MUNICIPAL WASTEWATER EFFECTS ON NITROGEN CYCLING
IN A MATURE HARDWOOD FOREST

by

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(ABSTRACT)

Land disposal of municipal wastewater is considered ecologically acceptable and cost effective. The success of land treatment systems, however, requires proper functioning of all ecosystem components. The impact of municipal wastewater irrigation on the structure and function of an Appalachian hardwood forest in Virginia was investigated. Four irrigation rates (17.5, 35, 70, and 140 cm yr^{-1}) were applied in this hardwood forest, and their effects on forest nutrient cycling were monitored for two years. Tree growth, seedling reproduction, tree mortality, species diversity, and N sequestering by vegetation were not changed significantly. Herbaceous ground cover increased due to irrigation, except for the 140 cm yr^{-1} treatment where the heavy spray caused physical damage to the cover. Depending on the rate applied, the mature hardwood forest system sequestered only -3.4 to 8.2 kg N ha yr^{-1} in the aboveground biomass. Therefore, the fate of added N to the system became a function of N transformation processes in the soil. Nitrogen mineralization and nitrification increased as irrigation increased. Denitrification rates were not affected by irrigation; the process of denitrification did not constitute a significant N output from the forest system. The additional soil nitrate (NO_3^-) was left to leach because of the low assimilation by the

plant/soil system and the low denitrification rate. Nitrogen storage decreased in the forest floor due to the increase in litter decomposition, and increased in the surface soil due to the increase in microbial N assimilation. Total soil N increased on the low irrigation sites and decreased on the high irrigation sites, indicating that high rates of irrigation stimulated N loss from the soil by enhancing soil N transformations. The health of the forest ecosystem was not adversely affected during this period, but the forest did not serve as a net sink for N. There was little opportunity for N sequestering in this mature hardwood forest. Without harvesting and regeneration, the system is likely to lose system N when wastewater is applied. When wastewater is applied to lands, N sequestering and denitrification should be maximized in order to minimize the pollution potential of NO_3^- leaching to groundwater systems.

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CHAPTER I. INTRODUCTION

Study Rationale

Since the invention of the central sewage collection system, people have been searching for ways to dispose of wastewater. The conventional "dilution approach" of wastewater disposal has already caused ecosystem degradation in many regions due to nutrient enrichment. Discharges of municipal wastewaters are often a significant source of nitrogen (N) in waterways. Typical N concentration of municipal wastewater ranges from 20 to 85 mg L^{-1} (Feigin *et al.*, 1991). The enrichment of waters with N and other nutrients undesirably affects water quality and use by causing excess growth of algae. Serious pollution of Lake Erie is a case in point. About 85% of the total N entering Lake Erie had been attributed to the discharge of municipal wastewater (Committee on Nitrate Accumulation, 1972). Lake Erie has undergone major renovation for the past two decades since a joint program to resolve the eutrophication problem was initiated by U. S. and Canadian governments in 1972 (Council of Environmental Quality, 1989). Nevertheless, N levels in Lake Erie and other Great Lakes have been increasing steadily (International Joint Commission, 1986). Even when nitrate (NO_3^-) levels are acceptable for drinking water, rising N levels could cause adverse environmental impacts such as subtle changes in phytoplanktons.

Chesapeake Bay is another important aquatic system that has been adversely affected by nutrient enrichment from agricultural and municipal wastewater (USEPA, 1983). Of the total nutrient load, 67% of the N and 37% of the phosphorus (P) were due to nonpoint sources. Pollution control efforts between 1980 and 1985 effected a 43% reduction (33% from point source and 10% from nonpoint source) of N and P loads into Chesapeake Bay (USEPA, 1988). In the Chesapeake Bay Agreement, signed in 1987, a goal was set for 40% reduction of N and P by the year 2000 (Chesapeake Bay Foundation, 1988).

Land disposal of reclaimed municipal wastewater is currently considered to be ecologically acceptable and cost effective (Cole *et al.*, 1983). Although irrigation with sewage effluent has been practiced for centuries on agricultural lands (Pound and Crites, 1973b), it has been only two decades since forested watersheds were seriously considered for wastewater recycling. It has been shown by several studies that forested areas efficiently remove contaminants from municipal wastewater (Red and Nutter, 1986; Reed and Crites, 1986; Sopper, 1971, 1986; Sopper and Kardos, 1972).

Forest lands have advantages over agricultural lands as sites for wastewater disposal. There are fewer public health concerns associated with irrigated forests because they are non-food crops. The scheduling of wastewater irrigation is more flexible in forest lands than in agricultural lands due to the perennial growth. Most forest soils are porous and have high infiltration rates in contrast to agricultural lands where soils are less permeable and compacted by cultivation. Furthermore, some tree species are tolerant to high concentrations of pollutants that may cause problems for some agricultural crops.

Despite the advantages of forest lands as wastewater disposal sites, lack of information in adequate design criteria limits the number of new irrigation projects being installed on forest lands. Early wastewater irrigation practices caused adverse effects on the survival and growth of trees because the application rates (1,000 to 1,500 cm yr^{-1}) were not matched with the hydraulic capacity of the soils or the moisture and nutrient requirements of the trees (Little *et al.*, 1959). Little and co-workers (1959) also found that inorganic N in the top 15 cm of soil in wastewater-sprayed plots had increased 20 times higher than that in control plots. Wastewater application rates have been reduced to much lower levels (130 to 350 cm yr^{-1}) in recent forest irrigation projects in order to prevent such adverse effects (Cole *et al.*, 1986).

Forest wastewater irrigation systems still need to be monitored even with low application rates. Wastewater irrigation at 5 cm wk^{-1} throughout the entire year on a mature forest system on a sandy soil caused an increase in NO_3^--N concentration in soil water that reached a peak of 42.8 mg L^{-1} within five years (Sopper, 1986). When irrigation was limited to the growing seasons, NO_3^--N levels remained below 10 mg L^{-1}.

For forest land treatment systems to be successful over time, they need to be designed properly, and their design must be based on the biological structure and function of the land and vegetation systems. Designers and managers need to know how wastewater applications affect ecosystem processes, because proper functioning of ecosystem components are required for the success of land treatment systems. Forest land treatment systems will not be adopted with full confidence until research and demonstrations are provided to convince the public of their effectiveness.

Development of Wastewater Irrigation Systems on Forest Lands

Land application of wastewater has been practiced throughout human history. Since the idea of using treated sewage sludge as a fertilizer emerged (Elsner, 1912), a system of recycling wastewater has been developed successfully for many agricultural crops (Page *et al.*, 1983; Pound and Crites, 1973b; Shuval *et al.*, 1986). Early sewage farms were established in Germany (1531) and Scotland (1650), and many other farms were established later in European countries and Australia (Feigin *et al.,* 1991). Modern land application of municipal and industrial wastewater is widespread in the United States and many other countries (Sullivan *et al.*, 1973); these modern systems have been operated successfully (D'itri *et al.*, 1981; Feigin *et al.*, 1991; Page *et al.*, 1983).

Irrigation of forests with wastewater has been recognized as an alternative for wastewater disposal after several comprehensive studies were reported in the 1970's and 1980's. Long-term studies of forest irrigation have been pioneered by the Pennsylvania State University since 1962 (Sopper, 1986; Sopper and Kardos, 1973; Sopper and Kerr, 1979; Sopper and Sagmuller, 1971). The "living filter" concept evolved from their studies, which is to use forest land to remove constituents in wastewater through microbial degradation, chemical precipitation, ion exchange, biological transformation, and plant uptake. These studies involved forest ecosystems of red pine, white pine, and mixed hardwoods irrigated at different rates (25 to 75 mm per week). Results indicated that potential groundwater contaminants were efficiently removed with a properly designed and managed forest and treatment system.

Another early forest irrigation study was conducted in Douglas-fir and Lombardy poplar plantations in Washington (Cole *et al.*, 1983; Schiess and Cole, 1981). These young plantations assimilated large amounts of N. Year-round irrigation of 50 mm wk^{-1} resulted in acceptable concentrations of N in leachate over a four-year period.

While secondary effluent produced from treatment plants was used in the above studies, pond-treated wastewater was also used in other studies. The major difference in the pond-treated wastewater is that most of the inorganic N is removed in the lagoon while P is still removed by the soil system after being land applied (Burton, 1982). Pond-treated wastewater, low in inorganic N and high in organic N, has been irrigated in a mixed pine and hardwood forest at the Unicoi State Park in Georgia over a twelve-year period (Nutter and Red, 1985). Results of this study have provided guidelines for wastewater irrigation systems in the southeastern United States. Effluent from sewage ponds was also used in experiments on young red pine plantations, northern hardwoods, and Christmas trees in Michigan (Urie *et al.*, 1984). The wastewater had very low inorganic N due to ammonia volatilization from the pond storage, and most N in the applied wastewater was in organic forms. There were substantial increases in growth of young plantations, while older stands showed a modest growth change.

By 1981, there were seven operational forest wastewater systems in the United States according to the 1981 EPA Design Manual (USEPA, 1981). By 1985, the number increased to 22 (Urie, 1986). Although the number of forest irrigation systems is increasing, many are experimental and small. One of the larger successful wastewater renovation programs on forest land is located in Clayton County, Georgia (Nutter, 1986). Wastewater from treatment plants has been irrigated over 1460 ha of loblolly pine plantations since 1982. Year-round operation with a 6.4 cm wk^{-1} loading rate increased annual stream flow by 93 % without significantly changing the quality of groundwater or surface water.

Several other examples of forest irrigation in New England were reported by Reed and Crites (1986). Their operations were put in place from 1971 to 1978 with hydraulic loading rates of 50 to 250 cm yr^{-1}. Most forests used in these systems were mature hardwoods with some areas mixed with conifers. Although the upland forest areas were formed by glacial influence and presented such negative aspects as irregular slopes, thin soils, shallow bedrock, and wet areas, the systems functioned well and effectively managed N and P. Results of the operations indicated that wastewater treatment by forest irrigation was also a reliable alternative in New England.

A forest irrigation system has been operated in mature stands of mixed oak and pine at Bennett Spring State Park in Missouri since 1972 (Barnett and Arnold, 1986). Lagoon wastewater was spray irrigated at a rate of 5 cm wk^{-1} for 28 weeks a year. During 14 years of wastewater irrigation, most of the nutrients in the wastewater were assimilated by plants and soils and nutrient leaching was very low. The Bennett Spring land application system has served as a model for many other parks, industries, and municipal treatment systems in Missouri.

Research Objectives

Based on these intensive experiments and successful operations, regulatory agencies have developed greater confidence in wastewater irrigation systems for forest lands. However, design criteria are strongly dependent on specific site conditions and the short and long-term effects on forest function. One overall conclusion that can be made from the above studies is that successful land application systems in forests depends on a high assimilation capacity for N. Aggrading ecosystems such as young plantations effectively accumulate large amounts of nutrients in their biomass, reducing nutrient loss by leaching. In mature forests, however, nutrient accumulation in vegetation is not substantial. Furthermore, nutrient retention by forest soils is limited for transient elements such as N. Mature forest systems are self-maintaining with balanced nutrient cycles; outputs are roughly equivalent to inputs. In these systems, small losses of soil N by denitrification and leaching are replenished by N inputs from the atmosphere and by symbiotic and non-symbiotic N fixation. Wastewater irrigation may or may not disturb the balance by introducing excess nutrients and water (Fig. 2.1). If the added nutrients can be assimilated by biomass expansion and accelerated cycling, the ecosystem will remain stable in terms of nutrient cycling, albeit at a new equilibrium. If the forest system does not sequester the added nutrients, transient elements such as N will leach from the forest and could become pollutants.

Many forest wastewater treatment projects use young and regenerating forests to maximize N assimilation by the fast growing vegetation. The N and other nutrients stored in the trees will be removed from the ecosystem by harvesting. In mature forests, however, there is little or no net vegetative uptake and removal of N. In these mature forests, N transformations (denitrification) and N storage (immobilization) are the only avenues of N dissipation or sequestering that would prevent leaching. Understanding the response of the forest N cycle to wastewater treatment will help ensure proper design

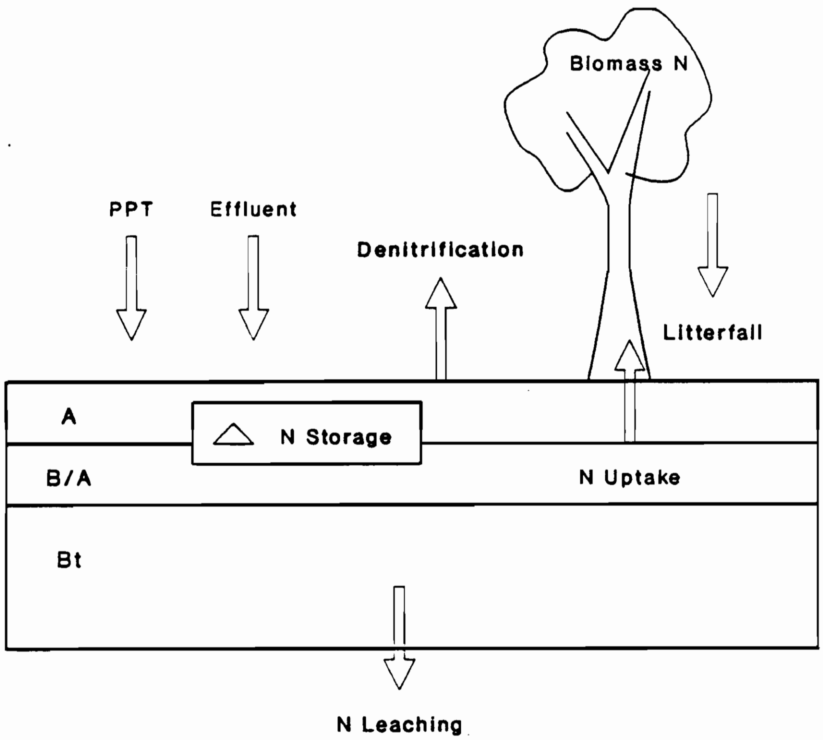


Figure 2.1. Simplified N cycling diagram showing major N fluxes and storage components.

criteria for forest irrigation systems.

The objectives of this study were 1) to evaluate the changes in biomass and N accumulation in a mature hardwood forest after two years of wastewater irrigation, and 2) to examine the effect of wastewater irrigation on soil N transformations and N cycling in the forest ecosystem.

In the context of these objectives, it is hypothesized that:

1. Ho: Irrigating the forest with wastewater at various rates elicits no forest response after two years (Chapter III).
2. Ho: Irrigating the forest with wastewater at various rates elicits no change in N transformation processes in the soil (Chapter IV).
3. Ho: After two years, wastewater irrigation has had no effect on N cycling in this mature hardwood forest (Chapter IV).
4. Ho: Denitrification is not an important flux for N removal from this system (Chapter V).

CHAPTER II. GENERAL METHODS AND PROCEDURES

The general methods described here are applicable to all of the following chapters; chapter-specific methods are presented chapter by chapter.

Study Area

The study area is located in a forest preserve owned by Mary Moody Northen, Inc. of Mountain Lake in Giles County, Virginia. It is in the Valley and Ridge Province of the folded central Appalachians (Parker et al., 1975). Elevations of the study area range from 1220 to 1270 m. Mountain Lake occurs at an elevation of 1178 m. The soil of the study area is deep to moderately deep, well drained, and very stony. It is a fine-loamy, silicious, mesic, Typic Hapludult, developed from colluvium of soil and rock fragments derived from hematite sandstones. Surface water and groundwater of this area are drained by springs and streams which flow north until they join Salt Pond Drain, the only drain from Mountain Lake. The Salt Pond Drain joins Little Stony Creek, which is a tributary of the New River. Little Stony Creek is a cold-water stream containing native and stocked trout. It is heavily used by recreationists where it flows through the Jefferson National Forest and across Cascade Falls.

The forest in the study area is dominated by 80 to 100-year-old hardwoods, northern red oak (*Quercus rubra* L.), white oak (*Q. alba* L.), and chestnut oak (*Q. prinus* L.). Northern red oak is the predominant species in this area. Basal areas of major species in each plot and in individual blocks are illustrated in Table 2.1. Understory species are composed of red maple (*Acer rubrum* L.), striped maple (*A. pensylvanicum* L.), shagbark hickory (*Carya ovata* (Mill.) K. Koch.), black birch (*Betula lenta* L.), and eastern hemlock (*Tsuga canadensis* (L.) Carr.). Herbaceous ground cover is comprised of cinnamon fern (*Osmunda cinnamomea* L.), New York fern (*Thelypteris noveboracensis* (L.) Nieuwl.), blueberries (*Vaccinium* spp.), and sedges (*Carex* spp.).

Average annual precipitation is 132.8 cm including an average snowfall of 142.2 cm. Precipitation occurs relatively uniformly throughout the year with typical monthly rates of 7.6 to 15.2 cm per month. Average monthly precipitation was 8.2 cm in 1989 and 7.8 cm in 1990. Temperature varies greatly throughout the year. Average daily temperatures range from -7.1°C in January to 24.7°C during July and August. Average monthly temperatures ranged from -5.2 to 20.0°C in 1989 and 3.1 to 20.2°C in 1990.

Mountain Lake Hotel is a seasonal resort developed around a natural lake on Salt Pond Mountain in Giles County. Wastewater generation from 116 rental rooms and a 125 seat dining room is estimated at 85,428 L day^{-1} when 100% occupied. The failure of the hotel's existing septic drainfield system was reported in 1985. A repair system was installed near the existing absorption system, but its use was limited to the 1986 summer season. From several alternatives to discharge wastewater from the hotel, forest irrigation was considered to be the most feasible. A forest wastewater treatment system was designed by Anderson and Associates, Inc. of Blacksburg, VA, that covers a total spray area of 6.4 ha with 96 sprinklers (Anderson and Associates, Inc., 1986). After construction in 1987 and 1988, spray irrigation started in June 1989.

Wastewater was primarily treated with comminution and screening (Fig. 2.2).

Table 2.1. Basal area of major tree species in each plot in 80 to 100-year-old upland hardwood stands in Giles Co., Virginia (1988).

Plot	QURU	QUAL	AMAR	ACRU	Other	Total
	m2/ha					
Block 1	18.1	1.7	4.6	3.3	2.0	29.7
Control	21.9	0	3.4	1.0	2.9	29.3
17.5	17.5	0	5.4	2.2	3.0	28.1
35	19.1	3.7	3.9	1.8	0.3	28.7
70	19.3	0	5.4	5.7	3.0	33.4
140	12.7	4.8	5.1	5.6	0.6	28.8
Block 2	17.6	3.8	3.2	1.4	2.0	27.9
Control	9.7	16.0	2.1	2.2	2.8	32.7
17.5	22.0	0	2.9	0.8	1.4	27.2
35	18.0	0	3.2	1.7	2.8	25.6
70	20.5	0	3.5	2.1	2.2	28.3
140	17.6	3.1	4.0	0.2	0.7	25.6
Block 3	16.9	4.3	3.3	1.3	1.6	27.4
Control	21.0	0.3	5.5	2.2	0.8	29.7
17.5	11.0	7.1	1.8	1.6	3.7	25.2
35	21.3	3.3	2.7	0.1	0.6	28.0
70	13.9	8.4	1.7	2.2	1.4	27.6
140	17.4	2.6	4.8	0.1	1.4	26.2

QURU: Quercus rubra (northern red oak)
QUAL: Quercus alba (white oak)
AMAR: Amalanchia arboretum (downy serviceberry)
ACRU: Acer rubra (red maple)

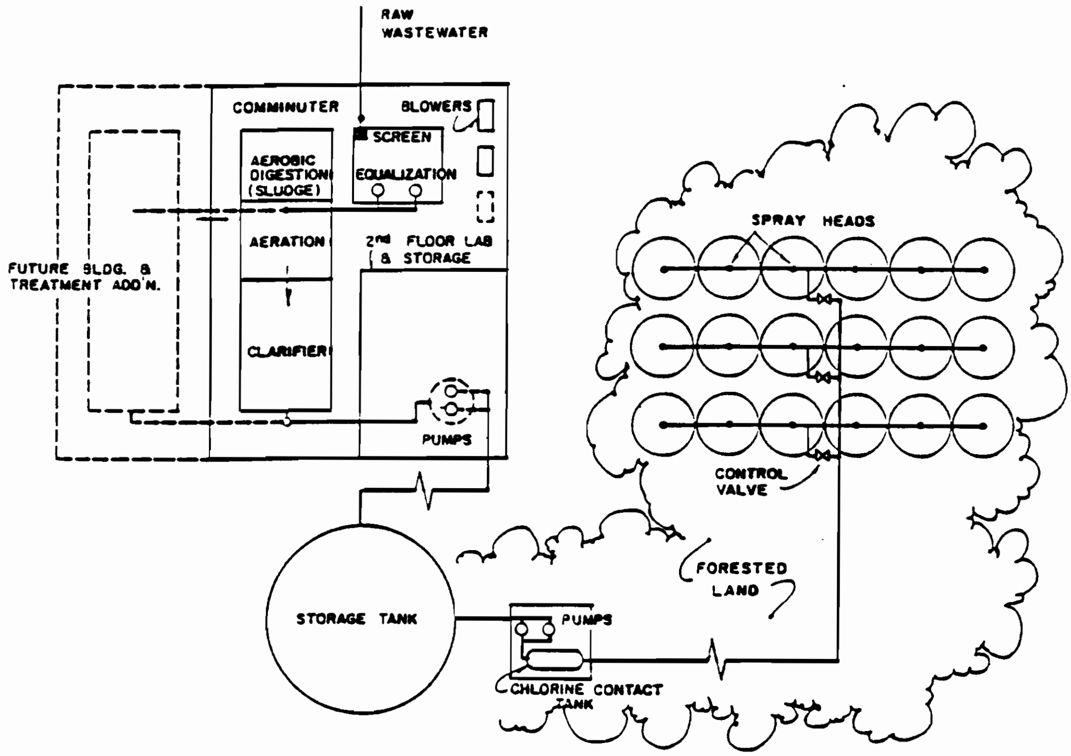


Figure 2.2. Layout of wastewater treatment system (Anderson and Associates, Inc., 1986).

Secondary treatment was provided using the extended aeration modification of the activated sludge process. Treated wastewater was stored in a storage tank and chlorinated at a pump station before it was sprayed in the forest.

Study Design and Installation

The stated hypotheses were tested by measuring forest and soil responses to increasing rates of applied wastewater. Secondary sewage effluent was irrigated in the study area with four application rates (17.5, 35, 70, and 140 cm yr^{-1}) and one control. Three replications of each treatment were included. The study was made possible by superimposing the experimental design on the operational spray field (Fig. 2.3, 2.4). The irrigation system is divided into 16 subsystems which consist of 6 risers (depicted as spray circles in Fig. 2.4). Four subsystems are included in block 1 and 2, and two subsystems in block 3. Block layout was based on topographic features of the spray field. Wastewater was operationally irrigated at the 35 cm yr^{-1} rate. The loading was increased to 70 and 140 cm yr^{-1} on three treatment sites each by installing two and four risers per point, respectively. At the three 17.5 cm yr^{-1} treatment sites, wastewater loading was reduced to half the operational rate by installing sprinkler heads equipped with a smaller size nozzle. Within the nozzle spray areas, 20 x 20 m experimental plots were established. Within these plots, three 4 x 4 m understory vegetation subplots were randomly located (Fig 2.4). Within each 4 x 4 m subplot, two 1 x 1 m sub-subplots were established at fixed locations for herbaceous ground cover measurements.

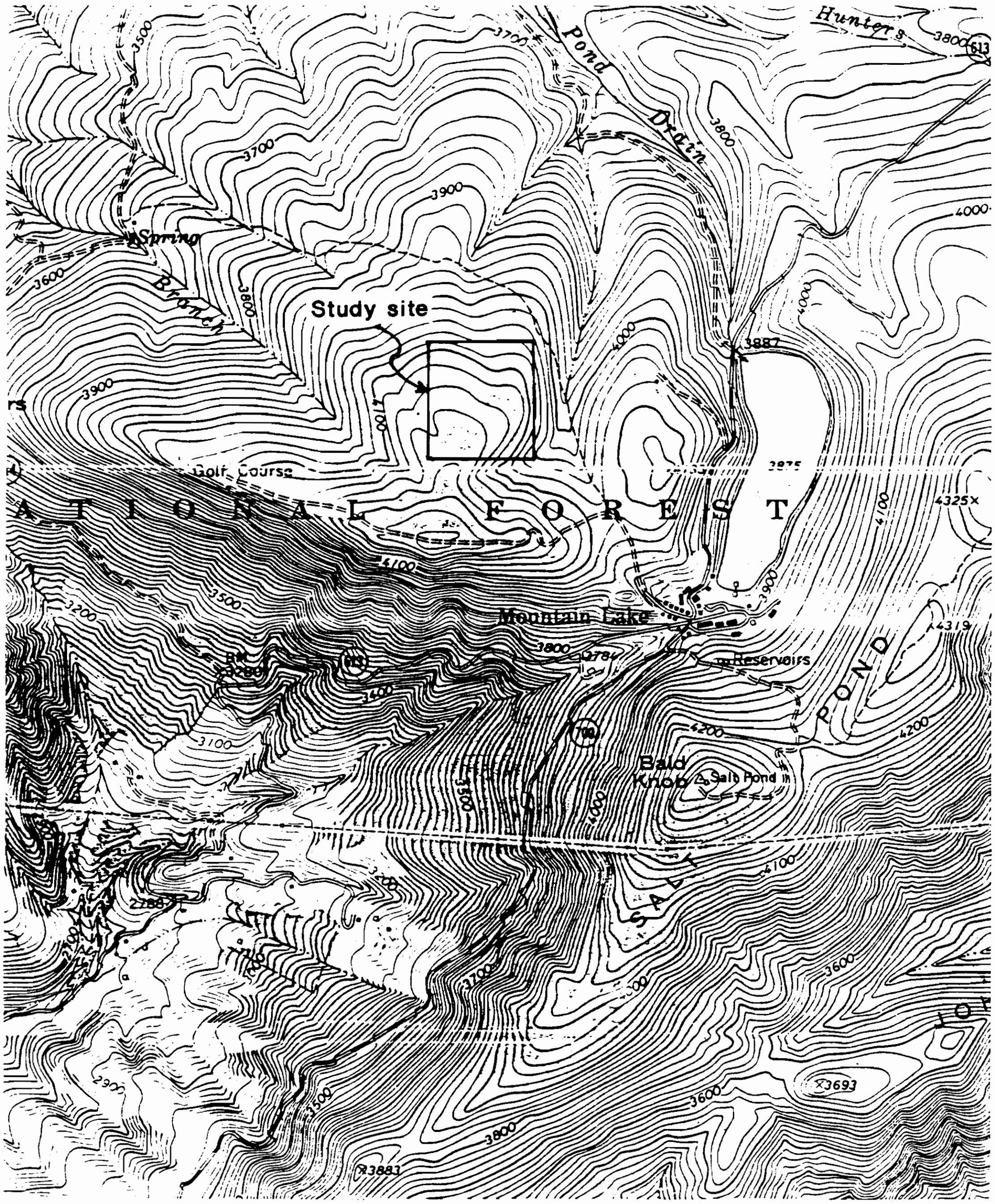


Figure 2.3. Location of the study site in the Mountain Lake area.

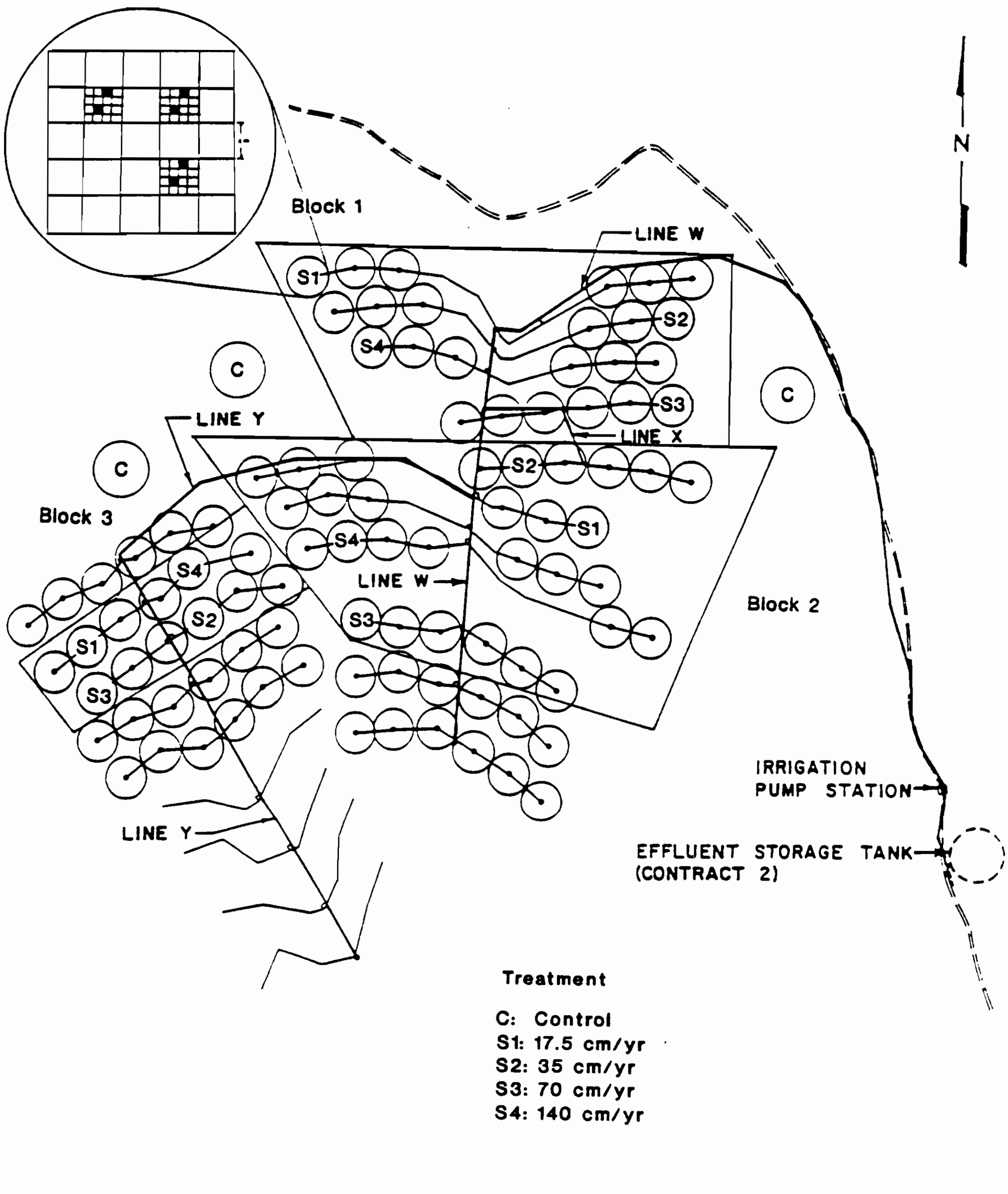


Figure 2.4. Spray irrigation layout showing the experimental design superimposed on the operational system.

Wastewater Composition and Application

Both effluent and throughfall samples were collected weekly during the spray operation period of 1989 and 1990. The effluent samples were collected from the pump house before it was sprayed. Chemical composition of the effluent and throughfall is presented in Table 2.2. Most inorganic constituents were below the range of typical secondary municipal effluents (Asano *et al.*, 1985; Pound and Crites, 1973a; Thomas and Law, 1977). Nitrogen concentration of the effluent used in this study is compared with those of other forest irrigation systems in Table 2.3. Most of the NH_4^+-N in the treated wastewater was converted to NO_3^--N by aeration during the storage period and was applied as NO_3^--N (Figure 2.5). Other inorganic constituents in the effluent did not vary much during the storage period. The amount of N-P-K applied operationally (16.8, 9.0, 26.8 kg ha^{-1}, respectively) was much lower than the average annual application (208, 87, 189 kg ha^{-1}, respectively) by Sopper and Kardos (1973).

Wastewater loading was limited by the amount generated from the hotel, which was not enough to meet the planned loading. Actual standard loadings were 21.5 cm yr^{-1} in 1989 and 35.2 cm yr^{-1} in 1990. Wastewater was applied for one hour a day during the growing season of 1989 and for two and one half hours every other day in 1990. After the operation had been stopped on rainy days to prevent surface runoff, the rates were doubled on dry days until the scheduled application was fulfilled, or as long as there was enough treated wastewater to be irrigated. The wastewater irrigation schedule was also occasionally interrupted by mechanical problems or insufficient amounts of effluent. Actual amounts of wastewater irrigated to the forest is illustrated in Figure 2.6.

Table 2.2. Chemical composition of the applied wastewater and throughfall, and total nutrient loading during 1989 and 1990.

Nutrients	Wastewater	Throughfall	Annual loading** 1989	Annual loading** 1990
	---------- mg/L	---------	------- kg/ha	-------
NO3-N	4.45 (5.04)*	0.45	8.3	17.8
NH4-N	1.35 (0.51)	0.40	4.7	1.8
Org. N	0.25	0.05	0.5	0.5
Total N	5.85 (5.60)	0.85	13.5	20.1
Total P	3.15	0.41	6.8	11.1
K	9.45	2.54	20.3	33.3
Ca	16.01	1.18	34.4	56.4
Na	33.68	-	72.4	118.6
Mg	1.97	0.22	4.2	6.9
Mn	0.01	-	0.02	0.04
Zn	0.03	-	0.06	0.11
Fe	0.01	0.01	0.02	0.04
B	0.02	-	0.04	0.07
pH	7.3	5.1		

* Values in the parentheses represent average of 1990

** Based on operational loading of 35 cm/yr

Table 2.3. Concentrations of N in secondary effluents used for forest irrigation.

Study site	Min. N	Org. N	Total N	Reference
	-------	mg/L	-------	
Penn. State Univ.	20.2	5.2	25.4	Sopper 1986
Pack Forest, WA	17.1	1.5	18.6	Schiess and Cole 1981
Harbor Springs, MI	2.1	3.3	5.4	Harris and Urie 1983
Middleville, MI	7.6	5.0	12.6	Urie et al. 1984
East Lansing, MI	10.6	3.2	13.8	Brockway et al. 1979
Unicoi State Park, GA	7.6	10.4	18.0	Nutter and Red 1984
Clayton County, GA	13.0	5.0	18.0	Nutter 1986
Coastal plain, GA	2.7	1.8	4.5	Red and Nutter 1986
Mountain Lake, VA	5.7	0.3	6.0	This study

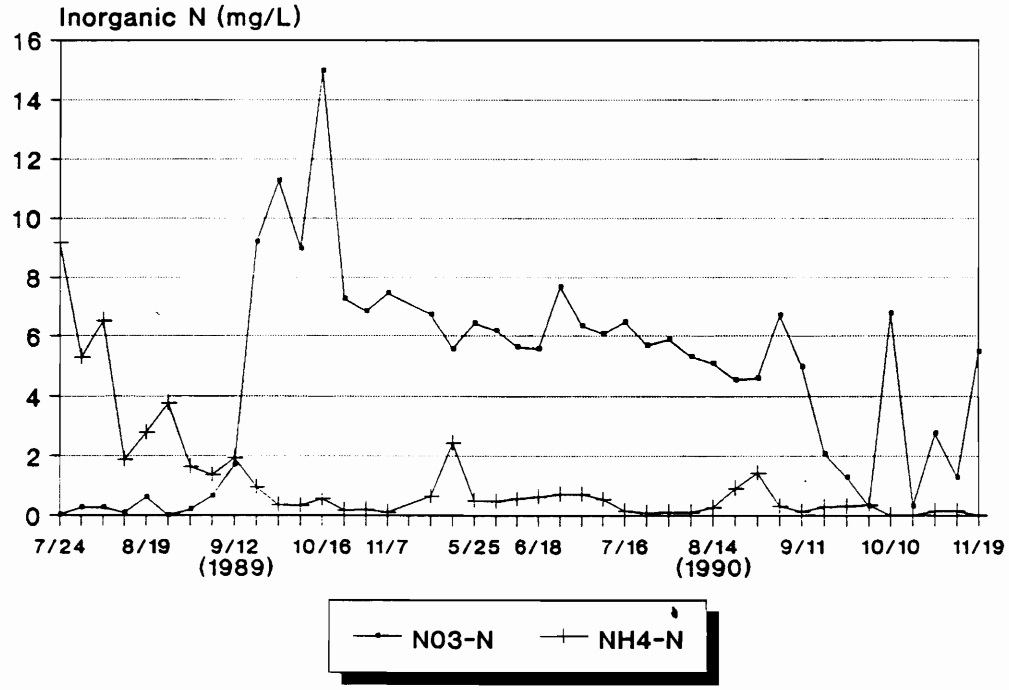


Figure 2.5. Concentrations of inorganic N in secondary effluent.

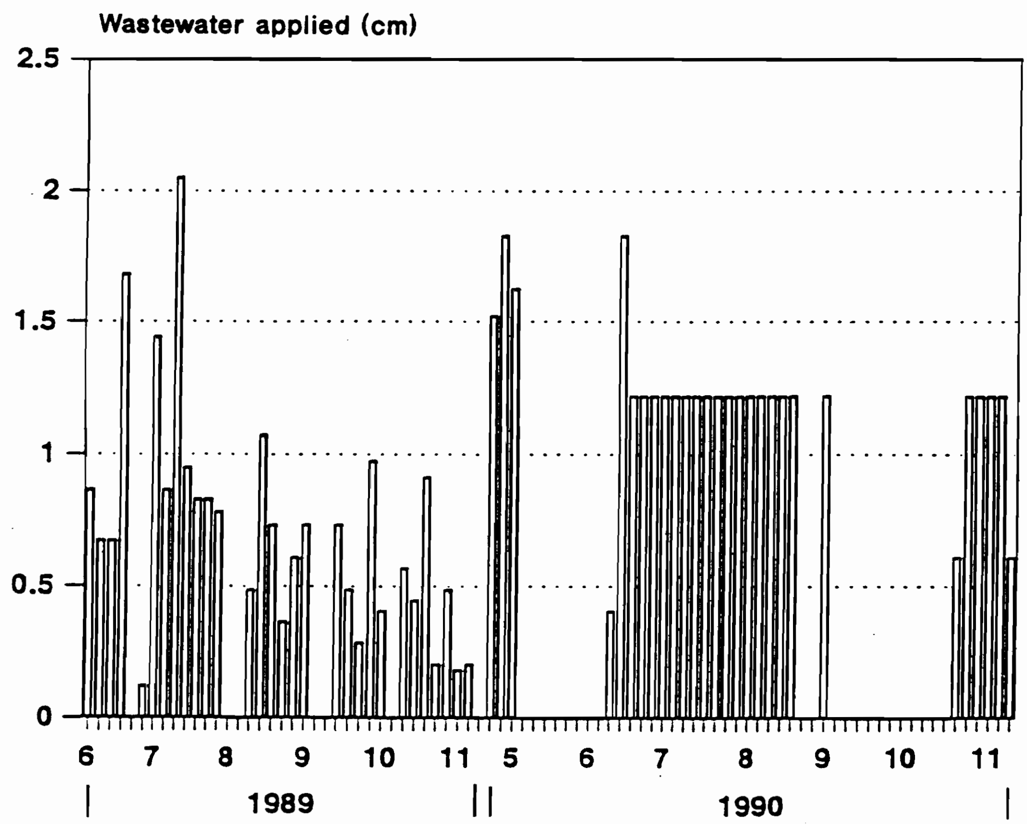
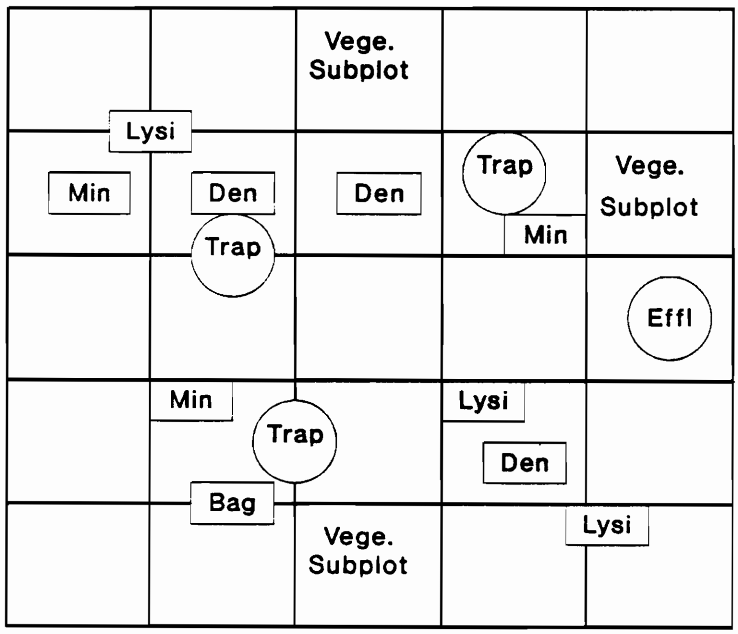


Figure 2.6. Wastewater irrigation on the study site in 1989 and 1990. Each bar represents a four-day irrigation period.

Experimental irrigation rates were always 0.5 x, 2 x, and 4 x the operational rate on any given day.

System Monitoring and Management

Nitrogen flux in the ecosystem and the changes of N in the soil were monitored throughout two years of wastewater irrigation. An example of equipment installation and location in a 20 x 20 m plot is shown in Figure 2.7. All samplings and measurements were carried out during the growing seasons of 1989 and 1990. Litterfall and sewage effluent were collected to measure N input to the soil. Litterfall was collected on a two-week interval during fall, and on a two-month interval during the rest of the year. Throughfall collections from control sites were used to estimate atmospheric inputs. Soil solution from lysimeters and denitrification activity were measured for the estimation of N output. Soil water at a 1 m depth was collected at two week intervals using continuous tension lysimeters. Denitrification activity was measured in the field once a month. Potential denitrification activity was measured by laboratory incubations under controlled conditions. Plant N uptake was also measured by biomass change and N concentrations in the biomass. Litter decomposition was measured with litter bags buried in the litter layer. The buried bags were collected after 6, 9, 12, 18, and 24 months. Net N mineralization was measured by incubating fresh A-horizon soil in plastic bags during a one-month period. Mineralized N was estimated from the difference in inorganic N before and after the incubation.



Vege. Subplot: Vegetation subplot for shrub and herbaceous ground cover

Lysi: Continuous tention lysimeter with hanging water column

Min: Nitrogen mineralization bags

Den: Denitrification chamber with four diffusion tubes

Trap: Litter trap

Effl: Effluent spray gauge, 9 m from sprinkler

Bag: Litter decomposition bags buried in litter layer

Figure 2.7. Layout of equipment installation in the 20 x 20 m plot.

Statistical Analysis

Plant and soil data were collected from the study site before and after the irrigation. The data collected before the irrigation (1988) showed spatial variation between plots. There was also year-to-year variation in control plots. In order to test the effect of wastewater irrigation for objectives 1 and 2, the temporal and spatial variations had to be eliminated. The 1990 data collected after two years of irrigation were adjusted to remove temporal variation by subtracting the change in control plots from the change in treatment plots. The spatial variation was accounted for in an analysis of wastewater treatment effect by using the 1988 values as covariates in multiple regression (Smith and Rose, 1989). The relationships between forest process response and wastewater irrigation were re-evaluated after removing the covariate term from the equation using stagewise regression (Hirsch *et al.*, 1982; S. Rheem, Department of Statistics, Virginia Tech, personal communications, 1992).

A general multiple regression equation which includes 1988 values as covariates is described by:

$$\mathrm{Y} = a + b\,\mathrm{X1} + c\,\mathrm{X2} \tag{1}$$

where Y is a dependent variable, X1 is a wastewater treatment, X2 is a covariate, and a, b and c are regression coefficients. After the wastewater treatment effect was tested with the muntiple regression, a regression equation between Y and X2 was obtained as follows:

$$Y = a' + c' X2 \qquad (2)$$

A regression between Y and residuals from equation (2) provided another equation (stagewise regression) which shows the wastewater treatment effect:

$$Y = a'' + b' X1 \qquad (3)$$

Equation (3) was used to illustrate the relationships between wastewater treatment and ecosystem variables in the tables and figures of chapter III and IV.

CHAPTER III. MUNICIPAL WASTEWATER EFFECTS IN A MATURE APPALACHIAN HARDWOOD FOREST IN VIRGINIA

Abstract

Two years of wastewater irrigation effects on stand growth, seedling reproduction, mortality, species diversity, and N accumulation in forest vegetation were determined. Irrigation rates of 0, 17.5, 35, 70, and 140 cm yr^{-1} were applied. Stand growth response to irrigation was negligible. Herbaceous ground cover increased with irrigation rate, except for the 140 cm yr^{-1} treatment where the heavy spray caused physical damage. Seedling reproduction was not changed by wastewater treatments for any species. The number of northern red oak seedlings increased up to 100 times in 1990, which was due to the large increase in acorn production in the fall of 1989. Tree mortality was not related to the treatments in this study. Species diversity, represented by Simpson's index, ranged from 0.24 to 0.33 for overstory trees and from 0.26 to 0.54 for understory trees. Species diversity was not affected by the irrigation in the overstory after the two-year period, whereas the understory showed a trend of increasing species diversity with increasing irrigation. Annual N balance showed a net plant N uptake that ranged from 4.7 to 16.2 kg ha^{-1}. Negligible plant N uptake observed in this study was consistent with other studies, showing that there is little opportunity for sequestering large amount of added N in mature hardwood forests.

Introduction

Trees often respond in a positive fashion to wastewater irrigation (Brockway, 1982; Sopper and Kardos, 1973). However, the process of tree response is not the same for all species, nor is it the same at various stages of their life cycles. Wastewater irrigation is more likely to increase the growth of young trees than mature trees (Cooley, 1982; Nutter, 1986). Because growth rate is a major determinant of nutrient uptake and assimilation, young trees sequester more N than old trees on an annual basis. In young, aggrading forests, large amounts of wastewater N is taken up and stored. Reported N uptake rates range from 90 to 400 kg ha^{-1} yr^{-1} in various forests receiving wastewater or sludge applications (Brockway *et al.*,1986). The greatest assimilators of N are young poplars with uptake rates between 200 to 400 kg ha^{-1} yr^{-1} (Cooley, 1979, Schiess and Cole, 1981). Young hardwood stands regenerating after a clearcut are also rapid N assimilators. Sopper (1986) reported that NO_3^--N in soil water was reduced significantly in the wastewater irrigation sites where a 30-year-old stand had been clearcut. With reforestation and the development of herbaceous and shrub covers in the irrigation sites, NO_3^--N in the soil had been maintained below 10 mg L^{-1} for over 10 years.

Nitrogen-enriched wastewaters affect the growth of N deficient forests in the same manner as fertilization. Nitrogen-fertilized forests reach a maximum biomass earlier than unfertilized forests (Figure 3.1). This higher rate of assimilation amounts to a significant flux of N from the soil that can then be removed via biomass harvests. In mature forests, however, biomass is already at a maximum level and there is little chance

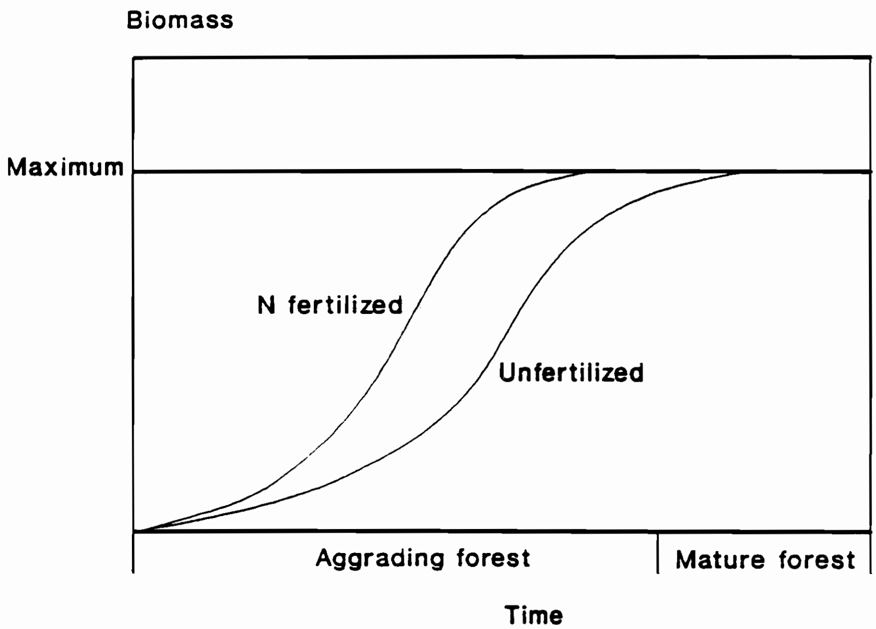


Figure 3.1. Nitrogen fertilization effect on forest growth.

of increasing it with additional N (Fig. 3.1). Therefore the effects of wastewater on the growth and health of mature forests are uncertain. With an irrigation rate at 5 cm wk^{-1}, Burton (1982) observed no significant growth of a late successional maple-beech forest. In another study, a hydraulic loading of 50 cm per application season did not impose any significant stress on mature hardwood forests in New England (Reed and Crites, 1986). In Georgia, a similar application had no significant impacts on forest growth or community structure (Red and Nutter, 1986).

Species composition may be changed by wastewater irrigation if the species are sensitive to wet conditions. In a study of wastewater irrigation on forest land, Barnett and Arnold (1986) found that many oaks and pines died out in favor of more hydric and mesic species. Seedlings of eastern cottonwood, white ash, Scotch pine, tulip poplar, and black walnut exibited good performance under irrigaton, while black cherry and northern red oak seedlings were adversly affected (Brockway, 1982). Cooley (1982) also showed that hybrid poplar, Scotch pine, white spruce, and balsam fir grow successfully with wastewater irrigation. *Populus* species and hybrids showed the greatest growth response, whereas pines showed the least. Northern red oak, which had been found to respond negatively to wastewater irrigation, was originally a dominant overstory species in the irrigation area.

Given the various site- and species-specific responses to wastewater irrigation, the purpose of this study was to determine if and how a mature Appalachian upland hardwood forest responded to wastewater irrigation. Total biomass, species change, and N sequestration in response to four rates of effluent applied for two years were studied.

Methods

The vegetation inventory system used in this study was similar to the methods developed by Zedaker and Nicholas (1990) for the U.S. Environmental Protection Agency. Vegetation on each plot was separated into four major categories; overstory (>5 cm DBH), understory (>1.4 m ht and <5 cm DBH), seedling (<1.4 m ht), and herbaceous ground cover. A coordinate (x,y) grid system (4 x 4 m) was used to locate and measure the trees in each plot. All trees were numbered by nailing aluminum tags at the tree base 15 cm above ground. Trees were recorded by location (x,y), number, species, DBH, and dominance. Qualitative estimates of tree vitality such as crown class, tree decline, disease and insect damage, and miscellaneous damages were also recorded. Above-ground tree volume and above-ground biomass were estimated from DBH and regression equations developed for different species (Clark and Schroeder, 1986). Tree diameter growth was measured by diameter tape and increment borer samples.

Understory trees in 4 x 4 m subplots were recorded by species and diameter at 15 cm above ground. Seedlings and herbaceous ground cover were measured from 1 x 1 m sub-subplots. The number of seedlings in the sub-subplot was counted by species. The herbaceous stratum was recorded as a percent cover by species. Leaf litter, live stems, rocks, moss/lichen, and soil were also recorded as a percent cover. All the vegetation measurements were repeated in the summer of 1990 and compared with pre-irrigation records of 1988. Species richness and diversity were estimated (Simpson, 1949). The equation used for species richness was:

$$d = S/\log N$$

where S is number of species and N is total number of individuals.

The equation used for Simpson's index was:

$$C=\sum_{i=1}^{S}\left[\frac{ni}{N}\right]^2$$

where C is Simpson's index, S is the number of species, ni is the number of individuals of S individual species, and N is the total number of individuals. Simpson's index has a value between 0 and 1. An index approaching 1 indicates that the site is occupied by large populations of a few species. Simpson's index becomes smaller when the species are distributed more evenly.

Litterfall was collected from three litter traps placed in each plot. The number of litter traps was increased to six in the second year. Litter collection was done biweekly during fall, and bimonthly during the rest of season. The collected litterfall was sorted by leaves, acorns, and other materials and oven-dried at 65°C for 48 hours. The dry weight of leaf litter collected during a one-year period was regarded as annual leaf production from the site.

Nutrient levels of leaf, branch, and stem tissue were determined with the samples collected in August of 1989 and 1990. Three trees of each species located closest to the plot center were selected. Leaf and branch samples were collected from the branches of the upper middle third of the tree crown. Stem tissue samples were collected at DBH with an increment borer. The collected vegetation samples were oven-dried at 65°C for

48 hours and composited for each plot. Total N was analyzed using a block digester and Technicon Auto Analyzer. Total P, K, Ca, Mg, Al, and Fe were determined by a dry ash method (500°C, 12 hours); the ash was extracted with 10% HCl and analyzed using an inductively-coupled plasma spectrometer.

The data were collected before (1988) and after (1990) two years of irrigation. The data collected before irrigation showed spatial variation between plots. There also was year-to-year variation in control plots. The data collected after the two-year irrigation treatments were adjusted for the temporal variation by subtracting the change in control plots from the change in treatment plots. The spatial variation was accounted for by using the 1988 values as covariates in multiple regression (Smith and Rose, 1989). The relationships between forest response variables and wastewater irrigation were re-evaluated after removing the covariate term from the equation using stagewise regression (Hirsch *et al.*, 1982; S. Rheem, Department of Statistics, Virginia Tech, personal communications, 1992).

Results and Discussion

Vegetation growth response

The basal area of overstory trees, adjusted for temporal and spatial variation, showed no wastewater treatment effect (Table 3.1). The large decrease in basal area on the 17.5 cm yr^{-1} treatment was due to the damage from hurricane Hugo in 1989. Overstory volume growth was not related to the wastewater treatment. Understory basal

Table 3.1. Basal area of overstory and understory trees and herbaceous ground cover in 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia.

Treatment (cm/yr)	Basal area 1988	Basal area 1990	Adjusted [1] BA change	Adjusted stand volume change
		(Overstory)		
	m2/ha			(m3/ha)
Control	30.6	31.3	0.00 [2]	0.0 [3]
17.5	26.8	26.4	-1.14	-17.3
35	27.4	28.2	-0.03	-0.7
70	29.7	30.1	-0.39	-3.7
140	26.9	27.4	-0.24	-4.0
		(Understory)		
	m2/ha			
Control	3.1	3.0	0.00 [4]	
17.5	1.7	1.8	0.16	
35	2.2	1.8	-0.32	
70	2.7	2.7	0.08	
140	2.1	1.5	-0.57	
		(Herbaceous ground cover)		
	%			
Control	16.0	10.9	0.0 [5]	
17.5	42.8	52.0	14.3	
35	17.9	31.0	18.2	
70	21.6	29.4	12.9	
140	11.7	11.4	4.8	

1 Adjusted BA change: (1990 BA - 1988 BA) - (BA change in control)
2 Overstory basal area = 28.5283 - 0.011548 EFFL (P>.457)
3 Stand volume change = -6.0027 + 0.0164 EFFL (P>.815)
4 Understory basal area = 2.5197 - 0.005924 EFFL (p>.332)
5 Aboveground cover change = -0.3995 + 0.3877 EFFL - 0.0028 (EFFL)2 (p>.149)
where EFFL is wastewater treatment (cm/yr)

area was also unaffected by wastewater treatment. Although a two-year response period may be too short for definitive results, these preliminary data are consistent with other observations that mature forests are relatively unresponsive to moderate levels of wastewater irrigation. At an early stage of wastewater irrigation in a late successional sugar maple-beech forest, Burton (1982) observed no tree growth responses. Although only significant at the $p > .15$ level, moderate wastewater treatments appeared to increase herbaceous ground cover, except for the 140 cm yr^{-1} treatment where physical impact of the spray was damaging (Fig. 3.2).

Seedling reproduction

Seedling reproduction was measured in sub-subplots for 9 species (Table 3.2). Northern red oak was the most numerous seedling species in the second year, followed by red maple and striped maple. There were 50 to 100 times more northern red oak seedlings on all sites during a two-year period, and it was strikingly different from other species. Northern red oak had a heavy mast year throughout the region in 1989. Annual acorn production, collected by litter trap, was 2,212 kg ha^{-1} in 1989 and 272 kg ha^{-1} in 1990. However, neither mature trees nor seedlings of northern red oak responded in any way to wastewater irrigation based on the regression analysis. It may be too early to observe a potential negative response of northern red oak to wastewater irrigation as found by Barnett and Arnold (1986) and Brockway (1982). Other species showed little change in seedling numbers from 1988 to 1990. Multiple regression analysis with 1988 values used as a covariate showed no significant wastewater treatment effect on seedling reproduction.

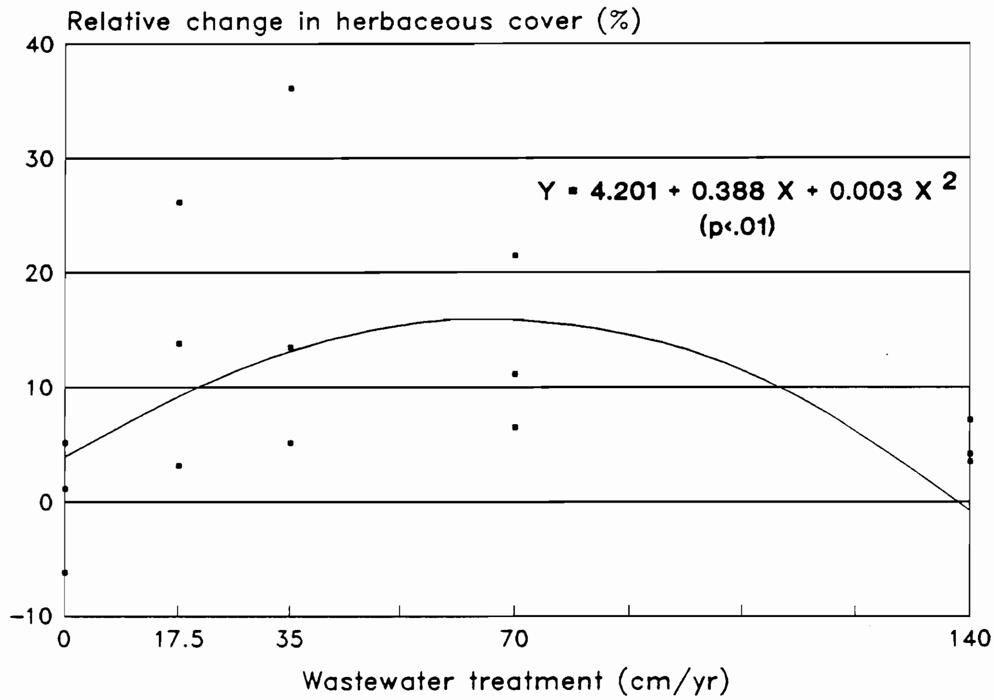


Figure 3.2. Relationship between wastewater irrigation and relative change in herbaceous ground cover in 80 to 100-year-old upland hardwood stands in Giles Co., Virginia.

Table 3.2. Seedling reproduction in 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., virginia (numbers/m^2).

Treatment (cm/yr)	ACRU 88	ACRU 90	ACPE 88	ACPE 90	QURU 88	QURU 90	CADE 88	CADE 90	AMAR 88	AMAR 90	QUAL 88	QUAL 90	COAM 88	COAM 90	RHCA 88	RHCA 90	ILMO 88	ILMO 90
Control	2.2	2.6	1.5	1.7	0.3	23.6	0.3	0.3	0.5	0.6	0.0	0.0	0.1	0.1	0.2	0.0	0.2	0.0
17.5	3.2	3.8	0.6	0.3	0.5	20.0	0.7	0.3	0.0	0.0	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1
35	0.4	3.2	1.0	2.1	0.4	31.8	0.0	0.1	2.4	2.5	0.1	0.1	0.2	0.2	0.2	0.4	0.2	0.2
70	1.3	2.8	0.8	0.8	0.1	22.3	0.0	0.0	0.1	0.8	0.0	0.0	0.1	0.1	0.1	0.0	0.2	0.2
140	2.9	3.1	1.6	2.4	0.2	20.2	0.3	0.2	2.4	1.7	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.0

ACRU: Acer rubra (red maple)
ACPE: Acer pennsylvanicum (striped maple)
QURU: Quercus rubra (northern red oak)
CADE: Castanea dentata (American chestnut)
AMAR: Amalanchia arboretum (downy serviceberry)
QUAL: Quercus alba (white oak)
COAM: Corylus americana (American hazelnut)
RHCA: Rhododendron calendulaceum (flame azelea)
ILMO: Ilex montana (deciduous holly)

Mortality

The number of overstory and understory trees decreased during the two-year period (Table 3.3); however, the tree mortality was not a function of wastewater treatment. The little wastewater irrigation effect on tree mortality might be due to the time lag or the low wastewater loading. One tree in the 17.5 cm yr^{-1} irrigation site was damaged by hurricane Hugo in 1989. All the other trees died due to the other causes. While tree number was reduced, stand volume and basal area increased in the study site (Table 3.1). This indicates that the stand volume increase was distributed in fewer individual trees. Tree mortality was less than 4% for the overstory and approximately 12% for the understory trees.

Species diversity

Species diversity is represented by species richness (d) and Simpson's index (C) (Table 3.4, 3.5, 3.6). Species richness showed a decreasing trend in understory trees over a two-year period (Table 3.5). However, species richness of overstory trees increased due to the decrease in tree number (Table 3.4). There were no significant wastewater treatment effects on species richness for overstory or understory trees. Herbaceous species richness had a positive relationship with wastewater irrigation.

Simpson's index for overstory trees remained unchanged for the two-year period, whereas those of understory trees increased. The increase in Simpson's index indicated that understory tree number increased on fewer dominant species such as striped maple. The changes of Simpson's indices, however, were not related to the wastewater treatments.

Table 3.3. Number of overstory and understory trees in 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia.

Treatment (cm/yr)	Overstory 1988	Overstory 1990	Overstory Change*	Understory 1988	Understory 1990	Understory Change*
	#/0.04 ha					
Control	49.0	48.7	0.0	212.5	212.5	0.0
17.5	39.0	37.7	-1.0	122.2	119.4	-2.8
35	40.7	40.0	-0.3	179.2	162.5	-16.7
70	46.0	44.7	-1.0	150.0	133.3	-16.7
140	51.0	50.3	-0.3	108.3	94.4	-13.9

* Change adjusted by subtracting the change in control sites from the change in the treatment sites

Overstory change = -0.188 - 0.02 EFFL + 0.0001 $(EFFL)^2$ (P>.175)
Understory change = 1.061 - 0.483 EFFL + 0.002 $(EFFL)^2$ (p>.186)
where EFFL is wastewater treatment (cm/yr)

Table 3.4. Species diversity of overstory trees in 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia (with standard error).

Treatment (cm/yr)	Species richness 1988	Species richness 1990	Simpson's index 1988	Simpson's index 1990
Control	4.8 (0.6)	4.8 (0.6)	0.24 (0.06)	0.24 (0.06)
17.5	3.6 (0.3)	3.6 (0.3)	0.28 (0.04)	0.29 (0.03)
35	4.4 (0.7)	4.4 (0.4)	0.33 (0.01)	0.33 (0.00)
70	5.2 (0.5)	5.1 (0.3)	0.24 (0.04)	0.24 (0.03)
140	3.9 (0.4)	3.9 (0.2)	0.33 (0.02)	0.33 (0.02)

Species richness change = 0.016 + 0.0001 EFFL ($p>.997$)
Simpson's index change = 0.004 - 0.001 EFFL ($p>.168$)
where EFFL is wastewater treatment (cm/yr)

Table 3.5. Species diversity of understory trees in 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia (with standard error).

Treatment (cm/yr)	Species richness 1988	Species richness 1990	Simpson's index 1988	Simpson's index 1990
Control	3.4 (0.5)	3.4 (0.4)	0.51 (0.14)	0.57 (0.14)
17.5	5.6 (0.5)	5.5 (0.4)	0.26 (0.02)	0.30 (0.04)
35	3.9 (1.3)	4.3 (1.4)	0.48 (0.18)	0.51 (0.15)
70	3.2 (0.8)	3.3 (0.6)	0.54 (0.14)	0.62 (0.05)
140	4.2 (0.6)	4.4 (0.7)	0.42 (0.14)	0.47 (0.18)

Species richness change = -0.009 + 0.001 EFFL ($p>.285$)
Simpson's index change = -0.008 + 0.0001 EFFL ($p>.948$)
where EFFL is wastewater treatment (cm/yr)

Table 3.6. Species diversity of herbs in 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia (with standard error).

Treatment (cm/yr)	Species richness (d)* 1988	Species richness (d)* 1990	Simpson's index (C)** 1988	Simpson's index (C)** 1990
Control	16.3 (2.8)	13.3 (2.4)	0.59 (0.11)	0.62 (0.12)
17.5	19.3 (3.2)	18.0 (2.0)	0.45 (0.08)	0.51 (0.04)
35	20.1 (3.8)	18.4 (4.2)	0.46 (0.03)	0.50 (0.11)
70	16.7 (1.3)	16.3 (1.9)	0.67 (0.16)	0.61 (0.14)
140	15.9 (2.0)	15.4 (1.5)	0.64 (0.07)	0.56 (0.09)

* $d = S/\log A$ where S: number of species
A: unit area (6 sq.m)

** $C = \sum_{i=1}^{S} \left[\frac{pi}{P} \right]^2$ (Simpson's index)

where pi: ground cover (%) of S individual species

P: total herbaceous ground cover (%)

Species richness change = 0.414 + 0.032 EFFL ($p<0.003$)
Simpson's index change = 0.021 - 0.001 EFFL ($p<0.046$)
where EFFL is wastewater treatment

Species diversity of herbs decreased on all study sites after two years (Table 3.6). The largest change was observed on the control sites, with smaller changes on the irrigated sites. Simpson's index increased on control and low-level irrigation sites (17.5 and 35 cm yr^{-1}), whereas it decreased on high-level irrigation sites (35 and 140 cm yr^{-1}). Wastewater irrigation enhanced the growth of herbaceous species, and caused an equalization in species distribution.

Annual N balance

Aboveground biomass production, estimated by the difference in tree DBH between 1988 and 1990, is presented in Table 3.7. Wastewater-treated plots had less biomass than control plots. The aboveground biomass production, however, was not significantly affected by wastewater treatments.

Plant N uptake and return by litterfall during the two-year irrigation period is presented in Table 3.8. Nitrogen uptake was estimated based on the increases in aboveground tree biomass and leaf biomass. The increases in biomass during the two-year period were multiplied by N concentrations of woody and leaf tissue to estimate the amount of N assimilated by the trees. Root growth of this mature stand was assumed to be equal to root turnover.

There was a difference in N return by litterfall between 1989 and 1990, which may have been related to the hurricane Hugo which passed through the study area in September 1989. The litterfall in 1989 (8,138 kg ha^{-1}) was approximately twice that in 1990 (4,137 kg ha^{-1}). The amount of litterfall was not significantly affected by wastewater treatments in either year. As a result, net N uptake occurring in the

Table 3.7. Aboveground biomass of 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia (with standard error).

Treatment (cm/yr)	Aboveground biomass* 1988	1990	Adj. biomass production
	---- t/ha ----		
Control	232.5 (22.1)	239.2 (22.2)	0.0
17.5	192.1 (8.1)	198.0 (6.4)	-0.8
35	199.2 (12.5)	205.5 (11.1)	-0.4
70	207.9 (7.4)	213.0 (8.5)	-1.6
140	186.5 (16.9)	191.0 (17.4)	-2.2

* Based on biomass equations developed by Clark and Schroeder (1986)

Biomass production = -0.1 - 0.023 EFFL + 0.0001 (EFFL)2 (p>.058)
where EFFL is wastewater treatment (cm/yr)

Table 3.8. Estimated N uptake and return by 80 to 100-year-old upland hardwood vegetation in response to municipal wastewater irrigation in Giles Co., Virginia.

Treatment (cm/yr)	Plant N uptake*	Litterfall N 1988	1990	total	Adj. net N uptake**
	kg N/ha				
Control	186.7	108.7	61.8	170.5	0.0
17.5	174.5	109.2	59.4	168.6	-10.4
35	172.3	99.6	57.8	157.4	-1.2
70	176.3	116.9	54.6	171.5	-11.5
140	182.0	106.5	63.6	170.1	-4.4

* Based on N contents in aboveground biomass including leaf N The values indicate 2-year total N uptake

** Adj. net N uptake = -1.5 - 0.207 EFFL + 0.001 (EFFL)2 (p>.25) where EFFL is wastewater treatment (cm/yr) irrigation area ranged from 4.7 to 16.2 kg ha^{-1}. There was no significant wastewater irrigation effect on net N uptake at this early stage.

Overall, forest vegetation responded very little to the wastewater treatments in this study. Vegetation growth response was not evident even in the 140 cm wk^{-1} treatment sites. Since the wastewater was applied only during the growing season, the highest application rate in this study, 140 cm yr^{-1}, was approximately equivalent to 6.7 cm wk^{-1}. The application rate was slightly higher than the conservative rate, 5 cm wk^{-1}, suggested for safe irrigation in mature stands (Burton, 1982). Wastewater irrigation rates from 5 to 5.6 cm wk^{-1} did not have detrimental effects on the vegetation of mature forests and their environments for periods of 6 to 10 years (Barnett and Arnold, 1986; Reed and Crites, 1986). In conclusion, wastewater irrigation of 17.5 to 140 cm yr^{-1} in a mature hardwood forest had little effect on the growth or health of forest vegetation during the first two years following the start of wastewater irrigation. Wastewater effect on N uptake and retention was also minimal showing that N sequestering by biomass in this mature forest is not a viable avenue for dissipation of excess N.

CHAPTER IV. NITROGEN TRANSFORMATIONS IN WASTEWATER-IRRIGATED SOIL IN AN APPALACHIAN HARDWOOD FOREST IN VIRGINIA

Abstract

Wastewater renovation on forest lands is effective in many situations, but long-term success depends on adequate N dissipation from, or sequestration in, the forest system. Municipal wastewater irrigation effects on N transformations in a mature Appalachian hardwood forest were investigated. Forest soils were irrigated with secondary effluent for two years. Litter N decreased as a result of net N mineralization. Nitrogen mineralization potential (No) decreased greatly in soils irrigated at a rate of 140 cm yr^{-1} for two years. Net nitrification and relative nitrification (the amount of NO_3-N as a proportion of the total mineral N) was enhanced with increasing irrigation. Denitrification activity was not significantly affected by wastewater irrigation and was not different from the rates in control plots. The increase in NO_3^- production and limited N assimilation by the plant/soil system resulted in the loss of N by leaching. Nitrate concentrations in soil water increased in the irrigation sites, with the hightest rates at 11 mg L^{-1} in the 70 cm yr^{-1} treatment sites. Nitrogen leaching losses increased from 3 kg N ha^{-1} yr^{-1} to 14.8 - 105.0 kg N ha^{-1} yr^{-1} by wastewater irrigation.

Introduction

Wastewater renovation projects have been developed successfully in many forested areas (Cole *et al.*, 1986; D'itri, 1982; Sopper and Kerr, 1979). A basic design premise is that wastewater irrigation be based on the N assimilation capacity of the targeted forest, since N is a major source of environmental contamination (Committee on nitrate accumulation, 1972; Council of Environmental Quality, 1989; USEPA, 1983). However, maximum N loading rates are often based on annual stand uptake rates with no consideration of internal cycling, litterfall, root sloughing, decomposition rates, and net mineralization rates that are all site and forest specific.

The fate of effluent N added to the forest is mostly determined by plant demand and the N transformation processes in the soil. The N transformations in a forest ecosystem are the result of very complex processes such as plant uptake, litter decomposition, N mineralization, nitrification, immobilization, fixation, volatilization, denitrification, and dispersion. These processes are affected by soil physical, chemical, and biological properties. By changing the nature of the forest vegetation and forest soil properties, wastewater irrigation may influence the rate of soil N transformations.

Over 90 % of the soil N in the O and A horizons exists in organic form (Stevenson, 1982), and most of the rest exists as NH_4^+ bound or fixed to clay. Only a small fraction of soil N is present in inorganic forms (NO_3^- and NH_4^+) which are the products of N transformation processes. Inorganic N is also added from the atmosphere. The atmospheric input of N is an important long-term source of N. Additions of NH_4^+-N and NO_3^--N along the Eastern Seaboard of the United States vary from 0.3 to 5.0 kg

ha^{-1} yr^{-1} (Wolaver, 1972). A world average of N input is about 9 kg ha^{-1} yr^{-1} (Pritchett and Fisher, 1987).

Nitrogen accumulation and retention in forest vegetation is relatively small because a large portion of the absorbed N is returned to the forest floor by litterfall or by the sloughing of fine roots. Annual retention of N in forests of temperate regions ranges from 5 to 44 kg N ha^{-1} yr^{-1} (Pritchett and Fisher, 1987). Mature forests are considered steady-state forests; tree biomass is neither increasing nor decreasing. Once a steady-state condition is reached, very little additional N accumulates in the biomass of these forests. Atmospheric N added to forests that are in a steady-state condition is roughly equivalent to the amount released by way of N leaching and denitrification.

Adding N-enriched wastewater to a mature forest on a continuous basis will cause a shift in soil and plant processes. If the growth and production of the vegetation is not limited by water or any nutrients the wastewater contains, total biomass will remain the same; however, a shift in species composition and evenness could occur that favors more mesic or hydric species. Soil processes will also shift to the extent that they were limited by water or nutrients.

The rate of organic matter decomposition is key to the N balance in forest ecosystems. The addition of N by wastewater may accelerate litter decomposition. The litter layer has a high C:N ratio ranging from 40 to 60:1 (Keeney, 1980), which normally limits N mineralization. Sewage effluents commonly have a C:N ratio of 5:1 or lower; this results in the addition of available N to the soil N pool. Richenderfer and Sopper (1979) found that wastewater irrigation increased decomposition rates of forest floors in both hardwood and red pine forests. The accelerated decomposition was attributed to the added water and nutrients, and elevated soil temperature caused by the irrigation.

Wastewater irrigation also affects the mineralization of soil N. In undisturbed forests, the N mineralization activity is usually very low because of the acidic soil condition. Soils to which wastewater has been applied have a high pH and high NH_4^+ levels. These conditions stimulate N mineralization (Cassman and Munns, 1980; Focht and Vestraete, 1977; Gilmour, 1984; Terry *et al.*, 1981). Available NH_4^+ that is not immobilized by plants or microorganisms is readily converted to NO_3^- which is susceptible to leaching.

The amount of NO_3^- leached into groundwater depends on nitrification, N immobilization, plant uptake, and denitrification (Figure 4.1). Nitrate is very mobile in the soil and susceptible to leaching into groundwater aquifers unless it is denitrified or absorbed by plants. In mature forest systems, uptake is minimal (Chapter III); therefore, denitrification and leaching are the only possible fates for excess NO_3^-.

Denitrification occurs in forest soils at measurable rates (Melillo *et al.*, 1983; Robertson and Tiedje, 1984) when soil and forest environments are appropriate. Research results suggest that denitrification potential is higher in young and old-growth forests, but substantially lower in mid-successional forests. Plants in the mid-successional forests immobilize most NO_3^-, leaving less for denitrification. Reports of wastewater-irrigation effects on denitrification in forest areas are few; however, the changes in soil environments by wastewater irrigation should be favorable for denitrification if managed properly. Sopper and Kardos (1972) suggested that the irrigation sequence be altered to create a period of soil saturation, or that imperfectly drained soils be used. The anaerobic conditions thus provided will enhance denitrification in the irrigation field. In

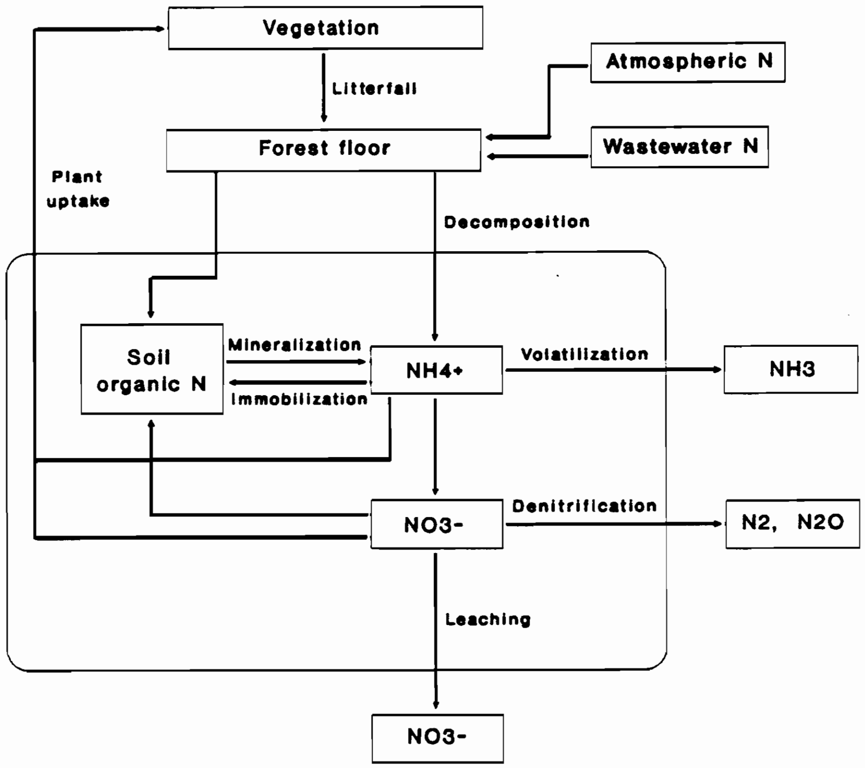


Figure 4.1. Nitrogen transformations and transport in a forest system.

a study by Sopper and Kerr (1979), an irrigation rate of 2.5 cm wk^{-1} during the growing seasons resulted in less than 10 mg L^{-1} of NO_3^- in soil water during the first 6 years, but concentrations did increase steadily. Long-term use of forest lands for wastewater recycling can be assured by converting them to young, aggrading stands and controlling irrigation rate and timing (Sopper, 1986). In young, regenerating stands, NO_3^- in soil water can be significantly reduced by rapid N assimilation and accumulation in the vegetation.

An apparantly successful wastewater renovation system has been developed in Georgia (Nutter, 1986). A hydraulic loading of 6.4 cm wk^{-1} with secondarily treated wastewater applied year-round in actively growing loblolly pine plantations has resulted in no change in groundwater quality and increased stream flow. This system is based on N removals from the site via whole-tree harvesting. However, in old-growth, steady-state forests that are not harvested, NO_3^- uptake is limited and could pose a threat to groundwater if not denitrified or otherwise immobilized in the plant/soil system.

Success of wastewater irrigation systems on forest lands with respect to N loading is usually judged by monitoring NO_3^- levels in groundwater after the system is put in place and is operating. This measure does not account for long-term forest health which will ultimately determine system success. The impacts of the irrigation on forest health and all N transformation processes should be used as criteria to determine N loading. Confidence in wastewater irrigation of forests on a long-term basis will depend on a full understanding of forest response and N cycling. The objectives of this study were to investigate the N transformations in the soil of a mature forest as affected by wastewater irrigation, and to determine if there is a net influx or eflux of N to or from the system.

Methods

Major N transformation pathways including litter decomposition, N mineralization, nitrification, denitrification, and NO_3^- leaching were measured. Nitrogen pools in forest floor and soil were also estimated before and after wastewater irrigation. O horizon biomass and soil samples were collected in late fall of 1988 and 1990 after the leaves had fallen from the trees. O horizon biomass was collected with a 30 x 30 cm frame from three random locations in each plot. The collected samples were oven-dried at 65°C and analyzed for total carbon (C) using a Leco C analyzer. Nutrient elements were analyzed by methods described in Chapter III. Soil samples were collected from A and B horizons at the same locations where O horizon biomass was sampled. The soil samples were air-dried and sieved with a 2-mm mesh screen for C and nutrient analyses. Soil C was measured using a Leco C analyzer. Total soil N was measured using Kjeldahl techniques (Bremner, 1965). For other nutrient elements such as total P, K, Ca, Mg, Fe, and Al, soil samples were extracted with double acid extract (0.05 N HCl + 0.025 N H_2SO_4) and analyzed using an ICP spectrometer.

Litter decomposition rate was measured by a litter bag method (Berg *et al.*, 1982; Edmonds, 1984; McClaugherty and Berg, 1987). Litter bags measuring 20 x 20 cm were made of polyethylene net with a mesh size of 1 mm. Fresh leaf litter was collected from the site in the fall of 1988 and air-dried for one week. Oven dry weight (65°C for 48 hr) was determined on a subsample. The equivalent of 4 g oven dry weight of the litter was placed in each bag. Nine litter bags were buried in the litter layer of a randomly selected point in each plot. The bags were collected after 6, 9, 12, 18, and 24 months.

The dry weight loss of the litter was determined by oven-drying the contents to constant weight at 65°C. The N and C concentrations in the litter were determined by methods used for the analysis of the litter layer in Chapter III.

Nitrogen mineralization potential was estimated using an aerobic incubation method (Burger and Pritchett, 1984; Stanford and Smith, 1972). The soil samples were collected from the site in November, 1988, before irrigation, and in November, 1990, after the irrigation was completed for the year. A subsample of 75 g of fresh, sieved soil was mixed with 100 g of dry sand, and packed in the incubation tubes (4.5 cm dia., 30 cm long). After leaching with 0.01 M $CaCl_2$ solution to remove initial mineral N, the soil columns were incubated at 35°C for two weeks. Then the columns were leached with 250 ml of 0.01 M $CaCl_2$ solution, followed by 100 ml of Hoagland minus-N nutrient solution (Hoagland and Arnon, 1950). The procedure was repeated for 16 to 20 weeks. Mineralized N (NH_4^+ and NO_3^-) was determined by a Technicon Autoanalyzer. An approximation of N mineralization potential (No) was obtained using the following equation:

$$1/Nt = (1/No) + (b/t)$$

where Nt is cumulative mineralization during a specified period of time (mg N/kg dry soil), t is time in weeks, b is the rate of mineralization, and No is the N mineralization potential (mg N/kg dry soil). The regression analysis assumes that N mineralization is a first-order reaction described by:

$$dN/dt = -kN$$

where N is mineralizable N, k is a rate constant, and t is time.

Net N mineralization and nitrification rates were measured using an *in situ* incubation method with polyethylene bags. Soil samples were collected from the surface horizon at three points in a plot. The fresh soil samples were composited and placed in three polyethylene bags (0.025 mm thick) sized 6 x 16 cm. The bags were buried in the A horizon at random locations where the samples were collected. The soil samples, after incubating for 30 days, were collected and air-dried. The soil was extracted with 2 M KCl and analyzed for inorganic N using a Technicon Autoanalyzer. Net N mineralization was estimated by subtracting the inorganic N concentrations in unincubated soils from the concentrations in field-incubated soils. The net increase of NO_3^- during the incubation period was considered net nitrification.

Denitrification was measured by acetylene inhibition with a sealed chamber method (Hauck, 1986; Ryden and Rolston, 1983). The N_2O which is a minor atmospheric constituent (300 ppb) is a product of denitrification along with N_2 gas. The inhibition of N_2O reductase activity by acetylene has been shown by Balderstone *et al.* (1976). Therefore, the amount of N_2O produced in the presence of acetylene is a direct measure of the total gaseous N produced by denitrification activity. The sealed chamber method was used in this study because the very stony nature of the soil was not conducive to column methods. Three bottomless PVC chambers were randomly located in each plot. The size and the installation of the chamber set is shown in Figure 4.2. To start denitrification measurement, acetylene gas was injected through the four diffusion tubes installed around the chamber. Acetylene gas was injected for fifteen minutes to establish at least 1 KPa of acetylene, which is a desired level to block N_2O conversion to

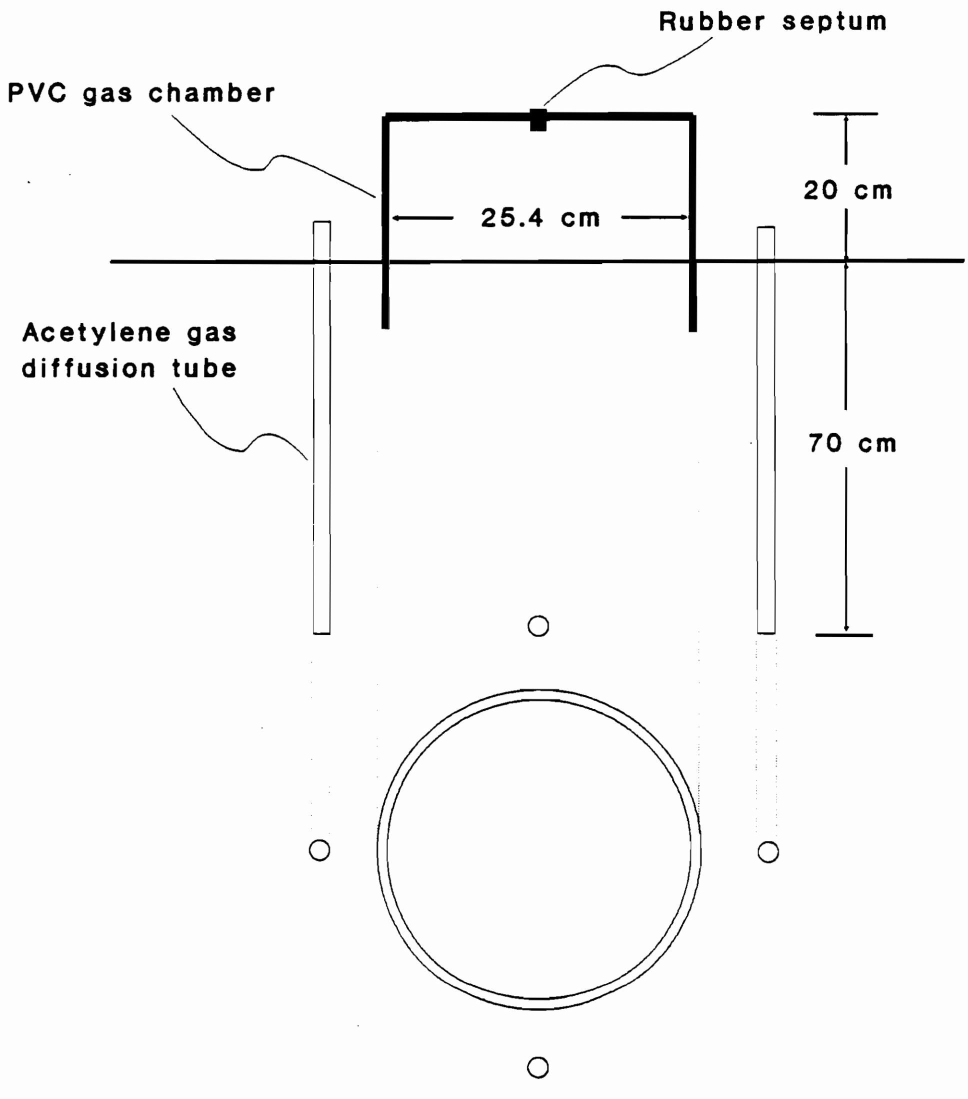


Figure 4.2. Chamber installation for the measurement of denitrification in the field.

N_2 gas. Burton and Beauchamp (1984) were able to establish the required 1 KPa acetylene partial pressure within 15 minutes at all moisture levels studied. After the acetylene injection, the chamber was covered and sealed with a plastic plate. The first gas sample was taken (t_0) with subsequent sample collection at 10 (t_1) and 20 (t_2) minutes. The acetylene remaining after incubation is consumed by soil microorganisms as an energy source (Germon, 1980); acetylene is degraded within 3 to 4 days after an anaerobic incubation (Watanabe and de Guzman, 1980). Soil temperature at the 15 cm depth was measured along with denitrification. Denitrification measurements were repeated every month during the growing season, from May to October.

The gas samples were analyzed for N_2O using a Varian 3400 gas chromatograph, equipped with ^{63}Ni electron-capture detector and a column packed with porapak Q. Nitrous oxide production was estimated by the following equation:

$$N_2O \text{ (kg ha}^{-1} \text{ day}^{-1}) = (N_2 - N_1)/(T \times A)$$

where N_1 and N_2 are N_2O concentration at time t_0 and t_1, respectively, T is incubation time in minutes, and A is the surface area covered by the chamber.

Soil water leachate was collected by suction cup lysimeters at a 1 m depth (Hansen and Harris, 1975). Since most denitrification activity has been found within a 1 m depth (Brar *et al.*, 1978; Firestone, 1982; Gilliam *et al.*, 1978; Rolstone *et al.*, 1976), it was assumed that NO_3^- leaching below 1 m stayed unchanged in the soil water and became a source of groundwater N. Three lysimeters were installed at random locations in each plot (Fig. 4.3). A continuous tension was provided by a hanging water column 3.5 m in height, which created approximately -35 KPa of water potential. Soil water

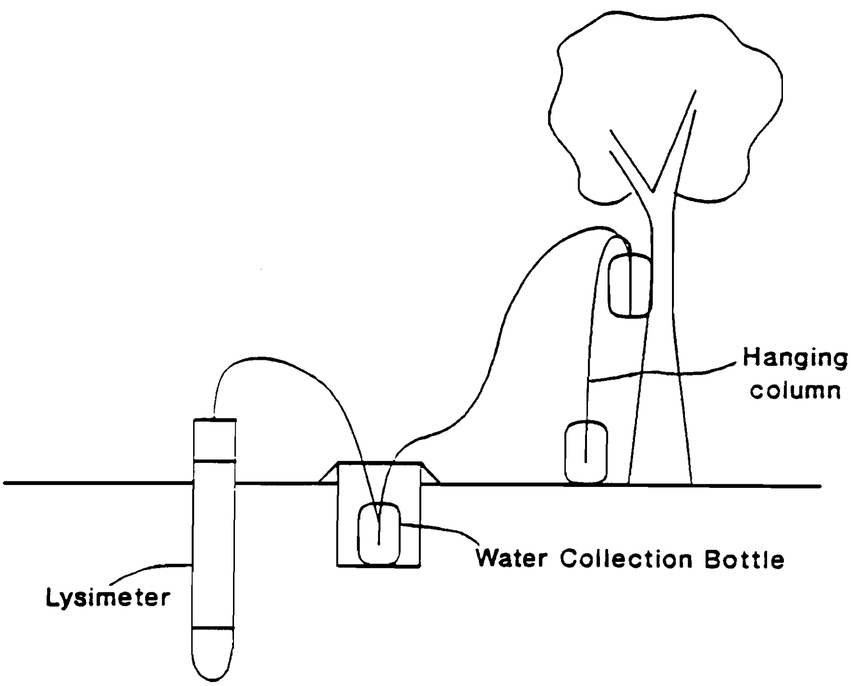


Figure 4.3. Lysimeter installation for soil water sampling.

samples were collected every other week during the spray period and stored in a freezer. The water samples were analyzed for inorganic N using a Technicon Autoanalyzer.

Water balance in the soil was described by:

$$\text{Precipitation} + \text{Irrigation} = \text{Runoff} + \text{Leaching} + \Delta S + ET$$

where S is the change in storage of soil water and ET is evapotranspiraton. Surface runoff was negligible in the study site. Rearrangement of the equation to calculate the amount of water leached from the system yields:

$$\text{Precipitation} + \text{Irrigation} - \Delta S - ET = \text{Leaching}$$

Precipitation was measured at a weather station located in the Horton Center of Virginia Tech near Mountain Lake. The amount of wastewater irrigated on the site was estimated from the irrigation operation record. Soil water storage change was estimated from the differences in soil moisture content. Evapotranspiration was substituted with potential evapotranspiration (PET) because actual ET is difficult to measure. Potential evapotranspiration was calculated using Thornthwaite's method (Thornthwaite, 1948). Although PET is normally greater than ET, ET would increase on the wastewater irrigation sites due to the added moisture and would be closer to PET. In any case, PET would overestimate actual ET. Nitrogen leaching was calculated by multiplying the N concentrations in soil water by the amount of leached water.

The response of each soil N transformation process to increasing rates of effluent was statistically analyzed by SAS using PROC REG (SAS Institute, 1982). The data collected had spatial and temporal variations. The data collected after the two-year irrigation treatments were adjusted for the temporal variation by subtracting the change in control plots from the change in treatment plots. The spatial variation was avoided by using the 1988 values as covariates in multiple regression. The relationships between wastewater irrigation and soil N transformation processes were re-evaluated after removing the covariate term from the equation using stagewise regression (Chapter II).

Results and Discussion

Nitrogen storage in the system

Nitrogen storage in ecosystem components of the study site are presented in Table 4.1. Nitrogen in vegetation was lower than that in litter and soil. It increased slightly after two years (1991), but no wastewater treatment effect was evident. Nitrogen storage in the O horizon was 17 to 34% less on wastewater-treated sites. Although the N in the O horizon of control plots also decreased, the litter N tended to decrease more with increasing irrigation. While the N pool decreased in the O horizon, total soil N increased, mostly in the A horizon. As a result, system N, including the upper 30 cm of soil, increased from 19.3 to 172.2 kg ha^{-1} after the two-year irrigation. Soil C and other nutrients also increased after irrigation (Table 4.2). Soil nutrients such as P, K, Ca, and Mg increased on the irrigation sites, whereas Fe and Al decreased slightly. Soil nutrients tended to increase with increasing levels of irrigation.

Table 4.1. Nitrogen storage in 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia.

Ecosystem component	Control	Wastewater treatment (cm/yr) 17.5	35	70	140
	kg/ha				
(1988)					
Vegetation*	157.9	121.4	111.8	135.3	128.0
O horizon	243.9	238.9	216.6	281.7	298.5
A horizon	1086.5	1017.4	1083.4	1145.4	1038.8
B horizon**	1434.4	1623.6	1750.7	1736.9	1657.1
Total	2922.7	3001.3	3162.5	3299.3	3122.4
(1990)					
Vegetation	162.5	125.0	115.2	138.7	131.1
O horizon	205.4	159.1	180.9	207.5	195.6
A horizon	1123.5	1212.5	1220.3	1268.3	1125.8
B horizon	1419.6	1610.1	1734.7	1704.1	1842.1
Total	2911.0	3106.7	3251.1	3318.6	3294.6
N storage change	-11.7	105.4	88.6	19.3	172.2

* N storage in stem biomass
** B horizon soil above 30 cm depth

Table 4.2. Carbon and nutrients in the soil of 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia.

Nutrients	Wastewater treatment (cm/yr)				
	Control	17.5	35	70	140
Total C (g/kg)					
A	110.1	131.3	128.2	145.0	120.9
B	29.7	40.7	43.6	48.0	49.7
Total N (g/kg)					
A	6.1	6.6	7.1	7.4	6.1
B	1.7	2.2	2.5	2.6	2.8
C/N ratio					
A	18.1	20.5	18.4	20.0	20.2
B	17.7	18.2	17.3	18.4	18.0
P (mg/kg)					
A	67.6	57.0	73.8	148.0	167.0
B	47.9	15.7	9.6	110.7	109.7
K (mg/kg)					
A	292.2	357.0	335.6	467.8	477.8
B	70.5	63.0	83.6	217.5	249.5
Ca (mg/kg)					
A	526.5	1558.2	1095.5	3118.0	4180.0
B	46.7	86.6	69.0	174.4	361.6
Mg (mg/kg)					
A	93.0	177.5	135.4	472.1	457.4
B	14.3	19.0	18.3	49.3	81.7
Fe (mg/kg)					
A	196.1	93.8	122.2	145.8	94.3
B	62.7	79.5	36.9	82.6	92.1
Al (mg/kg)					
A	3056.0	2731.0	3127.0	1879.0	2066.0
B	5229.0	5230.0	5910.0	5816.0	5447.0

Total C	A = 125.6933 + 0.043 EFFL	(p=.7465)
	B = 36.1775 + 0.1174 EFFL	(p=.0131)
Total N	A = 6.6948 + 0.0008 EFFL	(p=.9275)
	B = 2.0523 + 0.0064 EFFL	(p=.0310)
C/N ratio	A = 18.9124 + 0.0103 EFFL	(p=.5067)
	B = 17.8235 + 0.0021 EFFL	(p=.8591)
P	A = 76.4075 + 0.6566 EFFL	(p=.0574)
	B = 38.4733 + 0.2811 EFFL	(p=.3938)
K	A = 334.4408 + 1.0959 EFFL	(p=.1089)
	B = 61.8592 + 1.3872 EFFL	(p=.0013)
Ca	A = 690.56 + 24.8324 EFFL	(p=.0078)
	B = 26.7117 + 2.2117 EFFL	(p=.0151)
Mg	A = 122.8751 + 2.5618 EFFL	(p=.0145)
	B = 10.0167 + 0.4848 EFFL	(p=.0098)
Fe	A = 170.27 - 0.3411 EFFL	(p=.5958)
	B = 53.1467 - 0.3234 EFFL	(p=.2781)
Al	A = 3470.1667 - 11.0152 EFFL	(p=.0105)
	b = 5351.7 + 0.9314 EFFL	(p=.8025)

where EFFL is wastewater treatment (cm/yr).

Litter decomposition

The weight loss from the litterbags was 58% after the first year and 70% after the second year (Fig. 4.4). The rate of weight loss in the control sites tended to be slightly lower than that in the wastewater treated sites; however, no significant wastewater treatment effect was detected. Weight loss from the litterbags during the first six months suggested that decomposing microorganisms were active under the snow during the winter months. The rate of weight loss in this study was a little higher than the rates found in mixed oak forests in Ohio, which ranged from 30 to 50% over one year (Boerner, 1984), and was lower than the rates of N-rich alder leaves which lost 60 to 81% of the weight within 9 months (Kjoller and Struwe, 1980; Yamaya, 1968). Although there was no treatment effect on weight loss of fresh leaf litter, the treatment effect was evident in the litter mass on the forest floor, showing greater weight loss in the irrigation sites (Fig. 4.5). Decomposition constants (k), which are defined as the ratio of litterfall to litter mass, were not affected by wastewater treatments (Table 4.3). There were differences in annual litterfall between 1989 and 1990 (Table 3.8). The average of the litterfall in both years was used to determine k.

Nitrogen concentration of the litter in the bags, with an initial N concentration of 11.8 mg N g^{-1}, increased significantly during the first year on all sites (Fig. 4.6). The concentration did not change during the second year. While the weight loss was rapid during the first year, net N immobilization occurred in the litter of all sites except for the control (Fig. 4.7). Net increase of N in litter is common in early stages of litterbag experiments. Nitrogen accumulations in the litter also have been observed in other litterbag experiments and lasted for one to six years (Boerner, 1984; Edmonds, 1984;

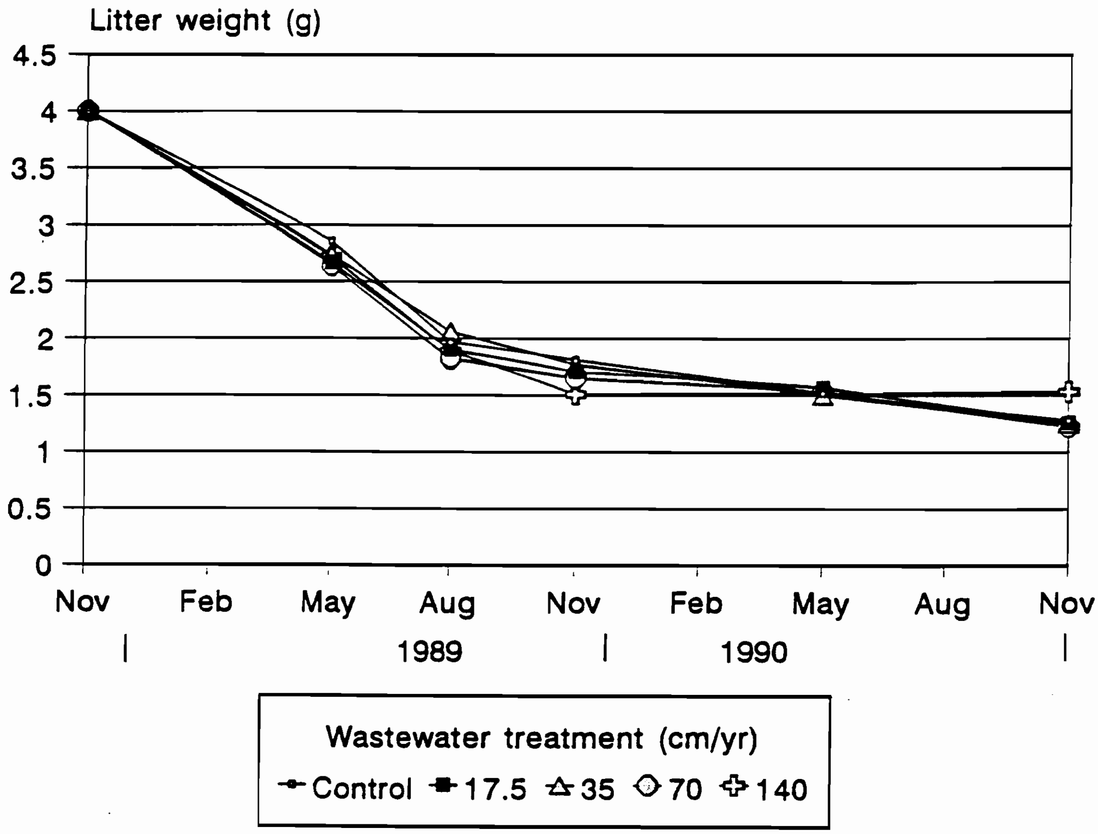


Figure 4.4. Mean weight loss of litter from litterbags in 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia.

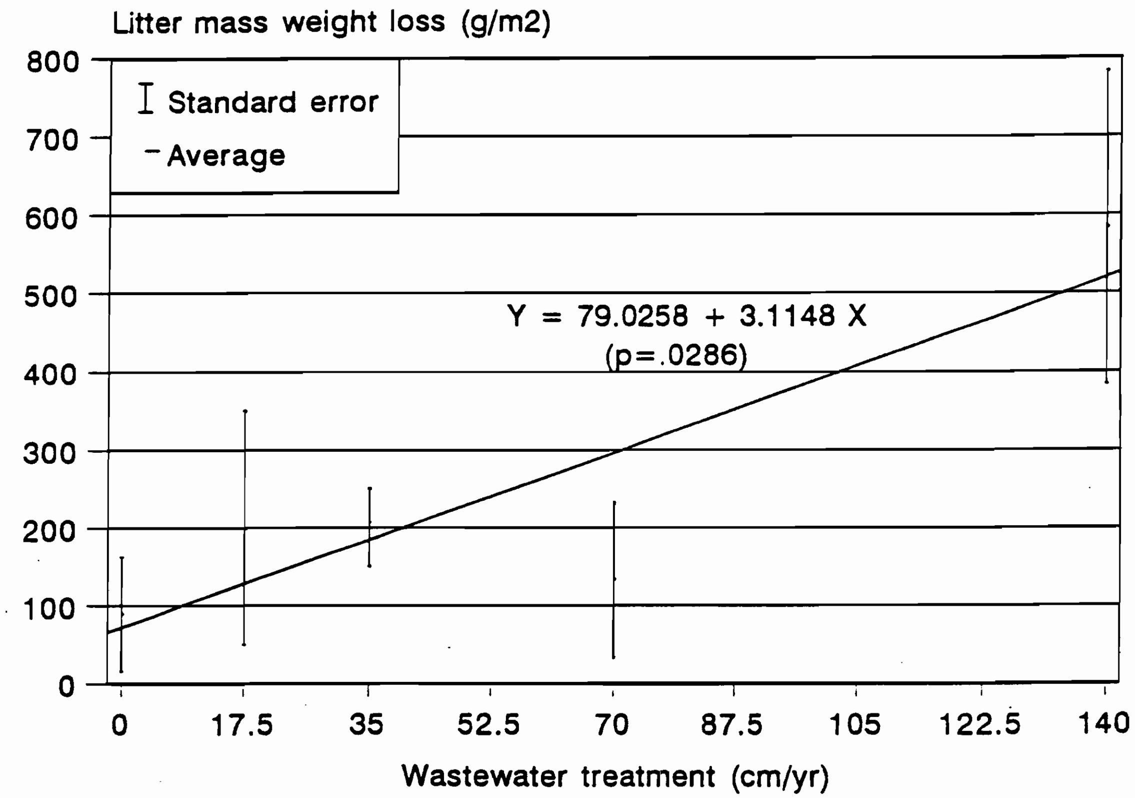


Figure 4.5. Relationship between wastewater irrigation and weight loss of litter in 80 to 100-year-old upland hardwood stands in Giles Co., Virginia.

Table 4.3. Decomposition constants and turnover time in 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia.

Treatment	Decomposition constants, k*	Turnover time (half life)**
		---- year ----
Control	0.36 ± 0.02***	1.91 ± 0.08
17.5	0.46 ± 0.09	1.61 ± 0.26
35	0.39 ± 0.04	1.80 ± 0.18
70	0.42 ± 0.04	1.70 ± 0.18
140	0.38 ± 0.03	1.82 ± 0.13

* k = litterfall/litter mass
k = 0.378 + 0.0001 EFFL (p>.556)
** Half life = 1.853 - 0.001 EFFL (p>.584)
where X is wastewater treatment (cm/yr)
*** ± standard error

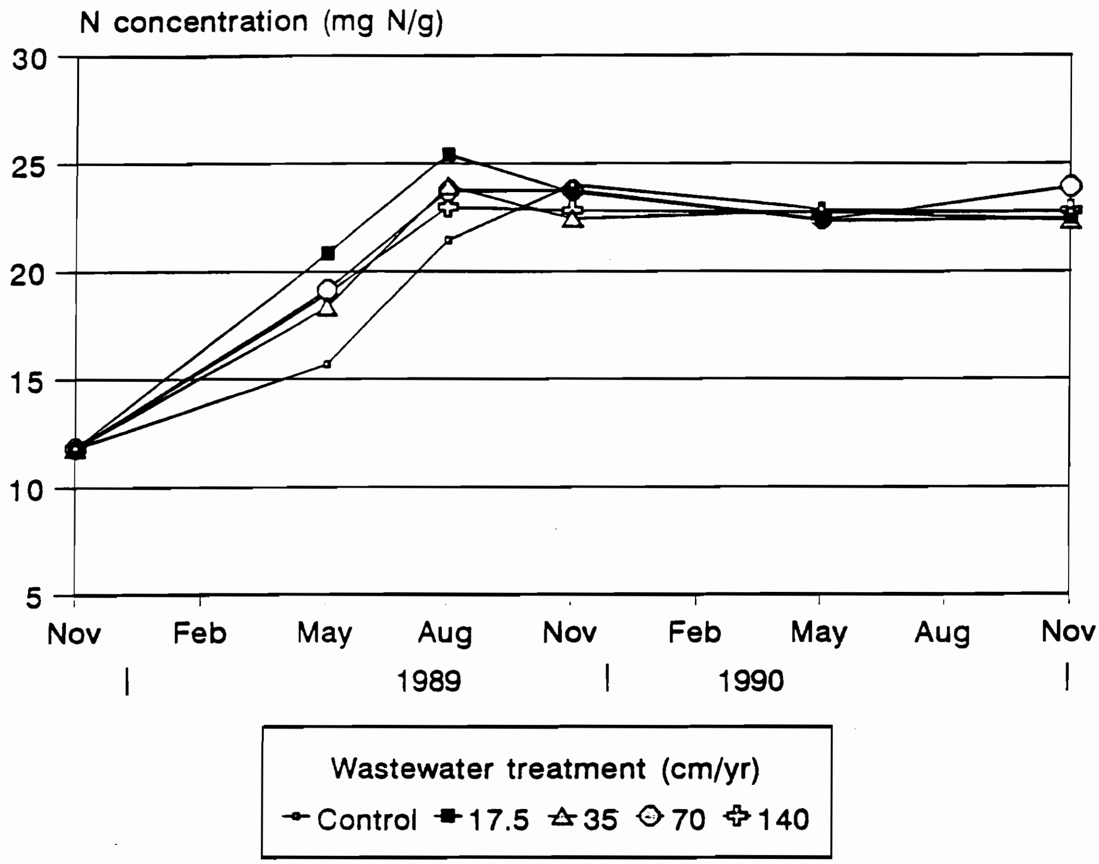


Figure 4.6. Nitrogen concentrations in leaf litter during decomposition in 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia.

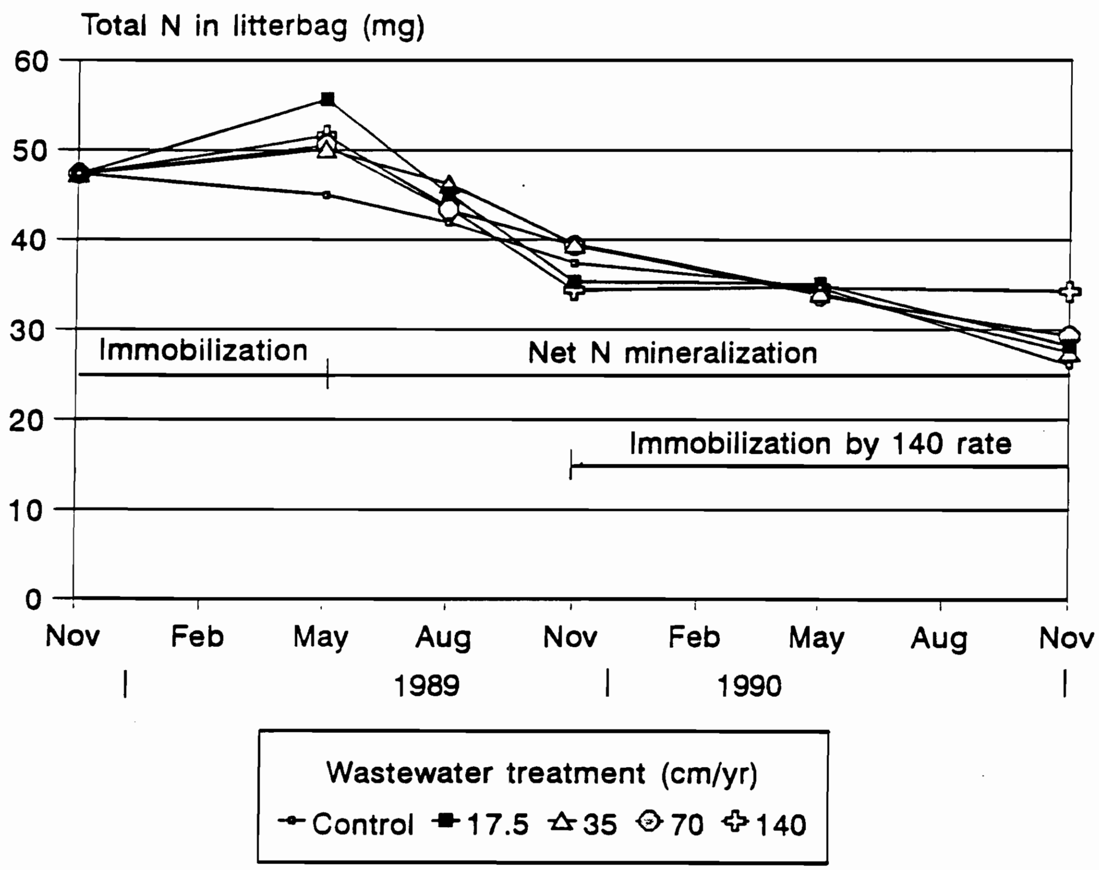


Figure 4.7. Nitrogen contents in leaf litter during decomposition in 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia.

Gosz *et al.*, 1973; Schlesinger and Hasey, 1981). Active heteroprophs with C:N ratio of 4:1 to 9:1 (Buckman and Brady, 1960) demand large amounts of N and retain most of it while C is consumed as an energy source. This causes an increase in N concentration in litter and a decrease in the C:N ratio (Fig. 4.8). The increase in N content in the litter indicates addition and accumulation from the environment. The large demand for N by heterotrophs sequesters N from wastewater, precipitation, insect debris, and green litter.

Net N mineralization started after 6 months (Fig.4.7). Nitrogen release in this study started several months earlier than those of other studies (Boerner, 1984; Edmonds, 1984; Gosz *et al.*, 1973; Staaf and Berg, 1982). Nitrogen release from the litter was highly correlated with weight loss after net N mineralization started (Fig. 4.9).

Net N mineralization in the surface soil

Net N mineralization is reported as the net production of total mineral N (NO_3-N and NH_4-N) (Fig. 4.10). The net production values were determined by subtracting the initial mineral N concentration from the concentration after a 30-day *in situ* incubation. Net N mineralization was not affected by the wastewater irrigation in the first year (1989), except for the 140 cm yr^{-1} treatment sites. The net N mineralization in the 140 cm yr^{-1} treatment sites was twice that of other treatments (Fig. 4.11). Net N mineralization more than doubled in the second year (1990), which might have been due to the early start of wastewater irrigation (early May in 1990 vs. late June in 1989), in addition to the cumulative wastewater effect on the site. Net N mineralization was also higher in control plots in 1990. The year-to-year variation of N mineralization was due to the differences in year-to-year climate. Average annual temperature in 1990 was

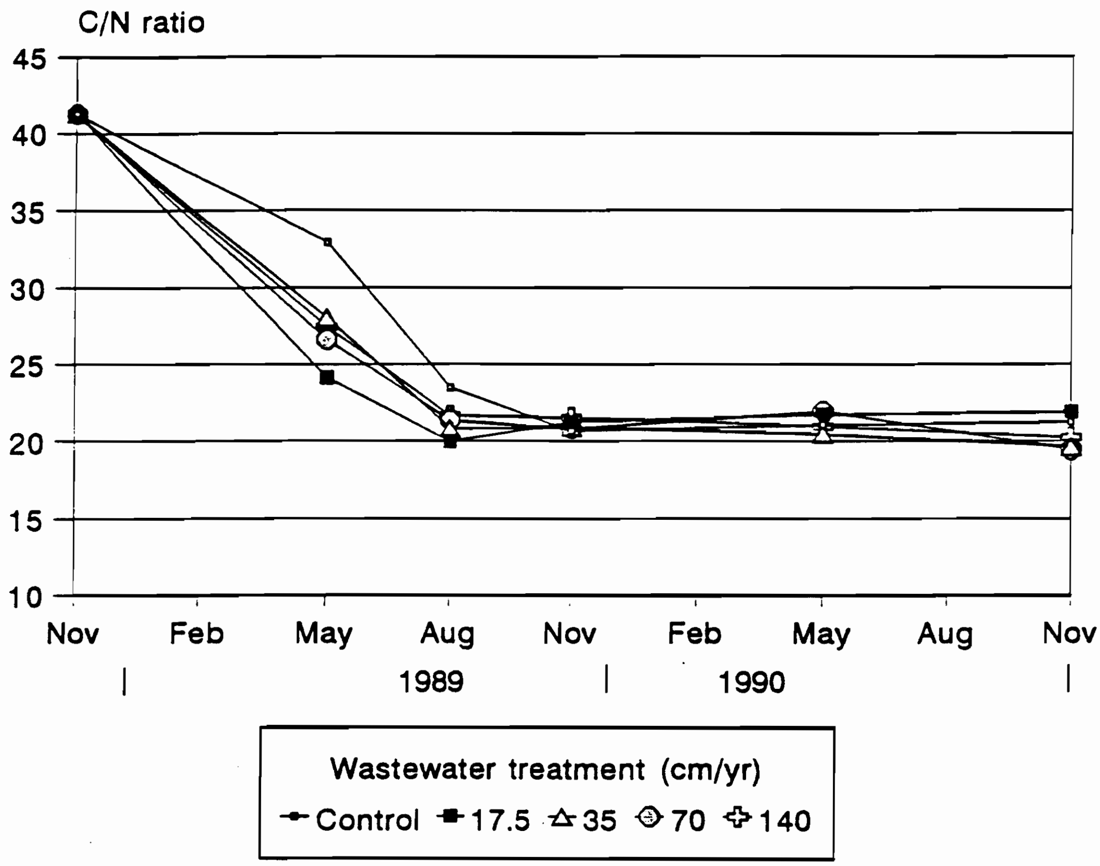


Figure 4.8. Change in leaf litter C:N ratio during decomposition in 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia.

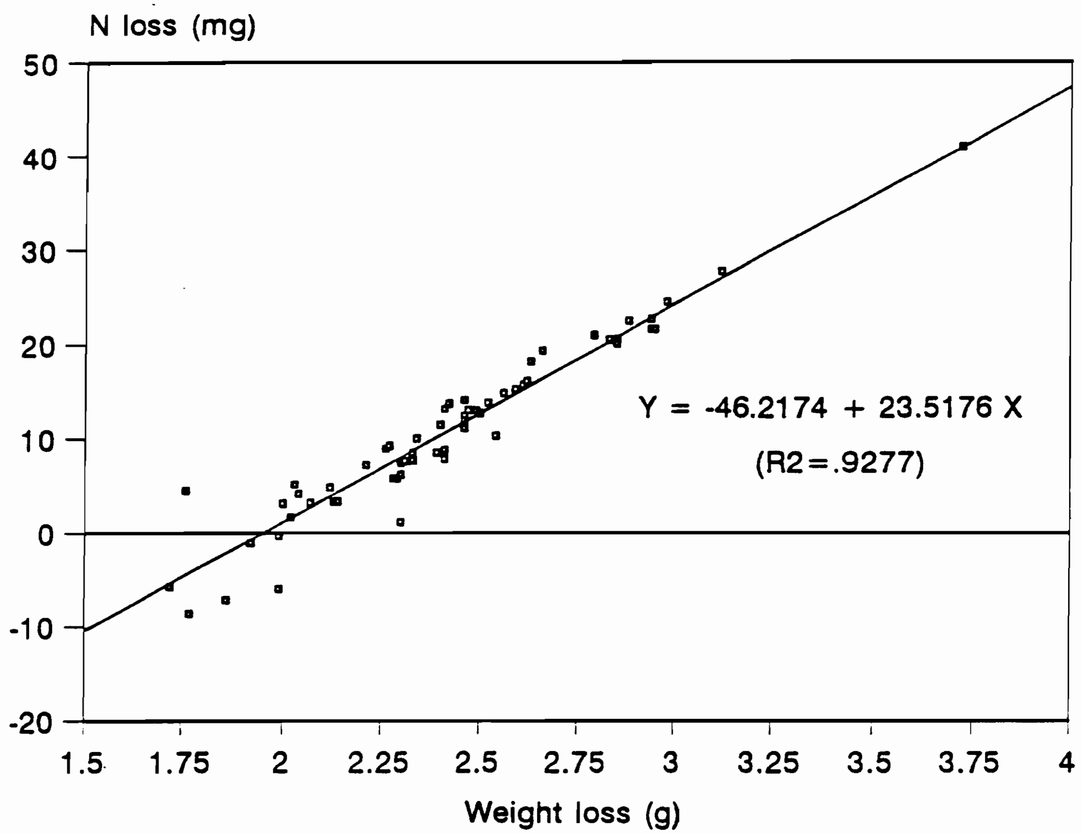


Figure 4.9. Relationship between litter weight loss and N release from litter in 80 to 100-year-old upland hardwood stands in Giles Co., Virginia.

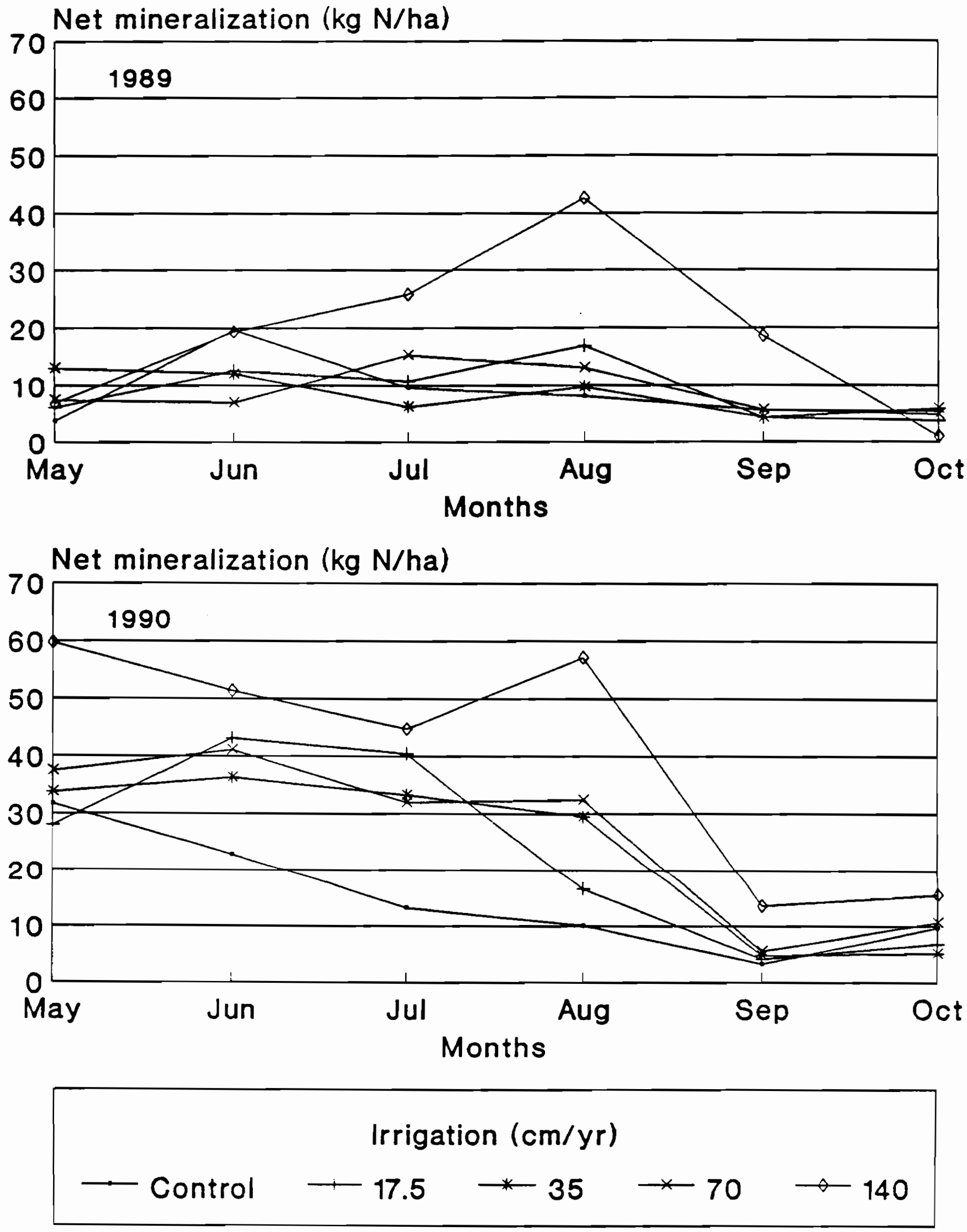


Figure 4.10. Net N mineralization in 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia.

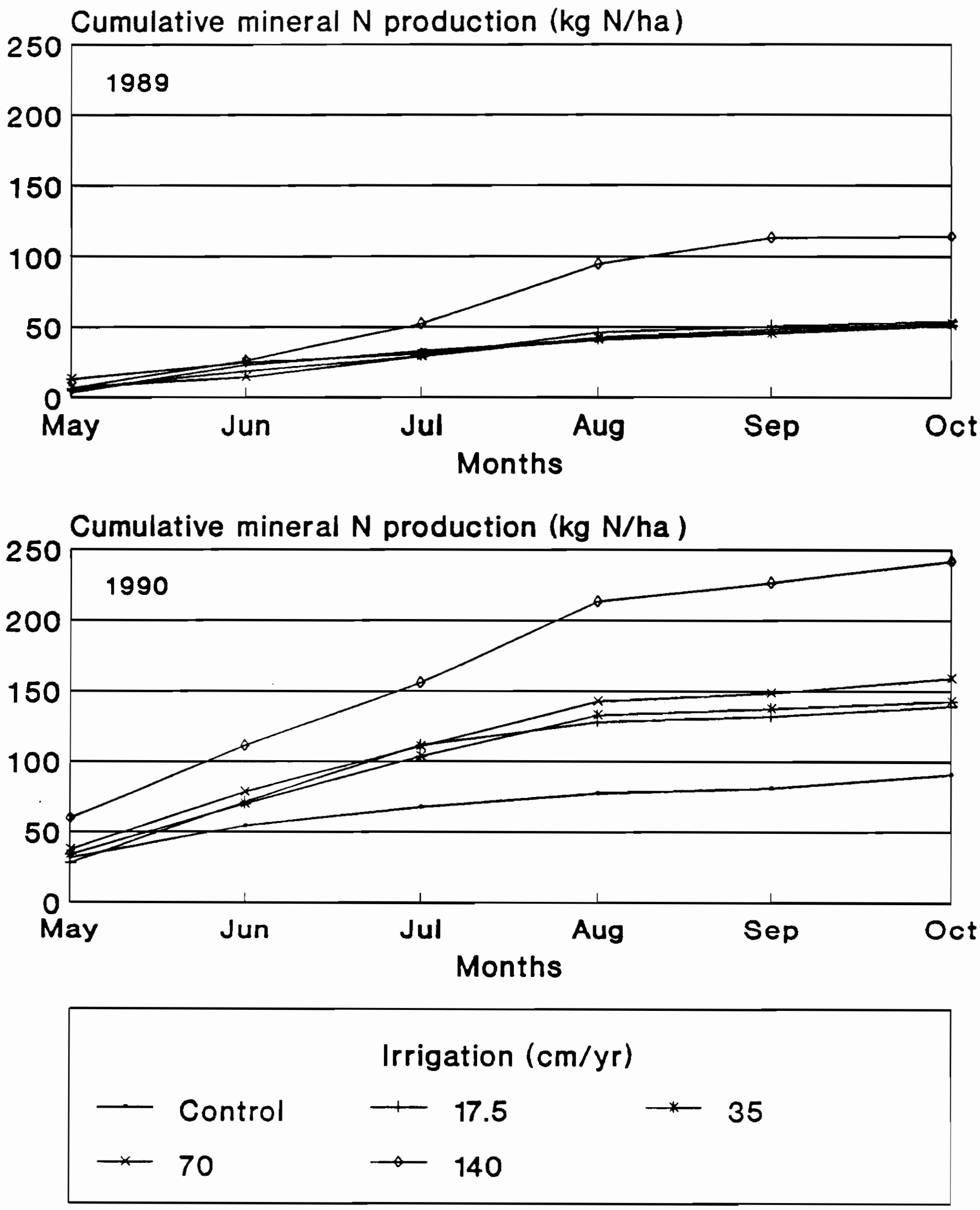


Figure 4.11. Cumulative N mineralization in 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia.

1.8°C higher than the average in 1989.

The net N mineralization was a function of irrigation rate during the first three years. An additional investigation in 1991 showed that net N mineralization rates on the 140 cm yr^{-1} treatment sites decreased to the first year level in 1991, while the rates on other sites were unchanged (Fig. 4.12). A stabilized rate of N mineralization similar to that of the control would probably be obtained at all sites after a few more years.

Soil N mineralization generally increases with increasing levels of soil moisture (Focht and Verstraete, 1977; Matson and Vitousek, 1981). This effect on N mineralization was evident in clearcut sites where soil moisture as well as soil temperature increased (Bormann *et al.*, 1974). This study also showed that net N mineralization was highest on the sites receiving the highest irrigation. Higher levels of added N and water stimulated the soil N transformation processes. Since potential N storage in vegetation in this mature forest is minimal (Table 4.1), leaching from the irrigation sites, as a final product of the N transformations, is likely.

N mineralization potential

Soil N mineralization potential (N_o) was significantly affected by the highest wastewater irrigation rate (Table 4.4). The N mineralization data were produced from the results of 16 weeks of aerobic incubation in 1988 and 20 weeks in 1990. The percent of total N mineralized during the two-year period increased with increasing irrigation intensities (Fig. 4.13). However, the N mineralization potential was reduced considerably in soils having received wastewater at a rate of 140 cm yr^{-1}. Levels of readily-mineralizable N were reduced in the soil due to higher levels of N mineralization. Net N

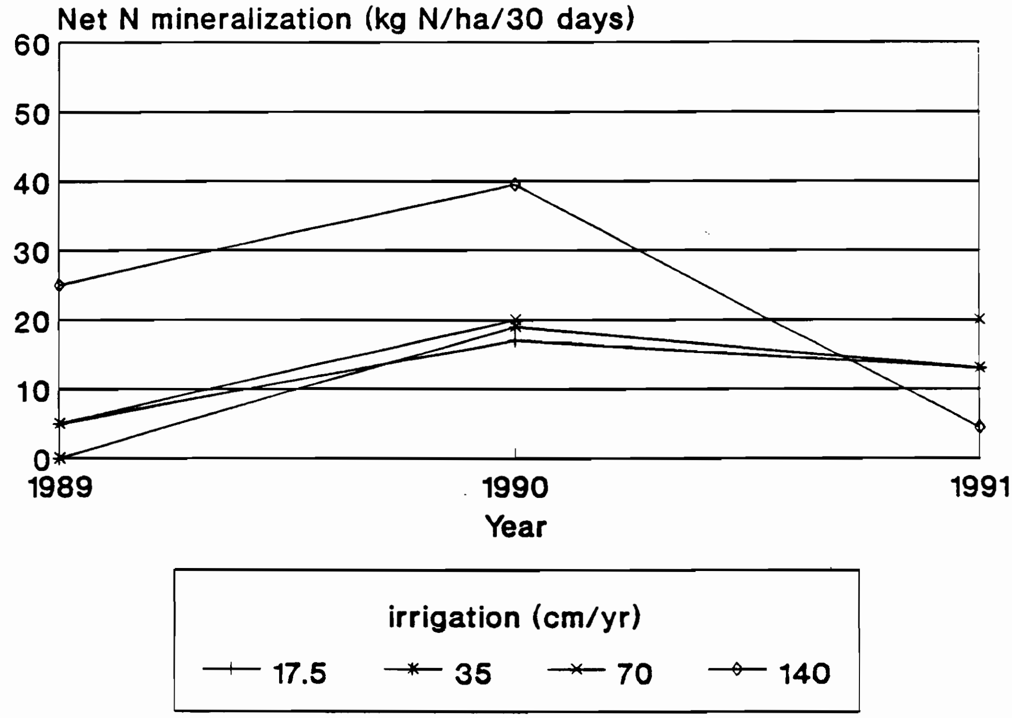


Figure 4.12. Average net N mineralization with time in 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia.

Table 4.4. Nitrogen mineralization potential (N_0) in the soil of 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia (with standard error).

Treatment (cm/yr)	N mineralization potential, 1988	1990	No* Adj. change
	mg N/kg		
Control	636 (36)	644 (41)	0
17.5	580 (16)	552 (24)	-36
35	563 (22)	547 (59)	-24
70	743 (21)	726 (45)	-25
140	733 (38)	587 (52)	-127

* No change = -16.846 + 0.308 EFFL - 0.009 (EFFL)2 (p>.064)
  where EFFL is wastewater treatment (cm/yr)

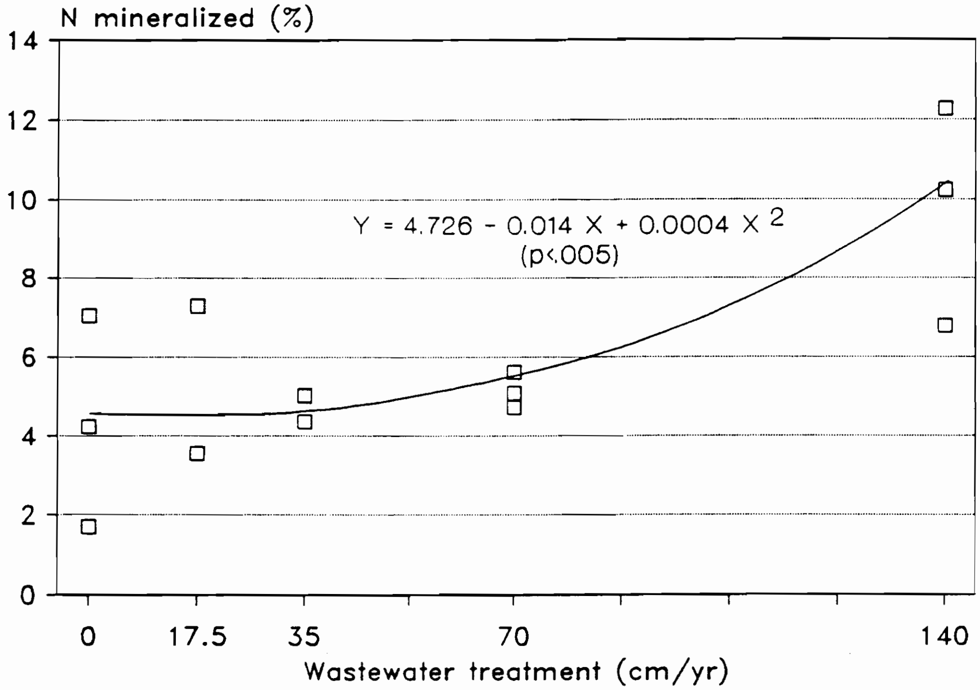


Figure 4.13. Percent of total soil N mineralized after 2 years as a result of wastewater irrigation in 80 to 100-year-old upland hardwood stands in Giles Co., Virginia.

mineralization on 140 cm yr^{-1} treatment sites culminated in 1990 and equilibrated in 1991 after the N_o values had been reduced by 6.5% of soil N (Fig 4.12). In other irrigation sites, where N_o values had been reduced by 2.4 to 2.8% of soil N during the two years, net N mineralization would also be equilibrated with continuous irrigation. Providing that soil N transformations occur at the same rates, net N mineralization would stabilize at all sites in three years.

Net nitrification

Net production of NO_3^- showed a similar pattern as net N mineralization (Fig. 4.14, 4.15, 4.16). Net nitrification is at least partly a function of N mineralization by which organic N is transformed to a form available to nitrifying bacteria. Relative nitrification (the amount of NO_3^--N as a proportion of mineralized N) was inconsistent with treatment during the first year (Fig. 4.17). In the second year, relative nitrification increased consistently with increasing irrigation. Almost all of the N produced in the 140 cm yr^{-1} treatment sites was NO_3^--N in the second year.

The results suggest that increases in wastewater treatment yielded significant increases in net nitrification. Matson and Vitousek (1981) found in a study, conducted in the Hoosier National Forest, Indiana, that the rates of N mineralization and nitrification increased with increased soil moisture. Nitrification processes were stimulated in this study by the increase in soil pH as well as soil moisture. Net nitrification was positively related to soil pH (Fig. 4.18).

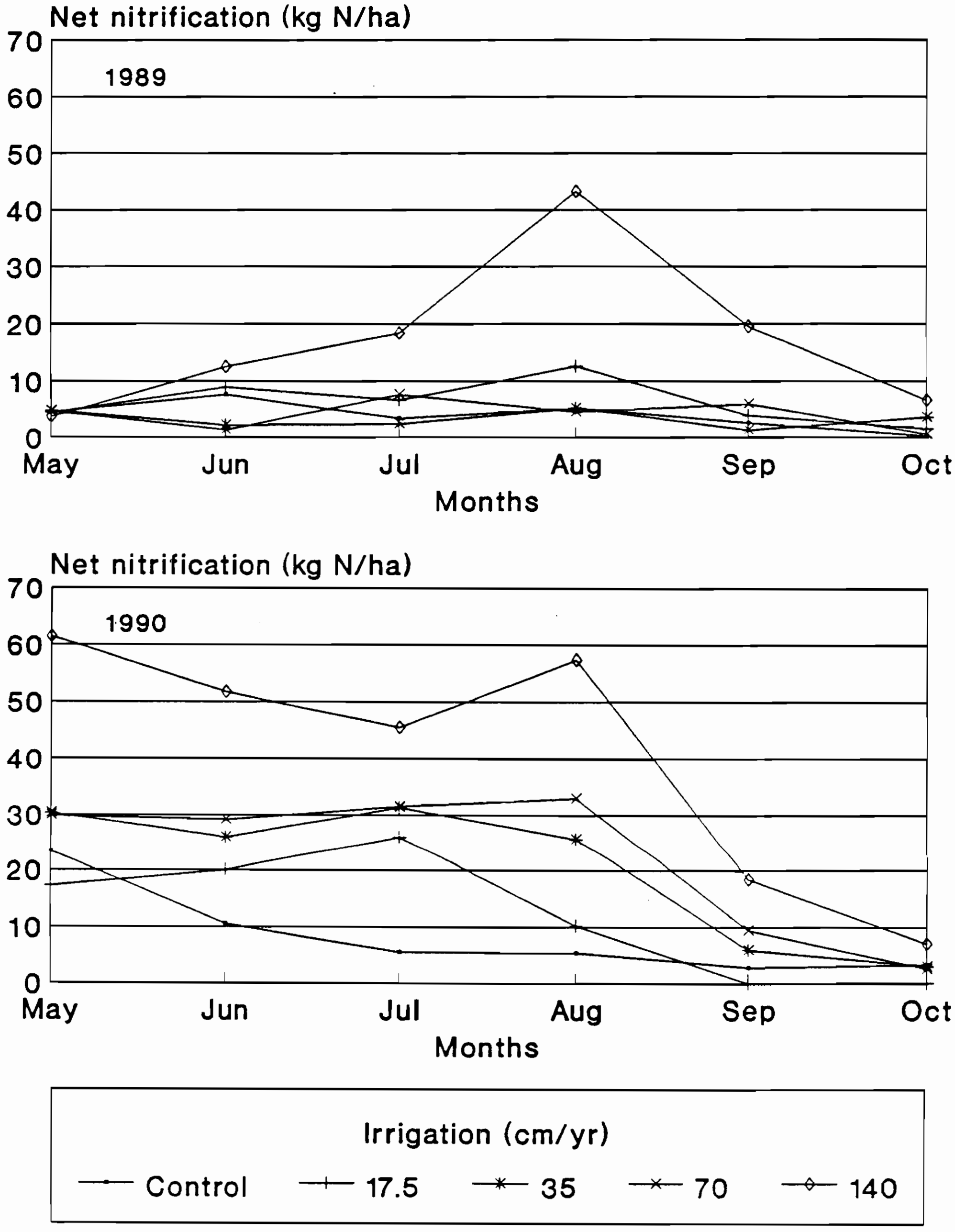


Figure 4.14. Net nitrification in the soil of 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia.

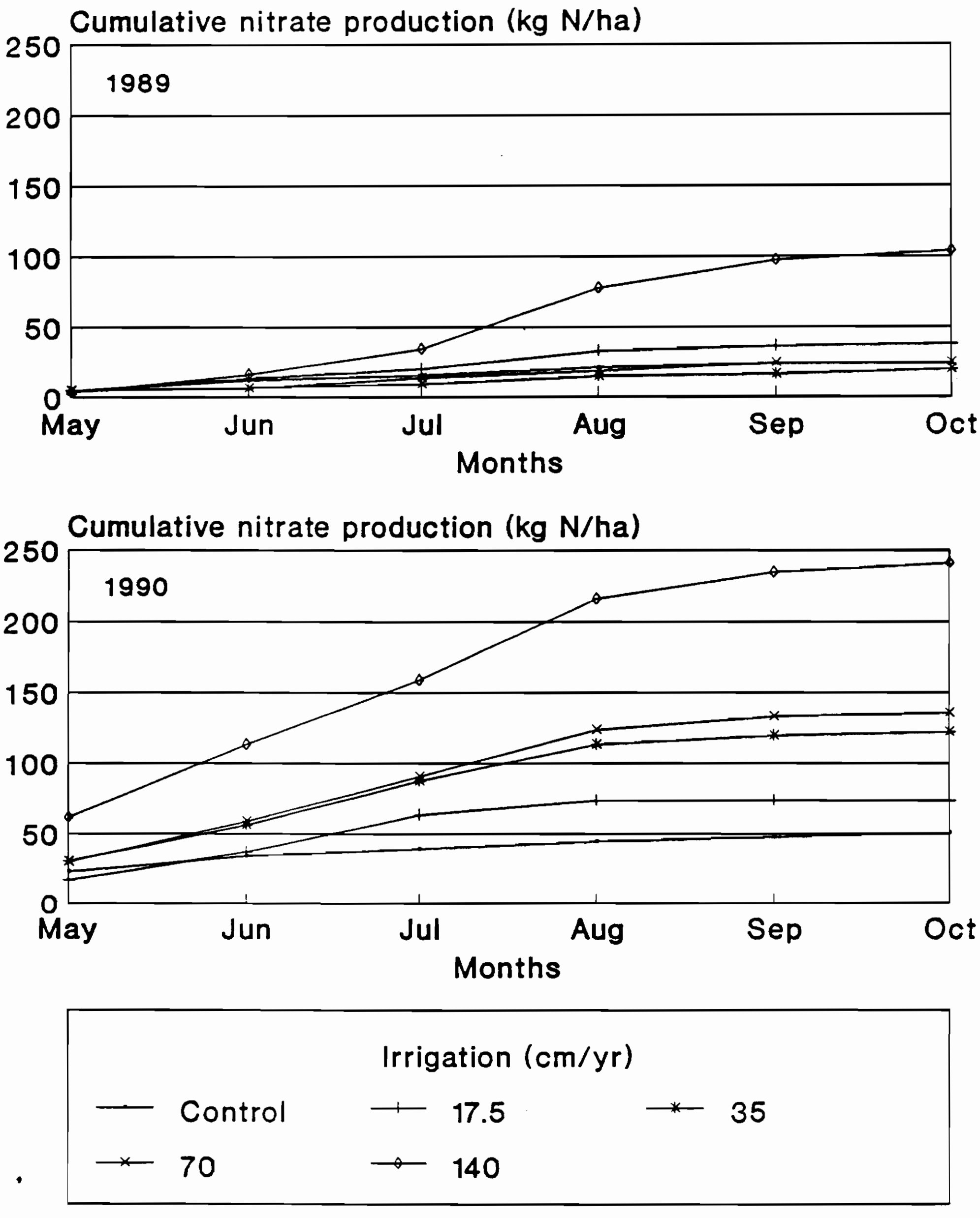


Figure 4.15. Cumulative nitrification in 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia.

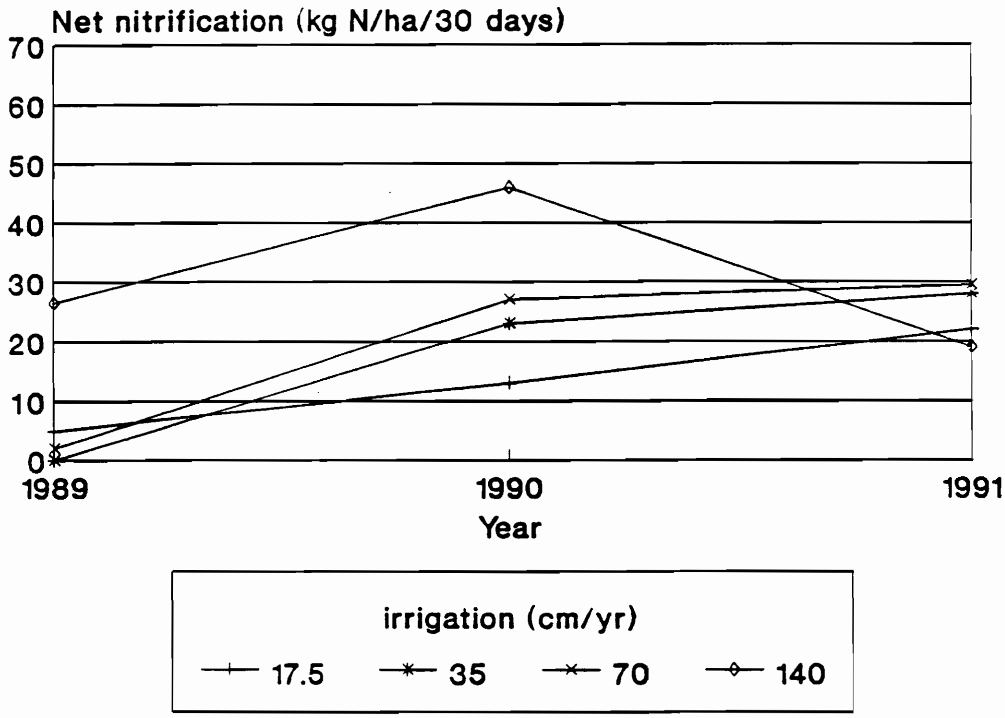


Figure 4.16. Average net nitrification with time in 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia.

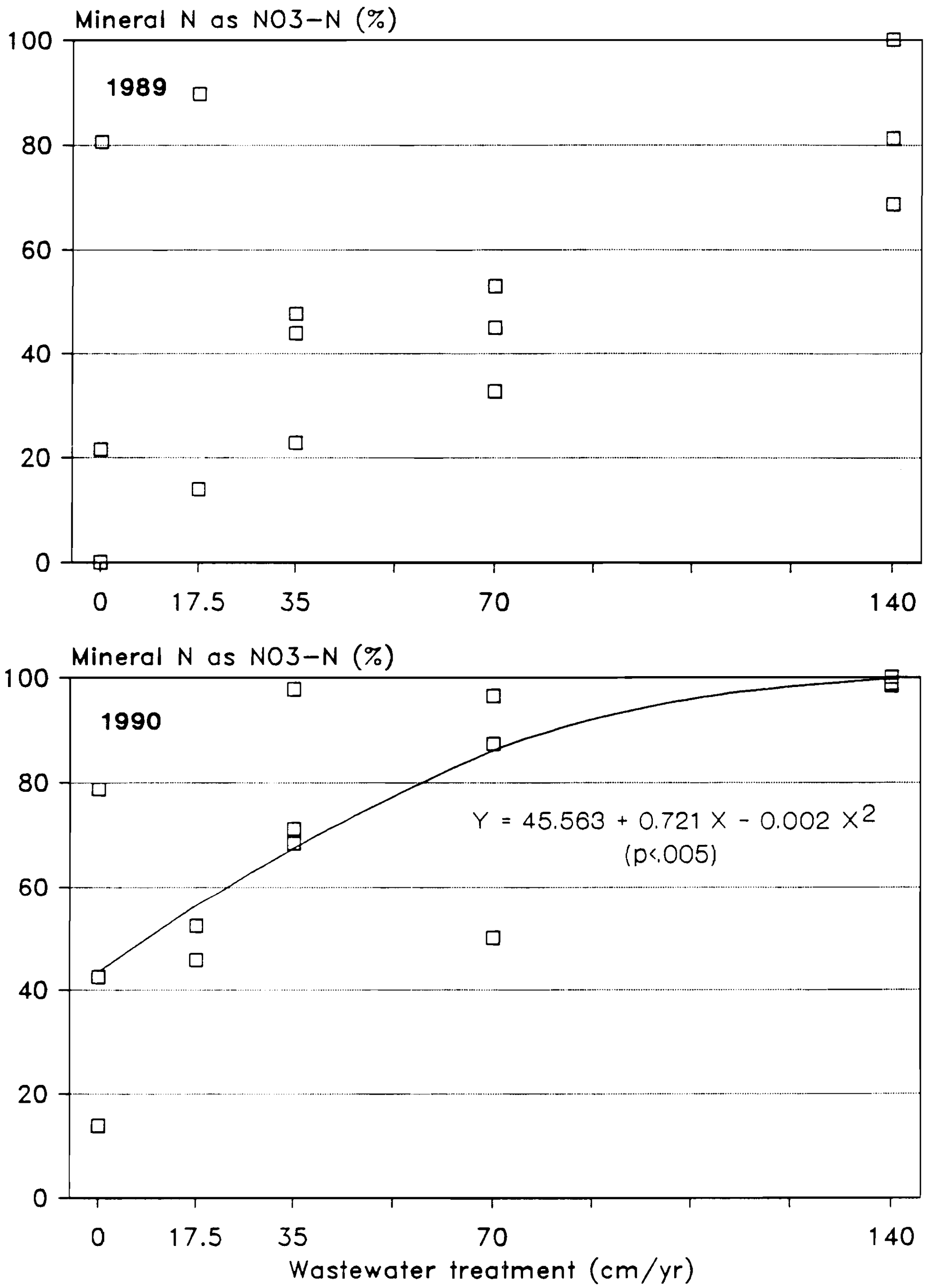


Figure 4.17. Relative nitrification (% of mineral N as NO_3^--N) in 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia.

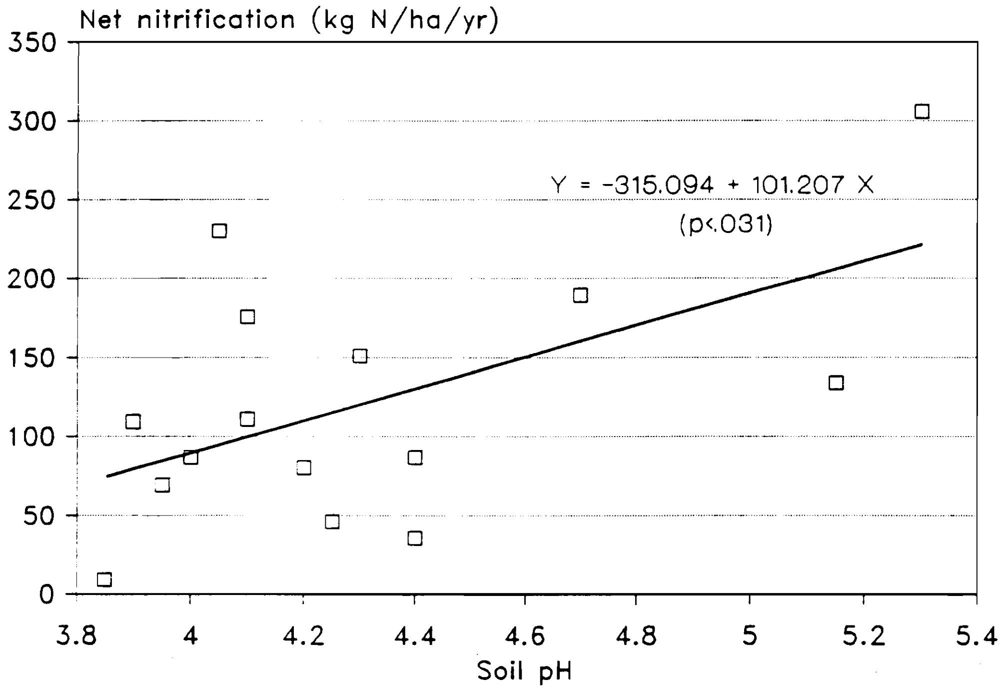


Figure 4.18. Relationship between net nitrification and soil pH in the soil treated with municipal wastewater for two years.

Denitrification

Denitrification activities in the soil of the study site ranged from 11.7 to 20.9 kg ha^{-1} yr^{-1} (Fig. 4.19). The activities were within the normal range of denitrification in deciduous forests in the United States, which is 0 to 50 kg ha^{-1} yr^{-1} (Davidson *et al.*, 1990). Denitrification increased with increasing irrigation intensities. Although the soil in the study site was in an aerobic condition as indicated by the high rates of nitrification (Fig. 4.20), denitrification increased in the soil probably due to the increased anaerobic microsites and/or the increased soil pH. Soil pH increased by 0.35 to 0.78 pH unit after the two-year irrigation period. Although the soil pH changes were not very great (0.7 pH unit increase), there was a positive relationship between soil pH and denitrification (Fig. 4.20).

Nitrogen leaching

Nitrate concentrations of the soil water at the 1 m depth increased on the wastewater-irrigated sites (Fig. 4.21). Fluctuations of concentration within each treatment level were probably due to irregular precipitation and wastewater irrigation. The N concentrations in the 70 cm yr^{-1} treatment sites were higher than those of the 140 cm yr^{-1} treatment during 1990 and 1991. However, the amount of N lost by leaching was greater in the 140 cm yr^{-1} treatment sites due to a larger amount of leaching water (Fig. 4.22). The amount of N leached increased greatly with increasing irrigation intensities. These increases in N leaching might be caused by enhanced nitrification rates and limited NO_3^- storage in the vegetation/soil system. Nitrate leaching loss increased after fertilization (Overrein, 1968) and sludge application (Riekerk and Zasoski, 1979) with

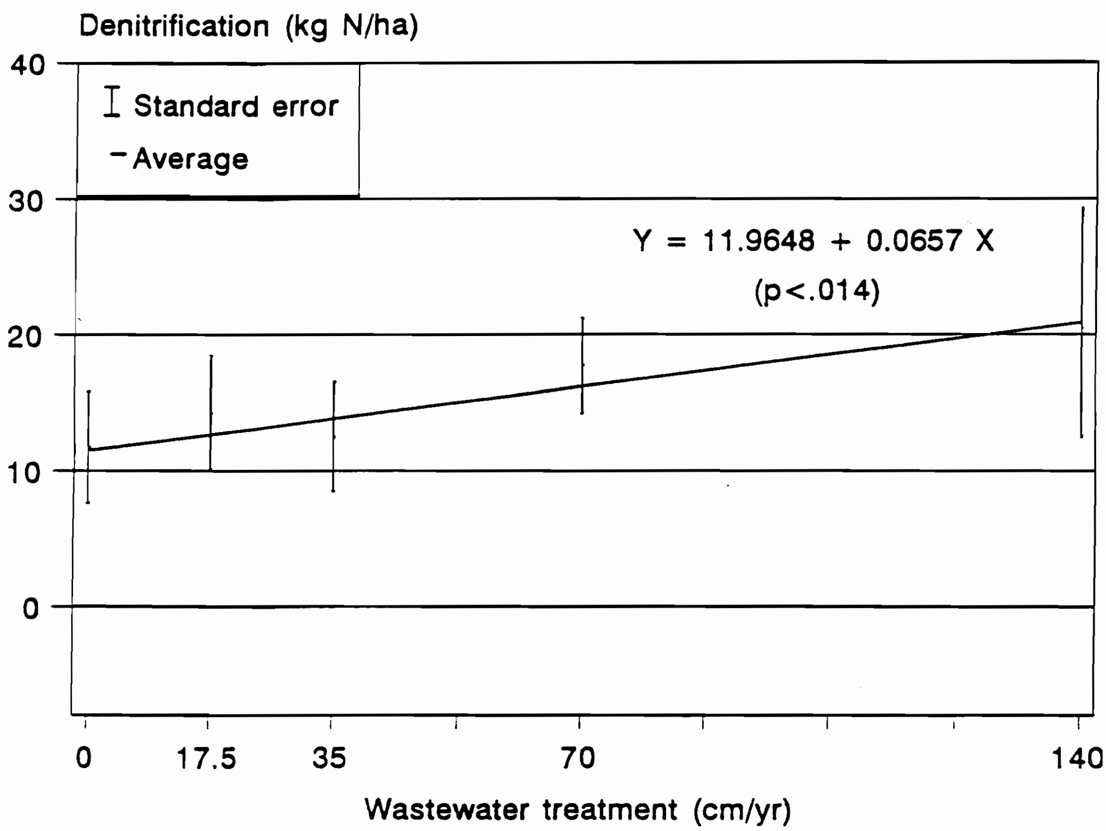


Figure 4.19. Relationship between denitrification and wastewater irrigation in 80 to 100-year-old upland hardwood stands in Giles Co., Virginia.

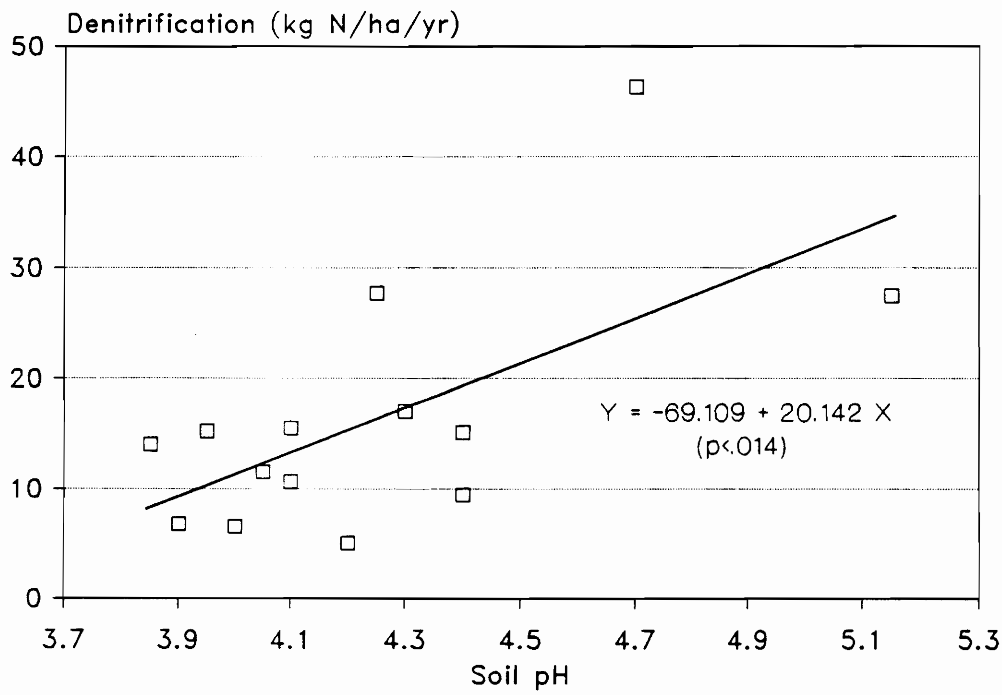


Figure 4.20. Relationship between denitrification and soil pH in 80 to 100- year-old upland hardwood stands in Giles Co., Virginia.

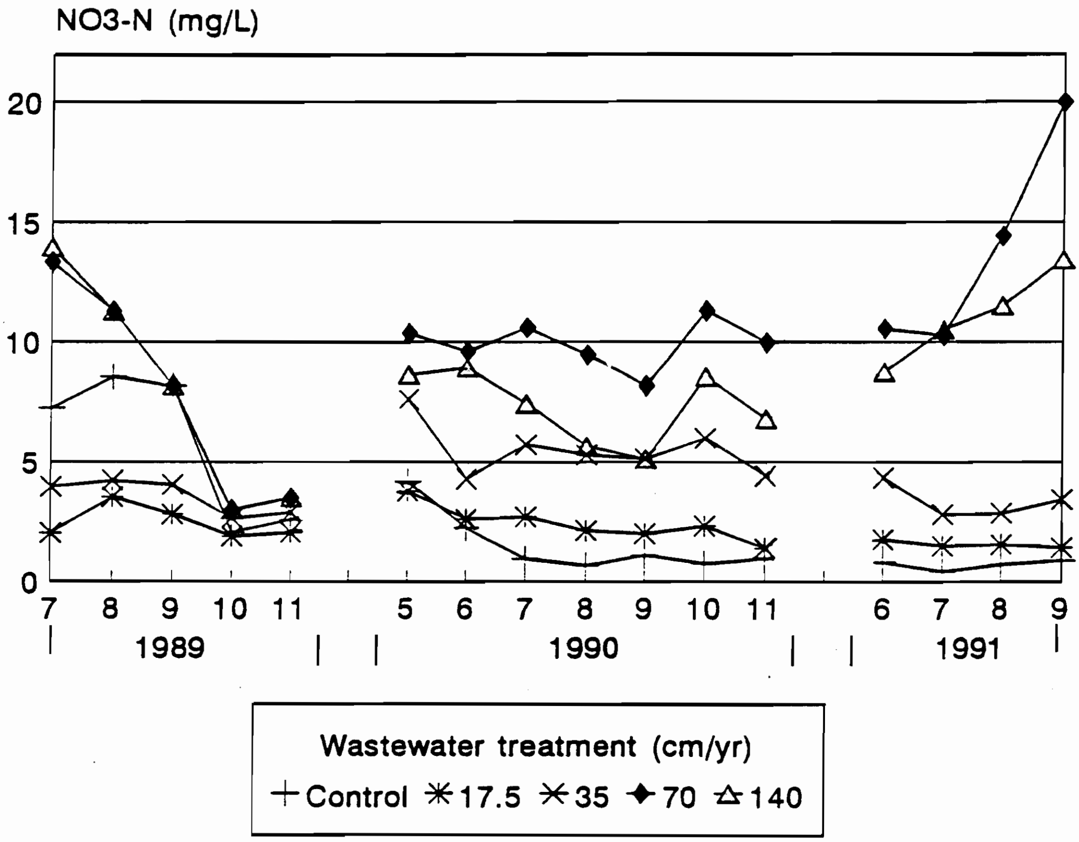


Figure 4.21. Nitrogen concentrations of soil water at 1-m depth in 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia.

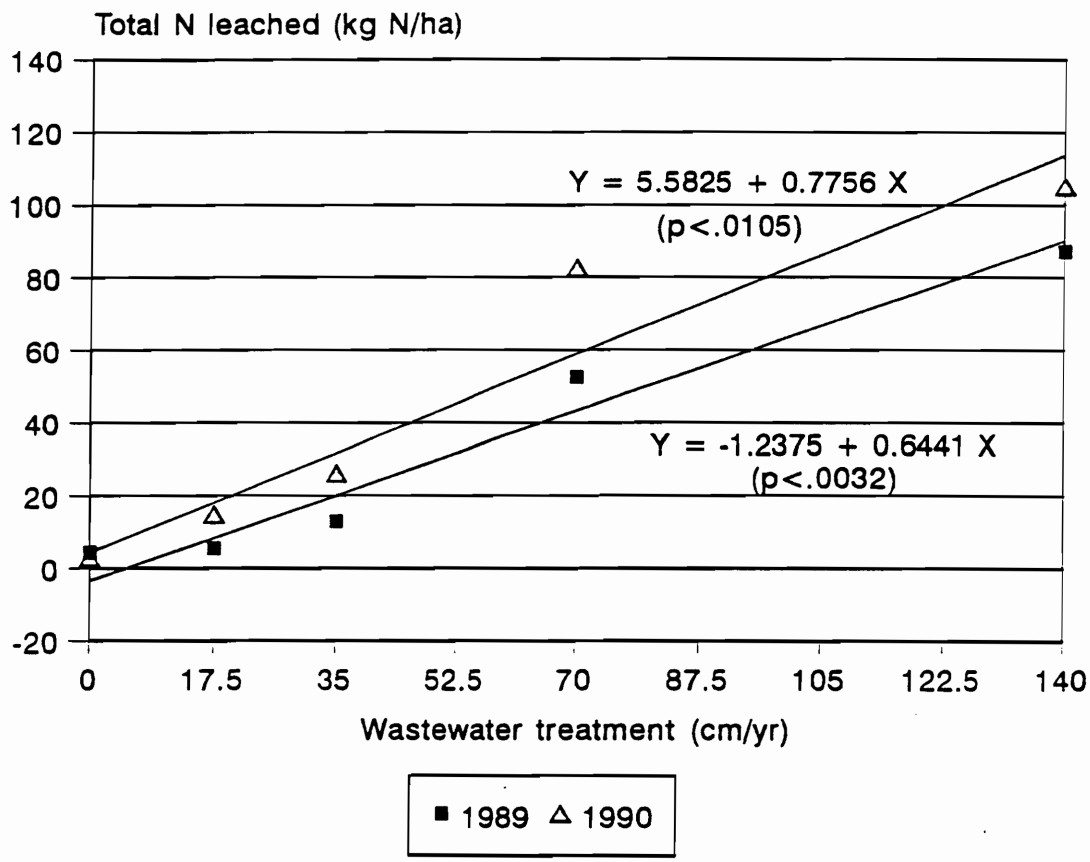


Figure 4.22. Relationship between N leaching loss and wastewater irrigation in 80 to 100-year-old upland hardwood stands in Giles Co., Virginia.

similar or larger magnitude than this study depending on the amount applied. Nitrate concentrations, however, were much lower in this study compared to other studies due to the dilution by the applied wastewater. The sites with low levels of irrigation (17.5 and 35 cm yr^{-1}) had soil water NO_3^- concentrations lower than that of the applied wastewater, while soil water on sites receiving high irrigation levels (70 and 140 cm yr^{-1}) had concentrations slightly higher than that of wastewater. The N assimilation capacity of the forest ecosystem appeared to be below the 70 cm yr^{-1} loading rate. The higher levels of N mineralization, and the large proportion of NO_3^- produced at the higher rates of wastewater application, probably contributed to soil water N levels higher than wastewater levels.

N balance in the ecosystem

Wastewater irrigation significantly increased N input to the forest by adding approximately 6 and 10 times atmospheric N input in 1989 and 1990, respectively (Table 4.5). The amounts of wastewater N added in this study (6.8 - 80.4 kg N ha^{-1} yr^{-1}), however, were relatively low, compared to the amounts applied in other hardwood forests in the eastern United States (240 - 540 kg N ha^{-1} yr^{-1}) and in the Great Lakes region (80 - 190 kg N ha^{-1} yr^{-1}) (Brockway *et al.*, 1986). The low N input in this study was due to low effluent loading and low N concentration in the effluent (Table 2.2).

Soil N transformation processes were affected by the increases in soil N and soil moisture. Nitrogen storage in forest vegetation increased from 2.1 to 10.3 kg ha^{-1} yr^{-1} except for the 70 cm yr^{-1} treatment sites in 1989. At the 70 cm yr^{-1} treatment sites, a large loss of vegetation N through litterfall that exceeded N accumulation in vegetation

Table 4.5. Nitrogen balance in 80 to 100-year-old upland hardwood stands in response to municipal wastewater irrigation in Giles Co., Virginia.

N transport	Control	Wastewater treatments (cm/yr) 17.5	35	70	140
	kg N/ha/yr				
(1989)					
Input	8.9	15.7	22.4	35.9	62.9
Atmospheric deposition	8.9	8.9	8.9	8.9	8.9
Wastewater	0	6.8	13.5	27.0	54.0
Retention	-7.3	-4.2	-3.1	-34.4	-45.2
Aboveground vegetation	10.3	3.8	9.4	-11.8	7.5
Forest floor	-7.0	-28.1	-13.1	-22.1	-38.5
Soil*	-10.6	20.1	0.6	-5.1	-14.2
Output	16.2	19.9	25.5	70.3	108.1
Denitrification	11.7	14.3	12.5	17.7	20.9
Leaching	4.5	5.6	13.0	52.6	87.2
(1990)					
Input	8.4	18.5	28.5	48.6	88.8
Atmospheric deposition	8.4	8.4	8.4	8.4	8.4
Wastewater	0	10.1	20.1	40.2	80.4
Retention	-7.9	-10.6	-10.1	-41.7	-37.1
Vegetation	5.9	2.1	5.5	4.9	4.4
Forest floor	-12.3	-51.7	-22.6	-52.1	-64.4
Soil*	-1.5	39.0	7.0	5.5	22.9
Output	14.3	29.1	38.6	90.3	125.9
Denitrification	11.7	14.3	12.5	17.7	20.9
Leaching	3.0	14.8	26.1	72.6	105.0

* Estimated from the difference in N balance in the ecosystem

resulted in net vegetation N loss. The vegetation N increases on irrigated sites were not significantly different from those of control sites. Nitrogen storage in forest litter was reduced over the two-year period. The forest litter tended to lose more N with increasing irrigation intensity. Soil N, estimated from the difference of system N balance, increased at the low irrigation sites and decreased at the high irrigation sites in 1989. Soil N increased on all irrigation sites in 1990. The soil N increase might be due to the organic matter translocation from forest litter and the increase in microbial N assimilation. Soil N reduction at high irrigation levels in 1989 was probably induced by inceases in N mineralization and nitrification activities with subsequent N leaching.

The forest ecosystem reacted to wastewater irrigation as a net N source by liberating N from the forest system. The system N loss was stimulated especially by high irrigation rates (70 and 140 cm yr^{-1}). In 1989, the amount of N leached was less than wastewater N input on sites receiving low irrigation rates (17.5 and 35 cm yr^{-1}), whereas N leaching exceeded wastewater N input on sites receiving high irrigation rates (70 and 140 cm yr^{-1}). Nitrogen leaching exceeded wastewater N input on all irrigation sites in 1990. The results show that the increase in N leaching on the low irrigation sites was due to the increase in N mineralization and nitrification. The mineral soil N, completely transformed to NO_3^- at the high irrigation sites, was not sufficiently assimilated by the plant/soil system, and composed approximately 35 to 50% of the N leached. The increased mineralization will stop when the system reaches a new equilibrium.

The N output results showed that N leaching was a dominant form of N loss from the system at the high irrigation rates. Nitrogen output was more than N input for all irrigation sites (Table 4.5). Excessive N leaching from the system was also found at an early stage of wastewater irrigation in old growth forests in southern Michigan (Burton, 1982). The results of this study suggested that the mature upland hardwood forest had a very low wastewater renovation capacity. After soil N processes equilibrate to new levels of hydraulic loading, little or no additional N will be sequestered by this forest system. Success of this system will simply be a function of adequate dilution in groundwater.

CHAPTER V. SOIL PH, TEMPERATURE, AND MOISTURE EFFECTS ON DENITRIFICATION OF WASTEWATER-IRRIGATED FOREST SOILS

Abstract

The effect of two years of municipal wastewater irrigation on the denitrification potential of soil taken from a forested land application site was investigated in this study. The effects of soil pH (control, pH 6.6), moisture (control, saturation), and temperature (15, 25, 35°C) on denitrification were measured in a 2 x 2 x 3 factorial laboratory experiment. The activity was measured in a laboratory using the acetylene inhibition method. Denitrification activity increased with increasing soil pH, soil moisture, and soil temperature. There was a three-way interaction among these three abiotic factors. Treatment effects were significant in the following order: flooding + soil pH > > > flooding > soil pH > control. Denitrification activity increased exponentially as temperature increased. Denitrification potential increased with increasing rates of wastewater irrigation. However, denitrification activities measured in the irrigation field were limited by soil temperature and soil moisture which had changed too little to significantly affect the activity. Wastewater irrigation effects on denitrification potential was primarily a function of increasing soil pH with continuous irrigation.

Introduction

Gaseous loss of N is an important mechanism by which N concentrations can be reduced in N-enriched soil systems. It can be especially important in mature forests serving as a land application site for wastewater. Ammonia volatilization may or may not occur in wastewater-irrigated forests depending upon the soil conditions and effluent quality. Overall, there is little chance of ammonia being volatilized from undisturbed forest floors due to their low soil pH. Applied N is more likely to be lost by denitrification which is stimulated by anaerobic soil conditions (Feigin *et al.*, 1981; Sommers *et al.*, 1979; Volz and Heichel, 1979). If neither volatilization nor denitrification occurs, inorganic N from wastewater is subject to leaching from mature steady-state forests.

Denitrification is a biological process which reduces NO_3^- to N_2O or N_2 by ubiquitous facultative heterotrophs. The microorganisms use NO_3^- as an electron acceptor in the absence of O_2 (Sikora and Keeney, 1975). Denitrification activity is controlled by three major factors: aeration, available C, and available NO_3^- (Firestone, 1982). When continuous soil pores are blocked by water, anaerobic conditions are favored because of low oxygen diffusion rates in water. Oxygen diffusion in soil becomes critical when approximately 85% of soil pore space is saturated by water (Wesseling and van Wijk, 1957). Denitrification flux, measured in an irrigated vegetable production field in Santa Maria Valley, California, showed peaks between water potentials of -5 to -10 KPa, and the rates were quite low below -25 KPa (Ryden and Lund, 1980). The activity ceased when soil became drier than -33 KPa (Focht and Verstraete, 1977). Nitrogen gas can be produced biologically in well-aerated soils when micro-anaerobic zones are present (Bremner and Blackmer, 1979; Gilliam *et al.*, 1978). Wastewater adds significant amounts of water to soil and denitrification activity is likely to increase with increased levels of soil water.

In soil, the oxidizable substrates are derived largely from soil organic matter. Denitrification is strongly correlated with the amount of readily available organic material (Burford and Bremner, 1975; Stanford *et al.*, 1975). The evolution of CO_2 is a good indicator of denitrification when sufficient NO_3^- is present (deCatanzaro and Beauchamp, 1985). Most forest soils are high in soil organic matter, and C is usually the least limiting factor of denitrification in the surface horizons of forest soils. Wastewater irrigation usually adds additional C which may increase the size of the available C pool.

Nitrate availability may be a dominant factor in controlling denitrification rates. High correlations between NO_3^- concentrations and denitrification activities have been found in forest systems (Melillo *et al.*, 1983; Robertson and Tiedje, 1984). Because forest soils have sufficient organic C in surface horizons, soil NO_3^- levels may play a greater role in denitrification in forest soils than in agricultural soils (Davidson and Swank, 1987).

Optimum temperature for denitrification is between 35°C and 65°C (Bremner and Show, 1958; Keeney *et al.*, 1979). The activity increases with increasing temperature between 15°C and 30°C (Fillery, 1983; Focht, 1974). The denitrification which occurs at high temperature probably reflects chemodenitrification reactions involving nitrite produced by thermophilic NO_3^- respirers (Keeney *et al.*, 1979). Chemodenitrification is probably insignificant relative to biological denitrification in forest soils. Measurable denitrification has also been reported at low temperatures beneath snow in the winter (Lueking *et al.*, 1986). Wastewater irrigation may increase the temperature in forest soils due to high temperatures of wastewaters. The forest canopy reduces heat dissipation from the soil surface and thermal conductivity increases by wetting the soil. This temperature increase will increase denitrification.

The rate of denitrification as a function of soil pH is reportedly variable. While some investigators were unable to demonstrate any soil pH effect on denitrification (Cooper and Smith, 1963; Fillery, 1979), other studies found denitrification activity highly correlated with soil pH in acid soils (Klemedtsson *et al.*, 1978; Mueller *et al.*, 1980). The increase in activity at higher soil pH levels indicates that microbial populations functioning poorly in acid conditions increased their activity when soil pH was increased. Although average denitrification activity is lower in acid soils than in soils of pH greater than 5, significant amounts of denitrification can occur in acid soils (Parkin *et al.*, 1985; Robertson and Tiedje, 1984, 1988; Sexstone *et al.*, 1986). Wastewater is normally above pH 7, and when it is applied to an acid forest soil, it will likely raise the soil pH regardless of initial soil acidity. Therefore, wastewater application could enhance potential denitrification activity by increasing soil pH.

Denitrification has been measured in forest soils by several researchers (Davidson and Swank, 1987; Egan and Sexstone, 1987; Groffman and Tiedje, 1989a, 1989b; Lueking *et al.*, 1986; Melillo *et al.*, 1983; Robertson and Tiedje, 1984). However, factors controlling denitrification activities in wastewater-irrigated forest soils are not well characterized. Wastewater irrigation of forest sites changes the soil conditions that regulate denitrification. Understanding the character of these soil changes is essential for predicting denitrification activities on wastewater-irrigated sites. The objectives of this study were to quantify the factors that limit or enhance denitrification activities in wastewater-irrigated soils, and to determine if wastewater-mediated changes in soil conditions after two years of irrigation effectively increased denitrification potential in an irrigated forest soil located in the Appalachian region of Virginia.

Methods

Soil samples were collected from the A horizon of the study sites after two years of irrigation. The soil was collected from three random locations in each plot that received wastewater at five different treatment rates (0, 17.5, 35, 70, 140 cm yr^{-1}). The fresh soil was passed through a 4 mm sieve and any visible plant materials were removed before and after the sieving. Twenty-five grams of the sieved soil were placed in 125 ml Erlenmeyer flasks.

Treatments were applied in a 2 x 2 x 3 factorial experiment with two soil pH (control, pH 6.6), two moisture (control, saturated with 20 ml water), and three temperature (15, 25, 35°C) levels. For control soils, pH ranged from 3.9 to 4.7 with a few exceptions of pH over 5; moisture content ranged from 40.4 to 49.6%; and temperature ranged from 16.0 to 16.7°C. All soil samples had been amended with KNO_3 (0.14 g per 25 g fresh soil) before treatments were applied in order to provide sufficient nitrate. The treatments were triplicated which seemed appropriate since compositing usually provides a reasonable estimate of the site mean (Keeney, 1980).

Adding approximately 0.3 g of $Ca(OH)_2$ to 25 g of fresh soil resulted in a soil pH increase to pH 6.6. In order to create an anaerobic condition, 20 ml of deionized H_2O was added to make a slurry. After the soil samples were treated for soil pH and moisture, flasks were closed with rubber stoppers. Ten percent of the head space was then replaced with C_2H_2 (Tiedje, 1982). The C_2H_2 gas was generated by adding water to calcium carbide (CaC_2); the gas was purified by passing it through a solution of $CuCl_2$ in concentrated HCl and then through water (Walter *et al.*, 1979). After the soil samples were incubated at 15°C, incubations were repeated with the same soil at 25°C and 35°C with intervals of at least one week so that remnant C_2H_2 was removed from the soil by microbial consumption. Water was added to the soil prior to each incubation to maintain the same soil moisture conditions .

Gas samples were withdrawn from the flasks by syringe and stored in 4 ml vacutainers. The gas was analyzed for N_2O, using a Varian 3700 gas chromatogragh, equipped with a ^{63}Ni electron capture detector, a column packed with Porapak-Q, and with a carrier-gas (90 % Argon, 10 % Methane) flow rate of 30 ml min^{-1}. The detector and column temperatures were 340°C and 60°C, respectively.

The head space N_2O concentration was calculated from the equation developed from the standard N_2O gas. Total amount of N_2O gas evolved was calculated by the following equation (Tiedje, 1982):

$$M = C_g (V_g + (V_l \times a))$$

where M is the total amount of N_2O in ml in the flask, C_g is the concentration of N_2O in the gas phase, V_g is the volume of the gas phase in ml, V_l is the volume of the liquid phase in ml, and a is the Bunsen absorption coefficient in ml of N_2O gas dissolved in 1 ml of water at a given temperature.

Wastewater treatment (17.5, 35, 70, and 140 cm yr^{-1}) effects were pooled when the 2 x 2 x 3 factorial experiment on soil moisture, acidity, and temperature was analyzed by SAS using PROC ANOVA (SAS Institute, 1982). The wastewater treatment effect on soils was examined across the main effects of the three abiotic factors.

Results and Discussion

Abiotic factor effects on denitrification

Flooding, soil pH, and soil temperature all interacted significantly to increase denitrification activities in a non-irrigated forest soil under controlled treatment conditions (Fig. 5.1). The three-way interaction of soil pH, temperature, and flooding caused a synergistic response. All two-way and three-way interactions are clearly illustrated in Figure 5.1.

Soil pH and flooding interacted significantly at all three temperatures (Table 5.1). Denitrification increased five fold under a flooded and neutralized soil condition compared to flooding alone or neutralization alone. The same magnitude of activity was found in flooded soils in a study conducted in mature and disturbed southeastern hardwood forests by Davidson and Swank (1987). The added $Ca(OH)_2$ was probably not evenly distributed throughout the soil when it was not flooded. It is possible that

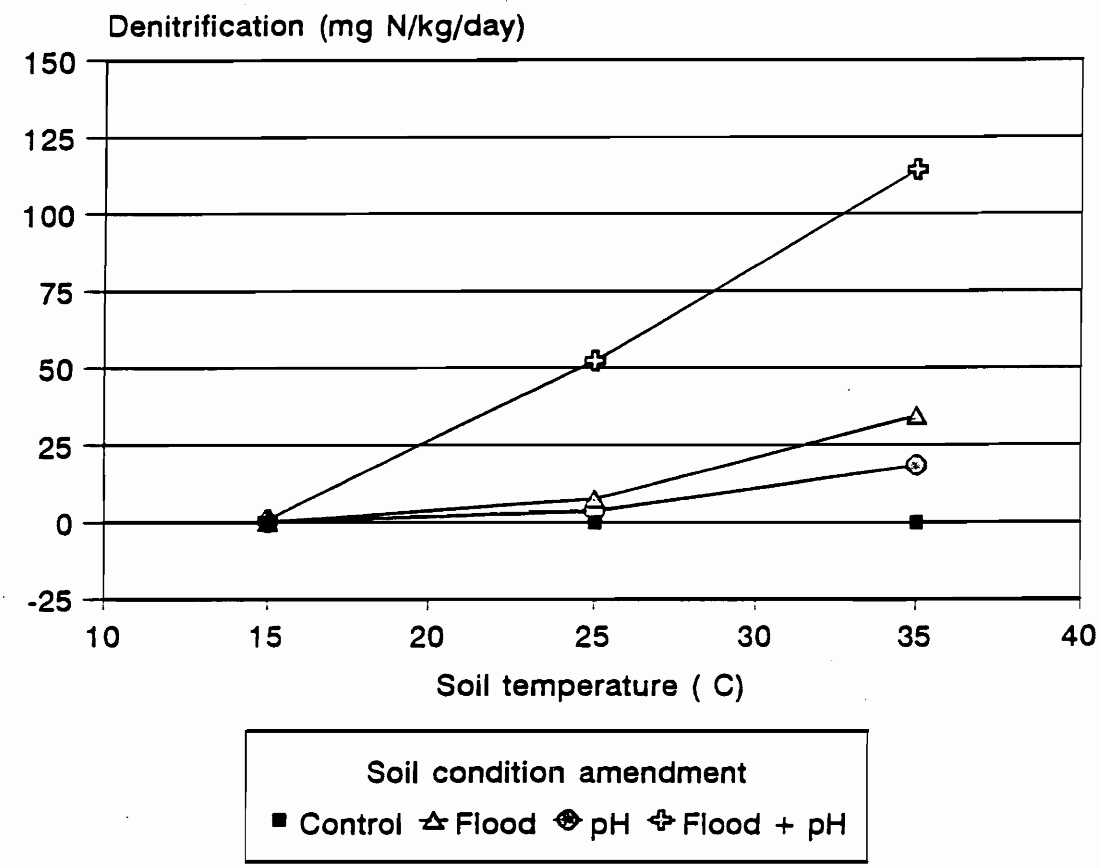


Figure 5.1. Effects of flooding, pH, and soil temperature on denitrification.

Table 5.1. ANOVA table showing the effects of flooding, pH, and soil temperature on denitrification.

Source	df	MSE	F value	P
Model	11	43156	40.24	.001
Flood	1	118751	110.72	.0001
pH	1	50245	46.85	.0001
Temp	2	69347	64.65	.0001
Flood x pH	1	23076	21.52	.0001
Flood x Temp	2	40108	37.39	.0001
Temp x pH	2	18399	17.15	.0001
Flood x pH x Temp	2	13466	12.56	.0001
Error	153	1072		
(at 15 C)				
Flood	1	133	6.02	.018
pH	1	149	6.74	.012
Flood x pH	1	115	5.19	.027
Error	44	22		
(at 25 C)				
Flood	1	23732	82.20	.0001
pH	1	9411	32.60	.0001
Flood x pH	1	4792	16.60	.0001
Error	51	289		
(at 35 C)				
Flood	1	184785	23.15	.0001
pH	1	89613	11.23	.002
Flood x pH	1	33485	4.19	.046
Error	52	7983		

$Ca(OH)_2$ became more accessible to the soil when the soil was flooded.

The interaction of temperature with flooding is due to the effects of temperature on O_2 diffusion, O_2 solubility, and O_2 consumption (Firestone, 1982). A temperature decrease would enhance an aerobic condition in the soil by suppressing O_2 consumption and increasing O_2 solubility. This was demonstrated by Misra *et al.* (1974) who showed that increasing O_2 concentration had a greater inhibitory effect on denitrification at low temperatures in a Columbia silt loam.

Denitrification activity increased with increasing soil pH at 25°C and 35°C. This suggests that there were microbial populations that functioned poorly in acid conditions. This response is consistent with the tremendous increase in denitrification activity observed by Klemedtsson *et al.* (1978) when they neutralized an acid peat soil. Firestone (1982) also observed the same response in laboratory culture studies.

Provided that other factors are not limiting, denitrification increases exponentially with increasing soil temperature (Focht, 1974). This temperature effect on denitrification was evident in this study (Fig. 5.1). At all three temperature levels, treatment effects were significant in the following order: flooding + soil pH >>> flooding > soil pH > control (Fig. 5.1). This shows that denitrification is potentially more limited on the study site by soil moisture and temperature than by soil pH.

<u>Characterization of wastewater-treated soils</u>

Soil moisture content within the surface 15 cm soil depth increased in the irrigation field sites by 4.6 to 11.2 % (Table 5.2). Increases were variable among treatment levels reflecting different water holding capacities of a draining soil (Fig. 5.2).

Table 5.2. Soil pH, moisture, and temperature conditions on the irrigation sites.

Soil condition	Wastewater treatment (cm/yr) Control	17.5	35	70	140
(Soil pH)					
A 1988	3.92 (0.28)*	3.87 (0.33)	3.80 (0.30)	3.62 (0.23)	3.90 (0.26)
1990	4.05 (0.30)	4.22 (0.20)	4.20 (0.10)	4.40 (0.65)	4.68 (0.63)
B 1988	4.47 (0.06)	4.28 (0.07)	4.37 (0.12)	4.12 (0.38)	4.40 (0.10)
1990	4.57 (0.12)	4.60 (0.05)	4.58 (0.08)	4.62 (0.16)	4.95 (0.26)
(% moisture)**					
1988	39.8 (1.59)	37.8 (7.13)	41.7 (0.99)	41.8 (2.36)	42.2 (5.37)
1990	40.4 (6.80)	49.6 (1.63)	47.3 (5.36)	48.6 (6.12)	47.4 (5.62)
(Temperature, C)***					
1990	16.0 (4.45)	16.1 (5.60)	16.3 (5.48)	16.3 (5.04)	16.7 (4.91)

* Standard deviations are denoted in the parentheses
** Upper 15 cm surface soil
*** measured at 15 cm depth every two weeks from June to October

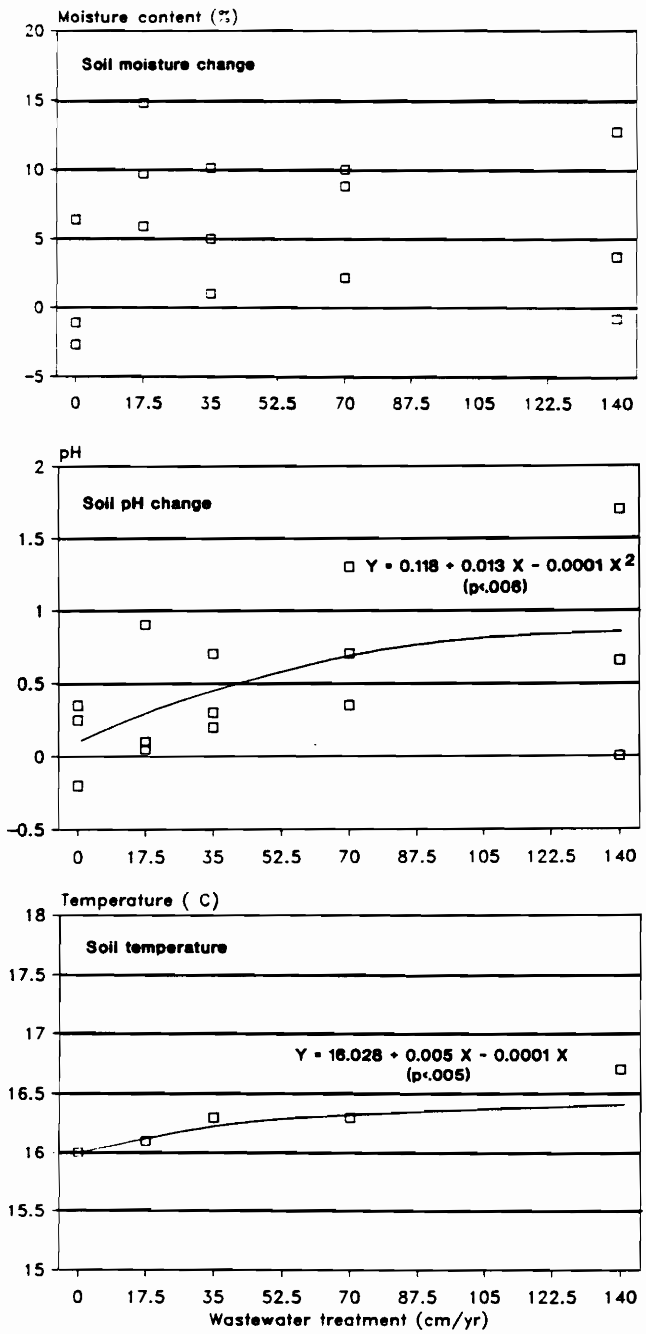


Figure 5.2. Changes in soil properties due to wastewater irrigation.

Soil pH increased in the A horizon with increasing irrigation (Fig. 5.2). Soil pH will increase more with time as wastewater with a pH of 7.3 is added. Soil temperature at the 15 cm depth increased slightly on the wastewater-irrigation sites (Fig. 5.2). However, the temperature change was not enough to affect field denitrification activities. Overall, the abiotic soil conditions, except for soil pH, were not significantly changed by irrigation.

Denitrification response to wastewater treatment

Flooding effect on denitrification was potentially greater in wastewater-irrigated soils than in unirrigated soils (Fig. 5.3). Irrigation during a two-year period changed the character of the soil in such a way that increased denitrification potential. The importance of soil moisture on denitrification has been demonstrated by high correlations between denitrification and rainfall patterns (Ryden, 1983) and between denitrification and irrigation (Rolstone *et al.*, 1984). On the study site, however, denitrification activities did not follow the pattern of soil moisture change; there was little relationship between the two. Although soil moisture was not related to wastewater irrigation, it is possible that anaerobic microsites increased on the irrigation sites. This could be one of the reasons that field denitrification increases with increasing irrigation (Fig. 4.19).

Wastewater treatment effect on denitrification potential was also observed in the main effects of soil pH and soil temperature (Fig. 5.3). The increase in soil pH with wastewater irrigation (Table 5.2, Fig. 5.2), and increase in field denitrification with increasing soil pH (Fig. 4.18), suggests that the positive relationship between field

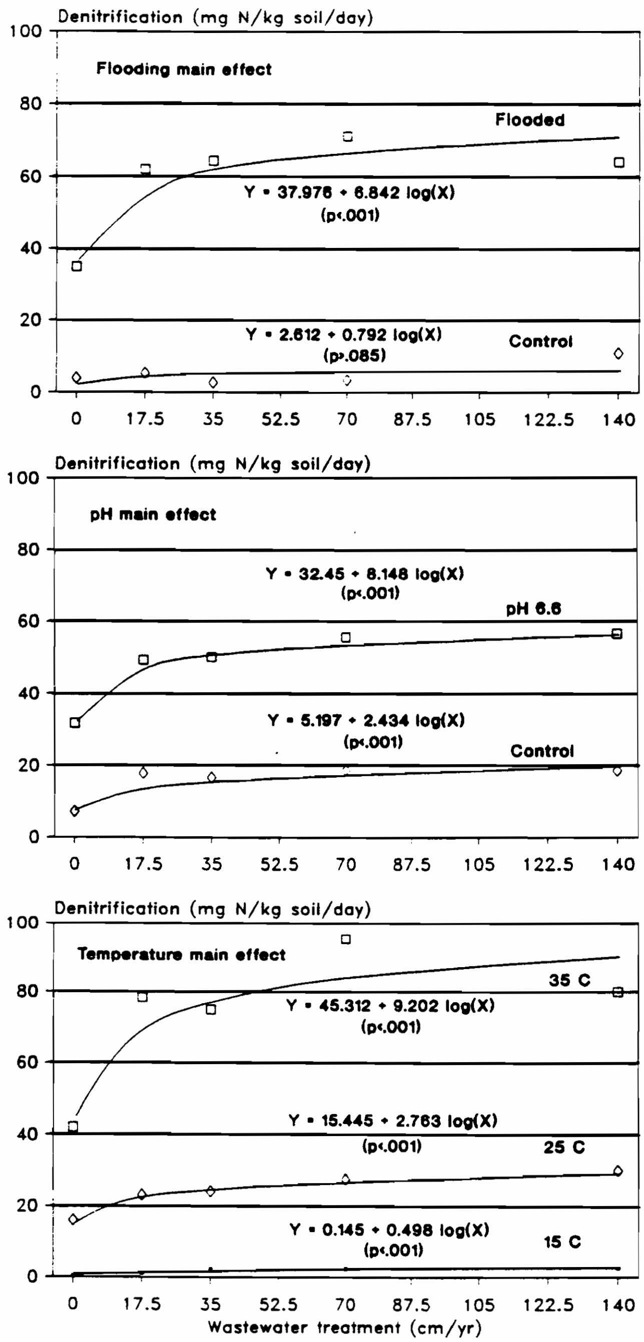


Figure 5.3. Denitrification response to added wastewater under different levels of abiotic factors.

denitrification and wastewater irrigation is due to the increase in soil pH.

The results show that denitrification potential increased with wastewater irrigation on the study site. However, field denitrification was well below the denitrification potential because of only small changes in abiotic soil conditions. The most significant change was wastewater-mediated change in soil pH which was reflected in an increase in field denitrification (Chapter VI).

Conclusion

Denitrification increased dramatically in soil taken from the wastewater irrigation site when soil pH, soil temperature, and soil moisture are increased simultaneously in a laboratory environment. After two years of wastewater irrigation, denitrification potential on the irrigation site improved due to a wastewater-mediated soil change. The increase in denitrification potential with wastewater irrigation was due primarily to the increase in soil pH. Future denitrification activity in the field will always be limited by soil temperature and soil moisture conditions because wastewater has little or no effect on soil heat capacity, and because relatively low rates of application and good soil drainage does not allow saturated conditions to predominate. Soil temperature will not increase significantly on the irrigation site because the soil always loses heat in the process of evaporation which is increased by irrigation. Soil pH will continue to increase with continuous irrigation and will be the main factor contributing to any increase in denitrification potential in the field.

DISSERTATION SUMMARY

Two years of wastewater irrigation on an Appalachian hardwood forest did not significantly affect stand growth, seedling reproduction, tree mortality, species diversity, or N accumulation in vegetation. Herbaceous ground cover increased with increasing irrigation except at the 140 cm yr^{-1} treatment rate where the heavy spray caused physical damage.

The mature upland hardwood vegetation has a very low N assimilation capacity. The fate of system N, including the applied wastewater N, mostly depended on N transformation processes in the soil. Nitrogen mineralization and subsequent nitrification increased on the irrigation sites due to higher levels of soil N, soil moisture, and a higher soil pH. Wastewater irrigation increased denitrification potential, but actual denitrification was not affected because denitrification activity was limited by low soil temperature and aerobic soil conditions. Denitrification amounted to only a small N flux from the system, especially on the high irrigation sites where N leaching was the dominant form of N loss. System N was efficiently transformed to NO_3^--N, but it was not sufficiently assimilated by the flora and fauna of the forest system or denitrified to reduce NO_3^- concentration in leaching water. Nitrogen concentrations in leaching water of high irrigation sites were higher than the concentrations in the applied wastewater.

Nitrogen storage in the forest floor litter layer was reduced as a result of irrigation. Litter N tended to decrease more with increasing irrigation. Soil N increased on the low irrigation sites and decreased on the high irrigation sites, indicating that high rates of irrigation stimulate soil N transformations and N loss from the soil. Overall, the forest ecosystem reacted to wastewater irrigation as a N source by losing system N. The loss of system N increased as the irrigation increased.

The operational irrigation rate of 35 cm yr^{-1} was low enough to be within the N assimilation capacity of this hardwood forest at this time. However, even at the lowest rates of irrigation, N will leach from this forest sytem when the system reaches a new equilibrium.

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APPENDIX A

Mountain Lake Hotel influent wastewater characteristics

Mountain Lake Hotel influent wastewater (Anderson and Associates, Inc., 1986)

Parameter	Conc. mg/L
BOD5	205
COD	429
Total solids	366
Volatile solids	248
Conductivity (umho/cm)	365
Alkalinity	98.6
pH	7.2
Organic N	15
NH_3/NH_4-N	10
NO_2/NO_3-N	1.03
Total P	6.2
Ortho P	3.2
K	21.9
SO_4	16.5
Ca	12.6
Mg	1.8
Na	78.9
As	.005
B	.18
Cd	<.006
Cu	.05
Cl	47.04
Ni	<.2
Pb	.3
Zn	.04

APPENDIX B

Botanical taxa found at Mountain Lake area, 1990.

Tree and woody plant species found on study sites at Mountain Lake area

Scientific name	Common name
Acer pensylvanicum L.	striped maple
Acer rubrum L.	red maple
Acer saccharum Marsh.	sugar maple
Amelanchier arborea (Michx.) Fernald	downy serviceberry
Betula lenta L.	black birch
Carya glabra (Mill.) Sweet	pignut hickory
Carya ovata (Mill.) K. Koch	shagbark hickory
Castanea dentata (Marsh.) Borkh.	American chestnut
Corylus americana Walt.	American hazelnut
Fagus grandifolia Ehrh.	American beech
Hamamelis virginiana L.	witch-hazel
Ilex montana (T. & G.) Gray	mountain holly
Magnolia acuminata L.	cucumber magnolia
Prunus serotina Ehrh.	black cherry
Quercus alba L.	white oak
Quercus rubra L.	northern red oak
Rhododendron calendulaceum (Michx.) Torr	flame azaelea
Robinia pseudo-acacia L.	black locust
Rubus spp.	black-, raspberry
Tsuga canadensis (L.) Carr.	eastern hemlock

Herbaceous species found on study sites at Mountain Lake area.

Scientific name	Common name
Amianthium muscaetoxicum (Walt.) Gray	fly-poison
Anemone lancifolia Pursh.	mountain anemone
Apiaceae spp.	parsley family
Aralia nudicaules L.	wild sarsaparilla
Aster acuminatus Michx.	whorled aster
Aster macrophyllus L.	aster
Athyrium asplenoides (Michx.) Desv.	lady-fern
Carex spp.	sedges
Clintonia borealis (Ait.) Raf.	yellow clintonia
Conopholis americana (L.) Wallr.	squaw-root
Convalaria majalis L.	lily-of-the-valley
Dennstaedta punctilobula (Michx.) Moore	hay scented fern
Dioscorea villosa L. var. *villosa* L.	wild yam
Disporum lanuginosum (Michx.) Nicholas.	yellow mandarin
Galium spp.	bedstraw
Gentiana decora Pollard	showy gentian
Lilium superbum L.	Turk's cap lily
Lythrum salicaria L.	whorled loosestrife
Maianthemum canadense Desf.	Canada mayflower
Medeola virginiana L.	Indian cucumber root
Osmunda cinnamomea L.	cinnamon fern
Panicum spp.	panic grass
Polygonatum biflorum (Walt.) Ell.	Solomon's seal
Potentilla spp.	Cinquefoil
Prenanthes spp.	rattlesnake-root
Ribes spp.	wild gooseberry
Scutellaria saxatilis Riddell	skullcap
Silene stellata (L.) Ait. f.	starry campion
Smilacina racemosa (L.) Desf.	false Solomon's-seal
Smilax herbacea L. var. *herbacea*	carrion flower
Solidago spp.	goldenrod
Stellaria pubera Michx.	star chickweed
Thelypteris noveboracensis (L.) Nieuwl.	New York fern
Uvularia perfoliata L.	bellwort
Vaccinium spp.	Blue-, Cran-, Huckle-, Deerberry
Viola spp.	blue violet
Viola rotundifolia Michx.	roundleaf violet

APPENDIX C

Soils in Mountain Lake area (Anderson and Associates, Inc., 1986)

Soils in Mountain Lake area were examined by Mattews Soil Consultants. The soil considered for wastewater irrigation are classified in three general units.

Soil unit #1

The soils of this unit are deep to moderately deep and well drained. They have developed from colluvium derived from hematite sandstones. Soil colors are predominantly dark red to reddish-brown and soil textures are predominantly loam in the upper 50 to 75 cm with clay loam predominating below 75 cm. Fragment content ranges from 10 to 15% to more than 70%. Permeability of the upper loam horizon is moderately rapid because of strong structural development and a high organic matter content. The permeability of the subsoil horizon is moderate primarily because of moderate to strong structural development. The soil of this unit are well suited for use as a wastewater irrigation site because of good permeability and the capability to infiltrate water at a rapid rate. They occur on slopes ranging from about 6% to slopes in the order of 20 to 25%. Depth to rock ranges from about 0.9 m to more than 1.8 m. Their good water movement characteristics are illustrated by the absence of red to dark red colors and strongly developed structural characteristics. Most of the wastewater irrigation area is covered with this type of soil.

Soil unit #2

The soils of this unit are deep to moderately deep and well to moderately well drained. They are developed from a thin veneer of dark deddish-brown soil materials associated with hematite sandstones which are underlain by soils developed from thinly bedded sandstones and shales of the Junieta Formation. The upper soil profile is loamy and has good infiltration characteristics similar to those of unit #1. The subsoils have developed from weathered sandstones and shales and contain higher clay contents and are somewhat less permeable than those of unit #1. Soil colors in the topsoil horizons are dark brown to dark reddish-brown and black with good permeability. Subsoil colors range from dark reddish-brown to yellowish-red, strong brown, yellowish-brown and sometimes contain gray and white mottles at depths ranging from about 30 to 48 inches below the soil surface. Permeability of the upper soil profile is moderately rapid and for the subsoil horizons is moderate to moderately slow. The soil of this unit are moderate to fair for use as wastewater irrigation sites. They are limited by occupying slope positions which range from about 3% to slightly more than 20%. Depth to bed rock ranges from 90 to 150 cm and permeability of the slowest horizon is in the order of 3.2 mm per hour. It is suggested that this area be utilized as a reserve irrigation site.

Soil unit #3

The soils of this unit have developed from a thin veneer of dark reddish-brown colluvial material associated with hematite sandstone which is underlain by clayey soils developed from stratified shale and sandstone of the Junieta Formation. The soils occupy gently rolling topography and have developed heavy clay subsoils. Gray mottles present are indicative of seasonal perched water tables which occur at depths ranging from 50 to 105 cm below the soil surface. These soils are not recommended for use as wastewater irrigation sites.

VITA

Dong Yeob Kim, the son of Il Hyun Kim and Do Won Yang, was born in Seoul, Korea on February 8, 1957. D. Kim received a B.S. degree in Forestry from Seoul National University in 1979. After serving two and a half years in the Korean Army, he worked in Chonju Paper Manufacturing Company in Korea from 1981 to 1982. He resumed his studies in forestry at Seoul National University Graduate School in 1983. He came to Oregon State University in 1984 and received his M.S. degree in Forest Ecology in 1987. He started a Ph.D. program at Virginia Polytechnic Institute and State University under the direction of Dr. James A. Burger in 1988 and received his Ph.D. degree in Forest Soils in 1992. D. Kim has been working in the Department of Forest Resources, University of Minnesota as a research associate since 1991. D. Kim and Lui Sook Chung married in 1985. They have a son, Aaron Yuhwan, born in 1987.

D. Y. Kim