphagnum behaves as a cation exchanger even when dead, and he concluded that these plants are the major source of bog activity. High concentrations of long chain polymers of unesterified uronic acids found in Sphagnum call walls irreversibly exchange the cations in rain and groundwater for the H^+ ions on the -COOH groups. New exchange sites are continually produced by growth at the apex of the plant. Production of these sites could serve as a means of limiting competition and also providing a

habitat favorable to the continuation of Sphagnum. Other acidophilic plants such as Utricularia may also possess this capacity. This mechanism may explain the lack of correlation between dissolved organic matter and pH for the dome waters.

Two aliquots of surface water (one purged with helium and the other unpurged) were titrated to pH 7.0 with a strong base in a vessel closed to the atmosphere, demonstrating that the effect of dissolved CO_2 ($=\text{H}_2\text{CO}_3^*$) on free acidity (i.e., measured pH) was negligible. The initial pH of unpurged samples was 4.38; initial pH of purged samples was 4.48. H_2CO_3^* does not contribute much to free acidity below pH 5 (Stumm and Morgan 1970). However, H_2CO_3^* has a large effect on total acidity. Purging the surface water of H_2CO_3^* decreased the total acidity from 726 $\mu\text{eq/L}$ to 239 $\mu\text{eq/L}$ (end point of titration at $\text{pH} = 8.35$, the stoichiometric neutralization of carbonic acid ($=\text{H}_2\text{CO}_3^*$) to bicarbonate). This is equivalent to 21.4 mg/L of CO_2 .

Eighty percent of the total acidity in the purged sample (titrated to $\text{pH} = 7.0$) was due to bound acidity (Figure 4-4) from nonvolatile weak acids (e.g., humic acids) and hydrolyzable metals (Bronsted acids) such as Fe^{+3} and Al^{+3} with $\text{pK}'\text{s} < 7.0$. Only 20 percent of the total acidity (33 $\mu\text{eq/L}$) was free acidity. Strong acids, such as nitric and sulfuric, which completely dissociate in aqueous solutions, always contribute to the free acidity. Aluminum, iron, and organic acids are high enough in the standing waters to influence the measured pH to some extent, since their $\text{pK}'\text{s}$ are between 3 and 5.

Major Cations

Levels of calcium, magnesium, sodium, and potassium in Austin Cary surface waters are low (Table 4-1), and in general similar to the

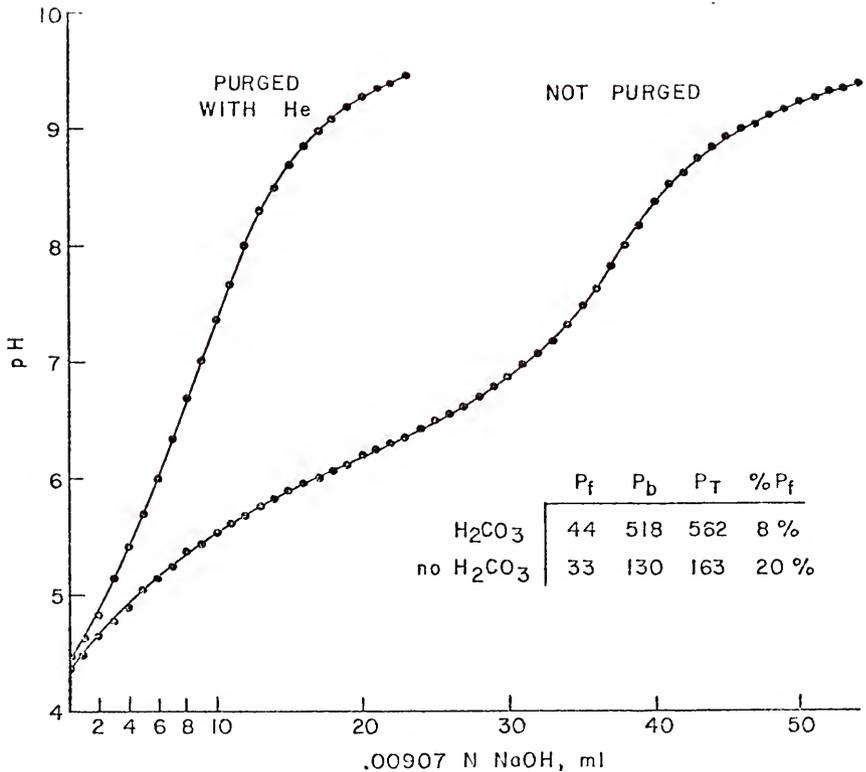


Figure 4-4. The effect of $H_2CO_3^*$ on the free, bound and total acidity of the surface water from Austin Cary cypress dome. The unpurged curve represents the presence of $H_2CO_3^*$. The purged curve represents an aliquot from the same sample from which the $H_2CO_3^*$ has been removed. The symbols P_f , P_b , and P_T represent, respectively, the concentration of free protons, bound protons, and total protons (in micro-equivalents per liter); P_b is determined by difference, $P_T - P_f$. The symbol $\%P_f$ represents the percentage of total protons that are free. Sample collected on March 15, 1979.

values reported for colored lakes in north-central Florida (Shannon and Brezonik 1972). The low concentrations reflect the lack of erosion (and erodable minerals) in the watershed. Coultas and Calhoun (1975) found low cation exchange capacities (CEC) (9.32 meq/100 g) in the surface horizons of the soils surrounding a cypress dome, but the CEC increased to 130 and 144 meq/100 g in the more extensive flooded soils in the dome interior. Extractable cations were low at all sites: 14.9 meq/100 g at the most frequently flooded site, 5.4 meq/100 g at the medium flooded site, and 0.3 meq/100 g at the least flooded site adjacent to the dome's edge. Thus the majority of the exchange sites are occupied by hydrogen ions. The low amounts of exchangeable bases at the site outside the dome is consistent with the weathered nature of the soils: the humic, well-drained surface horizons have been stripped of the cations by percolating water.

Major cation/chloride ratios in wet-only precipitation are similar to those in seawater and suggest a substantial marine influence on the concentrations found in rainfall (Table 4-2). On the other hand, Ca/Cl and K/Cl ratios in bulk precipitation (dry fallout plus rainfall) were higher than the ratios in seawater, indicating a significant terrigenous source (Table 4-2). The Ca/Cl and Mg/Cl ratios increased by a factor of two in throughfall, indicating that the cations are more readily leached from the canopy vegetation than chloride. The K/Cl ratio was the same in both sets of samples. The cation/chloride ratios for Ca and K in the standing (surface) water were lower than those in throughfall. Potassium had the largest reduction; cation exchange with the peat and/or biological assimilation could explain the lower ratios.

Table 4-2. Ratios of selected constituents to chloride (by weight) in the waters of Austin Cary cypress dome and comparison waters.

Water	Na/Cl	K/Cl	Mg/Cl	Ca/Cl	SO ₄ /Cl	F/Cl	Si/Cl	Fe/Cl	C/Cl
Seawater	0.55	0.02	0.07	0.02	0.14	7.0x10 ⁻⁵	2.0x10 ⁻⁴	5.3x10 ⁻⁷	
Austin Cary cypress dome									
Wet-only precipitation ^a	0.58	--- ^b	0.06	0.01	2.40	---	0.02	---	---
Bulk precipitation ^c	0.59	0.18	0.06	0.23	2.19	---	0.02	---	---
Throughfall ^c	0.48	0.18	0.10	0.40	0.96	---	0.02	---	---
Standing water ^c	0.67	0.03	0.15	0.34	0.43	3.7x10 ⁻³	0.29	0.05	6.91
Groundwater									
Austin Cary 1	0.26	0.005	0.16	0.31	0.04	1.2x10 ⁻³	0.19	---	0.67
Austin Cary 2	0.63	0.02	1.10	2.02	0.13	3.1x10 ⁻²	0.28	---	3.2
Papyrus swamps									
Uganda (Viner)	2.42	0.96	0.88	1.18	8.19	---	2.13	0.50	---
(Beck et al.) ^d	0.60	0.14	0.12	0.16	0.13	---	0.43	0.15	4.3 ^e

^a Based on one sample collected above dome on 4/20/78.

^b Below limits of detection.

^c Volume weighted means for one year (4/20/78 - 4/19/79).

^d Average of three swamp samples.

^e Based on carbon comprising 50 percent of the organic matter.

Larger increases in concentration were found for the divalent cations (5.6-6.2 x) in throughfall compared to levels above the tree canopy than were found for monovalent cations (2.9-3.4 x) (Figure 4-1). Higher concentrations (1.6-2.8 x) were found for Na, Ca, and Mg in the standing water than in throughfall. Potassium levels in the surface water were less than half those in throughfall, however.

Gibbs (1970) used the weight ratios of $\frac{\text{Na}}{(\text{Ca} + \text{Na})}$ versus total dissolved salts as a general indication of the major factors affecting surface water chemistry of various lakes, oceans, and rivers (Figure 4-5). The position of Austin Cary data on Gibbs' graph indicates that atmospheric precipitation controls dome water ionic composition.

Major Anions

The major inorganic anions in the surface water of the dome are sulfate and chloride, and these are derived primarily from the atmosphere via rainfall. In general, concentrations of these ions in rainfall, throughfall, and surface water (Figure 4-1) were a few mg/L or less, with chloride concentration increasing 3.5 times in throughfall compared to bulk precipitation. Standing water concentrations reflected a further increase (1.9 x) over the levels in throughfall (Figure 4-1). The chloride levels found in the standing waters ($\bar{x} = 8.2$ mg/L) (Table 4-1) are similar to values reported for Georgia swamps (Beck et al. 1974), but much higher than the levels (0.01-1.0 mg/L) reported for black water creeks of the Amazon (Sioli 1975). These differences reflect the distances from the ocean. Variations in the chloride concentrations of the standing water from 1977 to 1979 reflect hydrologic conditions (i.e., the balance between rainfall and evaporation).

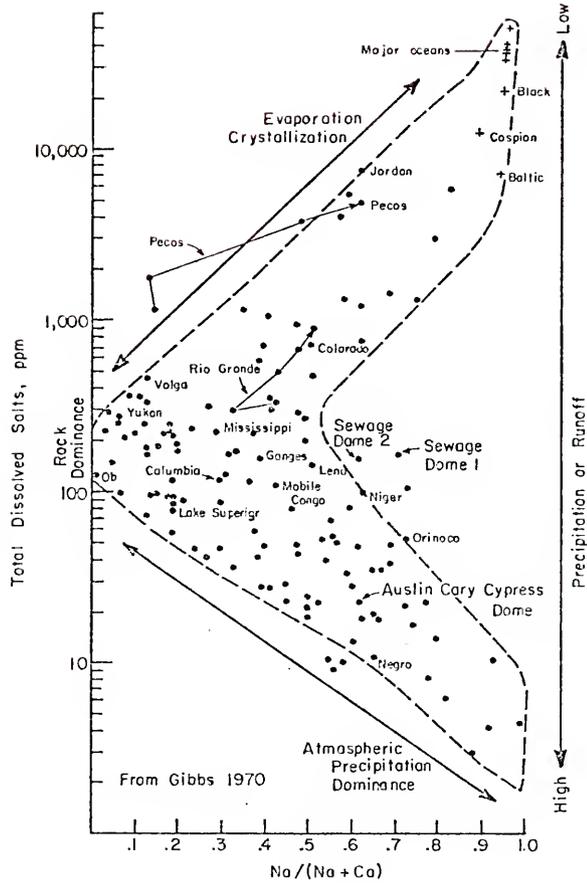


Figure 4-5. The relationship of Austin Cary cypress dome to other world surface waters with respect to the controlling mechanisms of their chemical compositions according to Gibbs' model.

Sulfate levels were slightly lower in the surface waters of the Austin Cary dome than in throughfall, but throughfall had 1.5 times more sulfate than rainfall (Figure 4-1). Temporal variations of sulfate concentrations in the surface water were not related significantly to standing water depth ($r = -0.21$). Sulfate is the major sulfur species for biotic assimilation, and its depletion in the surface waters (compared to throughfall) suggests that either sulfur is a limiting nutrient in these ecosystems, or it is reduced to H_2S by anaerobic bacteria. Ratios of SO_4/Cl in precipitation were much higher than the ratio for seawater (Table 4-2), indicating that much of the sulfate in rain is anthropogenic (i.e., SO_2 emissions from fossil fuel burning). Contributions from natural terrestrial sources are probably minor.

Sulfate concentrations in swamp waters are variable. Talling (1957) reported a large decrease in sulfate levels of the White Nile after it passed through a swamp region of the Sudan. Visser (1962) and Viner (1975) reported much higher sulfate levels (10-70 mg/L) for papyrus swamps in Uganda. Viner also found high sulfate levels in bog waters in the Ruwenzori Mountains. Sioli (1975) reported sulfate levels ranging from 0.0-2.7 mg/L for black water creeks of the Amazon. Beck et al. (1974) recorded sulfate concentrations between 0.6-0.9 mg/L for Georgia swamps and tributaries of the Satilla River. It appears that aqueous sulfate concentrations for swampy regions are generally lower than levels reported in lakes. Klinge and Ohle (1964) concluded that sulfur limitation is probable in Amazonia, based on the low sulfate levels. Because of the low pH of swamp waters, bicarbonate usually is not present. Although nitrate is a major ion in

rainfall (Figure 4-6), assimilation by plants and microorganisms depletes this ion in the surface water, leaving SO_4 and Cl as the only major anions in the water.

The sum of cation equivalents (5.75×10^{-4} eq/L) is appreciably larger than the sum of inorganic anions (3.3×10^{-4} eq/L) in the dome water. The occurrence of dissociated carboxylate anions associated with the dissolved organic matter accounts for the difference in the inorganic charge balance.

Dissolved Gases

Oxygen

Dissolved oxygen values were consistently lower than saturation and never exceeded 6.8 mg/L ($\bar{x} = 2.03$ mg/L) (Table 4-3). This is not unusual in swamp water. Beck et al. (1974) noted a reduction in dissolved oxygen percent saturation when the Satilla River was influenced by swamp waters. Talling (1957) reported that the oxygen content of the White Nile fell from 70-80 percent to 13-35 percent as the river flowed through a large swampy area. Carter (1955) found oxygen to be virtually absent in swamps near Lake Victoria. Beadle (1974) made the general comment that levels of dissolved oxygen in all shaded swamps in Africa are low.

Table 4-3. Dissolved gases in the surface waters of Austin Cary cypress dome over time period March 1976 to July 1979.

Gas	n	Range (mg/L)	Mean (mg/L)
Oxygen	21	0.25 - 6.75	2.03
Carbon dioxide	10	0.00 - 21.4	5.0
Hydrogen sulfide	21	---	<0.01

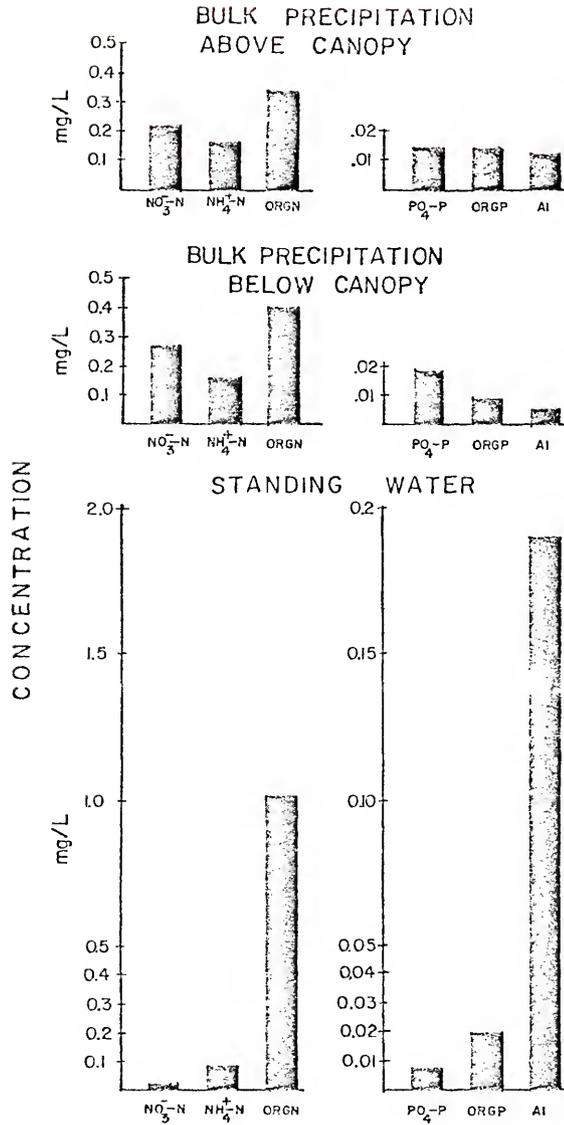
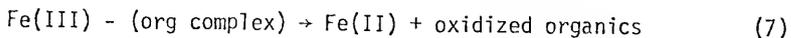
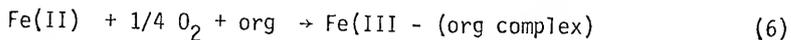


Figure 4-6. Nitrogen, phosphorus, and aluminum concentrations in the natural waters of Austin Cary cypress dome.

The low dissolved oxygen levels in humic-colored swamp water could be attributed to several processes. Heterotrophic respiration of organic matter in the shallow, shaded, colored swamp water and peat certainly contributes to oxygen consumption. However, a purely chemical mechanism may also be important. Lönnerblad (1931) and Juday and Birge (1932) noted that humic sediments and water could take up oxygen even after sterilization. Hutchinson (1957) suggested that purely chemical oxidation reactions may lower the oxygen content of surface waters in humic lakes, presumably by a photochemical mechanism. Flaig (1966) believed that polyphenols, amino acids and carbohydrates derived from decomposition of biopolymers constantly undergo autooxidation, resulting in polymeric phenolic substances that resemble soil fulvic acids.

Stumm and Morgan (1970) noted that organic substances containing hydroxyl and/or carboxylic functional groups (e.g., phenols, polyphenols, gallic acid, and tannic acid) such as are commonly found in the colored waters of swamps can both reduce ferric iron (to Fe II) and catalyze the oxygenation rate. The ferrous-ferric system thus acts as a catalyst for the oxidation of organic material by oxygen:



Miles (1979) has shown that the catalytic cycle involving Fe II/Fe III can produce O_2 consumption rates of $1.0\text{-}1.3 \times 10^{-6}$ M/L-h (0.03-0.04 mg/L-h) in samples of Austin Cary dome water incubated in the laboratory. Half times for dissolved oxygen (according to first-order kinetics) ranged from 17 to 23 h, indicating that this is a significant oxygen sink in humic-colored waters.

Carbon Dioxide

Inorganic carbon averaged 7.0 mg/L in Austin Cary dome water and was composed almost entirely of dissolved carbon dioxide. Examination of carbonate equilibria for the pH range in the dome water indicates that only trace amounts of bicarbonate exist in the water, and hence there is no titratable alkalinity. Dissolved CO_2 in equilibrium with the atmosphere results in a concentration of only 0.6 mg/L. The high levels of CO_2 in Austin Cary waters, along with the reduced dissolved oxygen, suggest respiration exceeds photosynthesis in the swamp waters. It is not unusual for swamp waters to have high levels of dissolved CO_2 . Talling (1957) found that CO_2 levels increased from 3 to 18 mg/L as the White Nile flowed through a large swamp. Fittkau (1964) found that free CO_2 averaged 20 mg/L in 12 streams of the central Amazon.

Hydrogen Sulfide

Hydrogen sulfide was not detected either in the dome surface water or in interstitial water up to 30 cm below the peat surface (limit of detection = 0.01 mg/L). Linero (1976) found no gases were emitted in Austin Cary dome during a 6-day test under the natural, quiescent conditions of the dome. Measurable quantities of gas were collected only after the sediments below the samples were stirred. Considering that the dome waters are always oxygenated and quiescent, H_2S produced in anaerobic peat probably has sufficient time to become oxidized before reaching the air-water interface. Beadle (1974) remarked that H_2S normally is not detectable by smell in papyrus swamps, though it is sometimes obvious for a short time after a sudden rise in water level following heavy rains.

Minor and Trace Inorganic Components

Fluoride

Fluoride normally was detected in the dome surface waters, but concentrations averaged only 0.030 mg/L (Table 4-4). The low levels of fluoride are as expected for soft, acidic swamp water that is hydrologically distant from fluorapatite deposits. The major source of fluoride to Austin Cary surface waters probably is precipitation, but most analyses of rainfall at the dome showed no detectable fluoride (limits of detection = 0.02 mg/L). Fluoride has been detected at low concentrations in rainfall near phosphate mining operations in central Florida and in extreme northern Florida (Brezonik et al. 1980b).

Table 4-4. Minor ionic composition of Austin Cary cypress dome surface water over the period January 1974 to June 1979.

	n	µg/L		µeq/L	
		Range	Mean	Range	Mean
Cations					
NH ₄ ⁺ -N	63	10 - 1200	139	0.7 - 85.7	9.9
Fe	18	150 - 600	370	8.1 - 32.2	19.9
Mn	18	9 - 96	23	0.3 - 3.5	0.8
Al	17	130 - 270	200	14.4 - 30.0	22.1
Anions					
F ⁻	24	< 19 - 50	30	< 1.0 - 2.6	1.6
NO ₃ ⁻ +NO ₂ ⁻ -N	63	< 5 - 1040	80	0.4 - 74.3	5.8
H ₂ PO ₄ ⁻ -P	61	< 4.8 - 480	74	< 0.2 - 15.5	2.4
Uncharged					
Si	18	20 - 5900	1960		

The ion product of the average molar concentrations of Ca^{2+} , F^- , and PO_4^{3-} ($10^{-74.5}$) is much lower than the solubility product of fluorapatite ($K_{\text{sp}} = 10^{-55}$). Use of activities instead of concentrations would not significantly change the calculated ion product, since the ionic strength is low. The high degree of undersaturation indicates that fluoride concentrations are not controlled by fluorapatite. Fluoride concentrations in the shallow groundwaters under the dome are higher ($\bar{X} = 0.17$ and 0.56 mg/L), reflecting the nearness of the fluorapatite-bearing Hawthorn formation (Gillespie 1976).

Iron

The equilibrium ratio of $\frac{[\text{Fe}^{+3}]}{[\text{Fe}^{+2}]}$ in the oxygenated surface water of the dome is very high ($\gg 10^2$). However, the observed ratio is about 10 because dissolved humic material reduces iron to the ferrous state (Miles 1979). Free ferrous iron is oxidized by dissolved oxygen to the ferric state, but because ferrous oxygenation is slow at low pH, appreciable Fe^{+2} can build up in the system.

It is commonly believed that colored water, such as Austin Cary surface water, has higher "soluble" Fe concentrations than predicted from hydroxide equilibria alone because functional groups associated with humic acid molecules form soluble chelates with ferrous and ferric iron and thus increase the solubility of iron. The observed mean "soluble" Fe_T in the surface waters of the Austin Cary dome was almost two orders of magnitude higher at the mean pH (4.5) than the equilibrium value, assuming the $\text{Fe}(\text{OH})_3(\text{s})$ controls the solubility of iron (Figure 4-7). Alternatively, the high concentrations of "soluble" iron could be explained by the formation of colloidal "lakes" between color bases and ferric hydroxide (Stumm and Morgan 1970). On the other hand,

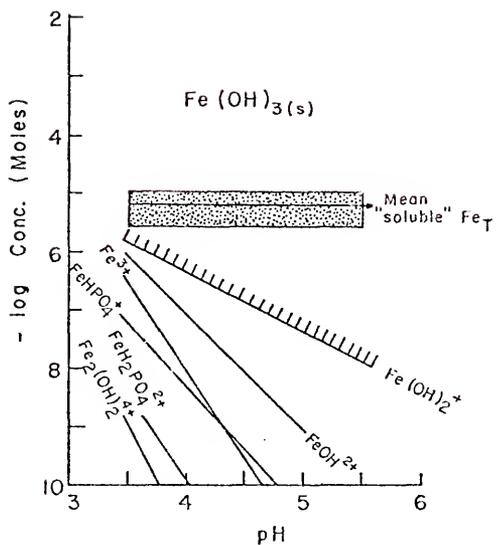


Figure 4-7. Equilibrium solubility domain of amorphous $\text{Fe(OH)}_3(\text{s})$ showing the Fe hydrolysis and Fe- PO_4 complexes in the surface water of Austin Cary cypress dome. The shaded area represents the range of "soluble" total iron (Fe_T) measured.

concentrations of "soluble" Fe_T (\bar{X} = 0.37 mg/L; range = 0.15-0.60 mg/L) were lower than levels often reported for humic-colored waters. Beck et al. (1974) found Fe concentrations ranging from 0.32 to 1.17 mg/L for three samples of swamp water in Georgia. Viner (1975) reported Fe concentrations of 0.02-7.2 mg/L in a papyrus swamp with most samples having concentrations >0.5 mg/L.

Dissolved Silica

Silica concentrations in the dome surface water are moderate (\bar{X} = 1.96 mg/L as Si) and range from 0.02 to 5.90 mg/L over the period 1977-1978 (Figure 4-8). Highest concentrations occurred in winter and lowest concentrations occurred during the summer for two successive years, suggesting that biological uptake was the cause of the observed seasonal variations. Replenishment of silica may depend primarily on runoff from surrounding uplands, since the atmospheric contribution of SiO_2 was negligible (\bar{X} = 0.02 mg Si/L in bulk precipitation and 0.05 mg/L in throughfall) (Figure 4-1).

Most of the dissolved silica in natural water results from the breakdown of aluminosilicate minerals in the process of weathering (Hem 1970; Stumm and Morgan 1970). Although silica solubility itself is independent of pH up to a value of 9.0, pH still plays a prominent role in weathering of soil because of its effects on the solubility of aluminum and iron compounds. Pedro (1961) noted that proportionately greater amounts of aluminum and iron relative to silica are leached in acid media than in neutral media. The acidic nature of the soils, peat, and surface water of the Austin Cary dome may explain the high iron and low silica concentrations in the surface waters. According to Stumm and Morgan (1970), increasing acidity, pE, and tendency for

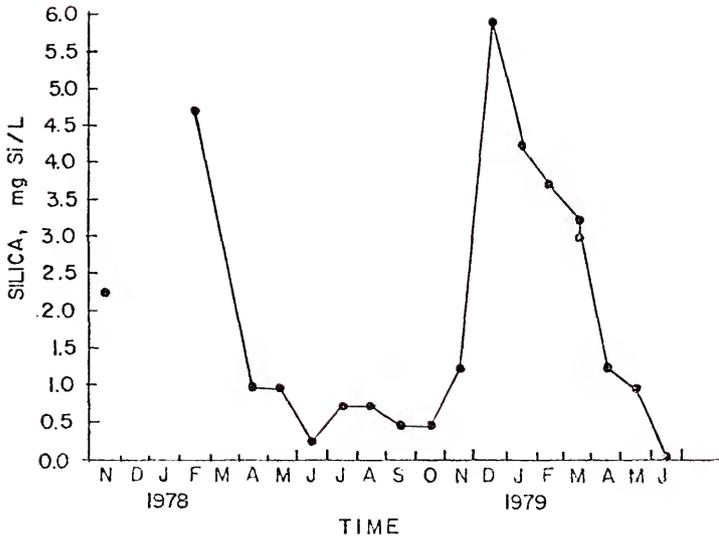


Figure 4-8. Monthly variations in the silica concentrations for Austin Cary cypress dome standing water during a 1½-year period.

complex formation all favor greater weathering intensity. Considering the low pH and the high concentrations of natural organic matter in the dome surface water, the low levels of soluble silica indicate the soil is thoroughly weathered. Coultas and Calhoun (1975) provided additional evidence that the soil is highly weathered in that quartz was found to be the dominant mineral in the surface horizons of all soil types in the watershed of a nearby cypress dome. They concluded that the extreme acidity of the surface horizons contributes to the breakdown and removal of silicate clays leaving the more resistant quartz. Kaolinite and metahalloysite were found to be the dominant clays in the deeper horizons. Considering the abundance of quartz in the soil, it is surprising that dissolved silica is not present at higher concentrations in the surface water. At 25°C, the solubility of quartz is 5.6 mg Si/L. This value was reached only once (December 1978) during an 18-month period. Uptake by the biota may serve to keep the water undersaturated. Also, the rate to reach equilibrium between silicic acid and quartz is extremely slow (Stumm and Morgan 1970).

Beck et al. (1974) found low silica levels (e.g., <1 mg/L in the Okefenokee Swamp, which is in a region of highly leached soils in the Georgia Coastal Plain. Sioli (1975) also reported low silica levels (0.8-2.7 mg/L) for black Amazonian waters, but African swamps have higher levels (up to 17 mg/L) (Rzóska 1974; Viner 1975).

Aluminum

The solubility of aluminum is pH dependent, and in the absence of organic complexing agents, the dominant soluble forms of Al are hydroxide complexes ($\text{Al}[\text{OH}]^{2+}$, $\text{Al}[\text{OH}]_2^+$ in acidic waters). At neutral

and slightly acidic pH values, Al is quite insoluble (<0.1 mg/L), but at pH <5, Al solubility increases markedly, and toxic concentrations (>0.2 mg/L) can be found in lakes that have been acidified by acid rain. In the presence of organic ligands, the solubility of aluminum can be enhanced by the formation of soluble organic-aluminum complexes, which are thought to be nontoxic (Driscoll et al. 1980). The average aluminum concentration (\bar{X} = 0.20 mg/L) in the standing water of the dome was high compared to values found in soft water lakes near Gainesville (Brezonik et al. 1980b), but nonetheless it was nearly three times lower than the equilibrium level based on aluminum hydroxide solubility at the pH of the dome water (4.51). The undersaturated condition may be explained by biological uptake, dilution by rain falling directly on the dome, or shielding by vegetation and high runoff. Higher aluminum concentrations (\bar{X} = 0.50 mg/L) were found in pore water from the peat (30 cm deep) under the standing water. According to Heimbürg (1976), about 1.5-2.0 times more water enters the dome as throughfall than as runoff. These findings support the last two possibilities as probable explanations for the difference between the observed and equilibrium aluminum concentrations in the standing waters.

Dissolved Carbon

Total organic carbon in the Austin Cary dome ranged from 16 to 75 mg/L (\bar{X} = 39.9 mg/L, n = 32) (Table 4-5). These values are comparable to levels found by Beck et al. (1974) for Georgia swamps, but are higher than the levels found in a mixed hardwood swamp at Wildwood, Florida (Boyt et al. 1977).

Table 4-5. Composition of organic matter in the surface waters of Austin Cary cypress dome.

Form	n	Range (mg/L)	Mean (mg/L)
Nitrogen	62	0.1 - 8.4	1.4
Phosphorus	61	0.0 - 2.3	0.11
Carbon	32	16.0 - 75.0	39.9
BOD ₅	28	0.9 - 6.5	2.9
Color (CPU)	43	195 - 789	456

As the high correlation between color and total organic carbon (TOC) in Figure 4-9 implies, most of the organic material in the surface waters of swamps is humic material that imparts the brown color to swamp waters. Organic color concentrations for the Austin Cary swamp ranged from 195 to 789 chloroplatinate units (CPU) with a mean of 456 CPU.

Humic materials are noted for their complexing properties. The concentration of dissolved iron was weakly correlated with both total organic matter ($r = 0.55$) and color ($r = 0.60$) in the dome surface water (Figures 4-10 and 4-11); Beck et al. (1974) reported a similar relationship for Satilla River water. Aluminum apparently complexes more strongly with humic material than does iron; correlation coefficients (r) for aluminum with organic matter and color were 0.74 and 0.93, respectively (Figures 4-12 and 4-13).

Konova (1961) and others have found dissolved humic substances to be resistant to microbial decomposition. The biochemical oxygen demand (5-day) of Austin Cary surface water consistently was low ($\bar{X} = 2.9$ mg/L)

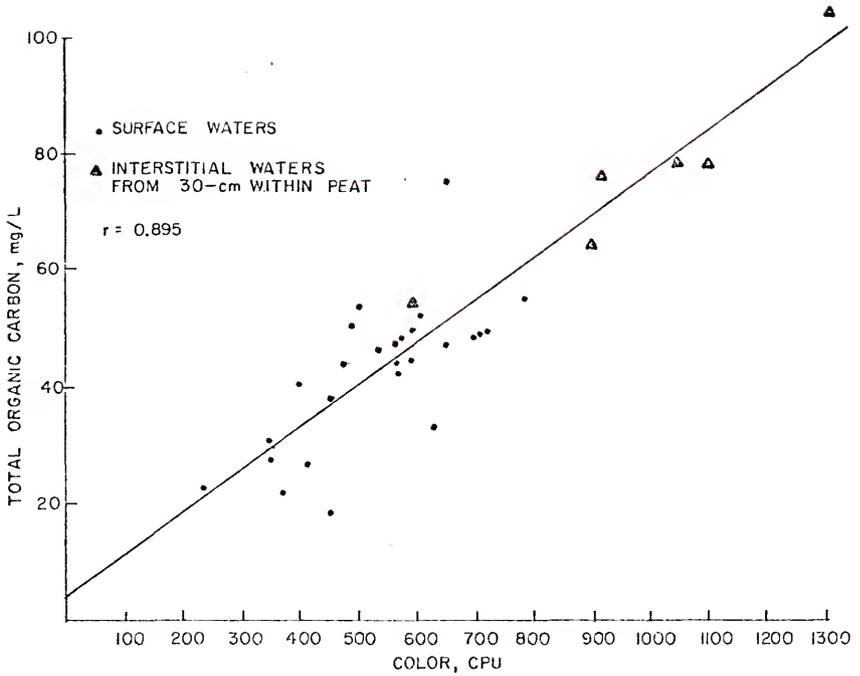


Figure 4-9. Correlation of total organic carbon with color for Austin Cary cypress dome.

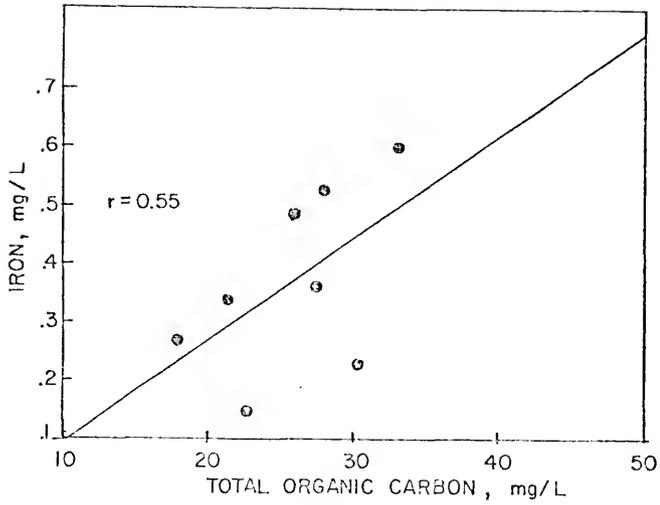


Figure 4-10. Correlation of iron with total organic carbon for Austin Cary cypress dome.

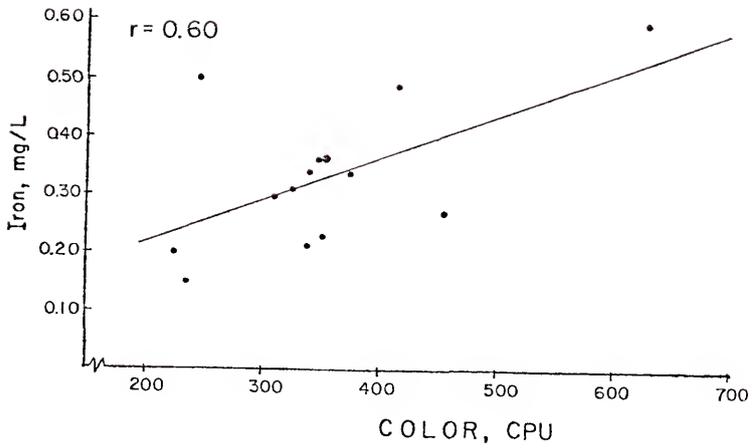


Figure 4-11. Correlation of iron with color for the standing water of Austin Cary cypress dome.

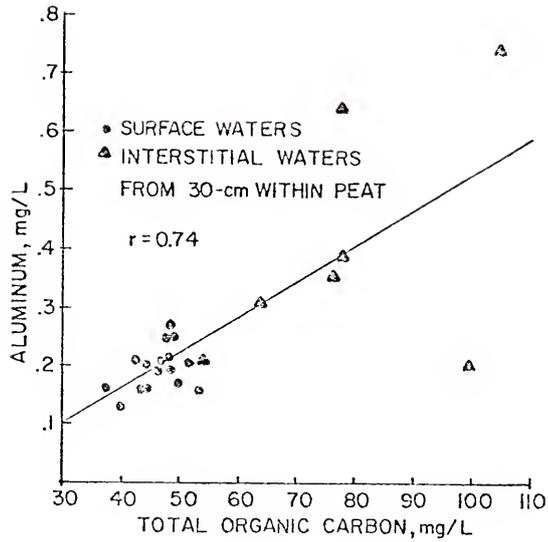


Figure 4-12. Correlation of aluminum with total organic carbon for Austin Cary cypress dome.

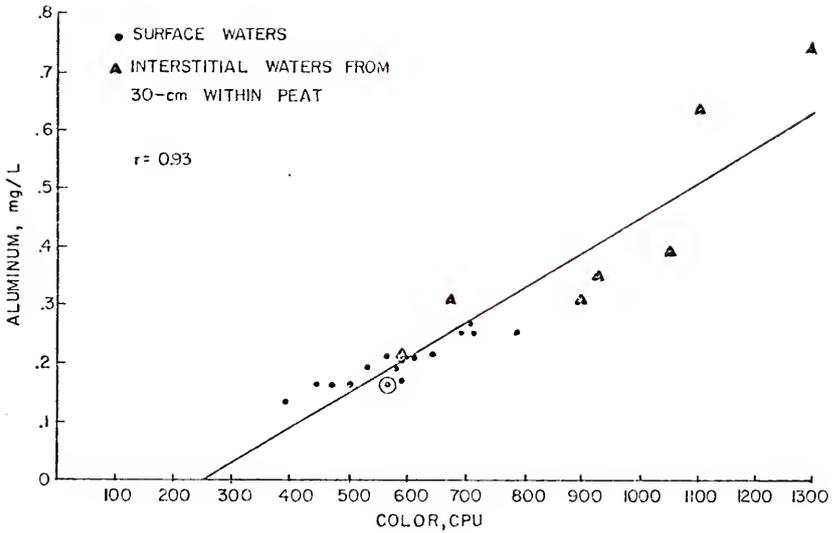


Figure 4-13. Correlation of aluminum with color for Austin Cary cypress dome.

(Table 4-5). Rates of cypress leaf decomposition (as measured by loss in dry weight) in the dome were also low (see Chapter 5). The low biological activity may possibly reflect toxic effects of polyphenols in humic or tannic substances. Such effects have been observed by some workers. For example, Rice and Pancholy (1973) found nitrification was inhibited by tannins in climax terrestrial systems, and Janzen (1974) discussed numerous observations implicating the toxicity of tannins in tropical wetlands. However, experiments reported in Chapter 6 showed that low rates of nitrification in Austin Cary surface water were caused by low pH and not by organic color.

Major Nutrients

Phosphorus

Soluble reactive phosphate (SRP) (i.e., orthophosphate) had relatively high concentrations (\bar{X} = 0.07 mg P/L) in the dome surface water (Figure 4-14). Levels of SRP were slightly influenced by the large water level fluctuations that occurred during the 5.5-yr period (r = -0.31).

High levels of phosphorus are common in Florida surface waters and groundwaters. For example, Odum (1953) reported mean values of total phosphorus of 0.046 mg/L for streams, 0.038 mg/L for lakes, and 0.045 mg/L for springs in Florida (excluding the phosphate mining area).

The levels of phosphorus in the bulk precipitation for the dome averaged 14 μ g P/L with only a slight increase occurring in through-fall (19 μ g/L) (Figure 4-6). However, standing water had a lower average concentration (7 μ g/L) for the same time period, indicating that the dome is a biological and/or chemical sink for SRP. Sources of phosphorus in rainfall are probably wind erosion of soil and dust

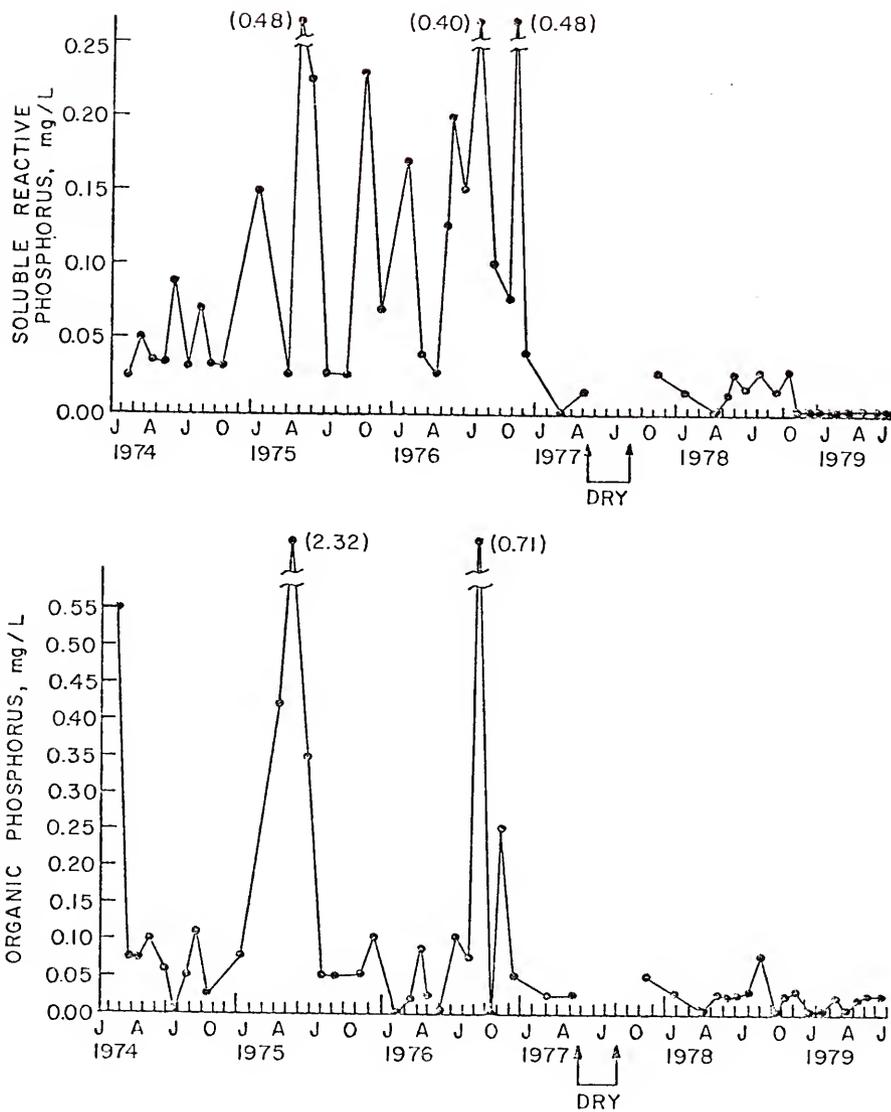


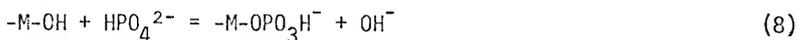
Figure 4-14. Monthly variations in the soluble reactive phosphorus and organic phosphorus concentrations for Austin Cary cypress dome standing water.

particles. The rainfall values found at the dome are comparable to levels found at other rural sites in Florida (Brezonik et al. 1980b).

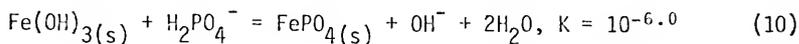
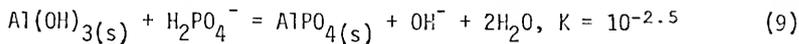
The shallow nature of the standing water in the dome (and hence the proximity of the peat and water) suggests that the phosphate content of the water may be controlled either by chemical equilibria with iron or aluminum compounds in the peat or by the rate of mineralization of organic phosphate in the sediment.

Inspection of the solubility equilibria of orthophosphate (Stumm and Morgan 1970) indicates that $\text{AlPO}_4(\text{s})$ is more likely to control the solubility of phosphate than $\text{FePO}_4(\text{s})$ and hydroxyapatite in the pH range of 4.0-5.5. At a pH of 4.0, the equilibrium concentration of orthophosphate is 0.1 mg P/L, and at a pH of 5.5, the solubility is only 0.02 mg/L. This range includes a large percentage of the observed orthophosphate levels (Table 4-4).

It is commonly thought, however, that surface complex formation with hydrous ferric and aluminum oxides plays a greater role in controlling the phosphate levels in natural waters than do simple solubility equilibria alone (Syers et al. 1973; Patrick and Khalid 1974); for example



Stumm and Morgan (1970) point out that reactions of this kind can be considered a special case of precipitation, in which metal ions on solid surface form bonds with solution species. For example in the equilibria:



Formation of aluminum phosphate and ferric phosphate is possible if the orthophosphate is greater than 3.1 $\mu\text{g/L}$ and 9.8 mg/L , respectively, at the mean pH for Austin Cary surface water (4.5). Since orthophosphate exceeded the former but not the latter value, aluminum phosphate could control phosphate solubility.

The mean concentration of organic phosphate (the difference between total phosphate and SRP) over the study period was 0.11 mg P/L ; but this value is influenced heavily by two periods of very high concentrations that occurred during dry periods in 1975 and 1976. Concentrations of organic P in the surface water generally were higher than SRP values (Figure 4-14). The organic phosphorus contents of rain was higher than that in throughfall (0.014 and 0.009 mg/L) (Figure 4-6).

Nitrogen

Nitrogen is frequently mentioned as the element limiting productivity in tropical fresh water and terrestrial environments (Date 1973; Viner 1975). The aqueous transformations and cycling of nitrogen are almost entirely microbially mediated, and the nitrogen cycle is complicated by gaseous transformations including N_2 fixation and denitrification.

Swamps are generally thought to act as nitrogen sinks. Carter (1955) found inorganic nitrogen was reduced to low levels after passage through swamps near Lake Victoria, and Viner (1975) similarly found inorganic N concentrations were much lower in a papyrus swamp than in other types of water in the same area of Africa.

Nitrate + nitrite

Levels of nitrate + nitrite in the surface waters of the Austin Cary dome ranged from <0.005 to 1.04 mg N/L, (\bar{X} = 0.08 mg/L) over the 5.5-yr period of the study (Table 4-4). Nitrate + nitrite concentrations were less than 0.12 mg N/L on all but three dates (Figure 4-15). The three peaks are not explainable in terms of either rainfall events or stage levels, and no seasonal or hydrologic trends are evident. Nitrate + nitrite levels were much higher in bulk precipitation and throughfall (\bar{X} = 0.22 and 0.26 mg/L, respectively) than in the surface water (0.02 mg/L) for the period of rainfall measurements (Figure 4-6). The rainfall values are similar to those reported for Gainesville by Hendry and Brezonik (1980). The atmosphere is the only significant source of nitrate and nitrite to the surface waters of Austin Cary dome. Because of the low pH of the dome water, microbial nitrification does not occur (see Chapter 6).

Ammonium

The mean ammonium concentration in Austin Cary over the entire study was 0.14 mg N/L, and a large range (0.01-1.2 mg/L) was found (Table 4-4). Variations in ammonium are more readily interpretable in terms of seasonal and hydrologic factors (Figure 4-15) than are the nitrate + nitrite levels. During the years when the water level remained high in the dome, peaks occurred from February to May. These peaks may result from the decomposition of the previous year's leaf fall; the decrease later in spring may reflect assimilation of NH_4^+ by plants during leaf growth. Elevated ammonium levels were also noted whenever the water level was low. The relationship between depth of water and ammonium concentration was weak ($r = -0.27$, $P < 0.05$).

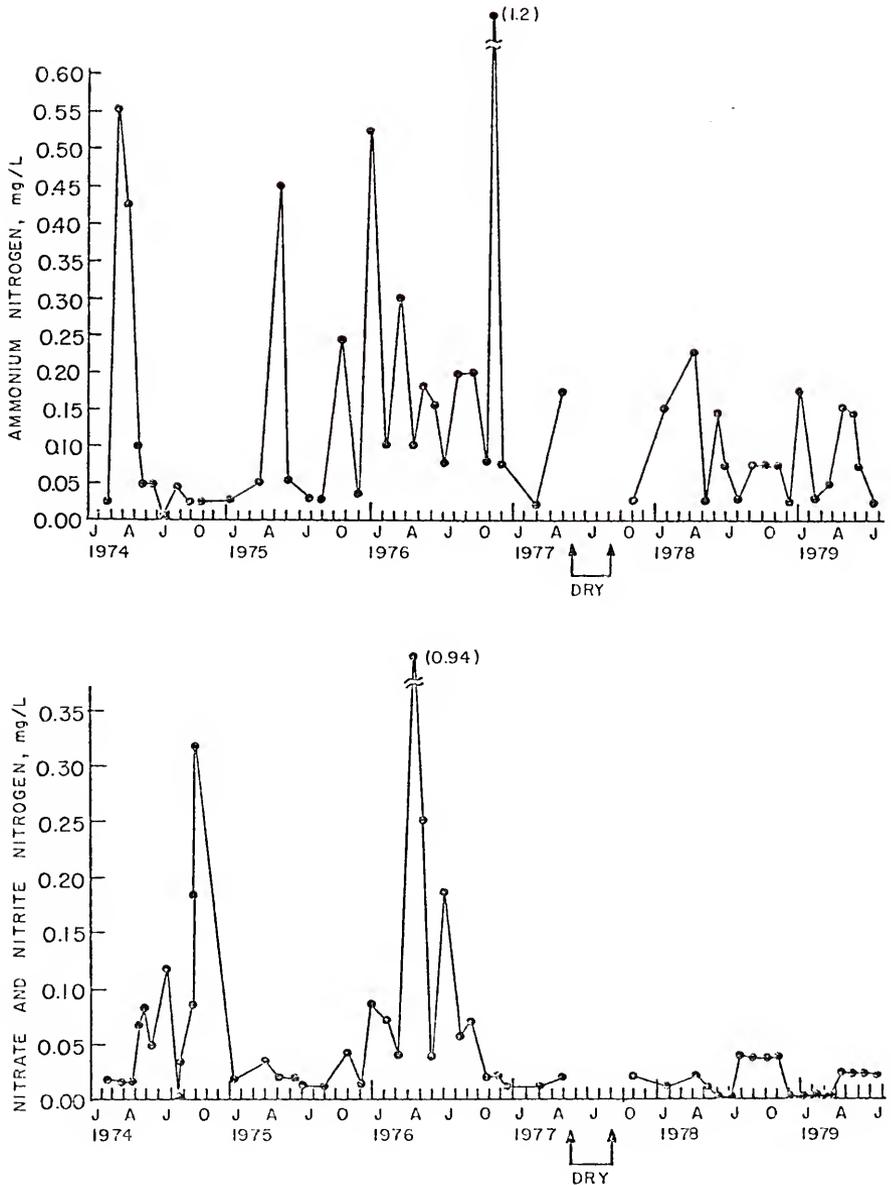


Figure 4-15. Monthly variations in the ammonium nitrogen and nitrate plus nitrite nitrogen concentrations for Austin Cary cypress dome standing water.

Ammonium concentrations were the same in throughfall and bulk precipitation (\bar{X} = 0.16 mg/L). The average concentration in the standing water for the same period of time was lower (0.08 mg/L), indicating that ammonium supplied by the atmosphere is readily assimilated by the biota in the dome.

Organic nitrogen

Organic nitrogen concentrations in the surface waters were high (\bar{X} = 1.4 mg/L), but quite variable (range 0.1-8.4 mg N/L) (Table 4-5). The mean concentration in bulk precipitation at the Austin Cary dome was high (0.3 mg/L) compared to the statewide average of 0.17 mg/L reported by Brezonik et al. (1980b). Organic N in throughfall was about 1.2 times that of bulk precipitation (Figure 4-6).

Fluctuations in organic N over the study period generally corresponded to oscillations in the water level (Figure 4-16). Standing water depth was weakly correlated (inversely) with organic N. Organic nitrogen concentrations were nearly an order of magnitude higher than inorganic nitrogen concentrations and comprised about 87 percent of the total nitrogen. The mean organic N level was comparable to levels reported in other swamp ecosystems. Viner (1975) found 2-4 mg of N/L in African papyrus swamps, and Boyt et al. (1977) found about 2 mg/L of organic nitrogen in a mixed hardwood swamp in Florida.

The C/N/P ratio in the surface water averaged 363:13:1. Hutchinson (1957) reported C/N ratios of 45/1 to 50/1 for allochthonous organic matter transported to lake or stream water. The high ratios reflect the relatively greater loss of nitrogen during decomposition. The average C/N ratio found in Austin Cary standing water (28/1) is considerably lower than the above ratios or even the C/N ratio in dead

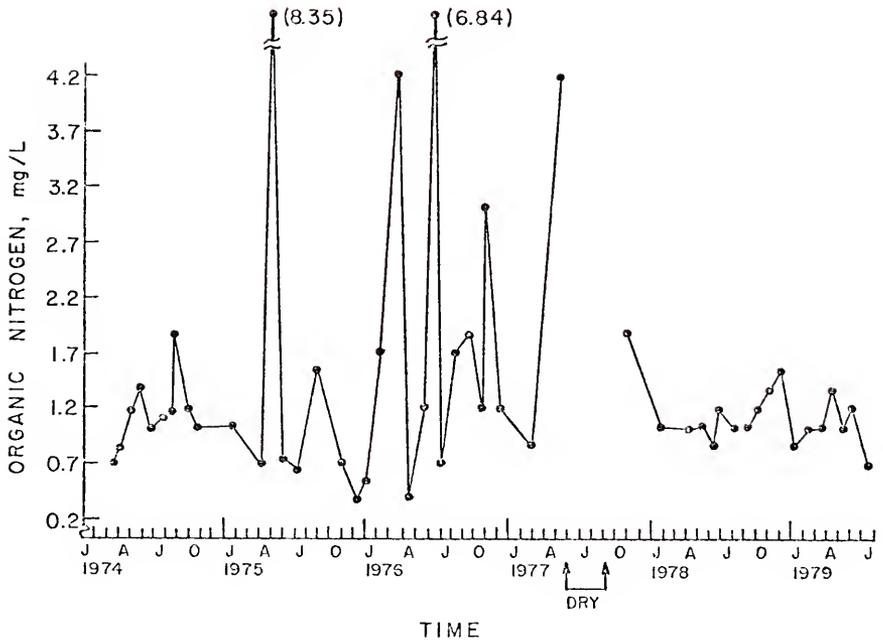


Figure 4-16. Monthly variations in the organic nitrogen concentrations for Austin Cary cypress dome standing water.

cypress leaves (50/1). The relatively low C/N ratio in the dome water suggests that nitrogen fixation occurs in the dome (see Chapter 6).

CHAPTER FIVE
DECOMPOSITION AND ORGANIC MATTER ACCUMULATION
ON THE SWAMP FLOORS OF A NATURAL AND SEWAGE-RECEIVING CYPRESS DOME

Litter decomposition rates are controlled by the susceptibility of litter to attack by decomposers. Some components of the litter are decomposed at faster rates than others (Waksman 1929, Cromack and Monk 1975, Fogel and Cromack 1977). According to Minderman (1968) the individual components of litter probably decay at rates that are exponential functions of time, but the observed rate (which is a combination of the losses of each component of the litter) may not be exponential. Foree and McCarty (1970) found that algae undergoing anaerobic decomposition followed first order kinetics (i.e., decayed exponentially) if the value of the refractory organic fraction of the algae was first subtracted.

Hunt (1977) divided substrates into rapidly and slowly decomposing components in a simulation model of decomposition in grasslands. Meentemeyer (1978) concluded that although the basic regulator of decomposition rates in terrestrial systems on a global basis is climate, lignin concentration (litter quality) is an excellent predictor of decay rates of different kinds of litters within a climatic zone and in climates having high actual evapotranspiration. Godshalk and Wetzel (1978) found that decomposition rates of aquatic angiosperms varied according to total fiber constituents (hemicellulose, cellulose, and lignin) in the tissue. Therefore, in analyzing decomposition rates of litter in aquatic ecosystems in warm climates, it seems clear

that failure to consider the different decomposition rates of the labile and refractory components in a complex substrate may affect the predictive capability of a decomposition model to a large extent.

Increased productivity rates (primarily due to the surface mat of duckweed) in sewage-treated cypress swamps raise the question of the longevity of the dome basin, since accumulation of organic matter would cause surface water storage capacity to be diminished. This chapter analyzes the effect of secondary sewage effluent on decomposition and organic matter accumulation in the sediments of cypress domes. A simple model is introduced whereby decomposition of leaf litter and duckweed is broken down into two components with different rates. These decomposition rates are then combined with productivity data for simulation of organic matter accumulation. The decomposition and organic matter accumulation on the swamp floor of a natural dome are presented for comparison.

Litter Decomposition

Goodness of Fit of Simple Exponential and Nonlinear Two-Compartment Models

The decomposition curves for cypress litter in the sewage and natural domes and the data from which they were generated are shown in Figures 5-1 and 5-2. A significant lack of fit ($P < 0.01$) was found when the data were fit to the linear exponential model (Figure 5-1). However, a nonlinear, two-compartment model provided a good fit of the data for both curves (lack of fit was not significant at the $\alpha = 0.05$ level) (Figure 5-2).

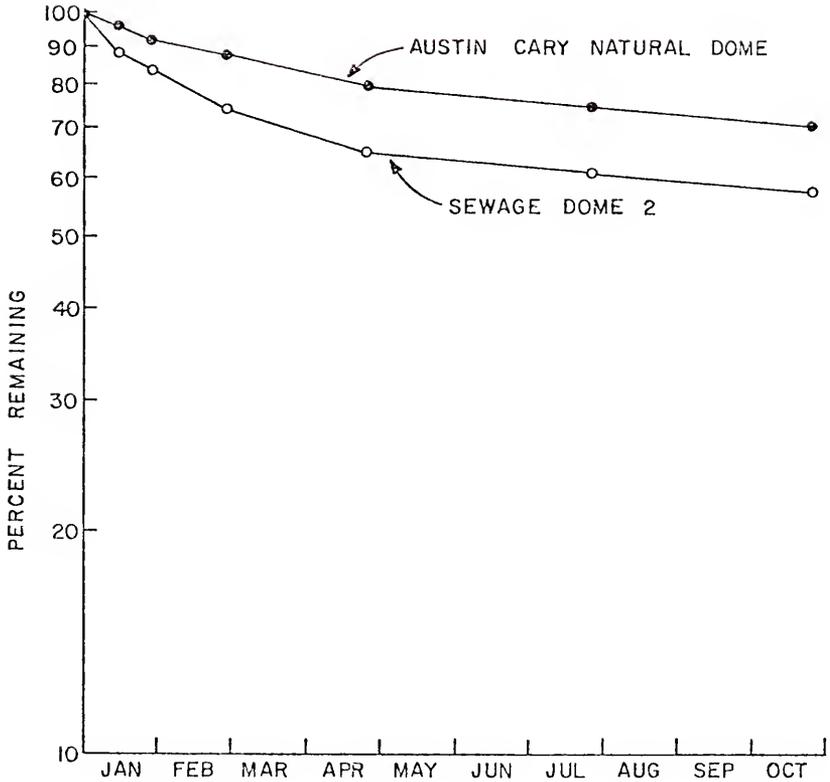


Figure 5-1. The decomposition curves of cypress leaf litter for sewage dome 2 and Austin Cary natural dome showing that decomposition is not accurately represented by a simple exponential decay model of the form $y = e^{-kt}$.

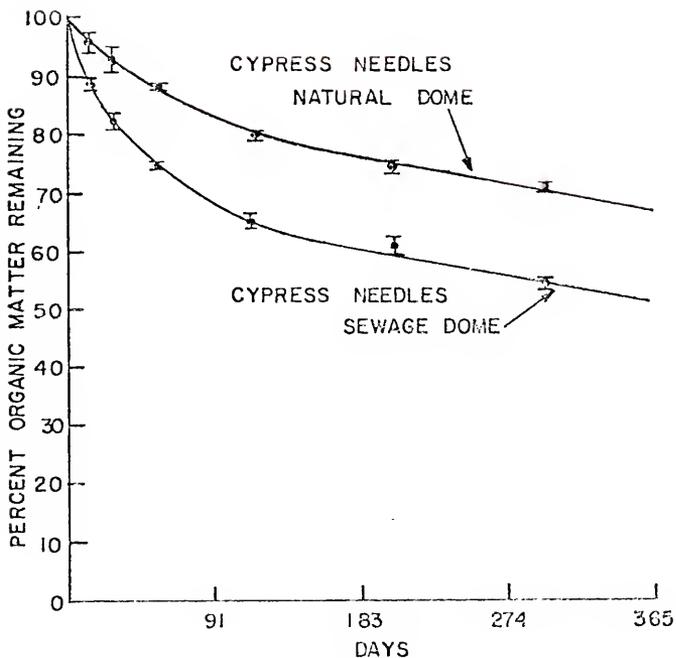


Figure 5-2. Decomposition values ($\bar{X} \pm \text{S.E.}$, $n = 5$) from litter bags at Austin Cary natural dome and sewage dome 2, and decomposition curves predicted from a two-compartment decay model of the form $y = pe^{-k_1t} + (1-p)e^{-k_2t}$.

Two-Compartment Parameter Estimates

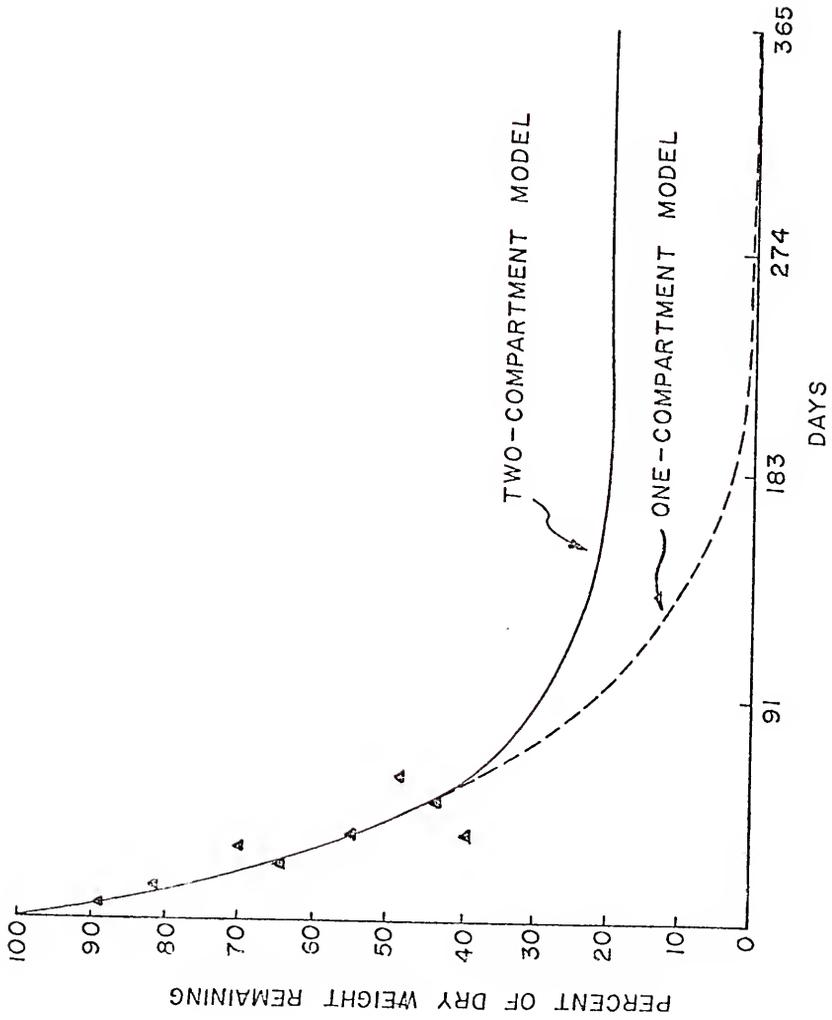
Cypress litter

Least squares estimates of the fast decomposition rate, slow decomposition rate, and the percentage of total biomass undergoing fast decomposition produced turnover rates of 5.70/yr and 9.74/yr for the fast component in Austin Cary (18 percent of the total) and sewage dome 2 (28 percent of the total), respectively. The slow component turnover rate was 0.17/yr in the natural dome and 0.32/yr in the sewage dome. Although the two-compartment curves fit the data much more closely than the exponential decay curves, the additional degrees of freedom generated by estimating the proportion of each component and their rate constants create wide confidence intervals for the estimated parameters.

Duckweed litter

The decomposition rate of the fast component of the duckweed litter was calculated using Price's (1975b) decomposition curves: 9.0/yr (80 percent of the biomass underwent fast decomposition). Because his study did not proceed long enough to provide a rate for the slow component, two alternatives were adopted. The first was to assume a value of 0.02/yr, the same rate that Foree and McCarty (1970) determined for algae under anaerobic conditions (Figure 5-3). The second alternative was to calculate a single decomposition rate based on the available data by using the simple exponential decay model (Figure 5-3). The decomposition constant, k , was 4.73/yr. Both methods were used to simulate the models (Figure 5-4).

Figure 5-3. Predicted course of decomposition for duckweed based either on the separation of the observed data into fast and slow components (two-compartment model) or on fitting the observed data to a simple exponential model of the form $y = e^{-kt}$ (one-compartment model).



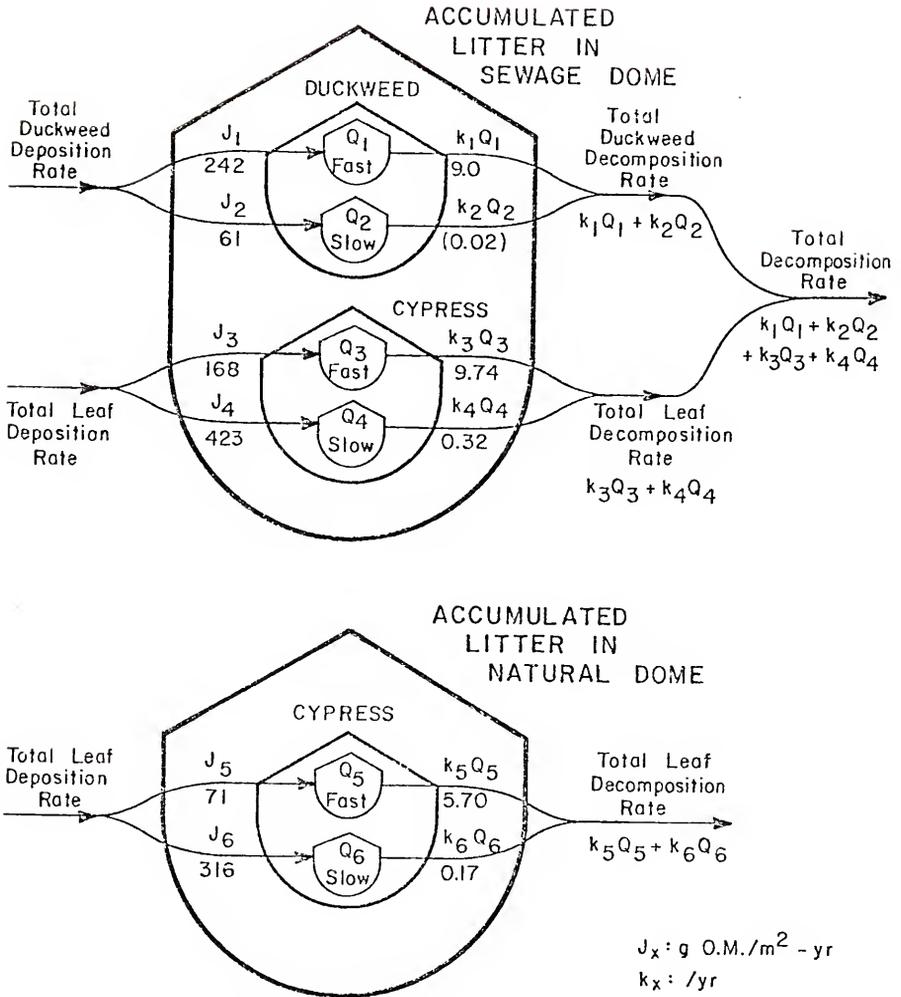


Figure 5-4. Values used to simulate model of litter accumulation on the swamp floors of sewage dome 2 and Austin Cary natural dome using the nonlinear two-compartment decomposition model ($y = pe^{-k_1 t} + (1-p)e^{-k_2 t}$). Initial conditions for the amount of slow decomposing cypress litter was 20.3 kg OM/m^2 for both domes; all other stocks were initialized at zero. See Appendix 1 for calculations, assumptions, and references used in evaluating this diagram.

Cypress Leaf Litter Decomposition

Approximately 70 percent of the cypress litter in the decomposition bags at the natural dome remained at the end of one year, and 50 percent remained in the sewage dome decomposition bags (Figure 5-2). Simple decomposition constants of these domes were 1.87/yr ($r^2 = 0.67$) and 2.12/yr ($r^2 = 0.68$), respectively.

Sewage effluent disposal into cypress domes apparently increases the rate at which cypress leaves decompose. This may be due in large part to increased nutrient levels in the leaves themselves (C:N ratios of 56:1 and 28:1 for the natural and sewage litter, respectively), although the creation of a more nutrient-rich and less acidic aquatic environment may be as important. The presence or absence of oxygen during the decomposition process may be of only minor importance compared with other variables, such as litter quality and temperature. Foree and McCarty (1970) found the rate and extent of algal degradation under anaerobic conditions to be similar to those found under aerobic conditions by other investigators. Moreover, although diversity in fungal and benthic invertebrate populations decreases after sewage addition, population levels of the remaining organisms increase (Drew 1975; Brightman 1976). The structure of the decomposer community therefore changes but the function remains the same.

Decomposition rates were measured in the four cypress domes (sewage dome 1, sewage dome 2, groundwater control dome, and Austin Cary natural dome) in 1974-1975 at the beginning of the project (Deghi et al. 1980). These rates did not differ significantly among the four cypress domes, and the simple decomposition rate constants averaged 1.28/yr ($r^2 = 0.83$). At the end of one year, 41 percent of the biomass

remained in the litter bags. Although The decomposition constant of Deghi et al. was smaller than the one reported in this study, less biomass remained at the end of the year, apparently because of more rapid decomposition rates at the beginning of the experiment. The smaller decomposition constant of Deghi et al., which represents the average of the calculated decomposition rates corresponding for each time the bags were withdrawn, was probably due to more frequent sampling during the end of the year. However, leaves for his experiment came from a different, undisturbed cypress dome, and the experiment started in mid-summer rather than late fall. The similarity of the substrate (i.e., the same nutrient levels in the leaves in litter bags at all four domes) accounts for the similarity in rates among the domes, and the earlier starting time accounts for the more rapid decomposition than was recorded in this study. The use of a decomposition constant calculated from a linearized model provides a reasonable comparison among studies only if the rates at which bags are withdrawn are similar.

Nessel (1978) performed a similar experiment at the Waldo cypress strand, which is sewage-enriched. Cypress leaves from the Waldo strand decomposed at a rate of 0.55/yr ($r^2 = 0.85$) in situ (58 percent remained at the end of the year), and at a lower rate (0.46/yr, $r^2 = 0.87$) in the Austin Cary natural dome. These leaves had a mean concentration of 1.755 mg P/g dry weight (S.E. = 0.178) in July 1977; the mean concentration of leaves at Austin Cary in June 1975 was 1.072 mg P/g dry weight (S.E. = 0.158) (Deghi et al. 1980). The higher concentration of nutrients in leaves from a sewage-enriched dome apparently increases decomposition rates over those found for native litter. The

faster rate calculated for the sewage-treated domes may again be attributed simply to less sampling after the early period during which decomposition is most rapid.

The greatly improved fit of data to a two-component model makes the more detailed model a useful tool in comparing the effects of a perturbation on the decomposition process, which clearly operates at different rates on different components. The results suggest that changes in needle composition, such as lignin content, may have occurred in response to sewage addition. Such a model may find most use for determining rates of nutrient loss, since nutrient concentrations do fluctuate considerably under such perturbations, and nutrient concentrations are notably refractory beyond an easily leached fraction.

Standing Stock of Organic Matter on Swamp Floor

Sewage dome 2 and the natural dome differed considerably in organic matter accumulation patterns (Figure 5-5). The organic horizon in the natural dome did not extend below 15 cm, and organic matter content of all cores except the center core was 5 percent or less below 25 cm. On the other hand, the cores obtained from the sewage dome contained considerable amounts of organic matter below 15 cm. The distribution pattern within the dome reflects the effect of sewage: the three replicate cores from the dome center show close agreement, while the cores to the north, the direction of predominant flow within the dome, show greater organic matter accumulation than the core taken to the south. Organic matter in the natural dome is less concentrated at the edges (Table 5-1).

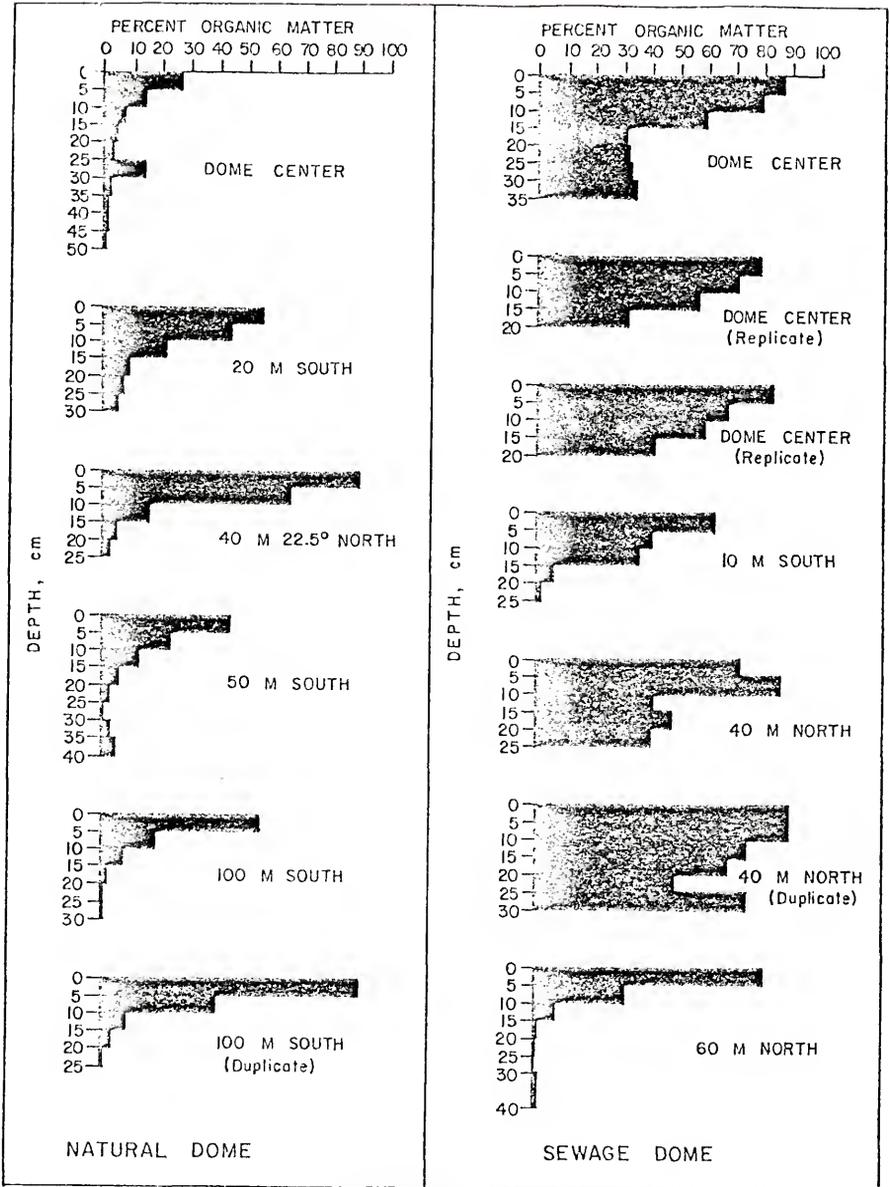


Figure 5-5. Percent organic matter in cores taken at various distances from the centers of Austin Cary natural dome and sewage dome 2.

Table 5-1. Accumulation of root-free organic matter on the swamp floor of Austin Cary natural dome.

Distance from Center of Dome (m)	Depth of Core (cm)	kg Organic Matter/m ² (Excluding roots) ^a
0	30	22.3
20	30	23.2
40	25	21.7
50	30	20.5
100	25	19.3
100	30	15.0
\bar{x}		10.3
S.D.		± 2.95

^a Based on 15 percent estimated root mat biomass from a nearby cypress strand (Lugo et al. 1978).

Simulation of Model

Simulation of models incorporating rates of litter fall and litter decomposition measured at the Austin Cary natural dome and sewage dome 2 (see Appendix 1 for calculation of transfer coefficients, scaled differential equations, and analog program) indicate a rapid decline in the standing biomass of organic matter in both domes in 10 years (Figure 5-6). A steady state of approximately 2 kg/m² is reached after 25 years in Austin Cary and 15 years in sewage dome 2 (one-compartment duckweed model). The fast rates of organic matter disappearance produced by the simulation probably resulted from an overestimation of the measured slow decomposition rate. Slower decomposing constituents (e.g., phenols, waxes, and lignins) would make up larger and larger

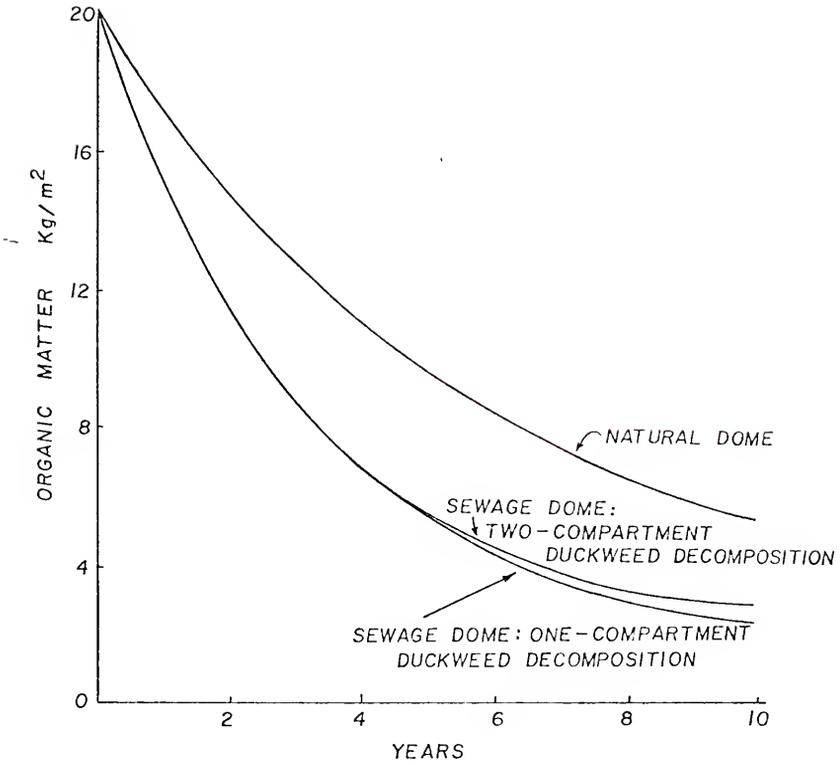


Figure 5-6. Simulated change in organic matter on floor of cypress domes over 10 years in the undisturbed dome in the Austin Cary Forest and in a sewage-enriched dome (sewage dome 2).

percentages of the undecomposed material once the more labile components have been removed, thereby lowering decomposition rates. Another phenomenon that may lower decomposition rates is a dry weight increase that occurs after the first year (see Chapter 6). Other workers have noticed the same (Brinson 1977; Deghi 1977). Brinson (1977) attributed it to accretion during heavy autumn leaf fall.

A two-compartment model cannot project decomposition rates far beyond the experimental period. Detection of a still more refractory component must rely on a more sensitive technique than year-long collection of litter decomposition bags. The results do indicate, however, that more rapid decomposition rates in a sewage-enriched cypress dome compensate for increases in productivity. This prevents rapid peat accumulation. Whether this effect continues after the first year cannot be answered at this time.

CHAPTER SIX
THE EFFECT OF SECONDARY SEWAGE EFFLUENT ON THE
NITROGEN TRANSFORMATIONS IN NATURAL CYPRESS DOMES

In this chapter, I discuss nitrogen fixation, ammonification, denitrification, and nitrification processes in natural and sewage-enriched domes. In each case, a brief literature review is presented on the microbial ecology of the process in wetlands systems. Methodology is given next, followed by a discussion of the experimental work.

Models of the Nitrogen Cycle

The generalized diagram of the nitrogen cycle for a swamp presented in Figure 6-1 identifies the important sources and sinks for nitrogen, as well as the major reservoirs. Common inputs and outputs that involve nitrogen in solution are bulk precipitation, surface runoff, and infiltration. Several pathways involve volatile forms of nitrogen: N_2 and NH_3 . Surface overflow (not shown in the model) is so minor that its effect is minimal in the nitrogen cycle of most cypress domes. Important internal, biologically mediated nitrogen transformations include assimilation, dissimilation (i.e., denitrification), fixation, deamination (ammonification), and nitrification. These may change nitrogen from a species that may not be capable of being exported from the ecosystem, to one that may. Each internal cycling reaction requires specific environmental conditions (Figure 6-2).

One way in which the pool of ammonium can be decreased is by nitrification, which occurs when the proper pH, temperature, oxygen tension (represented by a logic switch in the figure), and bacterial

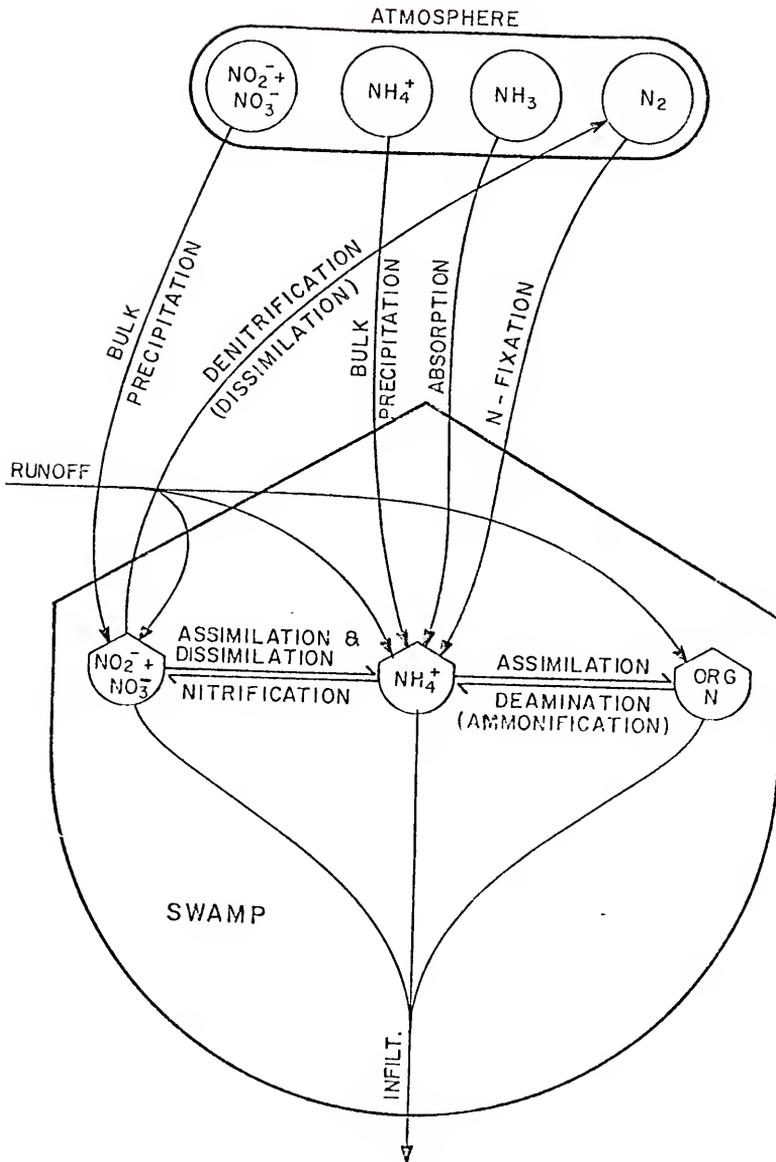


Figure 6-1. Generalized model of the nitrogen cycle for a swamp.

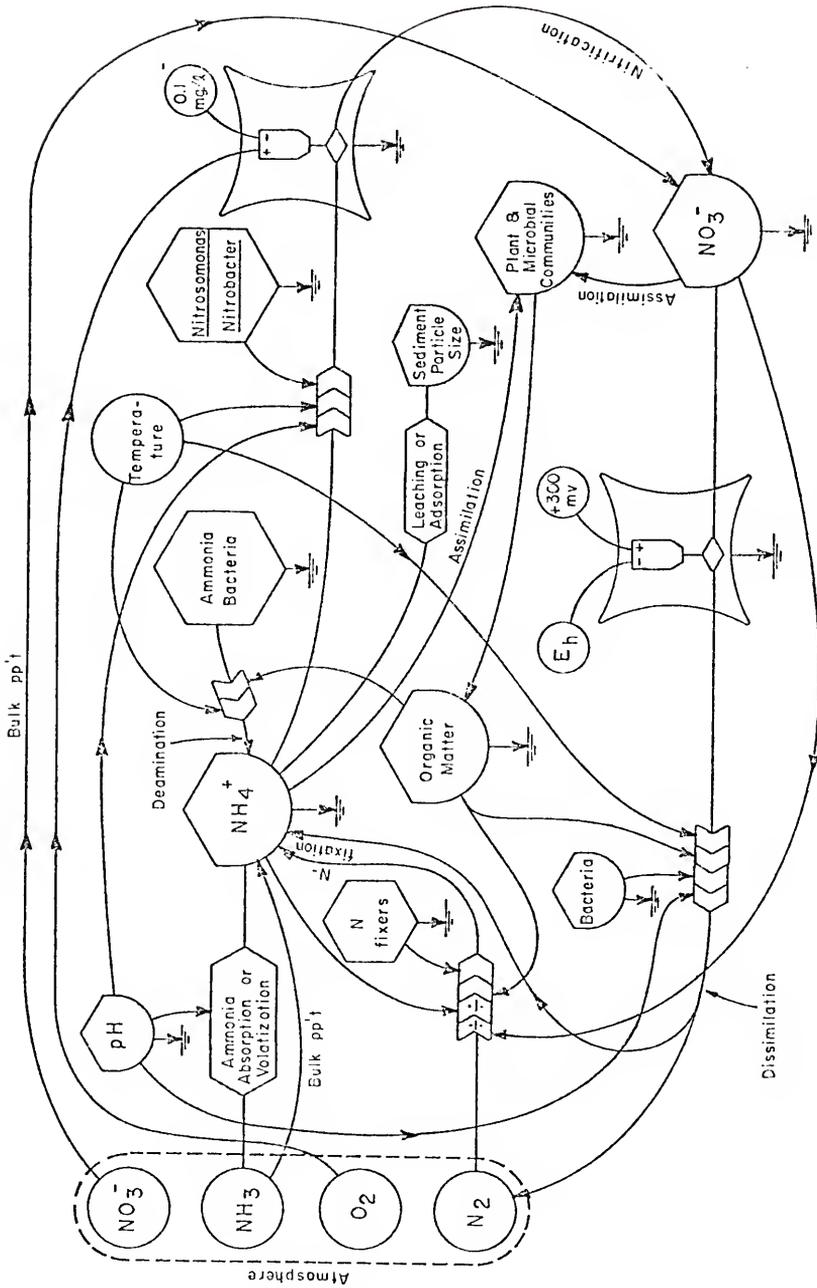


Figure 6-2. Systems model of the nitrogen cycle showing the major environmental factors controlling transformations. Symbols are from Odum (1971).

flora are present. The nitrate pool decreases by denitrification when the redox potential is less than +300 mV and if abundant organic matter, proper pH, and appropriate microorganisms are available. Nitrogen fixation occurs if nitrogen-fixing flora and sufficient organic matter are present, and if combined inorganic nitrogen is low. This process eventually results in an increase in the ammonium pool. Thus the systems model shown in Figure 6-2 forms the basis for a dynamic nitrogen model, which would require quantification of all storages and flows for solution.

The nitrogen cycle can also be presented as a simple series of forward and backward energy flows (Figure 6-3). An energy quality value is assigned to each nitrogen species depending on its oxidation state. The more oxidized species such as nitrite and nitrate can be thought of as representing lower quality energy forms since they contain low-energy chemical bonds and are susceptible to gaseous and leaching losses. On the other hand, ammonium and organic nitrogen represent high quality energy since they represent the non-equilibrium reduced condition where high energy chemical bonds produce higher free energies. Thus there exists a gradient of leachability, gaseous loss, free energy yields or losses, and oxygen/organic matter requirements between the most oxidized and reduced species of nitrogen. Ammonium is susceptible to gaseous loss as ammonia, but only under alkaline conditions (pH > 8.2).

Nitrogen Fixation

In situations where external supplies of nitrogen are low relative to other nutrients, natural selection favors the growth of nitrogen-fixing flora to convert atmospheric N_2 into fixed (organic)

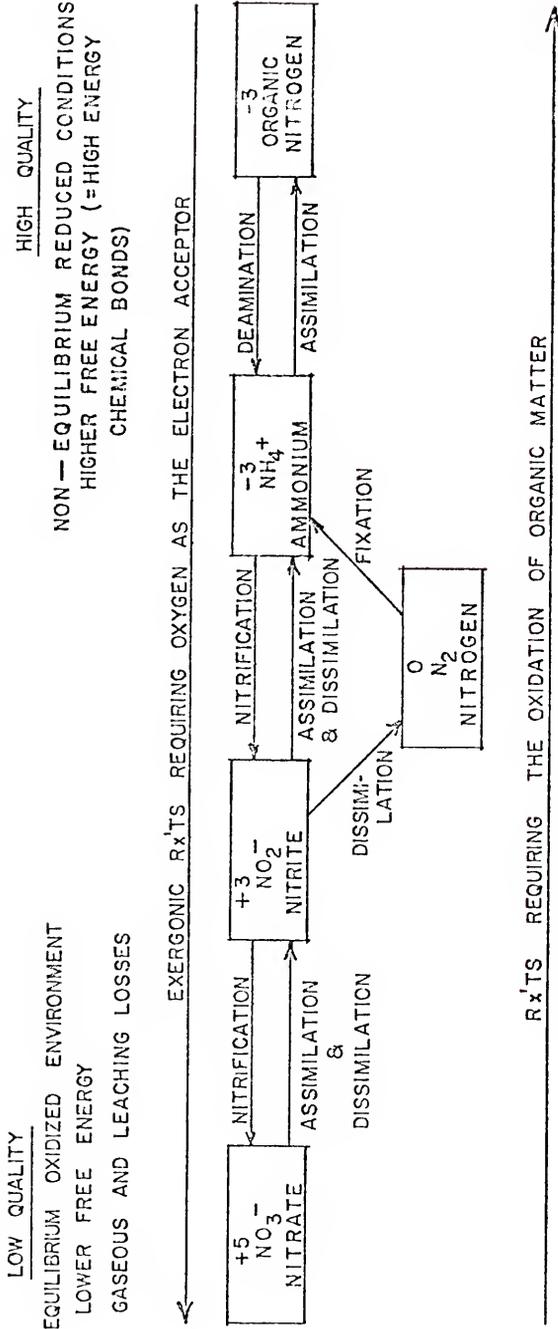


Figure 6-3. The nitrogen cycle presented as a simple series energy flow.

nitrogen. Because conversion of N_2 into organic forms is an energy-intensive process, fixation occurs only in nitrogen poor systems. Although nitrogen fixation is associated with many plants and animals, the actual process is limited to prokaryotic organisms, including various aerobic and anaerobic bacteria, blue-green algae, and actinomycetes. These organisms fix nitrogen either in free-living form or in varying degrees of association or symbiosis with higher organisms. Fixation of nitrogen by free-living blue-green algae in aquatic systems (lakes and the ocean) and by symbiotic bacteria (in legumes) is well-known (see reviews by Burns and Hardy 1975, NRC 1978).

Recent research has demonstrated the widespread occurrence of nitrogen fixation by free-living (non-symbiotic) bacteria and by bacteria in only loose association with higher organisms. The diverse habitats in which such non-symbiotic fixation has been found include: coral reefs (Wiebe et al. 1975); sea grasses (Bristow 1974); the rhizosphere of sea grasses (Patriquin and Knowles 1972); estuarine sediments (Brooks et al. 1971); fresh water sediments (Brezonik and Keirn 1971; Macgregor et al. 1973); rice soils (MacRae and Castro 1967; Becking 1976); grasslands (Paul et al. 1971; Döbereiner et al. 1972); the rhizosphere of Douglas fir (Jones et al. 1974); rain forests (Forman 1975); the rumen of cattle (Hardy et al. 1973) and the intestinal microflora of termites and sea urchins (Benemann 1973; Guerinet and Mann 1977).

Nitrogen fixation also has been assessed in several kinds of wetlands, e.g., salt marsh (Jones 1974; Hanson 1977; Whitney et al. 1975) and mangrove (Kimball and Teas 1974; Zuberer and Silver 1974 and 1978); and northern peatlands (Martin and Holding 1978; Waughman and Bellamy

1972; Moore and Bellamy 1974). Collins and D'Slyva (in Martin and Holding 1978) emphasized the need for more extensive sampling before a realistic estimate of the annual input of nitrogen through fixation could be made for British peatlands. Waughman and Bellamy (1972) suggested that the contribution of nitrogen by fixation could be at least as important as the input by precipitation to some accreting peat formations in Scotland. They found a relationship between the hydrology of mires (peat-producing ecosystems) and nitrogenase activity; higher acetylene reduction rates were associated with peat from rheophilous¹ mires than from ombrophilous² mires. This finding was supported by Moore and Bellamy (1974), who presented a summary of acetylene reduction in developing peat for ten mire complexes located in Europe and Canada. For five sites which had both ombrophilous and rheophilous mires, an absence of acetylene reduction was noted at all five ombrophilous mires. The authors concluded that the supply of nitrogen may well be a factor limiting the biological potential of ombrotrophic mires.

However, no estimates have been made for the role of nitrogen fixation in the nitrogen economy of freshwater forested wetlands (Granhall 1978). A few of the common plants in forested wetland ecosystems (e.g., wax myrtle: Myrica) are well-known as nitrogen fixers. Myrica is a non-leguminous shrub with root nodules that fix N_2 . The endophyte responsible for fixation in this plant is believed to be an actinomycete. Although some studies have been published on in situ

¹receive flowing water

²entire nutrient supply is contained in rain falling directly on them

rates of fixation by Myrica (Silver and Mague 1970; Bond 1951; Tjepkema 1978), no information is available on its contributions to the nitrogen budgets of wetlands. Based on a nitrogen mass balance, Gaudet (1976) believed nitrogen fixation was one of the most important processes in a papyrus swamp.

Several investigators have reported that the relative quantity of nitrogen in leaf detrital systems increases during in situ decomposition (Brinson 1977; de la Cruz and Gabriel 1974; Gaudet 1976; Kaushik and Hynes 1971; Mathews and Kowalczewski 1969; Odum and de la Cruz 1967). However, little work has been done to determine the quantitative significance that nitrogen fixation plays in this finding. Howarth and Fisher (1976), reported nitrogen fixation (acetylene reduction) associated with sugar maple discs in laboratory stream microecosystems, but they found that nitrogen fixation was an insignificant source of nitrogen for the litter. Gotto and Taylor (1976) stated that nitrogen fixation in decomposing leaves of the red mangrove (Rhizophora mangle) may contribute significantly to observed nitrogen increases, but did not provide a quantitative estimate.

Acetylene reduction activity (ARA) has been shown to be associated with the washed roots of such non-nodulated roots as forage grasses (Day et al. 1975; Döbereiner et al. 1972; Döbereiner and Day 1976), cereals (Balandreau 1975; von Bülow and Döbereiner 1975), and various non-woody wetland plants (Patriquin and McClung 1978; Patriquin and Keddy 1978; Tjepkema and Evans 1976; Zuberer and Silver 1978). Agents responsible for the nitrogenase activities are bacteria in the rhizosphere and "endorhizosphere" (root interior), and they are most active in reducing acetylene at low oxygen tensions (Barber and Evans 1976;

Day et al. 1975; Döbereiner et al. 1972; Döbereiner and Day 1976; Patriquin 1978). Possible nitrogen fixation in the rhizosphere and endorhizosphere of dominant woody plants has not been considered.

The small floating fern Azolla harbors a nitrogen-fixing endophyte, the blue-green alga Anabaena azolla (Moore 1969). The agronomic significance of this association, particularly in rice-growing regions of southeast Asia, has been long recognized (Mishustin and Shil'nikova 1971; Moore 1969). Nitrogen fixation rates associated with the fern in Indonesian rice culture have been reported using both ^{15}N (Moore 1969) and acetylene reduction (Becking 1978; Brotonegoro and Abdulkadir 1976).

Effectiveness of Various Metabolic Poisons on Azolla Caroliniana

Many metabolic poisons (particularly acids) have been shown to be abiotic sources of ethylene, which seriously impairs their utility as terminating agents in the acetylene reduction assays (Jones 1974; Thake and Rawle 1972). However, no systematic study has been done to compare the effectiveness of these metabolic poisons in terminating the acetylene reduction reactions.

The following poisons and their final concentrations were used to test their effectiveness in stopping ethylene production from Azolla caroliniana incubated under 0.1 atm C_2H_2 at 25°C and under 400 foot-candles of light: mercuric chloride (3,300 mg/L), H_2SO_4 (0.235N), HCl (0.192N), trichloroacetic acid (9 percent), NaOH (0.286N), buffered formalin (5 percent).

Not only did some metabolic poisons display significant ethylene production in the controls, but varying rates also occurred among the experimental groups (Table 6-1). For instance, HgCl_2 ($\sim 3,300$ mg/L final

Table 6-1. Ethylene production by Azolla caroliniana using various metabolic poisons.

Sample	Replicate	Poison	Final Concentration	Dry wt (g)	$\mu\text{M C}_2\text{H}_4$ $\text{g}^{-1}\text{h}^{-1}$	Mean and S.D.
Positive Control	1	None		83.	11.5	12.2 \pm 0.6
	2	None		69.1	12.5	
	3	None		76.1	12.6	
Control	1	HgCl ₂	2 ml saturated HgCl ₂ (\sim 3,300 mg/L)	92.8	4.2	3.8 \pm 0.3
	2			70.2	3.6	
	3			87.8	3.7	
Experimental	1			73.4	8.4	8.8 \pm 0.3
	2			62.1	8.9	
	3			67.7	8.9	
Control	1	H ₂ SO ₄	0.235 N	37.5	3.1	2.6 \pm 0.4
	2			48.8	2.3	
	3			52.0	2.4	
Experimental	1			69.0	13.7	15.8 \pm 1.8
	2			44.1	16.5	
	3			50.1	17.1	
Control	1	HCl	0.192 N	43.	1.5	1.5 \pm 0.2
	2			55.9	1.3	
	3			46.5	1.7	
Experimental	1			55.1	13.8	14.2 \pm 0.6
	2			83.5	14.7	
	3			41.1	--	
Control	1	Trichloroacetic acid	4 ml of 50 percent TCA (\sim 9 percent)	43.6	1.	1.0 \pm 0.1
	2			66.5	0.9	
	3			47.9	1.1	

Experimental	1						69.4	13.5	12.5 ± 0.8	
	2						70.5			11.8
	3						56.4			12.4
Control	1	NaOH	0.286 N				35.1	0	0	
	2						28.6			0
	3						29.6			0
Experimental	1						31.1	28.3	29.6 ± 1.7	
	2						25.2			31.6
	3						31.2			28.8
Control	1	Formalin	2 ml buffered formalin (~5 percent)				46.8	1.1		
	2						53.			0.9
	3						52.5			0
Experimental	1						52.3	19.5	19.5 ± 1.7	
	2						40.6			21.1
	3						51.1			17.8

concentration) was not only a poor control reagent (i.e., it did not stop the reaction when added prior to the acetylene injection), but it also yielded lower acetylene reduction rates on a dry weight basis than that of the positive controls. At the other extreme, although NaOH (0.29 N final concentration) completely stopped the reaction and thus served as an excellent control, it yielded the highest rates (about 2.5 times higher than the positive controls). The reason can be found by inspecting the dry weight values, which are low compared to those recorded for other reagents. A noticeable greenish hue was present in the water, and the Azolla plants had a chlorotic appearance, indicating leaching of cellular constituents into the medium (thereby reducing dry weights).

Neither HCl (0.19 N final concentration) nor H₂SO₄ (0.24 N final concentration) stopped the acetylene reduction. However, when the control rates were subtracted from the experimental rates, close agreement was found between them and the positive controls. Four milliliters of 100 percent trichloroacetic acid solution added to 40 ml of sample (~9 percent final concentration) yielded the best results. Formalin (~5 percent final concentration) yielded good control by stopping the reaction adequately, but the experimental values were higher than the positive control group. No obvious explanation can be offered at this time.

The most satisfactory way of terminating the incubation for assay purposes is removal of a sample of gas (Hardy et al. 1973). However, this is not always feasible in field-incubated assays and metabolic poisons are commonly employed instead of simple removal of a gas sample. Various metabolic poisons have been used at various strengths to stop

the acetylene reduction reaction and preserve the sample until gas chromatographic analyses could be determined. Among them are H_2SO_4 (Burris 1976; Horne 1972; Lean et al. 1978; Silver and Mague 1970; Vanderhoef et al. 1974), $(NH_4)_2SO_4$ (Lännergren et al. 1974), HCl (Toetz 1973a, 1973b), trichloroacetic acid (Brezonik and Harper 1969; Howard et al. 1970; Millbank and Kershaw 1970; Stewart et al. 1967), NaOH (Taylor et al. 1973), copper sulfate (Burris 1976; Mague et al. 1974), saturated mercuric chloride (Brooks et al. 1971), and formalin (Webb et al. 1975).

Failure of such reagents to rapidly quench the reaction as a result of slow penetration into the sample can cause errors. This is particularly true for the Azolla-Anabaena symbiosis where the nitrogen-fixing phycobiont is located inside a leaf cavity in the upper lobe of leaves of the fern. The only direct connection to the outside environment thus is through a pore on the underside of the fronds (Becking 1976).

The results of the comparison of common metabolic poisons used to stop the acetylene reduction reaction indicate that addition of either trichloroacetic acid or buffered formalin is the quickest, most accurate, and precise way to stop the reaction for the Azolla-Anabaena symbiosis. The concentration of these poisons is probably as important as the type. A concentration that is too weak will not effectively stop the reaction, whereas a too-strong concentration will extract soluble organics or hydrolyze cell constituents such that serious error in measuring dry weights will be introduced (see results for NaOH in Table 6-1). Thake and Rawle (1972) cautioned against using trichloroacetic, hydrochloric, and sulfuric acids as terminating agents.

They found considerable non-biological ethylene production (from either the rubber liner in the cap or the organic material in the sample) for samples terminated with trichloroacetic acid. Jones (1974) also reported that the addition of acid to samples of forest soils and leaves resulted in the production of large quantities of ethylene. Because of these considerations, a 5 percent solution of buffered formalin (pH = 8) was chosen as the best metabolic poison to use in the Azolla assays.

Aquatic Macrophytes

Blooms of the small floating fern Azolla caroliniana occurred sporadically in the control, natural, and sewage-treated domes during the period of investigation. This nitrogen-fixing macrophyte was found in the natural dome at Austin Cary only once (March 29 to July 20, 1977) during the four-year investigation, and the population was confined to a 300 m² area at the dome center. Acetylene reduction rates were variable during this period, ranging from 0.43 to 19.6 $\mu\text{M/g(dry wt)-h}$ (Table 6-2). The lower values were associated with the summer period, when the dome had no standing water and Azolla was disappearing. A rate of ethylene production of 6.79 $\mu\text{M/g(dry wt)-h}$ was found for a bloom during April, 1979 in sewage dome 2 (Table 6-2).

To relate the observed acetylene reduction values measured for Azolla caroliniana to quantities of nitrogen fixed, calculations were made using the following assumptions:

1. Nitrogenase activity occurs only during daylight hours (Brotonegoro and Abdulkadir 1976), and day length is 14 h.
2. One bloom occurs every four years.
3. The theoretical molar production ratio for $\text{C}_2\text{H}_4:\text{NH}_3$ is 3:2 (Stewart et al. 1967).

Table 6-2. Ethylene production associated with Azolla caroliniana blooms in Austin Cary natural dome, sewage dome 2, and groundwater control dome.

Date	Site	Standing Crop		Ethylene Production		Total N Fixed (g/m ²)
		(g(dry wt)/m ²)		(μ M ethylene/g (dry wt)-h)		
		\bar{x}	S.D.	\bar{x}	S.D.	
March 25, 1977	Austin Cary	38.0	3.2	1.81 ^a		0.36
March 25, 1977	Groundwater Control Dome	120.4	17.9	0.47 ^a		--
June 8, 1977	Austin Cary	24.7	2.6	19.63	2.93	2.53
June 28, 1977	Austin Cary	26.0	--	0.43	0.13	0.02
July 18, 1977	Austin Cary	26.0	--	0.59	0.35	0.04
April 10, 1979	Sewage Dome 2	16.0	--	6.79	1.39	0.37

^a Mean of two replicates; all others the mean of three replicates.

The rates of ethylene production, standing crop biomass, and total fixed nitrogen (dry wt) for Azolla caroliniana blooms in Austin Cary natural dome and sewage dome 2 are presented in Table 6-2. The area covered by the bloom in the natural dome was estimated to be 1,300 m², so a total of 3,835 g N was fixed for the entire dome. The area of Austin Cary natural dome is 42,000 m²; therefore, 0.081 g N fixed/m² was fixed. Since only one bloom occurred during the 4-year investigation, a rate of 0.023 g N fixed/m²-yr was found. The area covered by the bloom in sewage dome 2 was an estimated 5,249 m²; thus, a total of 1,942 g N was fixed for the entire dome. The area of sewage

dome 2 is 10,498 m²; therefore, 0.185 g N fixed/m² was fixed. Since 2.5 years elapsed since the last reported bloom (Ewel 1976), a rate of 0.074 g N fixed/m²-yr was found.

Duckweed (Lemna perpusilla and Spirodela oligorhiza) that occurred in the two sewage domes was also assayed for ethylene production, but no ethylene was produced over that of the controls. The aquatic macrophyte, Utricularia, did reduce a small amount of acetylene in the groundwater control dome (Table 6-3). The agent of fixation probably was the periphyton community associated with the Utricularia, but no effort was made to identify the organisms. Nitrogenase activity was not found associated with Utricularia from the natural dome.

Table 6-3. Ethylene production associated with Utricularia sp. in Austin Cary natural dome and groundwater control dome.

Date	Site	Standing Crop		Ethylene Production	
		(g(dry wt)/m ²)		(μ M ethylene/g (dry wt)-h)	
		\bar{x}	S.D.	\bar{x}	S.D.
March 25, 1977	Groundwater Control Dome	13.0	7.6	0.86 ^a	--
August 12, 1978	Austin Cary	--	--	0.0	--

Because of their infrequency, the episodic blooms of Azolla caroliniana that occurred in all the study sites did not contribute significantly to the overall nitrogen economy of the cypress domes (0.02 g N/m²-yr for the natural dome and 0.07 g N/m²-yr for sewage dome 2). However, the process may be important for short periods of

time when a surplus of phosphorus and other nutrients permits the establishment of this nitrogen-fixing symbiosis in order to provide the nitrogen needed by the ecosystem to assimilate the extra nutrients.

The maximum rate of nitrogen fixation found in the natural dome was 0.18 mg N/g(dry wt)-h in June. Brotonegoro and Abdulkadir (1976) reported a maximum rate of 0.30 mg N/g(dry wt)-h, and Moore (1969) reported a rate of 0.01 mg N/g(dry wt)-h for Azolla, using the ^{15}N technique. Becking (1976) calculated Azolla fixation rates in Indonesia to be approximately 0.55 mg N/g(dry wt)-h. Thus, the maximum rate obtained for the Azolla in the Austin Cary dome is comparable to the rates measured by other investigators for Azolla. The reasons for the infrequent appearance of Azolla in the standing waters of Austin Cary are not known, but Olsen (1970) considered iron toxicity to be the reason why Azolla did not develop in humic-colored lake water.

Leaf Litter, Peat, and Standing Water

Acetylene reduction activities (ARA) remained low (<2.2 nM ethylene/g(dry wt)-h) in both the sewage and natural domes from August 1970 until January 1979, when they increased in the natural dome to a high of 36.9 nM ethylene/g(dry wt)-h in March (Figure 6-4). After March, the rates decreased in the natural dome but increased in the sewage dome until June. Nitrogenase activities in both domes returned to the low levels measured initially by August 1979. At no time were the measured rates from the sewage dome higher than the rates in the natural dome. Integrating the monthly rates over the one year measurement period yields 1.01 mg N fixed/g (dry wt) and 0.21 mg N fixed/g (dry wt) for the natural and sewage-enriched domes, respectively.

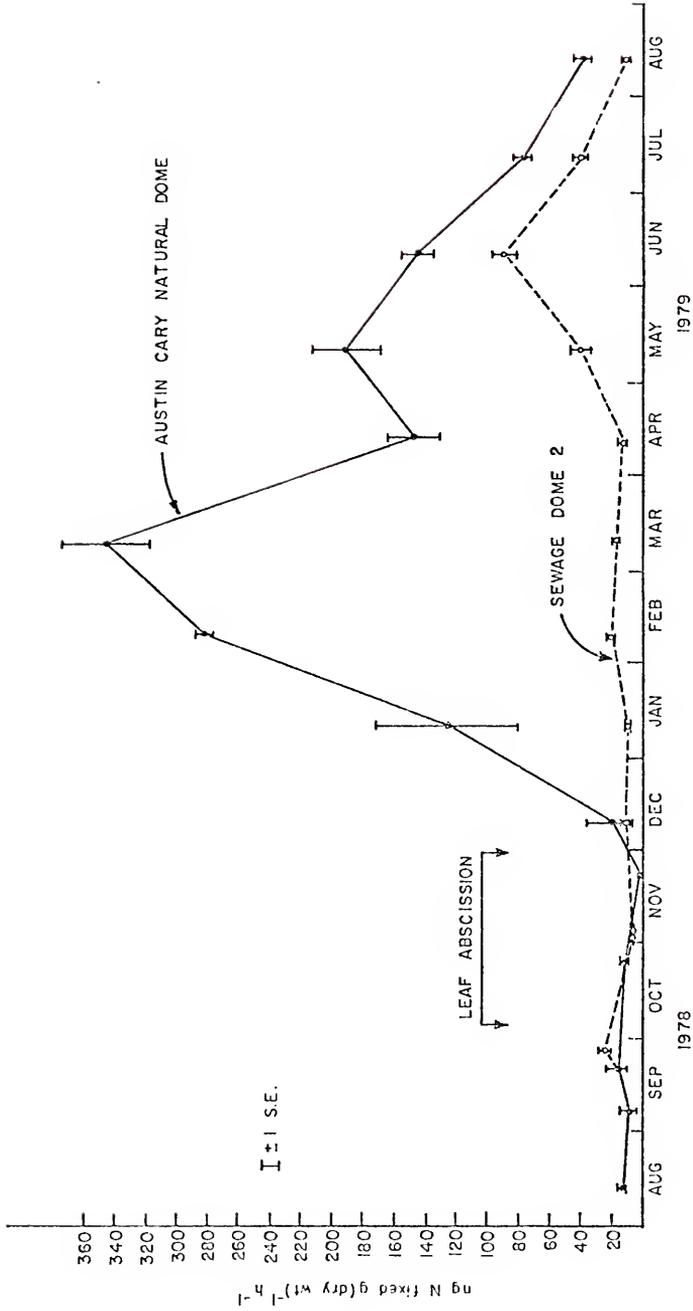


Figure 6-4. Seasonal variation in the nitrogen fixation rates (C_2H_2) by the surface layer of peat from Austin Cary natural dome and sewage dome 2 from August 12, 1978 to August 12, 1979. Each point is the mean of three to six replicates.

Rates were linear with no lag period over the range of incubation periods employed, as indicated in Figure 6-5 for leaf litter from sewage dome 2. The measured rates apply to only the surface layer of litter on the swamp floor since sectioned cores produced negligible amounts of ethylene (Figure 6-6) on two occasions.

Assays conducted on the dome standing water (collected at the bottom) consistently produced no ethylene.

Calculations were made to relate the observed acetylene reduction values measured for surface litter on the swamp floor of Austin Cary natural dome and sewage dome 2 to quantities of nitrogen fixed using the following assumptions:

1. Most of the observed nitrogen fixation occurred in the surface litter from the most recent litter fall (Figure 6-6). Deghi (1977) calculated the leaf litter fall (average of two years) to be 387 g(dry wt)/m²-yr for the Austin Cary natural dome and 591 g(dry wt)/m²-yr for sewage dome 2. The surface litter had an average specific gravity of .098 g/cc ± .009 S.D. (n = 5). Thus, the depth of litter fall in one year is 0.39 cm for Austin Cary natural dome and 0.60 cm for sewage dome 2. It is assumed that sampling of the surface litter for the acetylene reduction assay never exceeded those depths.
2. The theoretical molar production ratio of 3:2 for C₂H₄:NH₃ (Stewart et al. 1967) yielded a factor of 9.33 ng N fixed (as NH₄⁺) per nM C₂H₄ produced. Extrapolation of the results in Figure 6-4 to g N fixed/g(dry wt)-yr and gN fixed/m²-yr was done using a Hewlett-Packard Model 10 Digitizer System to integrate the areas under the yearly curves. For Austin Cary

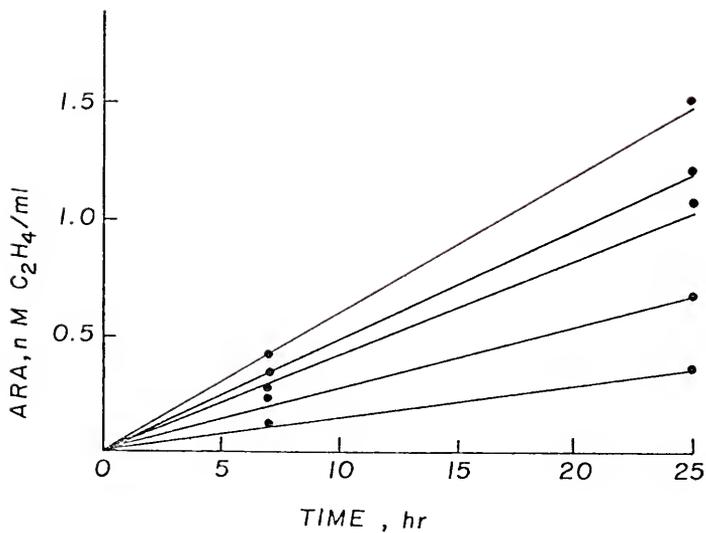


Figure 6-5. Acetylene reduction activity by five peat samples from sewage dome 2 during November 2-3, 1978. Incubations were initially aerobic and performed within one hour of sampling under 0.12 to 0.17 atm C₂H₂ at 17°C.

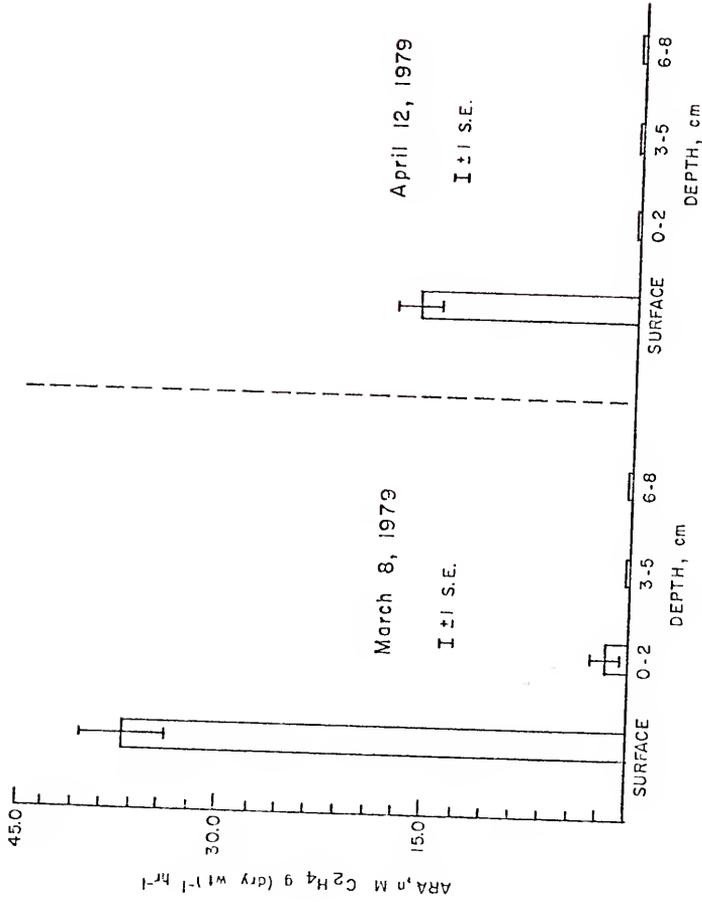


Figure 6-6. Acetylene reduction in peat from Austin Cary natural dome. Each depth interval is the mean of three subsamples from one core. The surface sample is the mean of six samples.

natural dome (from August 12, 1978, to August 12, 1979), the total fixation was 1.01 mg N fixed per g litter fall. Deghi (1977) found an average litter fall of 387 g organic matter/m²-yr over two consecutive years; thus, on an areal basis, fixation was 0.39 g N fixed/m²-yr. For sewage dome 2 (from August 12, 1978, to August 12, 1979), the total fixation was 0.2082×10^{-3} g N fixed/g-yr. Deghi (1977) found 591 g organic matter/m²-yr average litter fall for two consecutive years, yielding an areal fixation rate of 0.12 g N fixed/m²-yr.

A significant portion of nitrogenous compounds was leached from the pond cypress leaves in Austin Cary natural dome during initial decomposition (74 percent after one month). This loss later was replenished in excess of the original nitrogen concentration (Figure 6-7). Increase of a mineral during decomposition to concentrations greater than present initially indicates absorption of the mineral from the environment by fungi and bacteria colonizing the litter. The extent to which this occurs depends on the relative concentrations and availability of the mineral in the litter substrate and in the immediate environment.

Nitrogen, however, is unique in that an increase in concentration in decomposing litter may also be due to nitrogen fixation. In view of the nitrogen deficiency of senescent leaves and the potential availability of carbon substrate necessary for heterotrophic, nitrogen-fixing microbes (C:N = 56.5 for the natural dome), nitrogen fixation could be expected (a priori) to be a quantitatively important process in litter decomposition. Numerous investigations have shown that

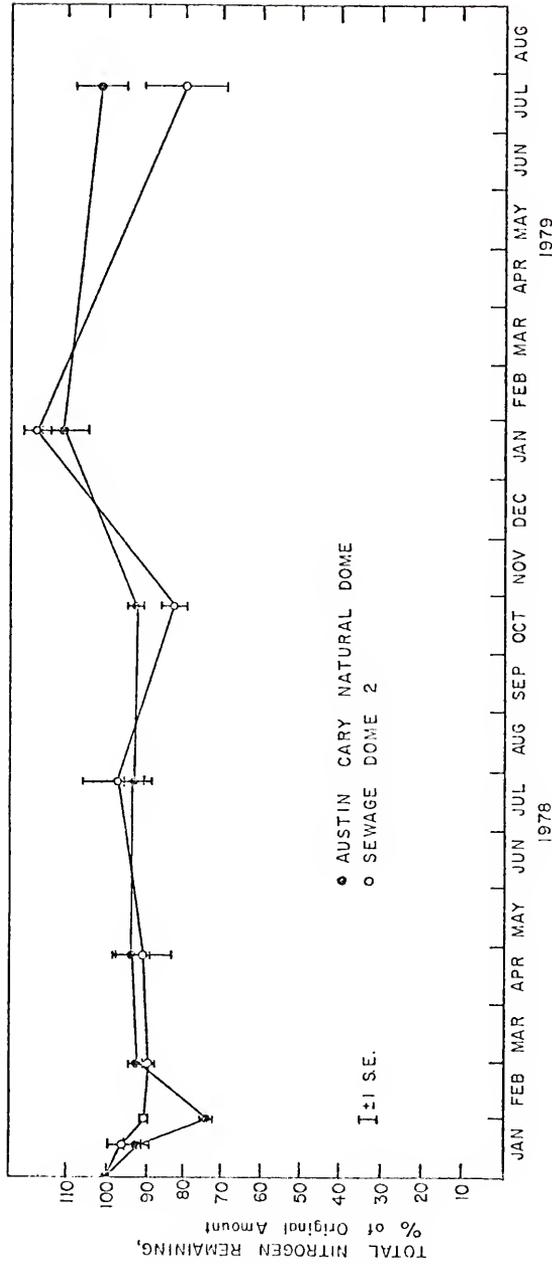


Figure 6-7. Exchanges of nitrogen in *Taxodium distichum* var. nutans leaves during decomposition in the standing waters of Austin Cary natural dome and sewage dome 2. Each point is the percent remaining of the amount in the original bag and is a mean of five replicate bags. The mean nitrogen concentrations at t_0 (as percent dry wt) were 0.81 percent and 1.50 percent for Austin Cary natural dome and sewage dome 2, respectively.

heterotrophic nitrogenase activity (C_2H_2) could be stimulated by adding simple carbon compounds (Brooks et al. 1971; Hanson 1977; Waughman and Bellamy 1972; Zuberer and Silver 1978).

This phenomenon (stimulation by added carbon) is indicated by the winter peak of nitrogenase activity in the natural dome, which followed the deposition of abscised leaves (during October and November) from the two most dominant trees (cypress and black gum). Todd et al. (1978) also reported a winter peak in nitrogenase activity (C_2H_2) for leaf litter in a deciduous forest.

The percentage contributed by heterotrophic diazotrophs (nitrogen fixing flora) to the increase in the relative nitrogen concentration in decomposing leaf litter of the natural dome is estimated as follows (all values expressed on a dry weight basis). After four weeks of decomposition the nitrogen content of the litter had decreased to 6.58 mg N/g (81 percent of its original level (8.12 mg N/g)), presumably due to leaching. After four months of decomposition the nitrogen content of the leaf litter increased to 9.50 mg N/g, or 117 percent of its original level. Thus, during the three months when the leaf litter was increasing in nitrogen content, a total of 2.92 mg N/g was added.

For the following year, a total of 0.408 mg N/g (dry wt) was estimated by integrating the area under the seasonal nitrogen fixation curve for the three-month period from December to March.

Thus, 14 percent $\left(\frac{0.408}{2.92}\right)$ of the nitrogen increment could be due to nitrogen fixation.

Considering the low levels of combined inorganic nitrogen in the surface water and sediment of Austin Cary (see Chapter 4), nitrogen

fixation apparently supplements the normal absorption of combined nitrogen by bacteria and fungi from the environment as they use the carbon-rich litter substrate. This nitrogen, while not forming a major part of the nitrogen requirement of the trees, may contribute significantly to an environment known to be short of available combined nitrogen (see Chapter 7 for quantitative justification). In addition, the 10 to 20-fold increase in nitrogenase activity during the cold and dormant part of the year occurs at a time when nitrogen compounds released from decomposing diazotrophs are readily available for spring growth of a new forest canopy. A maximum amount of nutrients would be required during this period of the year.

Nitrogen concentrations in the decomposing litter from sewage dome 2 never decreased to levels lower than what had been present originally because a constantly high concentration of combined nitrogen from the sewage provided a more favorable milieu for bacterial and fungal absorption. This fact, along with the low levels of nitrogenase activity (Figure 6-4), indicate that nitrogen fixation was negligible in contributing nitrogen to the decomposing litter (<1 percent).

The low acetylene reduction rates associated with peat from sewage dome 2 are attributable to the repressive effects that the increased concentrations of combined nitrogen from the sewage influent have on synthesis of the nitrogenase enzyme. The peak in nitrogenase activity during June is not readily explainable, but may represent an imbalanced N:P ratio. An extensive bloom of Azolla caroliniana occurred in sewage dome 2 two months prior to the measured increase in acetylene reduction of peat during June.

As with all assays based on indirect methodology, caution must be exercised when extrapolating from data based on the reduction of C_2H_2 to realistic nitrogen fixing rates. One group of nitrogen fixing bacteria, the methylotrophic bacteria, presents some unique problems when assayed for C_2H_2 reduction. These include: 1) inhibition of CH_4 oxidation by C_2H_2 , thus depriving the bacteria of their source of carbon and energy; and 2) co-oxidation of ethylene (DeBont and Mulder 1974). Thus, in routine C_2H_2 assays of sediments or other materials under aerobic conditions, the activity of methylotrophs would not be included, and reported rates of nitrogenase activity would be underestimated. However, this is not a likely source of error for either dome in the present study. Sewage dome 2 is anaerobic, and methylotrophic bacteria (obligate aerobes) should be absent. The very low levels of CH_4 produced in the oxygenated natural dome (Línero 1976) preclude a significant error associated with these bacteria.

The absence of standing water in the dome during November probably caused a lag in the increase in nitrogenase activity of the freshly fallen leaf litter in the natural dome. An earlier onset of nitrogenase activity (November-December) can be expected for those years when standing water is present during the litter fall period.

Nitrogenase activity associated with peat apparently is confined to the most recent litter fall (i.e., the surface layer). This conclusion is supported by the low ethylene production rates found for various integrated depth intervals taken from cores (Figure 6-6). On the other hand, Collins and D'Sylva (pers. comm. cited in Martin and Holding 1978) found acetylene reduction rates to be similar for all horizons of peat cores. However, they also stated that the numbers of

potential nitrogen fixers decreased down the profile, a finding contradictory to their acetylene reduction rates. Waughman and Bellamy (1972) sampled at the 15-20 cm depth interval in their C_2H_2 reduction assays and considered the rates calculated for these depths to be uniform up to the surface.

Considering the limited sampling periods and different climatic conditions for other sites where peat-associated nitrogen fixation (C_2H_2) studies have been conducted, the annual input of nitrogen by fixation ($0.39 \text{ g N/m}^2 \text{ yr}$) calculated for the natural dome agrees favorably with literature values (Table 6-4). Extrapolation of a few measurements of acetylene reduction rates for peat from systems that have a less well-defined period of litter fall (e.g., marshes, bogs, mires) to annual rates may be acceptable. However, due to the extreme seasonal variation in acetylene reduction rates associated with deciduous forested wetlands, yearly extrapolated rates based on just one or two months' data could drastically over- or underestimate the actual annual nitrogen input from fixation into these ecosystems.

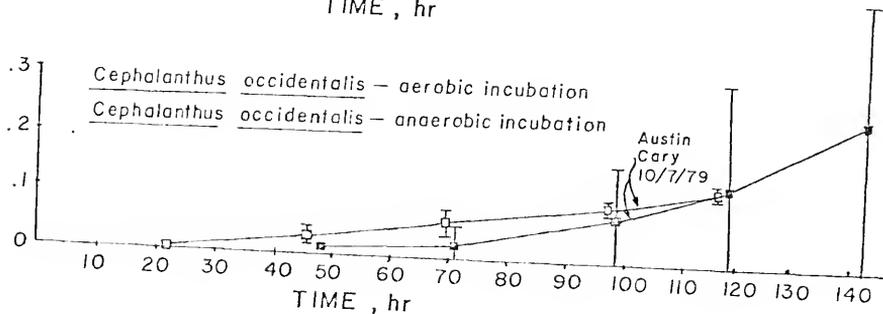
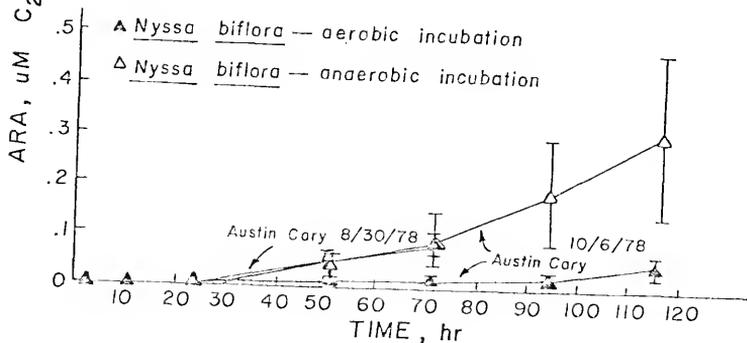
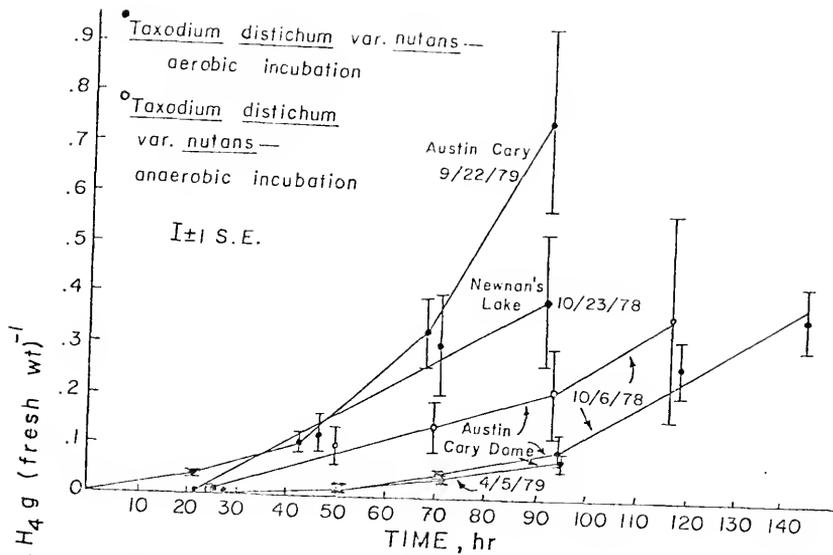
Rhizosphere-endorhizosphere of Woody Vegetation

Acetylene reduction activity occurred in washed, excised, non-nodulated roots of two dominant trees, black gum and pond cypress from the cypress dome in the Austin Cary Forest, and for pond cypress roots from the margin of a soft, colored lake (Newnans Lake) near Gainesville (Figure 6-8). In addition, low acetylene reduction activity was measured for roots of buttonbush (Cephalanthus occidentalis). The results shown in Figure 6-8 were each obtained with "replicate" samples from a single tree and they indicate that fixation does not occur uniformly in or on the roots of the trees.

Table 6-4. Estimated N_2 fixed (C_2H_2) by peat ($g\ N/m^2\text{-yr}$) for the Austin Cary natural dome, sewage dome 2, and other ecosystems.

Type and Location	N Fixed		Remarks	Reference
	Mean	Range		
Rheophilous and ombrophilous mires Scotland	0.37	(0.0 -1.2)	40 samples collected 15-20 cm below vegetation surface from 4 mire types; assumed activity continued at same rate for 20 cm	Waughman and Bellamy (1972)
Blanket peat England	0.70	(0.05-3.2)	1-3 cores sampled to a depth of 30 cm at 5 sites on one or two occasions	Collins and D'Slyva (in Martin and Holding 1978)
Cypress dome Florida	0.39	(0.0 -1.7)	3-6 monthly surface samples collected for 1 yr (1979); activity limited to surface litter layer; winter peak	This study
Sewage-enriched cypress dome Florida	0.12	(0.0 -0.3)	3-5 monthly surface samples collected for 1 yr (1979); activity limited to surface litter layer; no winter peak	This study

Figure 6-8. Acetylene reduction activity (ARA) associated with excised roots of pond cypress (Taxodium districhum var. nutans), black gum (Nyssa sylvatica var. biflora), and buttonbush (Cephalanthus occidentalis). The aerobic incubations were pre-incubated 23 h before adding C_2H_2 . (Nyssa biflora should be properly listed as Nyssa sylvatica var. biflora.)



For all experiments, C_2H_4 formation has been plotted as a function of time because of a lag before high activity was observed. Although the length of the lag was variable between experiments, it generally lasted less than 24 hours and was independent of whether incubations were carried out under an aerobic or anaerobic atmosphere (Figure 6-8). Similar results for other plants have been obtained for excised roots of grass, Paspalum notatum (Döbereiner et al. 1972); for excised roots of cord grass, Spartina alterniflora (Patriquin 1978); and for the rhizosphere of rice (Rinaudo et al. 1971). The exact cause of this delay is not well understood (Döbereiner 1977). Döbereiner et al. (1972) and Abrantes et al. (1975) eliminated the lag by pre-incubating excised roots of grass overnight at low oxygen tensions ($pO_2 = 0.02-0.04$ atm) followed by evacuating and incubating in the same low oxygen atmosphere with acetylene. These results suggest oxygen damage as the cause of the lag, but other reasons such as disturbance of the nitrogen metabolism of the roots (Baldani et al. 1977; Day et al. 1977), CO_2 depletion (Neyra and Van Berkum 1976), and bacterial multiplication due to liberation of organic acids from fermentation (Okon et al. 1977; Barber and Evans 1976) have been suggested.

Insignificant rates of acetylene reduction were found for the peat associated with the black gum roots (Figure 6-9). Essentially no acetylene reduction was observed over a 96-hour incubation of cypress roots obtained from the sewage-enriched cypress dome, but acetylene reduction occurred in a root sample from the natural cypress dome (Figure 6-10), suggesting that nitrogen fixation (acetylene reduction) is affected by the levels of combined nitrogen present in the pore water of the peat. The pore water taken at a 30 cm depth in the peat

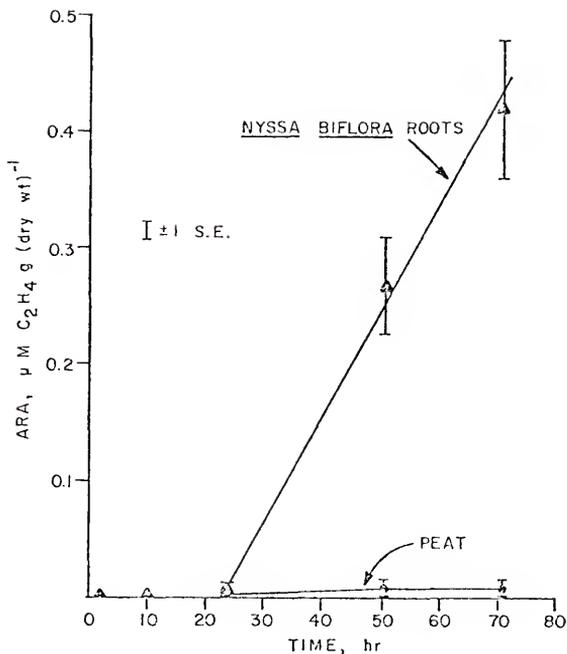


Figure 6-9. Acetylene reduction associated with excised roots of Nyssa sylvatica var. biflora (black gum) and surrounding peat from August 30 to September 2, 1978. Incubations were initially aerobic under 0.1 atmosphere C_2H_2 in the dark and at 24°C with 9 percent (vol) de-ionized water. Roots were excised, washed in de-ionized water, and incubated within 2 hours of collection from Austin Cary natural dome. Each data point represents the mean of three replicate samples. (Nyssa biflora should be properly listed as Nyssa sylvatica var. biflora.)

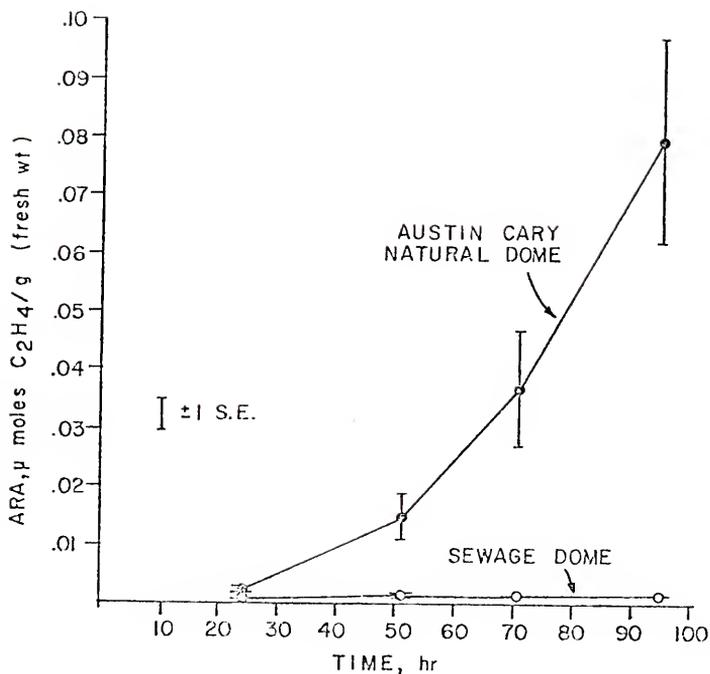


Figure 6-10. Time course of acetylene reduction associated with excised roots of *Taxodium distichum* var. *nutans* from April 5 to 9, 1979. Incubations were initially aerobic under 0.1 atmosphere C_2H_2 in the dark and at $24^\circ C$ with 7 percent (vol) surface water. Roots were excised, washed in the de-ionized water and incubated within 2 hours of collection from Austin Cary natural dome and sewage dome 2. Each data point represents the mean of four or five replicate samples.

in the natural dome contained low levels of combined nitrogen (0.10 mg N/L) and soluble reactive phosphorus (0.05 mg P/L). Sewage dome 2, on the other hand, contained high concentrations of combined inorganic nitrogen (20 mg N/L) and soluble reactive phosphorus (7.8 mg P/L) in the pore water 30 cm below the peat surface.

The repression of nitrogenase synthesis by high concentrations of inorganic nitrogen, certain amino acids, and amides is well documented (Burns and Hardy 1975; Hill et al. 1972). An inverse correlation between acetylene reduction of excised roots from coastal marsh angiosperms and concentration of ammonium in the groundwater (Patriquin and Keddy 1978) has provided field data for these repressive effects.

The occurrence of acetylene reduction activity in roots treated with chloramine-T (Figure 6-11) indicates that nitrogen-fixing organisms invade the root tissues or "endorhizosphere". However, most of the activity was associated with the root surface, since ARA was 43 and 27 percent of the untreated controls in roots treated with 1 and 10 percent chloramine-T, respectively. Microscopic observations of tetrazolium-reducing bacteria in the endorhizosphere of maize (Patriquin and Döbereiner 1978) and ARA in Spartina grass (Patriquin and McClung 1978) after surface sterilization attest to the endorhizosphere location of the nitrogenase. Other investigators (Okon et al. 1977; Zuberer and Silver 1978) used sterilization techniques, bacterial counts, and electron microscopy, and found no evidence that healthy, intact root cells of maize and mangrove were occupied by microorganisms. The symbiosis between roots and bacteria exists in the rhizosphere-rhizoplane in these cases.

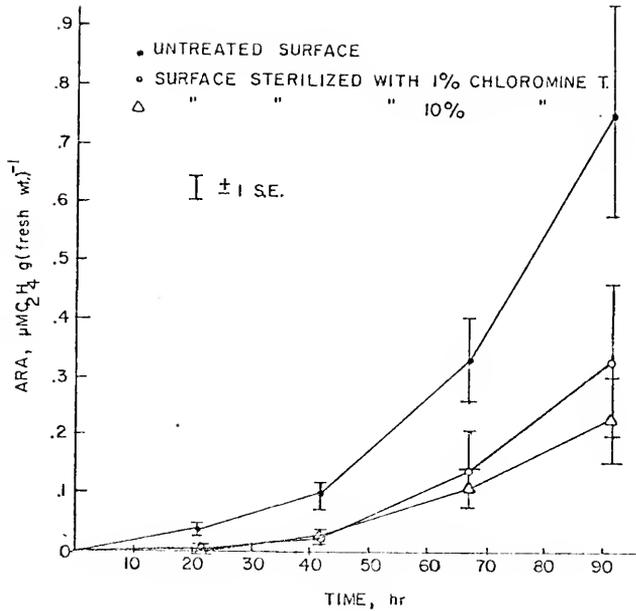


Figure 6-11. Time course of acetylene reduction activity associated with excised roots of pond cypress (*Taxodium distichum* var. *nutans*) from September 22 to 26, 1979. All roots were excised and surface sterilized with a 1 or 10 percent of chloramine-T or immersed in sterile phosphate buffer (pH 7.0) for 1.5 hours within 2 hours after removal from Austin Cary natural dome. Incubations were initially aerobic under 0.1 atmosphere C_2H_2 in the dark at 24°C with 36 percent (vol) *in situ* water. Each point represents the mean of five replicate samples.

It is interesting to note that the rates observed in the cypress roots from the natural dome when leaves were just starting to emerge in April (Figure 6-10) were up to an order of magnitude lower than those measured in cypress roots from the same dome near the end of the growing season (but substantially before leaf fall) in late October (untreated controls in Figure 6-11). This suggests a direct linkage between photosynthetic activity and root nitrogenase activity, as has been shown by a significant correlation between ARA and ^{14}C -activity in Spartina grass (Patriquin and McClung 1978).

Trees that are adapted to growth in flooded soils typically develop root adaptations to permit growth and nutrient uptake in the anoxic soil. These adaptations include: 1) morphological changes in secondary roots (those that are developed under flooded conditions may be succulent and less branched), and 2) physiological changes, such as the ability of the roots to oxidize their rhizosphere, increased tolerance of CO_2 , and higher anaerobic respiration rates (Hook and Brown 1973). In contrast, trees that are not adapted to flooding do not possess these attributes, or possess them to lesser extents. The implication is that flood-tolerant trees have developed mechanisms to bring oxygen to their roots. Consequently, even though the macro-environment may be anoxic, the rhizosphere may be oxygenated, and bacteria living in or on the roots may be able to function aerobically. Flood-tolerant trees that oxidize their rhizosphere include green ash (Fraxinus pennsylvanica), water tupelo (Nyssa aquatica) (Hook and Brown 1973), swamp tupelo (Nyssa sylvatica var. biflora), and several species of willow (Salix spp.) (Hook et al. 1972).

The three tree species dominant in the cypress domes have not been tested for morphological and physiological adaptation to flooding. No definite trend was seen in acetylene reduction activity of roots incubated under either oxygenated or anoxic conditions. However, the data are too scattered to be conclusive.

Previous investigations of acetylene reduction in the rhizosphere and endorhizosphere of non-woody, non-nodulated plants have shown bacteria to be the nitrogen-fixing agent (Day and Döbereiner 1976; Patriquin 1978; Patriquin and Döbereiner 1978), and it is likely that bacteria are the agents responsible for the nitrogenase activity found associated with these roots. Given the location of fixation and the possibility that these wetland tree species oxidize their rhizosphere, the agents of fixation are probably microaerophiles and a low partial pressure (~ 0.01 - 0.05 atm) is optimum (Döbereiner et al. 1972; Okon et al. 1977; Patriquin 1978).

The maximum acetylene reduction rate (calculated from the maximum rate of C_2H_4 production over any sampling period) is $17.3 \text{ nM } C_2H_4/g$ (fresh wt) $\cdot h$ for cypress roots. These rates are about an order of magnitude lower than acetylene reduction rates reported for non-leguminous nodules, but an order of magnitude higher than the rates reported for sediments and the rice root zone (Hardy et al. 1973). Insufficient data have been obtained to extrapolate the measured rates to annual rates on a per plant or areal basis. Temperature, nutritional status of the soil, physiological status of the trees (with respect to seasonal patterns of growth and dormancy), and soil moisture content are probably the most important variables affecting the rate of N_2 fixation by tree roots. In addition, data obtained by the excised

root and acetylene reduction method should be referred to as "potential nitrogenase activity" (Patriquin and Denike 1978) since transfer of fixed nitrogen to tree roots has not been demonstrated; N_2 fixation was assayed by an indirect technique; and estimates of N_2 fixation were based on ARA observed in washed, excised roots which there generally was a lag of 15-20 hours before ARA commenced.

These, and other, results (Patriquin and Keddy 1978; Patriquin and McClung 1978; Smith and Patriquin 1978; Tjepkema and Evans 1976) suggest that nitrogen fixation associated with roots of wetland vegetation may be widespread and that the locus of activity is both on the surface (rhizosphere) and within the roots (endorhizosphere). The reason for its occurrence in a natural cypress dome may be due to low availability of nitrogen. Although there are other sites (leaf litter, Azolla, Myrica) in the dome where nitrogen fixation does occur, these sources (along with bulk precipitation) are probably insufficient to support the nitrogen needs of the cypress dome. The colored, soft, acidic, canopy-shaded surface waters of the dome are unlikely habitats for nitrogen-fixing blue-green algae. Lichens (Usnea sp. and Parmelia spp.) associated with the trees do not belong to the genera that fix nitrogen. Data indicating that denitrification is an important nitrogen sink in this ecosystem emphasize the possibility for nitrogen shortage. Finally, mineralization studies have shown that the total nitrogen potentially made available each year from litter fall is not lost during the first 1.5 years of decomposition. It, therefore, cannot be available as a significant source for recycling back to the trees. Subsurface nitrogen fixation, on the other hand, appears to be intimately associated with the roots, thus facilitating transfer of fixed nitrogen to the trees.

Acetylene reduction associated with the roots of woody vegetation from forested wetlands has not been previously reported. Both the ecological and biogeochemical significance may be considerable, and fixation by this source may supply a significant portion of the nitrogen requirements of the dominant woody vegetation in forested wetlands. If fixation is found to be widespread in wetlands, estimates of the role of nitrogen fixation in the global nitrogen budget will have to be increased.

Ammonification

Kaushik and Hynes (1971) observed absolute increases in nitrogen with various leaves in freshwater streams, and Brinson (1977) noted the absolute amount of nitrogen in Nyssa aquatica leaves decreased in an alluvial swamp forest. In this study, the absolute amount of nitrogen in the cypress leaf material in the litter bags remained relatively unchanged (~90 percent of the original amount) at the sewage dome 2 and natural dome sites during the first year of decomposition (Figure 6-7 and Table 6-5). The lack of change resulted from a balance between the increases in the nitrogen content of the leaf litter and the loss of organic matter from the decomposing leaf litter (Figures 6-12 and 6-13, and Table 6-5). Thus, there was a net assimilation of nitrogen by decomposers in relation to other dry weight components which were lost from the litter. As a result, nitrogen from recent litter fall is not mobilized and lost, but tends to be retained inside the dome.

The relative nitrogen content of the leaf litter in the Austin Cary dome after 1.5 years approached the nitrogen levels measured in the top 5 cm of peat (compare Table 6-5 with Appendix 2), indicating

Table 6-5. Weight loss, carbon concentrations, nitrogen concentrations, and nitrogen content of decomposing leaf litter of *Taxodium distichum* var. *nuttans* in Austin Cary natural dome and sewage dome 2 for 1978-79. Each number represents a mean of five bags. The numbers enclosed in parentheses represent ± 1 S.E. of the mean.

Site	Date	Week	Percent Dry Wt Remaining	Percent C	Percent N	C/N	Observed Wt of N as Percent of Initial Wt	Percent Change in N Content
Austin Cary Natural Dome	1 Jan	0	100.0	45.8(0.9)	0.81(.03)	56.5		0
	16 Jan	2	95.8(0.6)	48.2(1.1)	0.78	61.8	92.3(3.6)	- 7.7
	30 Jan	4	91.5(2.6)	47.0(1.2)	0.66(.03)	71.2	73.8(1.5)	-26.2
	28 Feb	8	87.9(0.3)	48.2(0.7)	0.85	56.7	92.0(1.7)	- 8.0
	25 Apr	16	79.8(0.8)	49.9(1.8)	0.95	52.5	93.3(4.0)	- 6.7
	25 Jul	29	75.1(0.8)		2.00		92.9(2.4)	- 7.9
	25 Oct	42	71.0(0.7)		1.06		92.5(1.9)	- 7.5
	25 Jan	56	72.9(1.9)		1.22		110.4(6.0)	+10.4
	24 Jul	81	67.4(2.0)		1.22		101.7(7.0)	+ 1.7
	1 Jan	0	100.0	42.9(0.9)	1.50(.03)	28.6		0
Sewage Dome 2	16 Jan	2	88.5(1.1)	47.1(0.6)	1.62	29.1	95.5(3.7)	- 4.5
	30 Jan	4	83.5(0.5)	47.9(1.4)	1.62	29.6	89.9(1.0)	-10.1
	28 Feb	8	65.3(1.0)	50.2(0.7)	2.07	24.3	90.6(7.6)	- 9.4
	25 Apr	16	65.3(1.0)	50.2(0.7)	2.07	24.3	90.6(7.6)	- 9.4
	25 Jul	29	60.8(1.4)		2.31		97.4(8.6)	- 2.6
	25 Oct	42	57.6(0.8)		2.25		82.5(3.1)	-17.5
	25 Jan	56	67.4(1.6)		2.61		117.5(8.1)	+17.5
	24 Jul	81	53.5(5.8)		2.16		79.7(11.1)	-20.3

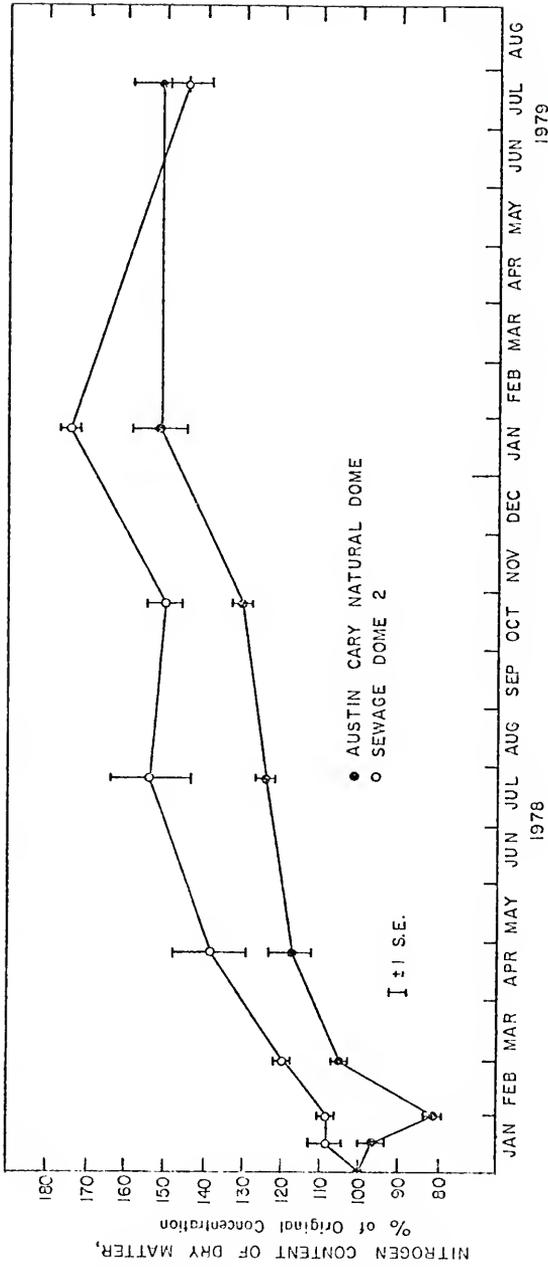


Figure 6-12. Exchanges of nitrogen in *Taxodium distichum* var. *nutans* leaves during decomposition in the standing waters of Austin Cary natural dome and sewage dome 2. Each point represents a mean of five values. The values plotted are percentages of the concentrations present at t_0 if there had been no loss of dry weight.

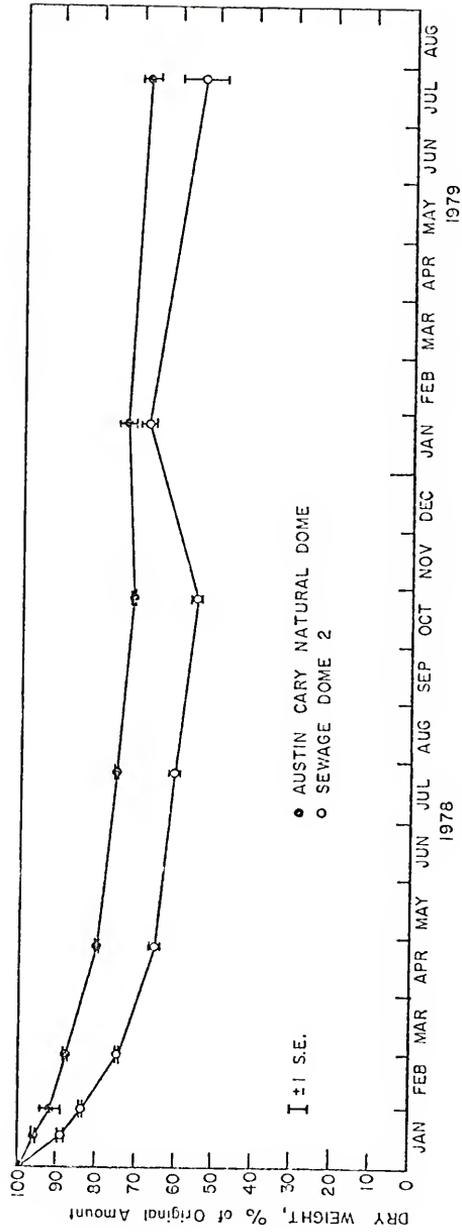


Figure 6-13. Dry weight loss of *Taxodium distichum* var. *nutans* leaves in the standing waters of Austin Cary natural dome and sewage dome 2.

that the majority of the nitrogen enrichment in the litter has been completed within 2 years.

In summary, the absolute nitrogen content of the leaf litter from the natural and sewage domes was not mobilized during decomposition. Thus, the nitrogen retained is either available for short-term internal cycling or for long-term storage in the domes.

Denitrification

Nitrate may be used by microorganisms either as a source of nitrogen for growth (assimilation) or as a terminal electron acceptor (dissimilation or anaerobic respiration). Assimilation involves a reduction of NO_3^- (oxidation state +5) to organic nitrogen forms (oxidation state -3) (Figure 6-3). Dissimilation includes the reduction of NO_3^- to: 1) NO_2^- or NH_3 and 2) nitrogen gas. The latter process, called denitrification, acts as a nitrogen sink in that the products (N_2 and N_2O) are relatively inert and are easily lost to the atmosphere. Recent investigations have indicated that dissimilatory reduction of nitrate to ammonium may be as prominent as denitrification in the turnover of nitrate in marine sediments (Koike and Hattori 1978; Sørensen 1978). The biochemistry, microbiology, and ecology of denitrification in aquatic systems have been extensively reviewed by Brezonik (1977). Payne (1973) provides a detailed review of the microbiological aspects in the reduction of nitrogenous oxides.

Various methods have been employed in determining the amounts and rates of denitrification in soil and aquatic ecosystems. Table 6-6 lists the relative advantages, disadvantages, and some references for the methods employed in measuring the denitrification process. Of all the methods presented, acetylene blockage possesses some unique

Table 6-6. Relative advantages and disadvantages for different methods used to measure the denitrification process.

Method	Advantages	Disadvantages	Examples
Nitrate disappearance	Simplicity of method and instrumentation Speed of analysis	Overestimates losses due to nitrate immobilization	Thomas (1966)
Mass balance	Measures the denitrification rate for the total ecosystem Integrates diurnal, spatial, seasonal, and yearly variations	Measurements of all other nitrogen flows and storages required Errors associated with above measurements will appear in the calculated denitrification rate	Vollenweider (1968) Andersen (1974)
^{15}N tracer	Direct measurement	Expensive Relatively insensitive Requires sophisticated instrumentation and expertise	Brezonik and Lee (1968) Goering and Dugdale (1966)
N_2/Ar	Direct measurement <u>In situ</u> determination	Tedious Difficult to quantify Applicable to only stagnant waters	Richards and Benson (1961)
N_2O reduction	Direct measurement	Relatively insensitive Variable according to flora and pH	Garcia (1974)
Acetylene blockage	Sensitive Inexpensive Simplicity of method and instrumentation Nitrogen fixation can simultaneously be measured	Indirect assay Uncertainties associated with its quantitative relationship to denitrification	Yoshinari, Hynes and Knowles (1977)

advantages, which, taken collectively, outweigh its disadvantages as well as the advantages offered by other techniques.

Federova et al. (1973) report that C_2H_2 inhibits the reduction of N_2O to N_2 during denitrification, and this finding has been confirmed by pure culture studies of denitrifying bacteria (Yoshinari et al. 1977; Klemetsson et al. 1977), and sediments (Sørensen 1978). In the presence of 0.1 atm of C_2H_2 , these workers found stoichiometric conversions of NO_3^- and NO_2^- to N_2O .

Peat plus standing water collected from sewage dome 2 and Austin Cary natural dome were incubated anaerobically with 0.1 atm (= 0.094 ml C_2H_2 /ml H_2O) of C_2H_2 . In addition to receiving 1.0 mg NO_3^- -N/L, one set of triplicate flasks from the natural dome received 0.2 percent glucose (w/w), another set was buffered to a pH of 6.2 (alkalinity = 300 mg $CaCO_3$ /L), while a third set received two percent glucose and was buffered to a pH of 6.2. A triplicate set of flasks containing natural dome peat and a duplicate set of flasks containing sewage dome peat received only 1.0 mg NO_3^- -N/L spike. Controls consisted of natural dome peat with 1 ml of saturated $HgCl_2$ solution added as a bactericide followed by injections of C_2H_2 to obtain a $pC_2H_2 = 0.1$ atm (0.094 ml C_2H_2 /ml H_2O) and 1 ml of 247 nM/ml of N_2O .

Anaerobically incubated peat from the natural dome showed a nearly constant production of N_2O for all the experimentally treated natural dome peat plus standing water samples during the first 19 hours (Figure 6-14; curves 1-4). A decrease in the production rates was observed for all curves during the following 8 hours. The formation of N_2O was complete by hour 27 h, with slight reductions occurring in the N_2O content of some flasks thereafter. Analysis of

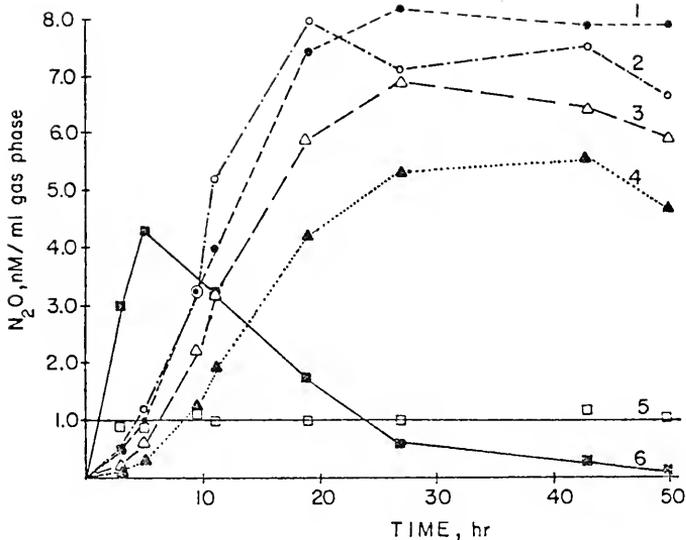


Figure 6-14. Production of N_2O by submerged peat incubated in a He atmosphere with 0.1 atm C_2H_2 . Curve 1, Austin Cary dome peat, nitrate, and C_2H_2 ; curve 2, Austin Cary dome peat, nitrate, glucose, and C_2H_2 ; curve 3, Austin Cary dome peat, nitrate, glucose, $NaHCO_3$, and C_2H_2 ; curve 4, Austin Cary dome peat, nitrate, $NaHCO_3$, and C_2H_2 ; curve 5, Austin Cary dome peat, nitrate, $NaHCO_3$, and C_2H_2 ; curve 6, sewage dome 2 peat, nitrate, and C_2H_2 . Each data point represents the mean of three replicate flasks, except for curve 5 where each data point is the average of duplicate flasks.

variance demonstrated that there was no significant difference in the mean N_2O produced among the four treatments after 43 h of incubation, and a chi-square test for differences among the rates of N_2O production (the slopes in Figure 6-14 between 5 and 19 h, the time of maximum rate) for the four treatments indicated that none of the slopes was significantly different from the others.

Leakage and/or absorption of N_2O by the rubber stoppers or septa did not occur, as shown by the horizontal line in curve 5 for the control. Of the 247 nM N_2O injected into each control flask, 160 nM was recovered in the gas phase. The remaining 87 nM (35 percent of the total) was dissolved in the liquid phase. Similar results were reported by Klemedtsson et al. (1977) for nearly the same aqueous:vapor phase volume ratio (0.71) as that used in this investigation (0.625).

In sewage dome 2 peat receiving 1.0 ppm NO_3^- -N (curve 6), only 30 percent of the NO_3^- -N was converted to N_2O by 5 hours, and the N_2O concentration subsequently declined, implying that C_2H_2 inhibition was not complete under these conditions. Although Yoshinari and Knowles (1976) and Klemedtsson et al. (1977) reported that 0.1 atm C_2H_2 was sufficient to block the reduction of N_2O to N_2 , other investigators using the technique in marine sediments have used considerably lower levels of C_2H_2 (e.g., 0.7 percent (Sørensen 1978); 0.01 to 0.02 atm (Balderston et al. 1976)) without any observed reduction of N_2O . Sørensen demonstrated that the reduction of N_2O to N_2 was completely blocked in the presence of 0.7 percent C_2H_2 since significant $^{15}N_2$ production was absent in the bottles with C_2H_2 . However, other investigators have indicated a slight decrease in the N_2O accumulation at

C_2H_2 levels as high as 0.1 atm (Yoshinari et al. 1977; Klemedtsson et al. 1977; Knowles 1979).

The N_2O decrease followed by its disappearance from the sewage dome 2 peat is the first such decrease reported under conditions indicated as sufficient for $N_2O \rightarrow N_2$ blockage by acetylene, and these results cast doubt on the use of the acetylene blockage technique to estimate denitrification rates under some conditions. The precise reason for the decline is not understood at this time. It may be that there are certain types of denitrifiers associated with the highly reduced sewage dome peat whose nitrous oxide reductase is not sensitive to blockage by acetylene. Whether higher amounts of C_2H_2 (e.g. ~ 0.4 atm) would overcome the reduction of N_2O , as reported by Knowles (1979), is not known. Certainly the results reported here using acetylene blockage on extremely reduced sediments and organic matter need to be investigated more fully in order to determine whether this technique for assaying denitrification is applicable under all experimental conditions.

The similarities in both the rates of N_2O production and the maximum N_2O accumulations among the four experimental groups of Austin Cary peat (Table 6-7; Figure 6-14, curves 1-4) were unexpected, since low rates of denitrification have been found associated with low pH in soils (Bremner and Shaw 1958; Focht 1974; Nömmik 1956). Klemedtsson et al. (1977) found only 5 percent of the $1600 \mu g NO_3^- - N$ added to 9.2 g peat (dry wt) at pH 3.5 could be detected as $N_2O - N$, even after additions of nutrient and/or energy sources. When the investigators increased the peat pH to 6.5, a much higher production of N_2O was recorded. Addition of an energy source and/or an increase in the pH

Table 6-7. Denitrification results in four experimental treatments of peat from Austin Cary natural dome.

	Nitrate ^a		Nitrate + Glucose ^b		Nitrate + Alkalinity ^c		Nitrate + Glucose + Alkalinity	
	\bar{X}	± 1 S.E.	\bar{X}	± 1 S.E.	\bar{X}	± 1 S.E.	\bar{X}	± 1 S.E.
Ave. pH at end of experiment	4.32		4.36		6.18		6.14	
Ave. ($\text{NO}_3^- + \text{NO}_2^-$)-N at end of experiment (mg/L)	0.093		0.043		0.10		0.067	
Ave. max. amount of N_2O ($\mu\text{g N/flask}$)	56.5	± 2.1	54.9	± 10.7	38.2	± 12.7	47.8	± 5.7
Ave. max. rate of N_2O ($\mu\text{g N/h}$)	2.68	± 0.51	2.89	± 0.56	1.52	± 0.72	2.13	± 0.30
Percent of total nitrate denitrified	61		56		41		50	

^a 1.00 mg NO_3^- -N/L final concentration or 0.10 mg NO_3^- -N/g peat (dry wt)

^b 20 mg glucose/L final concentration or 0.2 percent (w/w)

^c 300 mg/L as CaCO_3 final concentration

in the Austin Cary dome peat to the optimum range reported for denitrification (6-8) did not significantly increase either the denitrification rate or the total amount of N_2O produced above the rates and total amounts measured for unspiked peat at the ambient pH of 4.3. It appears that the denitrifying flora in cypress domes have become adapted to the prevailing acidic conditions, such that approximately 60 percent of the nitrate is denitrified and the remaining 40 percent is either assimilated or dissimilated to ammonium. Adequate levels of naturally-occurring, readily-oxidized organic material exist to serve as an energy source for the denitrification of low levels of nitrate entering the dome in atmospheric precipitation.

Nitrification

A low $NO_3^- - N / NH_4^+ - N$ ratio ($\bar{X} = 0.57$) in the standing water suggests the rate and extent of nitrification in the natural dome may be inhibited by the acid pH and high dissolved organic matter concentration of this water.

Numerous investigators have cited various organic compounds as being inhibitory to nitrification. Painter (1970) reviewed the literature and reported that thiourea, allyl-thiourea, 8-hydroxy quinoline, salicylaldehyde, histidine, and peptone inhibited nitrification to some extent. Patrick et al. (1968) used a known nitrification inhibitor, 2-chloro-6-(trichloromethyl) pyridine (N-Serve), in an attempt to prevent nitrification and thus subsequent denitrification in order to increase rice yields. Bremner and Bundy (1974) found that volatile sulfur compounds such as carbon disulfide, dimethyl disulfide, methyl mercaptan, dimethyl sulfide, methyl mercaptan, dimethyl sulfide, and hydrogen sulfide inhibit nitrification in soils. Tomlinson et al.

(1966) also found most of the potent inhibitors in activated sludge were sulfur-containing compounds that are metal-chelating compounds. These substances thus may inhibit enzymes requiring metals for activation. Rice and Pancholy (1973) reported that condensed and hydrolyzed tannins derived from plants could completely inhibit nitrification for three weeks in soils. Hockenbury and Grady (1977) screened 52 organic compounds (most contained nitrogen) likely to be found in industrial wastes for the degree of inhibition of ammonia oxidation by Nitrosomonas sp. They found that 12 of the 20 compounds inhibiting ammonia oxidation contained one or more benzene rings in their structure.

Surface Sediments

Autotrophic nitrifying bacteria from each of the four cypress dome sites were enumerated by the MPN method as a means of determining whether the environmental conditions of each dome were capable of supporting a large population of autotrophic nitrifiers.

A substantial range in the MPN (350 to >160,000) was found in sewage domes 1 and 2, while a smaller range occurred in the groundwater control dome (78 to 7,900) (Table 6-8). The MPN of nitrifiers in the Austin Cary natural dome showed the narrowest range (0-56). Using either cores or the sterile syringe methods to obtain samples yielded similar MPN values, indicating that both methods are comparable in sampling surface sediments for MPN estimates.

As expected, ammonium nitrogen levels were high in the pore water from surface sediments of sewage dome 2 (range of 9 to 22.5 mg/L). Sewage dome 1 surface sediments had lower levels of ammonium nitrogen, 0.65 to 24.5 mg/L (when sampled ten months after the cessation of sewage discharge). The groundwater control dome and Austin Cary natural

Table 6-8. Most Probable Number (MPN) of autotrophic nitrifiers, ammonium and nitrate and nitrite levels in the surface sediments of sewage dome 1, sewage dome 2, groundwater control dome, and Austin Cary natural dome.

Site	Date	Sampling Method	MPN (cells/ml)	NH ₄ ⁺ -N (mg/L)	(NO ₃ ⁻ +NO ₂ ⁻)-N (mg/L)
Sewage Dome 1	7/ 5/78	Syringe	350	2.2	0.005
	7/ 5/78	Syringe	> 1,600	1.5	0.03
	7/ 5/78	Core	9,200	24.5	<0.005
	7/30/78	Syringe	2,400	n.d.	0.04
	7/30/78	Syringe	>160,000	0.65	0.06
Sewage Dome 2	7/ 5/78	Syringe	1,600	9.0	0.02
	7/ 5/78	Syringe	> 1,600	12.5	0.02
	7/ 5/78	Core	350	n.d.	< .005
	7/30/78	Syringe	>160,000	20.0	0.05
	7/30/78	Syringe	5,400	22.5	0.03
Groundwater Control Dome	7/ 5/78	Syringe	350	< 0.05	<0.005
	7/ 5/78	Syringe	220	n.d.	<0.005
	7/ 5/78	Core	3,500	3.8	0.03
	7/ 5/78	Core	690	n.d.	<0.005
	7/30/78	Syringe	7,900	0.19	0.02
	7/30/78	Syringe	78	0.28	0.03
Austin Cary Natural Dome	7/ 2/78	Syringe	49	0.10	0.02
	7/ 2/78	Syringe	2.6	0.55	0.005
	7/ 2/78	Core	31	n.d.	n.d.
	7/ 2/78	Core	56	n.d.	n.d.
	7/30/78	Syringe	4.5	0.14	<0.005
	7/30/78	Grab sample using sterile flask	0	n.d.	n.d.

n.d. Not determined.

dome had low levels of ammonium nitrogen in their pore waters with ranges of <0.05 to 3.8 mg/L for the groundwater control dome and 0.10 to 0.55 mg/L for Austin Cary. Nitrate nitrogen in the pore water was consistently low at all sites.

The relatively favorable pH values associated with the waters of sewage dome 1, sewage dome 2, and the groundwater control (see Chapter 3) probably account for the high MPN values associated with them. The pH values of 6-7 are not quite optimum for nitrification, but they are not lethal (Engel and Alexander 1958). The low pH (4-5) of the Austin Cary natural dome surface water explains the near absence of nitrifiers from this dome. Latter et al. (1967) reported that nitrifying bacteria were virtually absent from British moorland peat because of their low pH and the presence of water.

The high MPN count associated with the surface sediments of anoxic sewage dome 2 (Table 6-8) does not necessarily indicate that nitrification was occurring. Painter (1970) states that some dissolved oxygen is an absolute requirement for growth, although absence of oxygen for long periods is not lethal. Considering the daily pumping of a sewage effluent whose treatment involved extended aeration, a steady supply of nitrifiers into sewage dome 2 would be expected. However, because of the continually anoxic conditions of the surface waters, nitrification would not be expected to occur.

The oxygen requirement for nitrification was met in the groundwater control dome and sewage dome 1 (when sampled 10 months after the cessation of pumping). The nitrifiers found in these two domes probably were nitrifying ammonium to nitrate.

Surface Waters

Colored and decolored standing waters from the natural dome at Austin Cary whose pH was ambient or adjusted to 8.5 were spiked with a population of nitrifiers. Only slight differences in the nitrification rates were observed between the colored, pH-adjusted and the decolored, pH-adjusted bottles (Figure 6-15). Onset of nitrification in the colored water with pH adjusted to 8.5 preceded that of the decolored water by two days, after which time the rates of the two treatments were equal. The delay in the onset of nitrification in the decolored, buffered samples could have been due to either the incorporation onto the exchange sites of the DEAE cellulose resin of (nutrient) anions such as phosphate (in addition to the color molecules), or the ability of the colored water to complex and thus make certain micronutrients more readily available to the nitrifiers.

The total amount of NH_4^+ (5 mg N/L) added was converted to nitrate and nitrite in both buffered waters, with the uncolored water showing a final concentration of 5.4 mg ($\text{NO}_2^- + \text{NO}_3^-$)-N/L, while the colored water yielded a final concentration of 4.85 mg ($\text{NO}_2^- + \text{NO}_3^-$)-N/L. Concentrations in excess of 5 mg/L for nitrate and nitrite are possible because of the potential source of ammonium added with the inoculum.

The colored, pH-unadjusted control displayed no increase in the nitrate plus nitrite level from the initial concentration, while the decolored, pH-unadjusted control showed a slight increase in the nitrate plus nitrite level. This reflected a difference in the initial pH of the two controls. The pH of the colored control was initially 4.5 and slowly increased to 4.8 at the end of the experiment (195 h). The decolored control had an initial pH of 6.1, which increased to 6.8

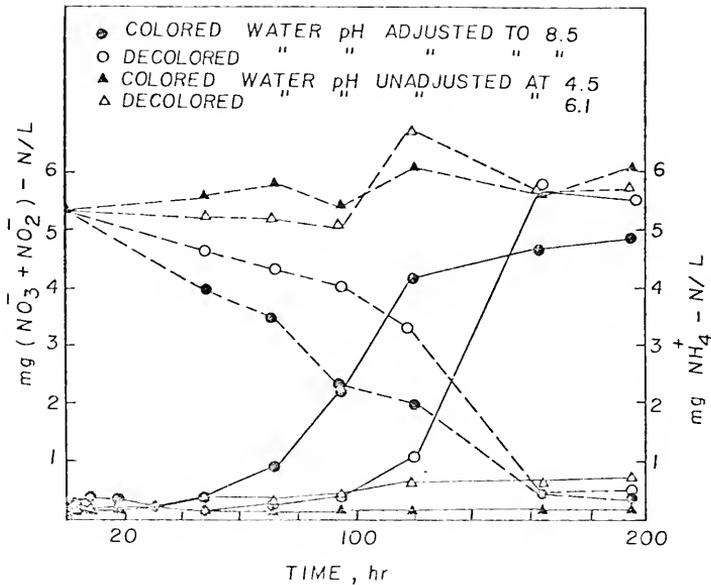


Figure 6-15. Nitrification in surface water in Austin Cary natural dome. Each data point is the mean of duplicate bottles. The solid lines represent nitrate plus nitrite nitrogen concentrations and the dashed lines represent the ammonium nitrogen concentrations.

in one of the duplicate bottles and to 6.3 in the other 9 h after adding the inoculum; thereafter, the pH in both replicates decreased to 4.8 as a result of the nitrification.

Tannins and their derivatives originate from various plant species (Rice and Pancholy 1973; Martin and Haider 1971) and are considered to be a major source of the organic colored water in swamps and marshes. The inhibitory effect of tannins and their derivatives on nitrification reported by Hockenbury and Grady (1977) and Rice and Pancholy (1973) was not supported by the above experiment using natural swamp surface waters whose normally acidic pH was adjusted to 8.5 (Figure 6-15). Hockenbury and Grady (1977) found that numerous aromatic and nitrogen-containing organic compounds of industrial importance severely inhibit nitrification. However, tannic acid acted only as a weak inhibitor of nitrification. A solution containing 50 mg/L tannic acid inhibited nitrification only 7 percent. The lower concentrations of tannic acid associated with swamp water evidently do not exert an inhibitory affect on nitrification. Erickson (1978) could not discern any effect on nitrification in inoculated marsh water after the removal of color by charcoal filtration. Rice and Pancholy (1973), however, found concentrations of plant-extracted condensed and hydrolyzable tannins as low as 2 mg/L to completely inhibit nitrification for three weeks in soil suspensions.

The inhibition of nitrification in the surface waters of Austin Cary natural dome is primarily a consequence of low pH and is not attributable to dissolved organic material in the humic-colored water. It has been demonstrated widely that the rate of ammonium oxidation declines with decreasing pH (Painter 1970). Nitrification in the

underlying peat also would be inhibited by the same mechanism, which explains the almost complete absence of nitrifiers in surface peat samples in July of 1978 (Table 6-8). A MPN range of 0-56 per cm³ of wet peat for six samples was enumerated; these may have been heterotrophic nitrifiers using the dissolved organic matter introduced with the inoculum in the low dilution tubes.

The implications these findings have on the conservation of nitrogen and energy in the cypress dome ecosystem are profound. By inhibiting nitrification, the ammonium ion becomes the dominant inorganic nitrogen species. This leads to the conservation of nitrogen since the ammonium ion is positively charged and is adsorbed on negatively charged clay particles and cell walls, thus preventing leaching beyond the root zone, which occurs readily with nitrate. Since denitrification is significant in cypress domes, the inhibition of nitrification would prevent losses of the converted ammonium via this sink.

A conservation of energy also is realized by the inhibition of nitrification, since use of ammonium by plants and microbes is more efficient than use of nitrate. Nitrate must be reduced to ammonium by energy-dependent processes before it is incorporated into protein (Figure 6-1). There is a preponderance of evidence that many plant species can use ammonium nitrogen as effectively or more so than nitrate nitrogen (Allison 1931; Ferguson and Bollard 1969; McFee and Stone 1968; Moore and Keraitis 1971; Shen 1969) and this certainly must be the case for the nitrate-depleted environment of the natural swamp.

CHAPTER SEVEN
NITROGEN AND PHOSPHORUS MASS BALANCE MODELS IN A
NATURAL AND A SEWAGE LOADED CYPRESS DOME

The purpose of this chapter is to describe nitrogen and phosphorus mass balance models for a cypress dome (sewage dome 2) that was receiving secondary-treated sewage effluent for a three-year period (1975-1977) and a natural cypress dome (Austin Cary) for a five-year period (1975-1979).

Methods and Data Sources

Nutrient Inputs

Treated sewage

Nitrogen and phosphorus loadings from the sewage effluent are based on mean monthly concentrations of total P, total N, and nitrate plus nitrite reported for the period April 1974 to March 1977 by Overman (1974 and 1975), Zoltek and Whittaker (1975), Zoltek (1976), and Zoltek and Neff (1978). Monthly hydraulic loading rates (cm/month) for the period 1975-1977 were obtained from Wang and Heimburg (1976) and converted to L/m²-month. Each monthly hydraulic loading rate was then multiplied with the corresponding month's mean nutrient influent levels to obtain loads in g/m²-month.

Surface runoff

Variations in surface water area are important to the calculation of runoff (surface inflow) because the depth of runoff added to the dome will appear higher at low water levels and lower at high water levels (because of the larger surface area). Using Heimburg's (1976)

water level records, the volume contributed by surface runoff to the increase in stage level was calculated for each rain event during the study period. Surface flow was considered to cease as soon as the water level recording of a storm peaked. The underlying infiltration rate was inferred from days immediately preceding and following the rainfall event and was usually negligible.

Because of seasonal changes in canopy cover, throughfall varied from a maximum of about 90 percent of the total rainfall in winter to about 60 percent in the summer rainy season (Heimburg 1976). The seasonal difference in throughfall significantly affects the quantity of runoff computed from the water level curves for rain events. A given increase in water level in winter thus is associated with a smaller amount of surface runoff to the dome than the same water level increase in summer, since the canopy intercepts rain that does not contribute to the surface water level increase. Thus, two sets of water level increase curves were used to quantify runoff contributed by each rain event during winter and summer. The increases in water depth from runoff were converted to water volumes using a stage curve relating water depth to water volume. The relationship is linear for depths >47 cm in sewage dome 2 (Wang and Heimburg 1976). Since volume/depth curves were not constructed for Austin Cary dome, the area of the dome was divided into thirds (corresponding to radii 50, 100, and 150 m), assuming the dome was a perfect circle. The depth of water at the center was divided into thirds also (0-0.24, 0.24-0.49, and >0.49 m) based on the maximum depth recorded for the five year period (0.76 m). The volume of water contributed by each surface runoff event was estimated from the equation for the volume of a cylinder

($v = \pi r^2 h$) by assuming the area covered by the standing water was circular and each rise in the water level within each of the three boundary depths corresponded to an area of standing water whose radius was equal to either 50, 100, or 150 m at the beginning of the runoff event.

In general, surface flow started after 1.0 or 1.2 cm of rain, and remained roughly constant to a storm depth of about 3.8 cm (Heimburg 1976). After 3.8 cm, it was assumed that the relationship between storm depth and surface flow was linear.

Nitrate plus nitrite, ammonium, organic nitrogen and total phosphorus concentrations in the surface flows into sewage dome 1 (ten separate rainfall events), sewage dome 2 (nine separate rainfall events), and Austin Cary dome (two separate rainfall events) were determined during a summer rainfall period (August 2 to August 13, 1976) and a winter rainfall period (December 16, 1977 to January 20, 1978). Station locations and variations in concentration with time, station, and antecedent conditions for the summer period were reported by Dierberg and Brezonik (1976). Annual loadings of nitrogen, phosphorus, and nitrate plus nitrite were estimated by multiplying the computed volume of water each runoff event by the average annual concentrations obtained from the summer and winter runoff studies.

Bulk precipitation

Loading rates ($\text{g}/\text{m}^2\text{-yr}$) for total P, total N, and nitrate plus nitrite were calculated by multiplying the volume weighted mean concentrations (mg/L) by rainfall amount for each constituent. Samples were collected weekly or biweekly from June 1976 to May 1977 atop a 30 m tower in sewage dome 1 (Hendry and Brezonik 1980) and from May

1978 to April 1979 atop a 30 m tower in the center of Austin Cary dome.

Nutrient Sinks

Infiltration

The nighttime slope of the water level record was considered to represent the infiltration rate, assuming that evapotranspiration is negligible at night, and that infiltration during the day was the same as that at night. Errors associated with both these assumptions are negligible (Heimburg 1976). Infiltration was computed weekly by multiplying the rate (cm/h) for a randomly chosen day of each week by 168 (h/wk). Weekly amounts were then summed to obtain yearly rates. For the water budget year (May 30, 1975 to May 30, 1976), Heimburg (1976) computed a surface water infiltration rate of 147 cm using daily infiltration rates; a rate of 141 cm was calculated from a subsample of the daily record consisting of one daily rate per week. The relative error thus is only 4.4 percent. The error associated with basing weekly infiltration on the measurement of a random day for each week instead of computing rates for each day is probably negligible. Infiltration during storm events was assumed to be the same as the rate immediately preceding or after the event. In instances where weekly water level data were missing, the mean infiltration rate was calculated from the rates for the previous and subsequent weeks. Infiltration rates calculated from the nighttime slope of the level record for the natural dome may represent net rates since there may be recharge of the surface water during high water tables from a hill on the northeastern side of the dome (Heimburg 1976).

Yearly amounts of water lost by infiltration (cm) for sewage dome 2 were multiplied by the mean levels of total P, total N, and nitrate plus nitrite over the same time period for the wells surrounding sewage dome 2 in order to calculate N and P losses by infiltration. For the natural dome in the Austin Cary forest, yearly infiltration rates from April 1975 to March 1979 were multiplied by the average total P, total N, and nitrate plus nitrite measurements made during April to December 1978 from well 2 (3.6-4.6 m deep; 50 m east of the dome center) (Figure 2-4).

Denitrification

Denitrification rates for sewage dome 2 and Austin Cary dome were calculated from the difference between measured loadings and sinks of $(\text{NO}_3^- + \text{NO}_2^-) - \text{N}$. These values were multiplied by the average percentages of the total nitrate reduced to N_2O using the acetylene blockage technique (75 percent for sewage dome 2 and 61 percent for the Austin Cary dome). Because the surface water usually is anoxic in sewage dome 2 and is acidic in Austin Cary, nitrification is negligible in both domes.

Surface overflow

Periods of overflow from sewage dome 2 were identified by sharp peaks above a 67 cm stage height in the "storm hydrograph." Below this stage height outflow did not occur. The duration of an overflow event was estimated by identifying the time when the nighttime slope of the water level recorder (representing effects of infiltration only). In the case of overflows of long duration, the time when the nighttime slope became zero was used to determine the duration of overflow, assuming that infiltration was negligible because of ground

saturation in such cases. Infiltration during overflow events was estimated from the average infiltration for the time preceding the rain and the time after the overflow period. The remaining slope during an overflow event (i.e., that not accounted for by infiltration) was then separated into overflow and evapotranspiration by using Gainesville pan evaporation data multiplied by pan coefficients (Heimburg 1976). When runoff continued during overflow, it was included with overflow rather than infiltration. Thus overflow was a net amount on those occasions. The lower set of N and P concentrations for the two edge stations in sewage dome 2 was selected from the closest sampling date to the overflow event to calculate the mass overflows. The lower concentrations are still considered to be an overestimate because of dilution by rain during overflow periods.

Storages

Cypress tree biomass

Net growth or production stored in aboveground cypress biomass was computed using regression equations relating the diameter at breast height (DBH) to the amount of biomass for the foliage, branches, and boles (Brown 1978). Data used in arriving at the regression equations originated from ten pond cypress trees harvested in Alachua County (Mitsch and Ewel 1979). Increases in computed biomass for each of one hundred trees in sewage dome 1, sewage dome 2, and groundwater control dome (two hundred trees in Austin Cary) from June 1976 to June 1979 were averaged and divided by three to obtain the average yearly aboveground net growth for a cypress tree (in g dry weight). The computer program used in calculating the net productivity for the aboveground biomass components (i.e., foliage, branches, and stumps plus

boles) is listed in Appendix 3. Multiplication by the number of trees within each dome and the nitrogen or phosphorus content for each biomass component (foliage, branches, or boles) (Straub and Post 1978) produced the total amount of nitrogen and phosphorus present in the aboveground cypress biomass.

Since diameter increments were not measured for black gum in Austin Cary, it was assumed that the increments in each biomass compartment computed for cypress in Austin Cary dome were the same for black gum. Each biomass increment was then multiplied by the ratio of hardwood basal area to cypress basal area (0.306) found in Austin Cary (Brown 1978). Multiplying by the number of trees within Austin Cary and the nitrogen or phosphorus content of each biomass component (Straub and Post 1978) produced an estimate of the total N and total P present in the aboveground black gum biomass.

Sediment

Sediment deposition of nitrogen and phosphorus in the sewage domes was obtained by the difference between total inputs and total losses and cypress storage. For phosphorus in sewage dome 2, phosphorus deposition in the sediments was estimated by multiplying average infiltration rates (1.176 m/yr) for a three-year period by the concentration of total phosphorus measured in the standing water ($\bar{X} = 6.62 \text{ g/m}^3$ for one center and two edge stations ($N = 94$)).

Results and Discussion

Sources

During the three-year study period (1975-1978), sewage dome 2 received loadings that averaged $14.9 \text{ g/m}^2\text{-yr}$ for N and $11.4 \text{ g/m}^2\text{-yr}$ for P (Figures 7-1 and 7-2). The natural dome in Austin Cary forest

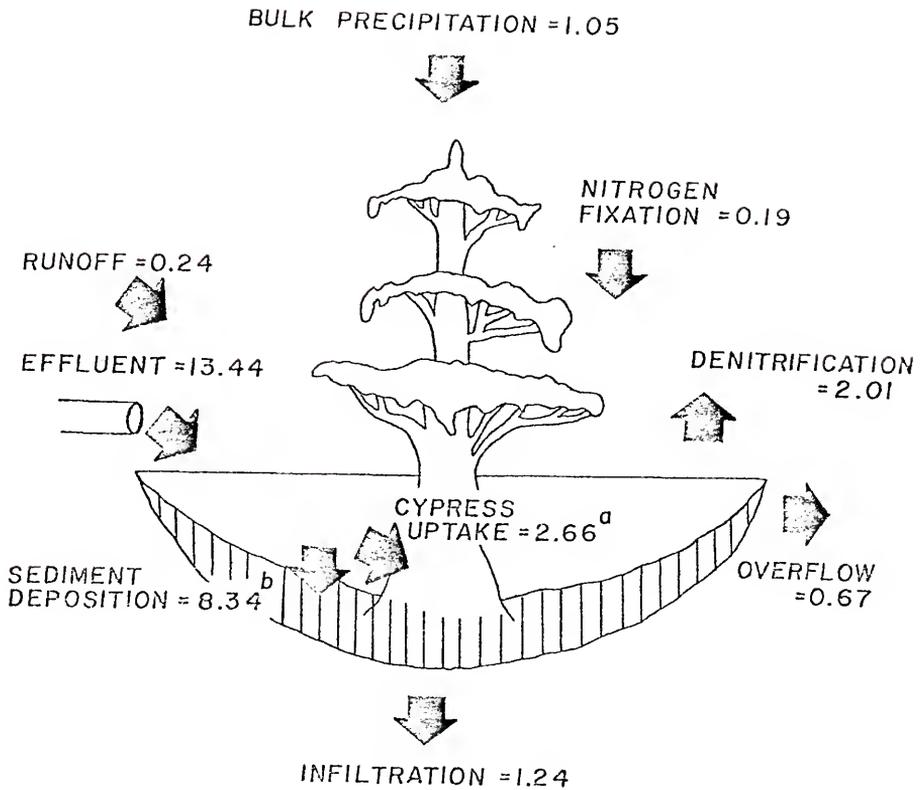
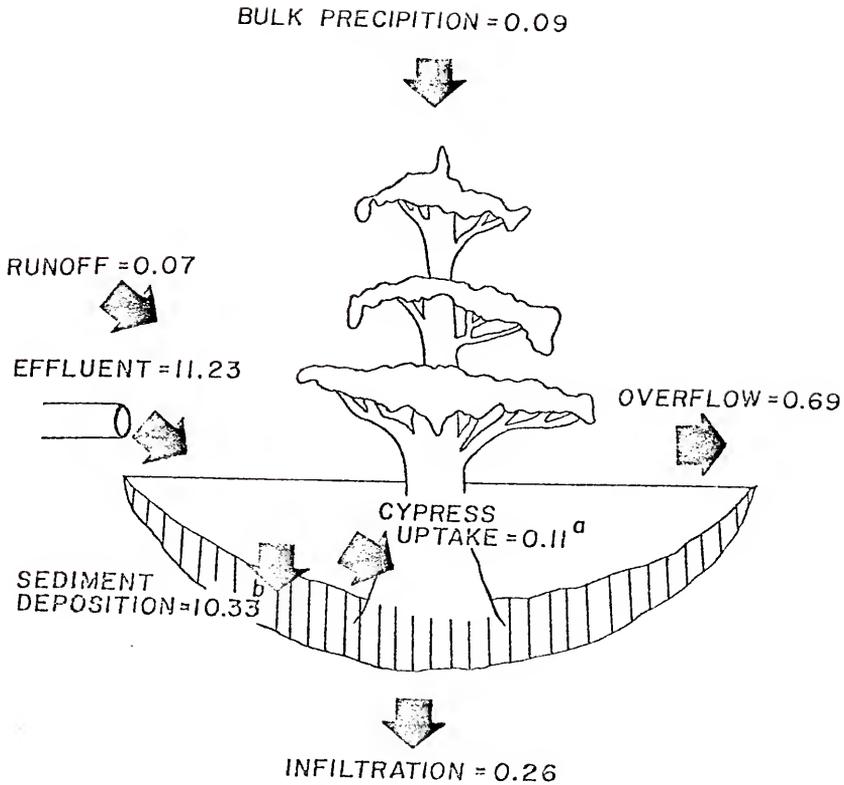


Figure 7-1. Nitrogen mass balance for sewage dome 2. All numbers are in $\text{g}/\text{m}^2\text{-yr}$ and are the averages of a three-year period (1975-1977).



^a Aboveground biomass only

^b Obtained by difference

Figure 7-2. Phosphorus mass balance for sewage dome 2. All numbers are in $\text{g}/\text{m}^2\text{-yr}$ and are the averages of a three-year period (1975-1977).

received 0.051 g P/m²-yr and from 2.19-4.26 g N/m²-yr for the five-year period (1975-1979) (Figures 7-3 and 7-4).

Treated sewage

Nitrogen and phosphorus loadings (13.4 g/m²-yr and 11.2 g/m²-yr, respectively) from the discharge of treated sewage accounted for 90 percent of the total P loading into sewage dome 2. The nearly equal amounts of nitrogen and phosphorus loadings from the sewage effluent resulted from a disproportionately greater removal of nitrogen than phosphorus by the package plant and oxidation pond (Chapter 3). The several relocations of the influent pipe relative to the package plant affected the amount of nitrogen loading from the sewage effluent (Table 7-1).

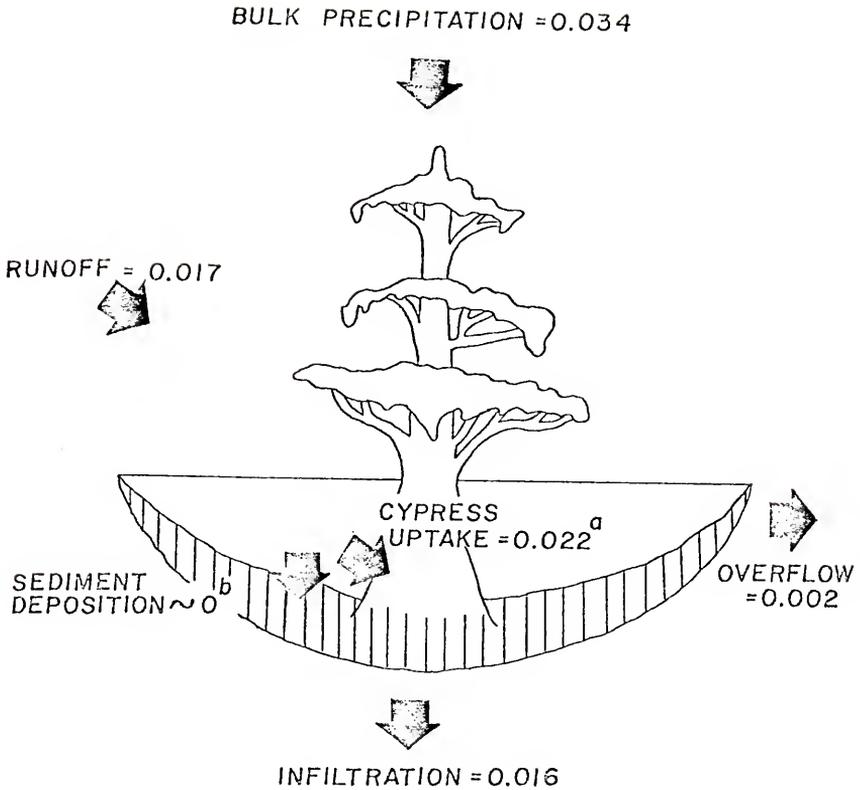
Table 7-1. Annual variations in the nitrate plus nitrite nitrogen and total nitrogen loadings from the sewage effluent piped into sewage dome 2 (April 1974 to March 1977). Nitrogen values are g N/m².

	Year 1 ^a	Year 2 ^b	Year 3 ^c	Total	Average
Nitrate plus Nitrite N	8.67	4.35	1.46	14.5	4.8
Total N	16.83	7.67	15.18	39.7	13.2
Percent	52	57	10		36

^a Effluent taken from within the package treatment plant.

^b Effluent taken from far side of oxidation pond (~10 days detention time).

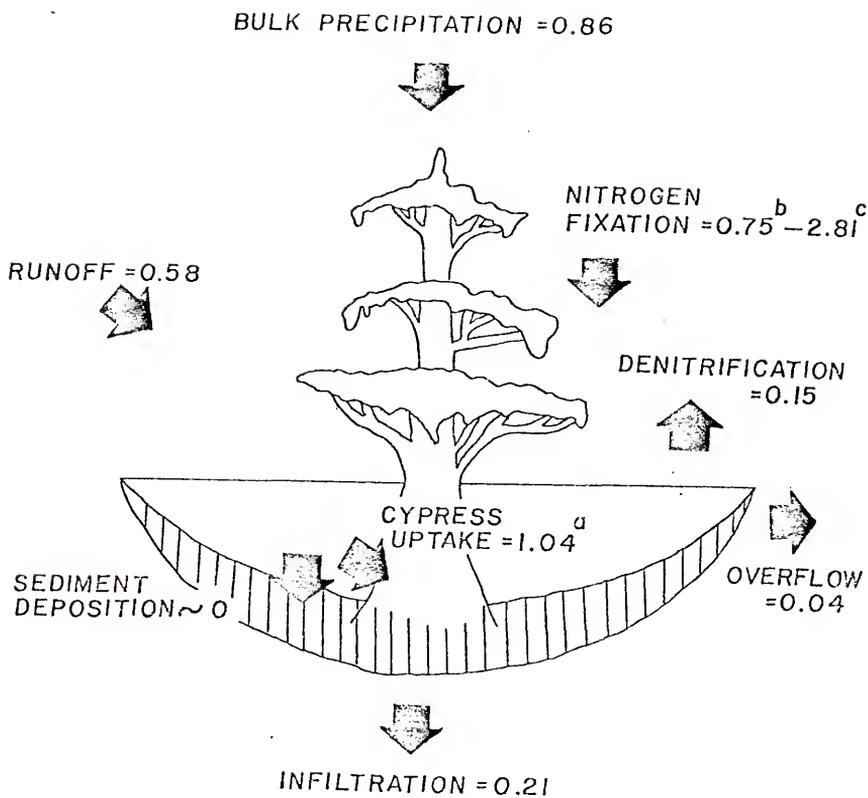
^c Effluent taken from oxidation pond adjacent to package treatment plant discharge.



^a Aboveground biomass only

^b Based on decomposition studies from Nessel (1976)

Figure 7-3. Phosphorus mass balance for Austin Cary natural dome. All numbers are in $\text{g}/\text{m}^2\text{-yr}$ and are the averages of either a four or five-year period (1975-1978 or 1979).



- a Aboveground biomass only
- b Excluding root-associated nitrogenase activity
- c Including root-associated nitrogenase activity (excised root method)

Figure 7-4. Nitrogen mass balance for Austin Cary natural dome. All numbers are in $\text{g}/\text{m}^2\text{-yr}$ and are the averages of either a four or five-year period (1975-1978 or 1979).

Nitrogen fixation

Negligible C_2H_2 reduction was measured in the duckweed populations and the rhizosphere of cypress roots in the sewage-enriched dome. Integrating monthly analyses of nitrogen fixation (C_2H_2 reduction) rates by surface litter during a one-year period yields $0.12 \text{ g N/m}^2\text{-yr}$ (see Chapter 6). The other significant nitrogen-fixing source in the dome was an episodic bloom of Azolla caroliniana that occurred during a 24-day period in 1979 (April 5 to May 1). The rate calculated for that bloom was divided by the number of years since the last reported bloom (2.5 yrs) (Ewel 1976), resulting in a contribution of $0.07 \text{ g N/m}^2\text{-yr}$. Thus, a total flux of $0.19 \text{ g N/m}^2\text{-yr}$ was estimated from N_2 fixation (Figure 7-1), constituting only 1.3 percent of the total nitrogen loading to the dome.

Although low N:P ratios generally favor nitrogen fixing blue-green algae in lakes (e.g., Schindler 1977), the low N:P ratios in the standing water of sewage dome 2 did not favor the growth of nitrogen-fixing organisms, probably because of inhibition by high levels of inorganic nitrogen (Hill et al. 1972; Stewart et al. 1968). Patriquin and Keddy (1978) found an inverse correlation of nitrogenase activity in excised roots of coastal marsh angiosperms with the concentration of ammonium in the groundwater.

Considerably larger amounts of nitrogen were fixed in the natural dome than in sewage dome 2. Besides the higher rates associated with the surface litter ($0.39 \text{ g N/m}^2\text{-yr}$) in the natural dome, the presence of a nodulated plant (Myrica cerifera) may also add approximately $0.34 \text{ g N/m}^2\text{-yr}$ (Silver and Mague 1970), but no direct measurements were made

on this plant. Including the Azolla bloom ($0.02 \text{ g N/m}^2\text{-yr}$) brings the total nitrogen fixed to $0.75 \text{ g/m}^2\text{-yr}$.

As previously discussed in Chapter 6, the relationship between the acetylene reduction activity of excised roots from woody species and in situ rates is uncertain. Consequently, the total annual nitrogen fixed in the natural dome was estimated conservatively without including the rates measured from excised roots (the rate given in the previous paragraph) as well as by including the average rate measured from excised roots after the lag for all species ($2.81 \text{ g/m}^2\text{-yr}$) (Figure 7-4).

The annual nitrogen fixation contribution by tree roots was estimated using an average acetylene reduction rate of 3.5 nM/g (fresh wt) $\cdot \text{h}$ (Figure 6-8), a standing crop of 2050 g (dry wt)/ m^2 in roots of trees with a diameter $< 10 \text{ mm}$ (Lugo et al. 1978), a factor of $4.8 \text{ g fresh wt/g dry wt}$ for roots, and an assumed period of fixation activity of 270 days, corresponding to the length of the growing season for deciduous trees in the domes. The resulting rate of $2.06 \text{ g N/m}^2\text{-yr}$ assumes that fixation is uniform throughout the entire habitat and throughout the period when the tree canopy is present. However, such large scale extrapolation may be misleading, particularly in view of the large variation among replicate samples.

Depending on whether the rates from excised roots are included in the total annual fixed nitrogen, approximately 34 to 66 percent of the total nitrogen entering the dome is accounted for by fixation. Nitrogen fixation plays an important role in meeting the nitrogen needs of the natural dome, which was not found to be the case in sewage dome 2. Figure 7-4 shows that losses plus aboveground storage in biomass of

cypress trees equal the sources of nitrogen, excluding fixation, but it should be pointed out that the belowground biomass storage has not been included. The aboveground net growth of the numerous black gum trees (estimated as $1.09 \text{ g N/m}^2\text{-yr}$) also is not included in Figure 7-4. Thus root-associated nitrogen fixation may play a prominent role in meeting the nitrogen demand of these trees.

Surface runoff

Winter concentrations in surface flows were slightly lower than corresponding values for summer for flows into sewage domes 1 and 2 (Table 7-2). This trend is likely due more to the difference in the time elapsed since previous rainfall for the two measurement periods than to seasonal differences in rainfall composition or vegetation. The summer runoff measurements were made during a period preceded by two weeks of dry weather, whereas the winter runoff measurements were made when another rain event had occurred only two days previously. Total nitrogen concentrations varied little among the stations for all domes; the mean levels for sewage dome 1, sewage dome 2, and the natural dome were 0.92, 0.88, and 1.03 mg N/L, respectively. Total P concentrations for stations adjacent to unpaved roads (S1R1 and S2R1) were both 0.43 mg P/L, while the other three sites had mean levels from 0.10 to 0.17. The trend was found for both summer and winter runoff. Total P levels at the Austin Cary stations were lower ($\bar{X} = 0.03 \text{ mg/L}$) than those recorded for the two sewage domes, but may reflect only a smaller sample size. Nitrate plus nitrite concentrations were unaffected by station location near sewage dome 1 ($\bar{X} = 0.09 \text{ mg N/L}$), whereas station S2R1 (near the unpaved road near sewage dome 2) showed more of an increase ($\bar{X} = 0.10 \text{ mg N/L}$) than the two other

Table 7-2. Total nitrogen, total phosphorus, and nitrate plus nitrite levels in the surface runoff entering sewage dome 1 (S1 stations), sewage dome 2 (S2 stations), and Austin Cary natural dome (AC stations). Number within parenthesis denotes the number of individual samples whose mean is reported.

Station	8/2/76	8/3/76	8/4/76	8/8/76	8/13/76	12/16/77
<u>Total N</u>						
S1R1	1.02(3)	0.71(2)	0.76(1)	0.86(4)	1.16(3)	
S1R2	1.58(2)	1.17(2)	0.74(1)	1.23(2)	1.18(1)	
S1R3	0.85(1)					
S2R1	0.94(4)	1.12(2)	0.80(1)	0.89(4)	0.92(3)	1.43(1)
S2R2	1.08(2)	0.81(2)	1.07(1)	0.72(2)	1.05(1)	0.62(1)
S2R3	1.30(2)	0.85(2)	0.72(1)		1.05(1)	0.88(1)
ACR1						
ACR2						
ACR5						
<u>Total P</u>						
S1R1	0.49(3)	0.36(2)	0.58(1)	0.38(4)	0.64(3)	
S1R2	0.18(2)	0.18(2)	0.08(1)	0.36(2)	0.08(1)	
S1R3	0.07(1)					
S2R1	0.71(4)	0.54(2)	0.64(1)	0.41(4)	0.33(3)	0.29(1)
S2R2	0.16(2)	0.18(2)	0.12(1)	0.12(3)	0.15(1)	0.05(1)
S2R3	0.08(2)	0.16(2)	0.18(1)		0.15(1)	0.04(1)
ACR1						
ACR2						
ACR5						
<u>Nitrate Plus Nitrite Nitrogen</u>						
S1R1	0.12(3)	0.04(2)	0.04(1)	0.15(4)	0.14(3)	
S1R2	0.10(2)	0.08(2)	0.03(1)	0.14(2)	0.18(1)	
S1R3	0.10(1)					
S2R1	0.04(4)	0.06(2)	0.13(1)	0.12(4)	0.17(3)	0.43(1)
S2R2	0.05(2)	0.04(2)	0.02(1)	0.07(3)	0.08(1)	0.01(1)
S2R3	0.02(2)	0.08(2)	0.02(1)		0.05(1)	0.02(1)
ACR1						
ACR2						
ACR5						

Table 7-2. - extended.

12/18/77	12/25/77	1/8/78	1/20/78	Station Mean	N	Dome Mean
0.79(1)	0.16(1)	0.36(1)	0.51(1)	0.82	17	0.92
0.93(1)	0.81(1)	0.71(1)	0.57(1)	1.08	12	
					1	
0.88(1)	0.16(1)		0.78(1)	0.91	18	0.88
0.93(1)	0.21(1)		0.68(1)	0.82	12	
0.79(1)	0.56(1)		0.76(1)	0.91	10	
	1.17(1)		1.03(1)	1.10	2	1.03
	0.94(1)		1.04(1)	0.99	2	
			0.95(1)		1	
0.36(1)	0.34(1)	0.24(1)	0.13(1)	0.43	17	0.31
0.14(1)	0.13(1)	0.09(1)	0.06(1)	0.17	12	
					1	
0.09(1)	0.23(1)		0.04(1)	0.43	18	0.26
0.37(1)	0.04(1)		0.05(1)	0.14	13	
0.03(1)	0.06(1)		0.03(1)	0.10	10	
	0.03(1)		0.01(1)	0.02	2	0.03
	0.10(1)		0.01(1)	0.05	2	
			0.02(1)		1	
0.01(1)	0.01(1)	0.01(1)	0.06(1)	0.09	17	0.09
0.01(1)	0.01(1)	0.01(1)	0.02(1)	0.08	12	
					1	
0.01(1)	0.01(1)		0.03(1)	0.10	18	0.06
0.01(1)	0.01(1)		0.03(1)	0.04	13	
0.01(1)	0.01(1)		0.03(1)	0.03	10	
	0.02(1)		0.03(1)	0.02	2	0.03
	0.04(1)		0.03(1)	0.03	2	
			0.05(1)		1	

stations receiving runoff from adjacent land ($\bar{x} = 0.04$ mg N/L). The Austin Cary stations (not influenced by roads) also had low levels of nitrate plus nitrite (0.03 mg N/L) associated with them.

The contribution of total P from surface runoff during the three-year study period was 0.071 g P/m²-yr for sewage dome 2 (Figure 7-2), which amounts to less than 1 percent of the total P entering sewage dome 2. On the other hand, while only 0.017 g P/m²-yr entered Austin Cary dome during the five-year period (Figure 7-3), this source comprised 33 percent of the total P loading. Surface runoff contributed 0.24 and 0.58 g/m²-yr of total N to sewage dome 2 and Austin Cary, respectively (Figures 7-1 and 7-4), equivalent to only 1.6 percent of the total N input into sewage dome 2 and from 14 to 26 percent of the total N loading into the natural dome, depending on whether the acetylene reduction activity of excised roots is included.

Bulk precipitation

Nitrogen and phosphorus loadings for sewage dome 2 from atmospheric sources averaged 1.05 g/m²-yr and 0.09 g/m²-yr, respectively. These are higher than the averages of 0.86 g N/m²-yr and 0.034 g P/m²-yr measured in the natural dome at Austin Cary forest, which made up 20-39 percent and 67 percent, respectively, of the total annual loadings. However, the measured precipitation flux of total P into sewage dome 2 accounted for only 0.8 percent of the total inputs, while atmospheric loading accounted for 7 percent of the total N loading.

Sinks

Infiltration

Annual losses of total nitrogen and phosphorus by infiltration were 1.24 g/m² and 0.26 g/m², respectively, for sewage dome 2, and

0.21 and 0.016 g/m², respectively, for the natural dome (Figures 7-1 through 7-4). The disparity in the infiltration losses between the domes is explained by both higher infiltration rates and higher concentrations of total N and total P in the wells surrounding sewage dome 2. Total nitrogen and phosphorus losses were highest for both domes during 1976 when higher infiltration rates and slightly higher nitrogen concentrations were recorded in the wells surrounding sewage dome 2. The lowest infiltration losses for nitrogen and phosphorus were in 1977 for sewage dome 2 because of an extended dry period that resulted in the absence of standing water for more than one month (Figure 7-5). On the other hand, a prolonged wet period in 1975 caused the groundwater surface gradient sloping away from the natural dome to remain high and thereby retard infiltration (Figure 7-6) (Heimburg 1976).

Infiltration loss accounted for 8 percent of the total nitrogen input to the sewage dome, but only 2.3 percent of the total phosphorus input. For the natural dome, from 5 to 10 percent of the total nitrogen and 31 percent of the total phosphorus supplied to this dome was lost by infiltration.

Denitrification

The total nitrate plus nitrite entering sewage dome 2 and the natural dome averaged 2.79 and 0.285 g N/m²-yr, respectively (Table 7-3). Nitrate plus nitrite associated with the sewage influent comprised 92 percent of the total input of oxidized nitrogen forms, although yearly variations were considerable due to relocations of the influent pipe within the package plant and oxidation pond several times during the study period (Table 7-1). A value of 2.01 g N/m²-yr

Figure 7-5. Fluctuations in the surface water level of sewage dome 2 during the study period. Sewage loading was initiated in December 1974.

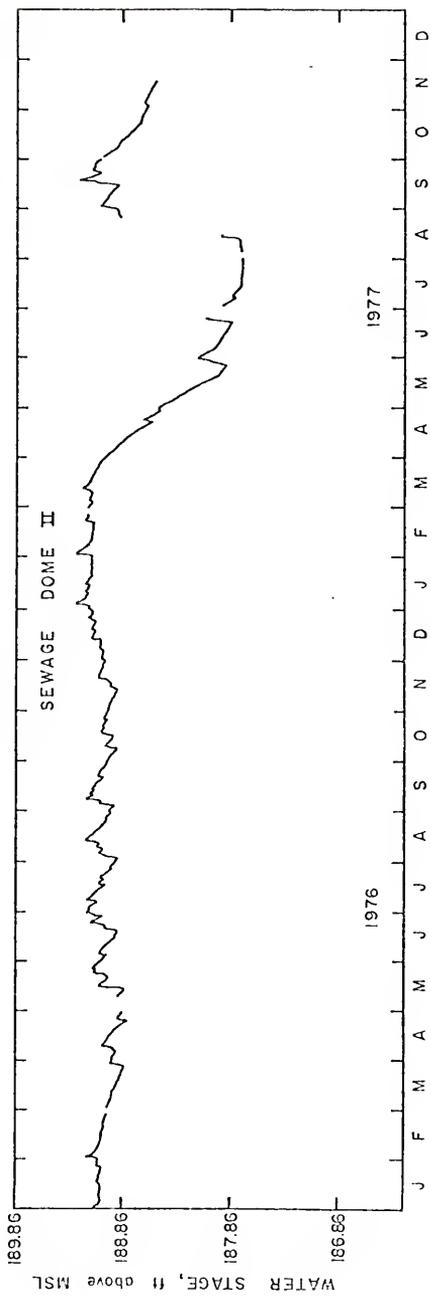
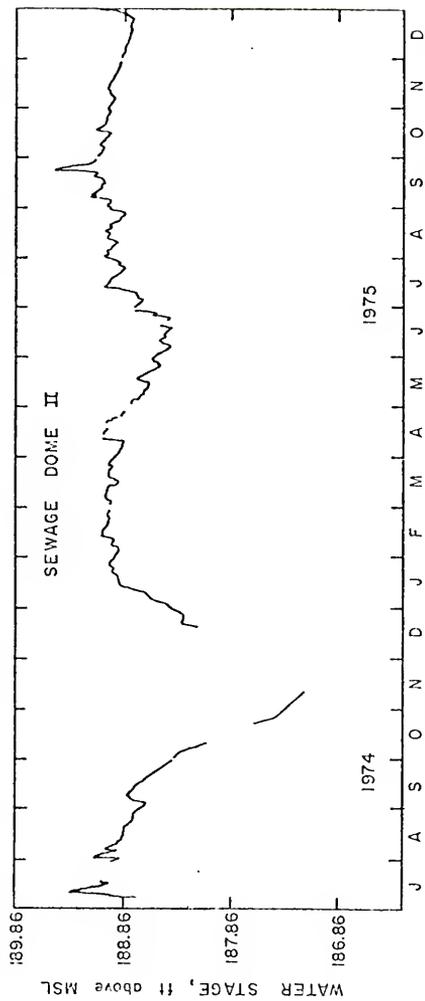


Figure 7-6. Water level fluctuations in the surface waters of the center of Austin Cary natural dome from 1974 to 1979.

Table 7-3. Average nitrate plus nitrite balances ($\text{g N/m}^2\text{-yr}$) for sewage dome 2 during a three-year period and the natural dome at Austin Cary during a four to five-year period.

	Sewage Dome 2	Austin Cary
Sources		
Treated sewage	2.56	---
Runoff	0.016	0.017
Bulk precipitation	0.214	0.268
Nitrification	n.s. ^a	n.s. ^b
Total	2.79	0.285
Sinks		
Infiltration	0.086	0.036
Overflow	0.02	0.001
Denitrification	2.013 ^c	0.151 ^d
Other	0.671 ^e	0.097 ^e
Total	2.79	0.285

^a Not significant because of anoxic surface waters

^b Not significant because of low pH

^c Based on 0.75 x the difference between the sum of all sources and the sum of infiltration plus overflow. Seventy-five percent represents the percentage of the total nitrate denitrified in sediment leaching columns (Chapter 3)

^d Based on 0.61 x the difference between the sum of all sources and the sum of infiltration plus overflow. Sixty-one percent represents the percentage of the total nitrate denitrified in batch experiments (Chapter 6)

^e Includes assimilatory and dissimilatory nitrate reduction to ammonium. Obtained by difference.

was computed for sewage dome 2 as the denitrification rate, representing a 14 percent loss of the total nitrogen flux into the dome. Denitrification losses for the natural dome were only $0.15 \text{ g N/m}^2\text{-yr}$, and represented a 4-7 percent loss in the total annual nitrogen entering the dome.

Even though conditions are near optimum for denitrification in the sewage-amended cypress domes ($\text{pH} > 5.5$, abundant carbon source, constant anaerobic conditions, favorable temperatures), the low levels of nitrate and nitrite pumped to the sewage domes limit the significance of denitrification in the total nitrogen budget. A more favorable design and efficient operation of the treatment plant, increasing the levels of nitrate in the effluent, could greatly enhance the potential use of the domes as nitrogen sinks.

Surface overflow

The average outflow of nitrogen in sewage dome 2 was $0.67 \text{ g/m}^2\text{-yr}$, which amounted to 4.5 percent of the total nitrogen loadings. Losses of total P averaged $0.69 \text{ g/m}^2\text{-yr}$, constituting 6 percent of the total P loadings. Water losses were primarily through a wide, shallow channel located at a break in the berm surrounding the dome on the north side. Surface water leaving the dome by this means flows through a small ditch into the surrounding pine flatwoods and is dissipated before reaching any permanent surface stream. Such losses would be avoided in full-scale use of domes for wastewater (tertiary) treatment by construction of a complete berm.

Surface overflow from the Austin Cary dome was diminished by the smaller edge relative to total area (4.2 ha). Consequently, only $0.04 \text{ g N/m}^2\text{-yr}$ and $0.002 \text{ g P/m}^2\text{-yr}$ were lost via this sink, representing

1-2 percent and 4 percent of the loadings for total N and total P, respectively.

Storages

Cypress aboveground biomass

Both the growth rates (Table 7-4) and nutrient content (Straub and Post 1978) of the aboveground cypress tree components increased as a result of sewage discharge. Storages of N and P in the aboveground cypress biomass of sewage dome 2 were 2.66 and 0.11 g/m²-yr, respectively. Only 1.04 g N/m²-yr and 0.022 g P/m²-yr were stored in the aboveground biomass of the cypress in Austin Cary. These calculations represent an underestimate of the total storage in woody vegetation since storages in roots and black gum trees were not taken into account. However, the number and biomass of black gum trees in sewage dome 2 is considerably less than that of cypress (Ordway 1976). In Austin Cary, black gum trees are a major subdominant tree species. Since diameter increments were not measured for black gum, an estimate of 1.09 g N and 0.026 g P/m²-yr was calculated based on the assumption that increments in each black gum biomass compartment were the same as computed for cypress.

Table 7-4. Increments in the aboveground biomass of cypress over a three-year period (1976-79). Each number is the mean of 100 trees for each cypress dome except Austin Cary, where 200 trees were sampled.

Dome	Foliage g	Branches kg	Stump & Bole kg	Total Aboveground kg
Sewage Dome 1	146.0	1.18	13.76	15.11
Sewage Dome 2	152.1	1.07	13.06	14.32
Groundwater Control Dome	208.3	1.75	20.13	22.12
Austin Cary Natural Dome	84.5	0.84	9.09	10.02

The annual rate of P storage in the aboveground cypress biomass was only 1 percent of the amount remaining in sewage dome 2 after losses were subtracted from all sources. On the other hand, 24 percent of the nitrogen remaining in the dome was stored in aboveground cypress. The disproportionate percent of N storage relative to P can be explained by the nearly equal inputs (by weight) of both elements into sewage dome 2, the additional loss of 14 percent of the total nitrogen influx from denitrification, and the larger N requirement by cypress (N:P ratios by weight are 8:1 for foliage and branches, and 58:1 for the stumps and boles).

The annual rate of P storage in the aboveground cypress biomass was 67 percent of the amount remaining in Austin Cary after all losses were subtracted from the inputs. Nitrogen storage in the aboveground cypress biomass ranged from 27-58 percent (depending on whether root-associated nitrogen fixation is included) of the total nitrogen remaining. Including the estimates for black gum aboveground biomass storage accounted for 145 percent of total P and 55-119 percent of the total N remaining in the dome, indicating that growth and standing crop of black gum are probably overestimated. Apparently most of the nitrogen and phosphorus remaining in Austin Cary dome is being used in net production of the woody biomass.

Sediments

By difference I calculated that 8.3 g N/m²-yr and 10.3 g P/m²-yr were deposited in the sediments in sewage dome 2. As previously discussed, this is probably an overestimate since the amounts stored in roots and black gum trees were not calculated. However, the error associated with phosphorus would be minor since only a small amount of

phosphorus probably was stored in the added biomass of cypress. When the average infiltration rate was multiplied by the concentration of total P in the standing water, a total loss of 7.8 g P/m²-yr was calculated. Little of the phosphorus penetrates the organic and inorganic sediment of the dome; calculated deep infiltration losses amounted to less than 0.3 g P/m²-yr. Hence, the difference (7.5 g/m²-yr) represents loss to sediment. This deposition rate is about 25 percent lower than the loss calculated by mass balance (i.e., by difference). The value calculated from infiltration rate times surface water concentration probably underestimates the rate of loss to sediments since it doesn't include deposition of phosphorus in leaves, duckweed, etc.

The underlying peat and clays in sewage dome 2 apparently retain the phosphorus and nitrogen loaded with the sewage effluent. The nitrogen and phosphorus lost with leaf abscission during the fall is also retained in the decomposing litter within the dome system (Nessel 1978; Chapter 6). Consequently, nutrient losses from overflow or infiltration are minimal.

An attempt was made to directly measure the amount of nitrogen stored in the peat of both sewage domes. Appendix 2 lists the specific density and nitrogen content for each 5 cm core depth from the four study sites. Since an inventory of the standing stock of nitrogen in the peat was not made prior to sewage discharge, comparisons based on the amount of nitrogen found after three years of sewage discharge into sewage dome 1 were made with both control domes (groundwater control dome and Austin Cary). Figures 7-7 and 7-8 show the location of the sampling sites and the areal distribution of sediment nitrogen for sewage dome 1, groundwater control dome, and Austin Cary dome. Although

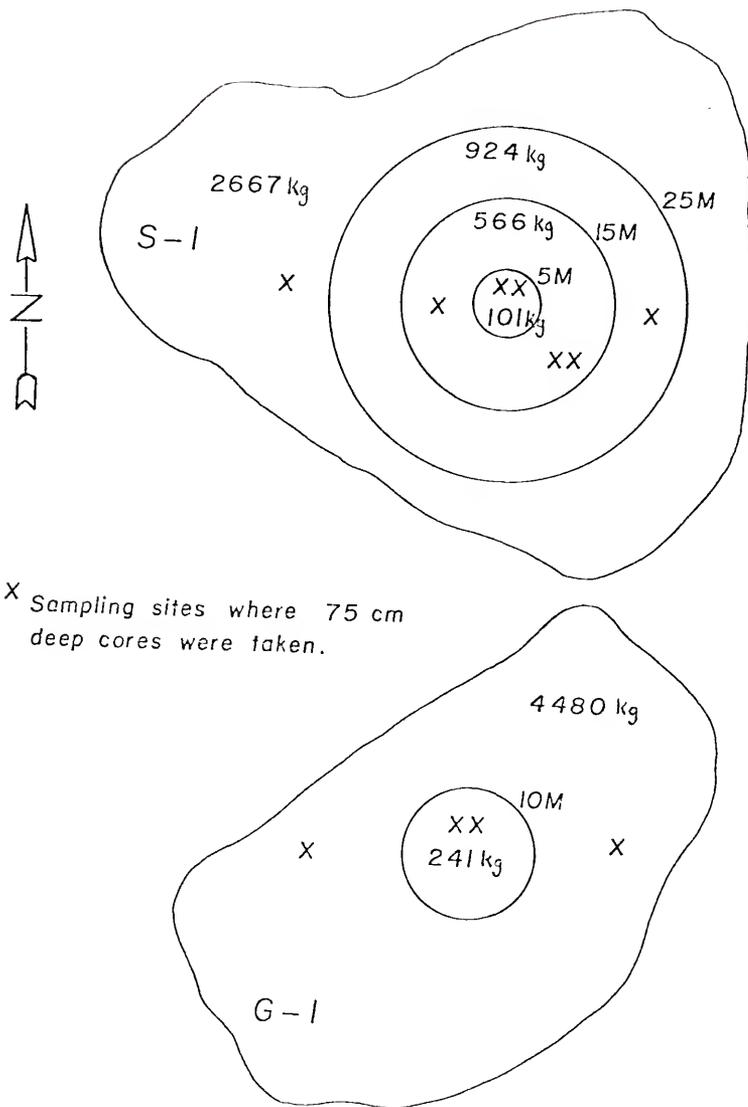
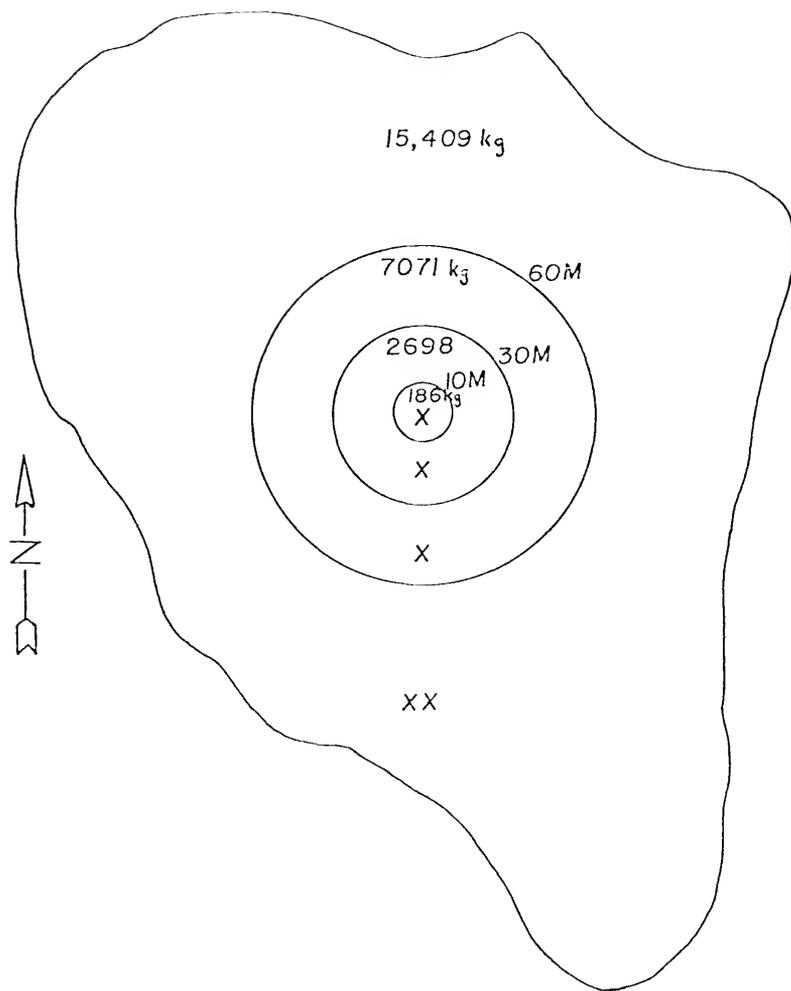


Figure 7-7. Areal distribution of sediment nitrogen measured in sewage dome 1 (S-1) and groundwater control dome (G-1). The average nitrogen storage in the sediments was 852 g/m² and 674 g/m² for sewage dome 1 and groundwater control dome, respectively.



X Sampling sites where 75 cm deep cores were taken.

Figure 7-8. Areal distribution of sediment nitrogen measured in Austin Cary natural dome. The average nitrogen storage in the sediments was 604 g/m^2 .

both control domes had comparable amounts of nitrogen in their upper 75 cm of peat (604 g N/m² in Austin Cary and 674 g N/m² in the ground-water control dome) and were considerably lower than the measured nitrogen values for sewage dome 1 (852 g N/m²), the elevated nitrogen levels in sewage dome 1 were not due entirely to sewage since only 42.6 g N/m² of sewage nitrogen were applied during the three-year period prior to coring.

Cypress Domes As Nutrient Traps

Nitrogen and Phosphorus Removal Efficiencies in Sewage Dome 2

The small losses of nitrogen and phosphorus found for the mass balances on the cypress dome system (Figures 7-1 through 7-4) indicate that cypress domes serve as efficient nutrient traps and hence as effective natural tertiary treatment systems for sewage effluent. Sewage dome 2 retained 87 percent of the total N and 92 percent of the total P entering the dome; even higher removal percentages would have occurred (~92 percent for nitrogen and 98 percent for phosphorus) if the dome had not had a surface overflow outlet. The removal efficiencies reported here are among the highest reported for any wetland receiving treated sewage effluent.

Even though the nitrogen and phosphorus removals found by both field and lab investigations in this study are high, any comparison to the removal efficiencies among different wetland types may be specious because of differences in methodology, accuracy of hydrological budget, analytical techniques, species measured, size of wetland, season(s) when samples were collected, and levels of influent nitrogen and phosphorus. Without standardized mass balance studies, it is not possible to: 1) determine whether wetlands accumulate, lose, or are in balance

with regard to nutrients; 2) compare the nutrient removal efficiencies of different types of wetlands; and 3) evaluate the effectiveness of management decisions.

Mechanisms Whereby Nitrogen and Phosphorus Losses are Minimized in a Natural Dome

The high nitrogen and phosphorus removal efficiencies found for the sewage domes may be an evolutionary strategy of natural cypress domes to minimize losses of the low nitrogen and phosphorus inputs in order to maintain the observed net production of woody biomass. Nutrient conserving mechanisms include slow mineralization of organic nitrogen and phosphorus in leaf litter, sorption of phosphorus to mineral and organic substrates, inhibition of nitrification, development of a possible diazotrophic association within the "endorhizosphere", and lack of significant losses from overflow.

CHAPTER EIGHT SUMMARY AND CONCLUSIONS

Water quality, nutrient cycling, mass balance, and organic matter decomposition aspects of secondary-treated sewage disposal into cypress domes were studied in an effort to determine the efficacy of using cypress domes as sewage treatment systems. In order to compare the effects of secondary-treated sewage effluent, a major portion of this work was focused on the aqueous chemical composition, nutrient cycles and mass balances, and organic matter decomposition occurring in a natural cypress dome.

Compared to the influent concentrations, only slight (<33 percent) reductions occurred in the surface water concentrations of nitrogen, phosphorus, BOD, sodium, potassium, chloride, and fluoride in the domes receiving treated sewage effluent. Larger reductions (~50 percent) occurred for calcium, magnesium, and sulfate. The conventional treatment plant/oxidation pond itself was effective in reducing the levels of BOD and total nitrogen, but was ineffective in reducing the levels of phosphorus and other minerals.

The potential of cypress domes to remove a high percentage (>90 percent) of the organic matter, nutrients and minerals was more obvious when the concentrations of these substances in the shallow groundwater (water table aquifer) below the sewage-enriched domes were examined. Concentrations of organic matter, nutrients, and minerals in shallow wells in and around the sewage domes were at background levels

throughout the study. High chloride values (compared to the control wells) indicated that treated sewage was percolating into the shallow aquifer.

Laboratory column leaching studies and field data on soil tubes corroborated the removal efficiencies for nitrate and phosphorus indicated by the monitoring data for wells within and surrounding both sewage domes. Nitrogen fixation may have accounted for the increase in nitrogen mass in the six laboratory leaching columns.

Addition of secondary sewage effluent to cypress domes changed the colored, oxygenated, acidic, soft water of natural domes to a more neutral, anoxic surface water containing higher dissolved solids and hydrogen sulfide. Both inorganic nitrogen and inorganic phosphorus levels were increased by two to three orders of magnitude in the domes receiving the treated effluent.

Sediments and vegetation continued to release inorganic ions to the standing water for more than 20 months after the cessation of sewage pumping. Hydrologic factors can ameliorate the buildup of inorganic ions in the surface waters by replacing the sewage-affected standing water with natural water derived from throughfall and runoff. On the other hand, parameters associated with sewage organic matter (BOD) and with the reduced environment (H_2S , NH_4^+) of the sewage dome water displayed a fast return to background levels.

Cypress leaf litter decomposed faster in the anaerobic sewage dome than in the aerobic natural dome. Since the observed decomposition rates were not a logarithmic function with time, a model dividing the observed decomposition rate into two or more separate rates incorporating labile and refractory components whereby each follows a logarithmic

decay rate over only a portion of the total decomposition time was employed. These separate rates can then be added together to yield the true total decomposition curve.

Simulation of the model indicated that the faster decomposition rates in the sewage dome counteracts the increased litter productivity from duckweed such that the volume of the dome basin available for storage and treating sewage will be undiminished.

Considerable alteration in the nitrogen cycle of natural cypress domes occurs when they are used for treated sewage disposal. Both the rates and the causal environmental factors are significantly changed in the major nitrogen transformation processes. A summary of the relative significance and underlying causal factors for each transformation in both the natural and sewage domes is presented in Table 8-1. Nitrogen is conserved in both domes by the inhibition of nitrification (for a different reason in each dome) and low rates of mineralization.

Despite the low pH of peat and standing water in the natural dome, significant denitrification did occur. Neither the total amount nor the rate of the N_2O produced was affected by the presence of an available carbon source or by buffering the ambient pH to a more neutral value. This suggests that the denitrifying flora in natural swamps are acclimated to the low pH present in their peat and standing waters. Field data and laboratory experiments indicate that cypress domes have the capacity to denitrify a highly nitrified effluent containing up to 30 mg NO_3^- -N/L. Because of the near neutral pH, the mole fraction of $N_2O:N_2$ for the products of denitrification is 0.17. Although acetylene blockage as an indirect assay for denitrification has

Table 8-1. Summary of the nitrogen transformations in natural and sewage-enriched cypress domes.

Transformation	Austin Cary natural dome	Sewage dome 2
Ammonification	Slight loss in dry wt. counteracted by nitrogen fixation and absorption of aqueous inorganic material.	Large loss in dry wt. counteracted by absorption of aqueous inorganic nitrogen.
Nitrification	Not significant because of low pH.	Not significant because of low dissolved oxygen.
Nitrogen Fixation	Significant: Heterotrophic free-living bacteria; symbiotic actinomycetes in non-leguminous nodulated <u>Myrica</u> sp.; possible root associated endosymbionts in <u>Taxodium</u> and <u>Nyssa</u> .	Not significant because of the repression of nitrogenase synthesis by high levels of nitrogen.
Denitrification	Significant because of low D.O. and sufficient organic matter; low pH and added energy source does not affect rate or total amount dissimilated.	Significant because of low D.O., sufficient organic matter, and favorable pH. Rate faster than in natural swamp.

shown considerable promise in the literature, its use should not be regarded as applicable in all environments.

Negligible nitrification rates measured in the natural dome were due to the low pH, and not to any toxic effect originating from the dissolved organic matter.

Nitrogen fixation may be an important source of nitrogen in the natural dome, which may be nitrogen-limited. Fourteen percent of the total nitrogen increase observed in decomposing litterfall came from this source. Although the data indicated that most sites within a

natural cypress dome are not likely to fix significant amounts of nitrogen, the finding that roots of the dominant woody vegetation of cypress swamps are capable of fixation requires more work to be done to determine their ecological significance. Although all the appropriate controls have been run, the rates still need to be verified with ^{15}N , and the site of fixation (in or on the roots), as well as the agent(s) of fixation, needs to be determined.

Total nitrogen and phosphorus loadings to sewage dome 2 were 14.9 g/m²-yr for total nitrogen and 11.4 g/m²-yr for total phosphorus. Sources other than sewage accounted for 11 percent of the total nitrogen and 1-2 percent of the total phosphorus loading for sewage dome 2 during the period December 1974 to December 1977. This reflects the low N:P ratio found in the treated sewage influent. In Austin Cary dome, loading rates of 0.051 g P/m²-yr and 2.19-4.26 g N/m²-yr were measured.

Although runoff contributions of total P and total N to sewage dome 2 were negligible (<2 percent) compared to the sewage inputs, runoff was a significant source of phosphorus (33 percent) and nitrogen (14-26 percent) to the natural dome. Runoff from unpaved roads was three times more concentrated in total phosphorus than runoff from adjacent land. Total nitrogen and nitrate-nitrite levels were unaffected by unpaved roads.

Nitrogen and phosphorus loadings from bulk precipitation were a significant portion of the total annual inputs in the natural dome (67 percent for phosphorus and 20-39 percent for nitrogen), whereas the contributions to the sewage dome from this source were insignificant (<1 percent for phosphorus and 7 percent for nitrogen).

Significant sources of nitrogenase activity in the natural dome are associated with decomposing leaf litter, Myrica cerifera, and probably non-nodulated woody roots. Nitrogen fixation was estimated to contribute between 34 and 66 percent of the total nitrogen input to Austin Cary dome, but was an insignificant nitrogen source (<2 percent of the total) in sewage dome 2. Low levels of nitrogenase activity in sewage dome 2 were probably the result of the inhibitory effects that the high concentrations of combined nitrogen had on nitrogenase synthesis. The results of a nitrogen mass balance for Austin Cary indicate atmospheric deposition is an important source of nitrogen and that without fixation, the measured growth rates of woody biomass could not be supported. Potential nitrogenase activity measured from excised roots of woody species may indicate a significant source.

Because of higher total phosphorus and nitrogen levels in the wells and higher infiltration rates, sewage dome 2 lost 6 times more total nitrogen and 16 times more total phosphorus by infiltration than did the Austin Cary dome. However, because of the influence of sewage loading, infiltration losses in sewage dome 2 were only 8 and 2 percent of the annual loadings for total nitrogen and total phosphorus, respectively. Higher percentages were recorded for Austin Cary, where 5-10 percent of the total nitrogen and 31 percent of the total P entering this dome was lost by this sink.

Denitrification losses were more prominent in sewage dome 2 than Austin Cary in both absolute amounts (2.01 vs 0.15 g N/m²-yr) and percentages of the total nitrogen loaded (14 percent for sewage dome 2 and 4-7 percent for Austin Cary) because of the higher nitrate-nitrite loadings in the effluent (accounting for 92 percent of the total

nitrate-nitrite influx). Both domes, however, are capable of denitrifying most (75 percent for sewage dome 2 and 61 percent for Austin Cary) of the nitrate-nitrite entering into them. Losses from surface overflow were minor in both domes: 1-6 percent of the annual total P and total N entering into them.

Although larger aboveground cypress uptake rates for nitrogen and phosphorus were calculated for sewage dome 2 than for the Austin Cary dome, the Austin Cary rates comprised 67 percent and 27-58 percent of the remaining phosphorus and nitrogen, respectively. When the major sub-dominant, black gum, was included, the phosphorus retention was 145 percent and nitrogen retention was 55-119 percent of that remaining, suggesting that the crude approximation method employed in calculating black gum net productivity resulted in overestimating the amounts of nitrogen and phosphorus stored.

Whereas nitrogen uptake by cypress in sewage dome 2 resulted in a significant aboveground storage (24 percent of the amount remaining in the dome), only 1 percent of the remaining phosphorus was stored in the aboveground cypress biomass. The majority of the nitrogen and phosphorus entering the dome is stored in the sediments: 10.3 g P/m²-yr (99 percent of the phosphorus not leaving the dome) and 8.3 g N/m²-yr (76 percent of the nitrogen not leaving the dome).

The most important factor in supplying new minerals and nutrients (except for nitrogen) to Austin Cary natural cypress dome is bulk precipitation from the atmosphere. Precipitation is also the agent responsible for the leaching of organic matter, iron, aluminum, and silica. Levels of minerals and nutrients in bulk precipitation are greatly increased upon passage through the canopy layer. The levels of most

chemical parameters were higher in surface water than in throughfall; sulfate, potassium, phosphate, ammonium and nitrate plus nitrite had lower levels in dome water. These elements may collectively limit the productivity in these domes. Anthropogenic sources may be significant for phosphate, sulfate, nitrogen, potassium, and calcium levels in the bulk precipitation. Surface water chemistry and the piezometric surface of the groundwater indicate that transport of minerals and nutrients from the mineral-rich deeper groundwaters to the surface waters does not occur. The influence of clay minerals is small because of the highly weathered conditions and low clay content in the soil.

The soft, acidic, low-ionic-strength surface waters contain approximately three times as much dissolved organic matter as dissolved inorganic matter. Dissolved organic matter is important with respect to iron and aluminum solubility and controls the acidic pH of these waters. Orthophosphate, ammonium, and organic N concentrations were correlated with the depth of the standing water.

Dissolved oxygen was always undersaturated in the surface waters. Although respiration undoubtedly contributes to the oxygen deficit, oxygen consumption by a ferrous-ferric catalytic cycle may be important in these humic-colored waters.

Although significant changes in the nitrogen and phosphorus cycles occur in cypress domes receiving secondary-treated sewage effluent, efficient removal of these elements remains unaltered. Furthermore, organic matter will not accumulate at a faster rate on the floors of cypress domes receiving treated effluent than on the floors of natural domes. Cypress domes reduce the pollutant levels of major water quality parameters to levels comparable to those of more conventional

tertiary treatment processes. Sewage disposal into cypress domes will neither affect their long-term capacity to treat secondary effluent nor contaminate the surrounding environment with chemical pollutants.

APPENDIX 1
 SUPPLEMENTARY INFORMATION ON THE
 ORGANIC MATTER ACCUMULATION SIMULATION MODEL

Table A-1. Documentation of storages and flows used in the organic matter accumulation model for natural and sewage-enriched cypress domes (Figure 5-4).

Notation	Description	Calculation and Assumptions	Reference
J_1	Yearly amount of duckweed undergoing fast decomposition in sewage dome 2	$J_1 = \text{Total duckweed deposition multiplied by percentage lost during initial fast decomposition period}$ $= (303\text{g}/\text{m}^2\text{-yr})(.8) = 242\text{g}/\text{m}^2\text{-yr}.$	Price 1975
k_1	Fast duckweed decomposition rate	$k_1 = \text{Turnover rate}$ $= \frac{365 \text{ days/yr}}{40 \text{ days}} = 9.0/\text{yr}.$ Forty days is the estimated time required for the disappearance of the rapidly decomposing duckweed component.	Price 1975
Q_1	Rapidly decomposing duckweed organic matter present in the sediments of sewage dome 2	$Q_1 = 0$ as initial condition	
J_2	Yearly amount of duckweed undergoing slow decomposition in sewage dome 2	$J_2 = \text{Total duckweed deposition multiplied by percentage lost during slow decomposition period}$ $= (202\text{g}/\text{m}^2\text{-yr})(.2) = 61\text{g}/\text{m}^2\text{-yr}.$	Price 1975
k_2	Slow duckweed decomposition rate	$k_2 = 0.02/\text{yr}.$ Assumed slow decomposition rate comparable to that found for algae under anaerobic conditions.	Foree and McCarty 1970

Table A-1. - Continued.

Notation	Description	Calculation and Assumptions	Reference
Q_2	Slowly decomposing duckweed organic matter present in the sediments of sewage dome 2	$Q_2=0$ as initial condition	
J_3	Yearly amount of cypress, hardwood and understory leaves undergoing fast decomposition in sewage dome 2	J_3 =Total cypress, hardwood, and understory leaf litter production multiplied by percentage lost during initial fast decomposition period for cypress only $= (591\text{g}/\text{m}^2\text{-yr})(.285) = 168\text{g}/\text{m}^2\text{-yr}$. Assumed percentage lost from hardwood and understory leaf fast decomposition equalled that of cypress.	Deghi 1977; This study
k_3	Fast cypress, hardwood, and understory leaf decomposition rate for sewage dome 2	k_3 =Yearly loss of the rapidly decomposing component of cypress leaves = $9.74/\text{yr}$. Assumed fast decomposition rate of hardwood and understory leaves equalled that of cypress.	This study
Q_3	Rapidly decomposing cypress, hardwood, and understory leaves present in the sediments of sewage dome 2	$Q_3=0$ as initial condition	
J_4	Yearly amount of cypress, hardwood, and understory leaves undergoing slow decomposition in sewage dome 2	J_4 =Total cypress, hardwood, and understory leaf litter production multiplied by percentage lost during slow decomposition period for cypress only $= (591\text{g}/\text{m}^2\text{-yr})(.715) = 423\text{g}/\text{m}^2\text{-yr}$.	Deghi 1977; This study

Table A-1. - Continued.

Notation	Description	Calculation and Assumptions	Reference
k_4	Slow cypress, hardwood, and understory leaf decomposition rate for sewage dome 2	<p>Assumed percentage lost from hardwood and understory leaf slow decomposition equalled that of cypress.</p> <p>k_4=First year's loss of the slowly decomposing component of cypress leaves = 0.32/yr. Assumed slow decomposing rate of hardwood and understory leaves equalled that of cypress. Assumed the rate found for the first yr (0.32 yr⁻¹) was valid for succeeding yrs.</p>	This study
Q_4	Slowly decomposing cypress, hardwood, and understory leaves present in the sediments of sewage dome 2	<p>Q_4=20.3 kg OM/m² as the initial condition. Assumed standing stock of organic matter found for Austin Cary was the same for sewage dome 2 before effluent discharge.</p>	This study
J_5	Yearly amount of cypress, hardwood, and understory leaves undergoing fast decomposition in the Austin Cary natural dome	<p>J_5=Total cypress, hardwood and understory leaf litter production multiplied by percentage lost during initial fast decomposition period for cypress only = (387g/m²-yr)(.185)= 71g/m²-yr. Assumed percentage lost from hardwood and understory leaf fast decomposition equalled that of cypress.</p>	Deghi 1977; This study

Table A-1. - Continued.

Notation	Description	Calculation and Assumptions	Reference
K_5	Fast cypress, hardwood, and understory leaf decomposition rate for Austin Cary natural dome	k_5 =Yearly loss of the rapidly decomposing component of cypress leaves = 5.70/yr. Assumed fast decomposition rate of hardwood and understory leaves equalled that of cypress.	This study
Q_5	Rapidly decomposing cypress, hardwood and understory leaf biomass present in the sediments of Austin Cary natural dome	$Q_5=0$ as initial condition	
J_6	Yearly amount of cypress, hardwood and understory leaves undergoing slow decomposition in the Austin Cary natural dome	J_6 =Total cypress, hardwood, and understory leaf litter production multiplied by percentage lost during slow decomposition period only = $(387\text{g}/\text{m}^2\text{-yr})(.815) = 316\text{g}/\text{m}^2\text{-yr}$. Assumed percentage lost from hardwood and understory leaf slow decomposition equalled that of cypress.	Deghi 1977; This study
k_6	Slow cypress, hardwood, and understory leaf decomposition rate for Austin Cary natural dome	k_6 =First year's loss of the slowly decomposing component of cypress leaves = 0.17/yr. Assumed that the rate found for the first yr (.17/yr) was valid for succeeding yrs.	This study
Q_6	Slowly decomposing cypress, hardwood and understory leaves present in the sediments of Austin Cary natural dome	$Q_6=20.3$ kg OM/ m^2 as the initial condition	This study

Table A-2. Scaled differential equations used in the simulation of the organic matter accumulation model on the swamp floors of a natural and sewage-enriched cypress dome.

Duckweed (Two-compartment Decomposition)

$$\left[\frac{Q_1}{20,000} \right] = \frac{J_1}{20,000} - k_1 \left[\frac{Q_1}{20,000} \right] \frac{20,000}{20,000}$$

$$\left[\frac{Q_2}{20,000} \right] = \frac{J_2}{20,000} - k_2 \left[\frac{Q_2}{20,000} \right] \frac{20,000}{20,000}$$

Duckweed (One-Compartment Decomposition)

$$\left[\frac{Q_1}{20,000} \right] = \frac{J_1}{20,000} - k_1 \left[\frac{Q_1}{20,000} \right] \frac{20,000}{20,000}$$

Cypress Leaves (Sewage Dome 2)

$$\left[\frac{Q_3}{20,000} \right] = \frac{J_3}{20,000} - k_3 \left[\frac{Q_3}{20,000} \right] \frac{20,000}{20,000}$$

$$\left[\frac{Q_4}{20,000} \right] = \frac{J_4}{20,000} - k_4 \left[\frac{Q_4}{20,000} \right] \frac{20,000}{20,000}$$

Cypress Leaves (Austin Cary)

$$\left[\frac{Q_5}{20,000} \right] = \frac{J_5}{20,000} - k_5 \left[\frac{Q_5}{20,000} \right] \frac{20,000}{20,000}$$

$$\left[\frac{Q_6}{20,000} \right] = \frac{J_6}{20,000} - k_6 \left[\frac{Q_6}{20,000} \right] \frac{20,000}{20,000}$$

Table A-3. Scaled coefficients (pot settings) for initial simulation of the organic matter accumulation model on the swamp floors of a natural and sewage-enriched cypress dome.

Coefficient	Pot Setting	Coefficient	Pot Setting
$\left[\frac{Q_1}{20,000} \right]$ i.c.	0.001	$\left[\frac{Q_4}{20,000} \right]$ i.c.	1.01
$\left[\frac{J_1}{20,000} \right]$	0.012	$\left[\frac{J_4}{20,000} \right]$	0.021
k_1	9.00	k_4	0.317
$\left[\frac{Q_2}{20,000} \right]$ i.c.	0.001	$\left[\frac{Q_5}{20,000} \right]$ i.c.	0.001
$\left[\frac{J_2}{20,000} \right]$	0.003	$\left[\frac{J_5}{20,000} \right]$	0.004
k_2	0.020	k_5	5.70
$\left[\frac{Q_1}{20,000} \right]^*$ i.c.	0.001	$\left[\frac{Q_6}{20,000} \right]$ i.c.	1.01
$\left[\frac{J_1}{20,000} \right]^*$ i.c.	0.015	$\left[\frac{J_6}{20,000} \right]$	0.016
k_1 *	5.73	k_6	0.174
$\left[\frac{Q_3}{20,000} \right]$ i.c.	0.001		
$\left[\frac{J_3}{20,000} \right]$	0.008		
k_3	9.74		

*One-compartment duckweed decomposition

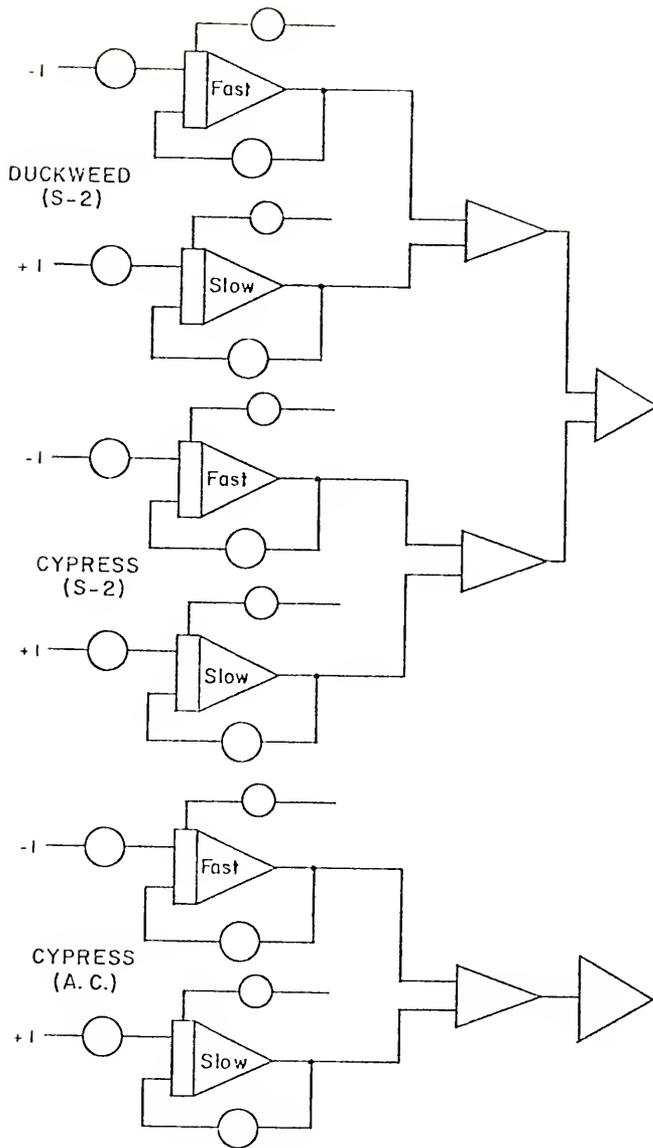


Figure A-1. Analog program for simulating the organic matter accumulation model on the swamp floors of a natural and sewage-enriched cypress dome. (S-2 and A.C. denote sewage dome 2 and Austin Cary natural dome, respectively.)

APPENDIX 2
 NITROGEN CONTENT AND DENSITY OF THE PEAT FROM
 AUSTIN CARY, GROUNDWATER CONTROL, AND SEWAGE DOMES

Site	Station	Depth Interval (cm)	Density (g dry wt/cc)	Concentration (mg N/g dry wt)
Austin Cary	Center	0-5	0.39	8.3
		5-10	0.56	5.0
		10-15	1.16	1.1
		15-20	1.53	0.8
		20-25	1.58	0.6
		25-30	0.63	1.9
		30-35	1.79	0.6
		35-40	1.79	0.1
		40-45	1.75	0.1
		45-50	1.73	0.4
Austin Cary	20 m South of Center	0-5	0.20	23.5
		5-10	0.27	14.9
		10-15	0.44	8.3
		15-20	0.92	2.6
		20-25	1.03	2.4
		25-30	1.36	1.6
Austin Cary	50 m South of Center	0-5	0.32	14.0
		5-10	0.49	8.3
		10-15	0.74	3.5
		15-20	1.22	1.9
		20-25	1.50	1.0
		25-30	1.65	0.4
		30-35	1.63	0.4
		35-42	1.44	0.8
Austin Cary	100 m South of Center	0-5	0.18	17.1
		5-10	0.64	5.0
		10-15	1.11	1.8
		15-20	1.40	0.4
		20-25	1.58	0.0
		25-30	1.61	0.0

Site	Station	Depth Interval (cm)	Density (g dry wt/cc)	Concentration (mg N/g dry wt)
Austin Cary	100 m South of Center (replicate)	0-5	0.09	21.0
		5-10	0.27	17.2
		10-15	1.12	2.2
		15-20	1.45	1.1
		20-25	1.57	0.4
Austin Cary	40 m North Northeast of Center	0-5	0.07	20.9
		5-10	0.19	24.8
		10-15	0.62	9.9
		15-20	1.11	1.6
		20-25	1.23	0.5
Groundwater Control Dome	Center	0-5	0.68	7.0
		5-10	0.78	5.1
		10-15	0.81	1.5
		15-20	1.05	1.5
		20-25	1.43	1.7
		25-30	1.49	0.6
		30-35	1.66	0.4
Groundwater Control Dome	Center (replicate)	0-5	0.36	10.8
		5-10	0.56	6.9
		10-15	0.56	3.8
		15-20	0.56	-
		20-25	0.66	3.3
Groundwater Control Dome	20 m East of Center	0-5	0.20	17.0
		5-10	0.45	6.6
		10-15	0.49	4.9
		15-20	0.88	2.0
		20-25	1.25	1.0
		25-29	1.57	0.7
Groundwater Control Dome	20 m West of Center	0-5	0.19	18.5
		5-10	0.40	8.0
		10-15	0.91	2.2
		15-20	1.02	1.8
		20-25	1.15	1.5
		25-30	1.54	0.5

Site	Station	Depth Interval (cm)	Density (g dry wt/cc)	Concentration (mg N/g dry wt)
Sewage Dome 1	Center	0-5	0.67	7.2
		5-10	0.66	6.2
		10-15	0.77	8.7
		15-20	0.77	9.2
		20-25	0.75	3.9
		25-33	0.59	10.5
Sewage Dome 1	Center (replicate)	0-5	0.76	5.0
		5-10	0.91	3.8
		10-15	0.99	3.1
		15-20	0.84	3.3
		20-25	0.96	2.4
		25-30	1.36	1.7
		30-35	1.56	0.7
		35-40	1.65	0.7
Sewage Dome 1	20 m East of Center	0-5	0.53	7.1
		5-10	0.80	4.7
		10-15	1.19	1.8
		15-20	1.33	1.4
		20-25	1.57	0.7
		25-30	1.60	0.6
		30-35	1.59	0.5
		35-39	1.67	0.2
Sewage Dome 1	10 m West of Center	0-5	0.57	4.4
		5-10	0.89	3.1
		10-15	0.77	2.1
		15-20	0.89	3.5
		20-25	1.07	2.3
		25-30	1.44	0.7
		30-35	1.58	0.6
		35-42	1.64	0.4
Sewage Dome 1	30 m West of Center	0-5	0.34	13.8
		5-10	0.84	7.4
		10-15	1.19	2.7
		15-20	1.48	1.2
		20-25	1.63	0.6
		25-31	1.71	0.4
Sewage Dome 1	10 m Southeast of Center	0-5	0.42	11.9
		5-10	0.66	4.7
		10-15	0.75	3.5
		15-20	0.99	3.3
		20-25	0.99	3.1
		25-31	0.96	2.7

Site	Station	Depth Interval (cm)	Density (g dry wt/cc)	Concentration (mg N/g dry wt)
Sewage Dome 1	10 m Southeast of Center (replicate)	0-5	0.44	8.7
		5-10	0.62	8.3
		10-15	0.68	4.3
		15-20	1.01	1.7
		20-28	0.98	2.5
Sewage Dome 2	Center	0-5	0.14	18.4
		5-10	0.23	17.7
		10-15	0.27	12.5
		15-20	0.25	10.1
Sewage Dome 2	Center (replicate)	0-5	0.14	20.4
		5-10	0.23	21.1
		10-15	0.20	13.5
Sewage Dome 2	Center (replicate)	0-5	0.17	20.0
		5-10	0.21	19.0
		10-15	0.24	14.3
		15-20	0.35	3.4
Sewage Dome 2	Center (replicate)	0-5	0.09	24.3
		5-10	0.12	20.8
		10-15	0.32	21.6
		15-20	0.20	19.3
		20-25	0.40	12.4
		25-30	0.37	4.6
		30-38	0.28	6.8
Sewage Dome 2	10 m South of Center	0-5	--	15.0
		5-10	--	6.5
		10-15	--	3.3
		15-20	--	1.6
		20-25	--	0.6
Sewage Dome 2	20 m South of Center	0-5	--	20.5
		5-10	--	4.9
		10-15	--	18.9
Sewage Dome 2	20 m North of Center	0-5	--	21.2
		5-10	--	22.1
		10-15	--	14.8

Site	Station	Depth Interval (cm)	Density (g dry wt/cc)	Concentration (mg N/g dry wt)
Sewage Dome 2	40 m North of Center	0-5	--	18.1
		5-10	--	18.1
		10-15	--	22.1
		15-20	--	18.4
		20-25	--	13.4
		25-30	--	14.8
Sewage Dome 2	40 m North of Center (replicate)	0-5	--	19.7
		5-10	--	23.5
		10-15	--	3.5
		15-20	--	6.9
Sewage Dome 2	40 m North of Center (replicate)	0-5	--	15.0
		5-10	--	14.3
		10-15	--	4.2
Sewage Dome 2	60 m North of Center	0-5	--	17.5
		5-10	--	8.6
		10-15	--	1.9
		15-20	--	0.8
		20-25	--	0.0
		25-30	--	0.1
		30-39	--	0.1

APPENDIX 3
 FORTRAN IV COMPUTER PROGRAM USED IN THE CALCULATION OF THE
 NET GROWTH OF ABOVEGROUND CYPRESS BIOMASS COMPONENTS

```

0001          DIMENSION DATA(500,2),SUMBIO(4),AVEBIO(4)
0002          READ(5,11)NO
0003          11 FORMAT(12)
0004             KNT=0
0005             2 KOUNT=0
0006                I=1
0007                KNT=KNT+1
0008                1 READ(5,3,END=10)DATA(I,1),DATA(I,2)
0009                3 FORMAT(9X,F7.2,8X,F7.2)
0010                WRITE(6,101) DATA(I,1),DATA(I,2)
0011          101 FORMAT(1X,F7.2,8X,F7.2)
0012             KOUNT=KOUNT+1
0013             I=I+1
0014             GO TO 1
0015          10 DO 5 J=1,4
0016             5 SUMBIO(J)=0
0017             DO 4 I=1,KOUNT
0018                DO 105 J=1,4
0019                   GO TO (20,30,40,50),J
0020          20 A=1.237
0021             B=1.566
0022             GO TO 70
0023          30 A=-2.648
0024             B=2.65
0025             GO TO 70
0026          40 A=-0.90
0027             B=2.258
0028             GO TO 70
0029          50 A=-0.928
0030             B=2.272
0031             GO TO 70
0032          70 X1=ALOG10(DATA(I,1))
0033             X2=ALOG10(DATA(I,2))
0034             Y1=10**(A+B*X1)
0035             Y2=10**(A+B*X2)
0036             BIO=Y2-Y1
0037          105 SUMBIO(J)=SUMBIO(J)+BIO
0038             4 CONTINUE
0039             DO 110 J=1,4
0040          110 AVEBIO(J)=SUMBIO(J)/KOUNT
0041             WRITE(6,100) ((SUMBIO(J),AVEBIO(J)),J=1,4)
0042             IF(KNT.LT.NO) GO TO 2
0043          100 FORMAT(4(1X,E12.2,F12.2))
0044             STOP
0045             END

```

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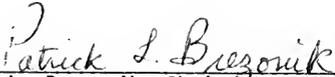
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BIOGRAPHICAL SKETCH

Forrest Edward Dierberg was born March 27, 1944, in St. Louis, Missouri. He attended Southeast Missouri State University in Cape Girardeau, Missouri, graduating in June 1967 with a B.S. in Education degree (major: biology). Following that, he served as a Peace Corps volunteer for two years in environmental sanitation in the Kingdom of Tonga in the South Pacific where he lived in complete harmony with his environment. After a brief period working on a sugar mill plantation in Hawaii, he returned to St. Louis and worked as a medical technician in immunology at Washington University School of Medicine during 1970. It was during that year he consented to enter into matrimony with the former Sharon Jeanne Fox. He entered the Graduate School at the University of North Carolina at Chapel Hill in 1971 and received the Master of Science in Public Health degree in 1972. Immediately following a brief period of employment as an immunologist at the University of North Carolina School of Medicine, he and his wife spent six months backpacking in the South Pacific. Upon return to the United States in 1974, he was gainfully employed as an algologist for Aquatic Control, Inc., in Seymour, Indiana. He returned to graduate school at the University of Florida in September 1975, where he obtained the Doctor of Philosophy degree in 1980 in environmental engineering sciences. He has subsequently been at Florida Institute of Technology where he presently teaches.

Forrest and Sharon are the proud parents of Jason Edward, born on July 17, 1975.

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Professor of Environmental
Engineering Sciences

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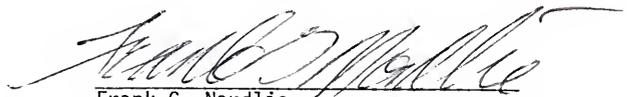
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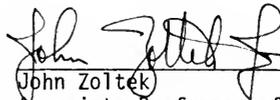
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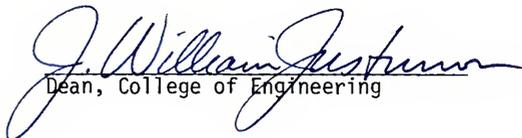
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This dissertation was submitted to the Graduate Faculty of the College of Engineering and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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