WATER RESOURCES research center

Publication No. 48

DENITRIFICATION IN WETLANDS AS A MEANS OF WATER QUALITY IMPROVEMENT

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PUBLICATION NO. 48

FLORIDA WATER RESOURCES RESEARCH CENTER

RESEARCH PROJECT TECHNICAL COMPLETION REPORT

OWRT Project Number B-035-FLA

Matching Grant Agreement Number
14-34-0001-8073

Report Submitted: May, 1980

The work upon which this report is based was supported in part by funds provided by the United States Department of the Interior, Office of Water Research and Technology as Authorized under the Water Resources Research Act of 1964 as amended.

ACKNOWLEDGEMENTS

The authors wish to extend appreciation to the Office of Water Research and Technology, United States Department of the Interior, for financial support of this work. The administrative assistance of Dr. W. H. Morgan (former Director) and Dr. James Heaney (present Director) of the Florida Water Resources Research Center is greatly appreciated. Thanks are also due Ms. Mary Robinson for accounting assistance. Supplemental financial support of the Center of Environmental Programs, Institute of Food and Agricultural Sciences, University of Florida is gratefully acknowledged.

Thanks are also due Drs. S. E. Bayley and J. Zoltek, Jr. for allowing us to cooperate on their study of the Clermont marsh, and to Dr. K. R. Reddy for his constructive advice and assistance. The assistance of U.S.D.A. Soil Conservation Service personnel in locating sampling sites was extremely helpful. Special thanks go to Candace Cantlin and Diane Sompongse for their assistance in the field and in the laboratory.

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ABSTRACT

In recent years, much attention has been focused on the use of wetlands for nutrient removal from polluted waters such as secondarily treated sewage effluent and agricultural drainage waters. In the case of non-point sources such as agricultural runoff, wetland treatment may be the only feasible means of pollution abatement. Nitrification and subsequent denitrification can be an important route for the removal of nitrogen from water flowing through a wetland ecosystem. The objectives of this research were (1) to determine the extent of nitrification and denitrification occurring in a marsh ecosystem receiving secondarily treated sewage effluent, and (2) to measure denitrification and nitrous oxide evolution rates in a variety of flooded wetland soils.

Nitrification and denitrification rates were measured in simulated marsh ecosystems, i.e., soil:water columns containing approximately 30 cm of marsh soil and either 15 or 30 cm of overlying water (secondarily treated sewage effluent). Nitrification was apparently rate limiting when nitrogen was supplied in the form of ammonium. Therefore, efficiency of nitrogen removal would be increased by allowing the nitrogen in wastewater to nitrify prior to entering the marsh. First-order denitrification was observed in soil:water columns. Average nitrate removal rates, assuming a floodwater nitrate concentration of 10 mg N $liter^{-1}$, were 1.2 kg N $ha^{-1}day^{-1}$ without plants and 2.0 kg N $ha^{-1}day^{-1}$ with plants.

Denitrification potentials of Florida wetland soils were determined using 15 soils with a wide range of texture, organic matter content, and soil reaction (pH). Denitrification followed apparent first-order kinetics, with rate constants varying from 0.040 day^{-1} to 0.192 day^{-1}. Nitrate removal rates, assuming a 10 mg N $liter^{-1}$ floodwater nitrate concentration, ranged from 0.6 to 2.9 kg N $ha^{-1}day^{-1}$. Denitrification rate was significantly correlated with soil organic carbon content and with soil pH. Nitrous oxide accounted for from less than 0.2 to 6.5% of nitrate consumed.

The experimental data obtained in this work indicate that many Florida wetlands could function as efficient treatment systems for nitrate-bearing wastewater and drainage water. Soils high in organic matter with a pH range of 5 to 7 would provide the most rapid removal rates.

INTRODUCTION

Considerable concern exists over detrimental effects of human activities on natural waters. One major problem is excessive nitrogen input from such sources as domestic sewage, urban and agricultural runoff, and certain industrial wastes. In waters where nitrogen is the limiting nutrient, excessive input can accelerate eutrophication, thereby detracting from the aesthetic, recreational, and commercial value of the waters. Nitrogen pollution may also be a health hazard, as nitrate-N levels above 10 ppm in water for human or animal consumption are known to result in methemoglobinemia, a potentially fatal disorder.

A variety of methods exist for removal of nitrogen from wastewater with tertiary treatment, including ammonia stripping and induced nitrification-denitrification. These generally require substantial capital investment and energy expenditure. Diversion of wastewater into wetlands has recently attracted interest as a low-cost, effective alternative for removal of nitrogen and other undesirable materials. In the case of non-point sources of pollution such as agricultural runoff, wetland treatment may be the only feasible means of pollution abatement.

Nitrate is an important form of nitrogen in wastewater. Denitrification, the microbial reduction of nitrate to gaseous products (primarily denitrogen and nitrous oxide), is among the mechanisms for removal of nitrate by wetlands. An undesirable aspect of denitrification is release of nitrous oxide into the atmosphere, as this compound reacts with and destroys ozone in the stratosphere. Ozone is important in shielding the earth from solar ultraviolet radiation. Depletion of the stratospheric "ozone layer" and consequent high levels of ultraviolet radiation would be likely to have a number of negative effects on terrestrial lifeforms, including increased incidence of skin cancer in humans.

Knowledge of wetland denitrification rates and soil characteristics affecting these rates is incomplete, and knowledge of wetland nitrous oxide production is almost nonexistent. It is hoped that the results presented here may be useful in the evaluation of wetland sites for wastewater treatment, and perhaps contribute to the development of regional or global nitrogen budgets. Specific objectives include:

(1) To determine nitrification and denitrification rates in a marsh soil receiving secondarily treated sewage effluent.

(2) To measure denitrification rates in a variety of flooded wetland soils and to determine quantitative relationships between denitrification rate and selected soil characteristics in order to develop a predictive equation for denitrification rates in specific soils.

(3) To investigate nitrous oxide evolution from wetland soils in terms of amounts evolved and factors influencing evolution rates.

CHAPTER I

LITERATURE REVIEW

Wetlands as Wastewater Treatment Systems

A variety of wetland ecosystems, including freshwater marshes (Fetter et al., 1978; Zoltek et al., 1979), hardwood swamps (Boyt et al., 1977), and cypress domes (Odum and Ewel, 1977) have been investigated as wastewater treatment systems. Studies have included systems with primarily downward percolation (Odum and Ewel, 1977), primarily lateral flow (Boyt et al., 1977), and intermediate situations (Zoltek et al., 1979). In general, wetlands have been quite efficient in removal of nitrogen, phosphorus, B.O.D., and coliform bacteria from both secondarily treated sewage and agricultural and urban runoff. Removal of 90% or more of incoming nitrogen and phosphorus is not uncommon, although much lower efficiencies have been reported (Fetter et al., 1978).

Denitrification, plant uptake, and microbial assimilation (immobilization) are possible nitrogen removal mechanisms. The last two processes can remove both ammonia and nitrate, the primary inorganic nitrogen forms in wastewater. By definition, denitrification involves only nitrate. Ammonia entering a wetland must therefore be nitrified (biologically oxidized to nitrate) before it can be denitrified. Nitrification can occur in the aerobic floodwater or surface soil zone and the nitrate produced is subject to denitrification after diffusion into the deeper, anaerobic soil (Patrick and Reddy, 1976). The kinetics of this situation are quite complex, and either nitrification or denitrification may be rate limiting (Reddy and Graetz, 1980).

A significant portion of the nitrogen entering a wetland may already be in the nitrate form (Fetter et al., 1978; Boyt et al., 1977). In one study, an artificial wetland treatment system was specifically designed to maximize nitrification during flow of runoff through pasture prior to entering the actual wetland (Small, 1978). Thus, the nitrogen burden on the wetland was mostly nitrate, with denitrification the primary removal mechanism.

Nitrification

Nitrification is a biologically mediated two-step reaction in which ammonium is first oxidized to nitrite and then to nitrate (Alexander, 1977). The organisms responsible for these reactions are chemoautotrophs and therefore are not dependent upon organic carbon, but rather use carbon dioxide as the carbon source. Nitrifier numbers have been found to vary from zero to a million organisms per gram of soil, depending on conditions. Their numbers are generally less in water samples, with ammonium oxidizers ranging from zero to a few thousand and nitrite oxidizers from zero to a few hundred (Matulewich et al., 1975). Nitrifiers are reported to decrease to very low numbers in climax ecosystems (Rice and Pancholy, 1973). These low numbers have been partially explained by increasing concentrations of tannins (Rice and Pancholy, 1978) and other carbon containing compounds (EPA, 1973; EPA, 1975) that have been reported to have an inhibitory effect, although this has

been debated. Alexander (1977) attributed this "inhibition" to the depletion of inorganic nitrogen (i.e., ammonium and nitrate) which is assimilated by the proliferating heterotrophic microorganisms decomposing the carbonaceous compounds. Free ammonia at high pH values has been found inhibitory (Alexander, 1977) as well as accumulation of certain trace metals (Liang and Tabatabai, 1978) and certain pesticides (Alexander, 1965).

Although autotrophic microorganisms are most often responsible for nitrification, heterotrophic nitrification has recently been observed. Results indicate that under appropriate conditions of pH, sufficient carbon and ammoniacal nitrogen (and low competition), heterotrophic bacteria (and actinomycetes) can produce small quantities of nitrite as well as other more oxidized inorganic and organic nitrogen compounds, i.e., hydroxylamine, 1-nitrosoethanol (Alexander, 1977; Verstraete and Alexander, 1973). Heterotrophic nitrate formation is usually minimal but it has been shown that certain fungi (in culture) are capable of oxidizing nitrite to nitrate. Recently, work done by Tate (1977) on an organic soil in South Florida with a pH of 7.1, showed that the populations of Nitrosomonas and Nitrobacter (autotrophs) were only sufficient to account for 0.1% of the nitrate present. With selective culturing techniques, large populations of Arthrobacter (heterotrophs) were found. When these bacteria were inoculated into sterile soil, nitrite was produced. Thus, it appears that in some cases, heterotrophic nitrification can be an important source of nitrate, but the general significance of this process has not yet been determined.

Nitrification rates are very sensitive to environmental conditions. The most obvious consideration is that the nitrifiers exist under aerobic conditions with ammonium present. In flooded soils, oxygen supply is a potential limiting factor for nitrification since few nitrifiers are present in the overlying aerobic waters and oxygen diffusion into the soil is slow. White et al. (1977) suggested the coupling of photosynthetic oxygen production in surface water with nitrification since nitrification rates were higher at midday than morning and higher in July and August than any other months. Other work by White et al. (1977) showed daytime nitrate production three times that at night. Consequently, development of an oxidized surface soil layer is crucial to the nitrification process which requires 4.3 mg of O_2 to oxidize one milligram of ammonium nitrogen to nitrate (White et al., 1977). Reddy et al. (1976) described the development of an oxidized layer in initially anaerobic soil:water columns amended with 200 mg NH_4-N per gram of soil. In the first week a thin aerobic soil layer developed on top of the anaerobic soil, which increased to 0.5, 1.25, 1.5, 1.6, and 2.0 cm after 7, 15, 30, 60, and 120 days respectively, at 28°C. Patrick and Delaune (1972) also found the aerobic layer to increase to 1.5 and 2.0 cm with time. The rate of development and thickness of this layer is determined by the oxygen supply and amount of readily decomposable organic matter, being slower and thinner with more organic matter due to increased microbial activity and higher oxygen demand (Engler and Patrick, 1974; Patrick and Reddy, 1976). The

small size of this aerobic layer has been implicated as the limiting factor in nitrification (and consequently denitrification) in many flooded soils (Patrick and Tusneem, 1972). Ammonium is supplied to the oxidized soil layer by diffusion from eutrophic surface waters or from the anaerobic subsurface soil by diffusion and physical disruption (by benthic fauna and gas ebullition) (Chen et al., 1972). In experiments using flooded columns packed with ammonium amended soils, it was reported (Patrick and Reddy, 1976) that approximately half of the ammonium nitrified was initially present in the aerobic surface layer of soil and the remaining half diffused to the aerobic layer from the anaerobic subsurface soil. The diffusion coefficients calculated in the above experiments for ammonium moving in a saturated Crowley silt loam were 0.216 cm^2/day (Patrick and Reddy, 1976; Reddy et al., 1976).

Reddy et al. (1976) reported that nitrification followed zero-order kinetics (based on ammonium loss). Other work (based on nitrite plus nitrate production) indicated a sigmoid relationship where the peak production varied with the initial concentration of ammonium (Alexander, 1977).

Alexander (1975, 1977) reported a significant relationship between nitrate production and pH. In neutral to slightly alkaline soils, nitrifiers are present in the largest numbers. The optimum range for nitrate production has been reported to vary from 6.6 to 9.0 (Alexander, 1977; Keeney and Gardner, 1970; EPA, 1975). At high pH values, ammonium changes to ammonia which is detrimental to Nitrobacter populations, and as a result, oxidation of nitrite to nitrate is inhibited. Lime has been reported to stimulate nitrification (Alexander, 1977; Tilsdale and Nelson, 1975) by serving as a pH buffer and a carbon source. On the other end of the pH scale, nitrification has been reported below pH 4 but generally does not occur at a significant rate until a pH of 5.0 to 5.5 is reached (Alexander, 1977; Tilsdale and Nelson, 1975). The nitrification process also lowers pH due to the release of hydrogen ions as ammonium is converted stepwise to nitrate (Broadbent, 1973). The extent of the lowering depends on the amount of ammonium nitrified and the buffering capacity of the system.

Temperature has also been shown to have a marked effect on nitrification with an optimum range between 30 and 35°C (Alexander, 1977; Broadbent, 1973). Above 40°C and near freezing, the amount of nitrate or nitrite produced becomes negligible (Tilsdale and Nelson, 1975). White et al. (1977) reported nitrification (in stream bed sediments) to cease when the water temperatures dropped below 17°C.

Since the nitrifiers are obligate aerobes, they are more sensitive to excess moisture than dry conditions, although nitrate is not produced at very low moisture levels. Chemical breakdown of nitrite (and release as gaseous nitrous oxide) has also been implicated under well aerated,

low moisture conditions. Lance and Whisler (1972) showed the effect of varying cycles of flooding and drying on nitrification of ammonium in wastewater and found essentially all the ammonium was nitrified with short frequent cycles: 2 days flooded followed by 5 days of drying. Other factors such as season or depth in the profile are important only as they are related to factors such as temperature, pH, oxygen content, and nutrient availability.

Denitrification

Biological denitrification is generally regarded as the use of nitrate and other oxidized forms of nitrogen as alternatives to oxygen in the respiratory process. A large number of heterotrophic bacteria, and a smaller number of autotrophs are capable of this process (Payne, 1973). Virtually all denitrifiers can respire aerobically, and some can obtain energy through fermentation. Because of the diversity of denitrifying organisms, denitrifier populations are relatively high in most soil habitats. With a few possible exceptions (Delwiche and Bryan, 1976), denitrification occurs only under at least partially anaerobic conditions, since oxygen is used preferentially to nitrate when both are present. In flooded systems, anaerobic conditions exist below a surface layer of aerobic soil, the thickness of which is controlled by oxygen concentration in the overlying water and oxygen demand of the soil (Bouldin, 1968).

As with most biochemical processes, denitrification rate tends to increase with rising temperature until heat induced enzyme deactivation begins to occur (Focht, 1974). Slow denitrification has been reported at 3°C (Nommik, 1956), with rates increasing rapidly up to about 35°C (Bremner and Shaw, 1958; Bailey and Beauchamp, 1973). Rates increase somewhat more slowly from 35° to about 60-65°C, dropping off rapidly at higher temperatures (Nommik, 1956; Bremner and Shaw, 1958).

Amount and source of available carbon is perhaps the most important soil characteristic determining denitrification rate. Many reports link rapid denitrification with high levels of organic matter, in both flooded and unflooded systems (McGarity and Meyers, 1968; Stefanson, 1972; Engler and Patrick, 1974). Added organic material tends to stimulate the process more than endogenous organic matter because the latter is generally humified and thus resistant to degradation (Bremner and Shaw, 1958; Reddy et al., 1978). Alternation of aerobic and anaerobic conditions has been found to increase denitrification by increasing carbon decomposition (Lance and Whisler, 1972; Reddy and Patrick, 1975).

At least three studies have attempted to establish a quantitative relationship between organic carbon and denitrification. Stanford et al. (1975a) related hot water extractable carbohydrate to denitrification rate for 30 widely varying soils. An r^2 value of 0.82 was obtained for carbohydrate vs. apparent first-order denitrification rate, while total organic carbon vs. apparent first-order denitrification rate gave an r^2 of 0.69. Burford and Bremner (1975) studied 17 soils and obtained a 0.99 r^2 value for zero-order denitrification rate vs. "mineralizable" carbon as determined by CO_2 evolution. Total organic carbon

yielded only a 0.59 r^2 value. In both of these studies, denitrification was assessed in batch studies under total anaerobiosis. The relevance to *in situ* denitrification rates may therefore be limited because oxygen is usually present to some degree in natural systems. Andersen (1976) studied nitrate loss from aerobic floodwater overlying five lake sediment cores. Correlating "available carbon" (determined by O_2 consumption) with denitrification, he obtained r^2 values of 0.92 and 0.70 for low (2-3 ppm) and high (10 ppm) nitrate-N concentrations, respectively. These lower correlations than those obtained by Burford and Bremner (1975) may reflect such complicating factors as nitrate transport and the presence of oxygen.

Plant roots have been widely reported to enhance denitrification, particularly in soils with low endogenous carbon (Stefanson, 1972; Bailey, 1976). This has been attributed to increased organic matter or lower oxygen levels in the root zone, or both (Volz et al., 1976; Alexander, 1977). Sherr and Payne (1978) conducted an interesting study on the effect of *Spartina alterniflora* roots on denitrifying activity. Above ground parts of the plants were cut and roots pruned in order to terminate the below ground effects of living plants. Denitrifying activity was found to be unaffected five months after perturbation, but significantly lowered after 18 months. This was taken to indicate that root exudates were relatively unimportant and that the main effect of roots was due to long term maintenance of underground biomass. Another important implication of this study is that root pruning, such as would occur in the taking of soil cores, may have little short term effect on denitrification.

There is a definite relationship between soil pH and denitrification, but the strength of this relationship is subject to question. A number of reports indicate that nitrate loss rates are highest between pH 7 and 8, and drop off considerably below pH 6 (Wijler and Delwiche, 1954; Nommik, 1956; Bremner and Shaw, 1958). Some of these reports (Nommik, 1956; Bremner and Shaw, 1958) are suspect because pH variation was accomplished by amendment with acid or base, resulting in non-adapted microbial populations. Brezonik (1977) feels that the variety and adaptability of denitrifying organisms reduces the importance of pH in determining nitrate consumption rates. There are several reports of relatively rapid denitrification at pH values approaching 5.0 (Van Cleemput et al., 1975) and even below 5.0 (Ekpete and Cornfield, 1965; Erickson, 1978; Gilliam and Gambrell, 1978).

A factor which has not been widely investigated is duration of exposure of the soil to nitrate. As with any microbial substrate, addition of nitrate to soil results in certain adaptive and selective changes in the soil population (Nash and Bollag, 1974; Doner and McLaren, 1978). Insufficient nitrate adaptation time has been invoked as the cause of irregular nitrate consumption rates in short term studies (Starr and Parlange, 1975). In one of the very few truly long term denitrification experiments, a soil column was leached with a solution of 100 ppm nitrate-N for over 1,000 days (Day et al., 1978). Nitrate removal rate in the top 12.5 cm was found to decrease several fold over the first 30 days, and more slowly thereafter. This effect was

attributed to depletion of available organic matter. Such a dramatic effect might not occur under other experimental conditions. Leaching tends to maximize organic matter loss through removal of the soluble fraction (Doner et al., 1974) and would be expected to maximize oxidation by continually and rapidly resupplying nitrate. Reddy found relatively little change in nitrate removal rate from floodwater overlying an organic soil during a 25 day period (K. R. Reddy, personal communication).

Denitrification Kinetics: Real and Apparent

The literature regarding denitrification kinetics is somewhat confusing, with zero-order, first-order, and Michaelis-Menton kinetics reported. Zero-order kinetics are reported most frequently (Nommik, 1956; Broadbent and Clark, 1965; Cooper and Smith, 1965; Van Cleemput et al., 1975). Some authors (Focht, 1974; Doner et al., 1974) regard these reports to be indicative of Michaelis-Menten kinetics with very low Km values, rather than true zero-order kinetics. In sewage treatment systems, Michaelis-Menten kinetics with Km values of about 0.05 ppm nitrate-N have been reported for denitrification (Requa and Schroeder, 1973; Stensel et al., 1973). Doner et al. (1974) reported "very low" Km values for soil column studies but did not specify numbers. A much higher Km value of 125 ppm nitrate-N has been reported for a continuous flow soil column (Ardakani et al., 1975). This unlikely value may be due to failure to achieve a true steady state or failure to account for diffusion of nitrate into microsites (Galsworthy et al., 1978).

First-order denitrification kinetics have also been widely reported, primarily with experimental designs involving floodwater overlying soil (Bowman and Focht, 1974; Stanford et al., 1975). The relationship between presence of floodwater and first-order kinetics prompted a thorough study by Phillips et al. (1978) and Reddy et al. (1978). They determined both experimentally and theoretically that zero-order nitrate depletion in the soil together with continuous resupply by diffusion from floodwater results in what appears to be a first-order nitrate loss rate. The initial boundary conditions for the model of Phillips et al. (1975) involve an even distribution of nitrate in floodwater and soil solution. In such a situation, the nitrate diffusion front does not reach an equilibrium, and a quasi-steady state is not attained until after the majority of nitrate in the system has been denitrified. For this reason the model is applicable to batch type studies where the above condition is met, but not to column studies where initial soil nitrate concentration is zero.

Bouldin and coworkers addressed the conditions which would occur in soil columns more directly. First, a model was developed for oxygen consumption by sediments (Bouldin, 1968). Applying Fick's diffusion laws and assuming zero-order oxygen consumption within the sediment, it was determined that the second derivative of oxygen concentration with respect to depth is a constant. This results in a parabolic shaped, steady state diffusion front as shown in Figure 1(a). Since oxygen demand is constant in the volume of sediment exposed to oxygen, total

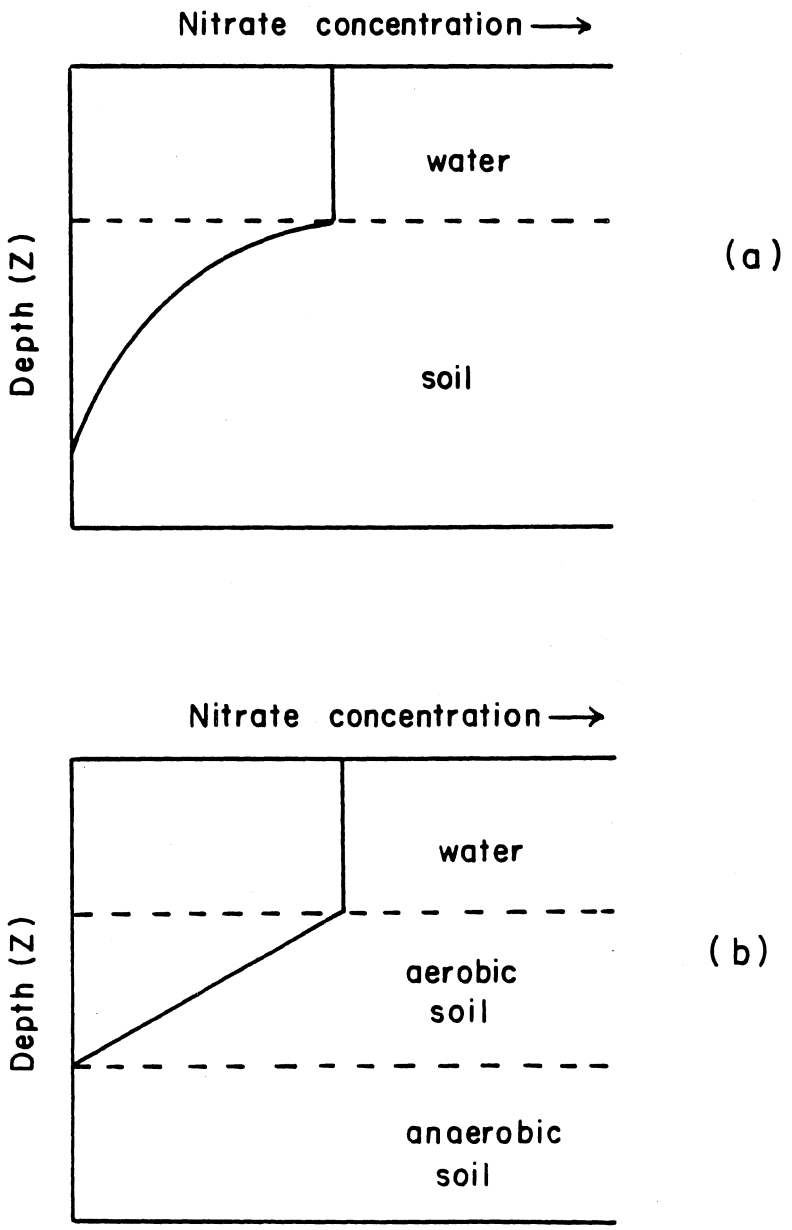

Figure 1. Nitrate diffusion fronts in flooded soil systems (a) with no aerobic zone, and (b) with aerobic zone.

consumption is proportional to depth of penetration. Both are proportional to the square root of oxygen concentration in the floodwater and also to the square root of volumetric sediment oxygen demand, as in the following equation:

$$\frac{dQ}{dt} = (2CAD)^{\frac{1}{2}} \qquad [1]$$

where Q is quantity of oxygen (mg) crossing the soil:water interface; C is floodwater oxygen concentration, mg liter^{-1}; A is rate of oxygen consumption, mg cm^{-3}day^{-1}; D is oxygen diffusion coefficient, cm^{2}day^{-1}; and t is time, in days. These relationships were later verified experimentally (Howeler and Bouldin, 1972).

The above model would be expected to apply to denitrification if it were assumed that nitrate concentration in the floodwater decreased fairly slowly (as would be the case with all but very shallow water depths) and that nitrate consumption is uniform from the soil:water interface downward. The second assumption is not valid when oxygen is present in the water, since denitrification is theoretically zero in the aerobic soil layer. Bouldin et al. (1974) addressed this situation by developing a model which assumes no nitrate consumption in the aerobic zone and maintenance of nitrate concentration at zero at the aerobic: anaerobic interface. In this case, the second derivation of concentration with respect to depth is zero, and the diffusion front is as shown in Figure 1(b). The total consumption is now directly proportional to floodwater concentration (i.e., apparent first-order) and directly proportional to the thickness of the aerobic zone. The assumption that nitrate reduction is rapid enough to maintain zero concentration at the aerobic:anaerobic interface is obviously and admittedly (Bouldin et al., 1974) erroneous, and is adopted for the sake of simplifying the model. As a result, there is no allowance in the model for volumetric nitrate consumption rate. This problem may be insignificant since oxygen consumption rate and denitrification rate are closely related. A truly accurate model would involve a combination of Figures 1(a) and 1(b) and would be somewhat complex, mathematically. A number of investigators (Van Kessel, 1976; Reddy et al., 1979) have obtained high r^2 values when applying apparent first-order denitrification kinetics to nitrate consumption data under boundary conditions similar to those assumed by the model of Bouldin et al. (1974), indicating that this model may be adequate. Apparently no attempts to correlate nitrate loss to square root of floodwater concentration (as in the 1968 Bouldin model) have been published.

Additional Mechanisms for Nitrate Loss

Although reduction of nitrate to nitrite in nature is thought to be a solely biological process, under certain conditions nitrite can be chemically decomposed into gaseous products. Four major types of chemodenitrification are generally recognized: (1) self-decomposition of nitrite into NO; (2) reaction of nitrite with ammonia, urea or amino acids yielding N_2 (Van Slyke reaction); (3) reaction with organic matter yielding mostly N_2; and (4) reaction with heavy metals resulting

in NO, N_2O or N_2 (Broadbent and Clark, 1965). The first three require a pH in the vicinity of 5.0 or lower. The last can occur at a pH as high as 8.0 (Moraghan and Buresh, 1977). There is evidence for the occurrence of all of these reactions in sterile, nitrite amended soils (Van Cleemput et al., 1976; Nelson and Bremner, 1970). Assessment of the natural significance of chemodenitrification is difficult due to inability to inhibit biological nitrite decomposition without inhibiting biological nitrite production (Van Cleemput et al., 1975). However, it is generally felt that chemodenitrification is insignificant in nature (Burford and Stefanson, 1973; Focht, 1974).

Dissimilatory reduction of nitrate to ammonia is analogous to denitrification, but the end product is ammonia rather than gaseous N compounds. As in denitrification, the reduction is for the purpose of obtaining respiratory energy rather than obtaining cell nitrogen. This process was first described over 20 years ago (Verhoeven, 1956) but has only recently received much attention. Although populations capable of this process may be large (Tiedje et al., 1979), the process tends to occur only under intensely reduced conditions (Buresh and Patrick, 1978). Since nitrate is produced under oxidizing conditions, the majority of nitrate would likely be denitrified before reaching conditions sufficiently reducing for ammonia production. For this reason, Buresh and Patrick (1978) feel that dissimilatory reduction to ammonia is insignificant in flooded soils. Sorensen (1978) feels that this process may be of equal significance to denitrification in marine sediments.

A significant portion of the nitrate entering soils can be assimilated by microorganisms and converted to organic nitrogen. Assimilation of added nitrate is usually low when soil C:N ratio is narrow, since nitrogen assimilation needs would be met by mineralized N. For this reason, assimilation is lower with a well humified carbon source than with fresher material (Alexander, 1977).

Reports of assimilation under saturated conditions range from about 10% to 37% of applied nitrate (Andersen, 1976; Chen et al., 1972; Chen and Keeney, 1974). There is some indication that percent assimilation is related to concentration of added nitrate, 37%, 14-26%, and 17% having been reported for concentrations of 2 ppm-N (Chen et al., 1972), 10 ppm-N (Chen and Keeney, 1974), and 100 ppm-N (Stanford et al., 1975b), respectively. This would be expected in view of the relatively low assimilation needs and high respiration needs of anoxic communities (Tusneem and Patrick, 1971). Doner et al. (1974) found that net assimilation was very low in relatively long term column studies with continuous nitrate supply. It was felt that once rapid increase in microbial biomass ceased and stable population developed, assimilation became nearly zero. An increase in assimilation relative to denitrification at low temperatures has been reported (Craswell, 1978), possibly indicating that denitrification is more affected by cold than is assimilation.

Nitrous Oxide Production

The amount of nitrous oxide (N_2O) evolved during denitrification varies greatly. Ratios of N_2:N_2O can range from greater than 100:1 (Denmead, 1979) to less than 1:10 (Stefanson, 1972). The majority of reports are in the vicinity of 10:1, and the Council for Agricultural Science Technology (C.A.S.T., 1975) has estimated an average worldwide ratio of 16:1 for agricultural soils.

Such factors as high organic matter content, high moisure content, fine soil texture, and low oxygen concentration have all been shown to decrease N_2O emission (Nommik, 1956; Stefanson, 1972; Focht and Verstraete, 1977), indicating that conditions of high electron acceptor demand result in more complete reduction of nitrate to N_2. Guthrie and Duxbury (1978) reported an exception to this trend with lower N_2:N_2O ratios occurring in flooded histosols than in the same soil when drained. Ratios of N_2:N_2O have been reported to decrease at low temperatures (Bailey and Beauchamp, 1973).

Nitrous oxide production tends to increase with decreasing pH (Wijler and Delwiche, 1954; Nommik, 1956; Burford and Bremner, 1978). Blackmer and Bremner (1978) have shown that this may be due to an indirect effect of pH. These investigators reported that high nitrate concentrations inhibited N_2O reduction, thereby increasing emission of this gas. The effect was greater at low pH than at high pH. In the absence of nitrate, N_2O was reduced as rapidly at low pH as at high pH. It was concluded that the effect of pH was to control the degree of nitrate inhibition of N_2O reduction.

Prior exposure to denitrifying conditions (i.e., anoxia and nitrate) can also influence N_2:N_2O ratios. Blackmer and Bremner (1979) observed that nitrate application resulted in an increase in N_2:N_2O ratio over a 48 hour period, presumedly due to induction of N_2O reduction activity in the denitrifier population. Guthrie and Duxbury (1978) noted indications of a more long term increase in N_2:N_2O ratio due to population selection in nitrate-amended soil.

Nitrous oxide can be produced from processes other than denitrification. As mentioned above, chemodenitrification can potentially result in N_2O production, but the extent of this in nature is unknown. Yoshida and Alexander (1970) reported that axenic cultures of *Nitrosomonas* can produce N_2O from nitrite during nitrification of ammonia. This had been thought to be insignificant in nature (Focht, 1974) until recently when it was found that considerable N_2O production can occur during nitrification in aerobic soils (Bremner and Blackmer, 1979; Denmead et al., 1979b). Dissimilatory reduction of nitrate to ammonia can also result in N_2O production in pure culture (Tiedje et al., 1979), but this has not been demonstrated *in situ*.

Summary

Pertinent literature on nitrification, denitrification and N_2O production in soils can be summarized as follows:

(1) Many of the factors affecting nitrification and denitrification in soils are known in a general, qualitative way. Nitrification is favored by high oxygen availability and near neutral pH, and may be inhibited by certain organic substances. Poor aeration, high organic carbon availability and neutral pH tend to accelerate denitrification. In flooded systems, transport of nitrate into the anerobic zone is an important rate-determining factor which many studies have ignored.

(2) In wetland systems, the potential exists for rapid nitrification of incoming ammonia in the upper, aerobic soil zone, and possibly in the water column.

(3) It is likely that denitrification accounts for the majority of nitrate consumption in wetland soils, particularly under conditions of heavy nitrate enrichment.

(4) Relatively little is known about rates of N_2O production during denitrification. Average reported N_2:N_2O ratios are in the vicinity of 10:1. Low electron acceptor demand and low pH tend to favor N_2O production. Soil processes other than denitrification, including nitrification and dissimilatory reduction of nitrate to ammonia, may be important N_2O sources, at least under certain conditions.

(5) The data base for nitrification, denitrification, and N_2O production in wetlands is at present quite narrow.

CHAPTER II

NITRIFICATION AND DENITRIFICATION IN A MARSH SOIL RECEIVING SECONDARILY TREATED SEWAGE EFFLUENT

The objective of this study was to determine the feasibility of using natural wetlands to remove nitrogen via denitrification from secondarily treated municipal sewage effluent. Since nitrogen in the form of nitrate is a prerequisite for denitrification, nitrification was included as part of the study. Nitrogen removal was investigated in laboratory and *in situ* experiments on a freshwater marsh in Central Florida.

METHODS AND MATERIALS

Soil and Water Sampling

Soil samples were obtained from a 32 hectare freshwater marsh located in the town of Clermont, Florida, about 100 miles (160 kilometers) south of Gainesville on Route 27. The soil was classified as a variant of Brighton peat (typic medifibrist) characterized by 1.5 meters of relatively undecomposed, acidic, organic soil overlying 1.5 meters of sand over clay of an undetermined depth.

Soil used in "batch" type studies was collected in bulk from a zone between 8 and 45 cm deep. Soil was not taken from the top 8 cm because of the dense network of roots near the surface. Bulk samples were stored in a polyethylene pail (with lid) until used. Soil used in the column nitrification and denitrification studies was collected as intact columns, some with plants and some without. This was done by selecting an appropriate site (i.e., a young, healthy plant, or no plant), using a shovel to make the initial circular cut through the peat, roots, and/or rhizome, then placing polyvinyl chloride (PVC) pipe (70 x 10 cm, length x diameter) over the plant and pushing it into the soil as far as the cut was made with the shovel. This was followed by hammering the column about 45 to 50 cm into the soil. Compaction was less than 10%. The next step involved rocking the column back and forth in place to break the bottom loose and then pulling the column up with a hand under the bottom to prevent the soil from falling out. A "knock-out test cap" was then pushed into the bottom of the column. Silicone rubber sealant was used around the cap to stop leaks. In the lab, the columns were allowed to stabilize for two to four weeks (with those containing plants placed under a window) while receiving only enough deionized water to keep the soil saturated.

Water used in these investigations was obtained from the following sources: marsh surface water, oxidation pond water and secondarily treated sewage effluent from the Clermont sewage treatment plant, and Lake Alice water (University of Florida campus). All water samples were collected and stored in polyethylene containers. Lake and marsh water

was used within 24 hours. Effluent was stored at 4°C for a maximum of three days. Water samples containing particulate matter were allowed to settle and the water used was siphoned off the top.

Plant species were in the process of change during the study period, due to changing weather patterns and lower water levels. Sagittaria lancifolia (arrowhead) and Pontederia cordata var. Lancifolia (pickerel weed) were the original dominant species which were invaded by Panicum sp. (maidencane) and Hibiscus sp. (marsh hibiscus).

Description of Experiments

Preliminary Nitrification Study

An initial study was designed to determine the effects of dissolved organic compounds (tannins) and bacterial inoculation on nitrification in marsh water. Yellow colored, dissolved organic compounds were removed from the marsh water by adding powdered activated charcoal, mixing for one hour, and filtering (#42 Whatman filter paper). A suspension of nitrifiers was obtained by shaking a mixture containing 100 grams of soil known to contain nitrifiers with 150 ml of deionized water for one hour and allowing the soil to settle out. Ten milliliter aliquots were used as inoculum for the appropriate treatment. All samples were amended with ammonium (as ammonium sulfate) to a concentration of about 50 ppm NH_4-N, placed in polyethylene containers (in triplicate) and incubated at 25°C for 30 days. After 25 days, it was noted that the pH had dropped considerably so it was adjusted up to 7.2 (±0.1) with 0.5N NaOH. Twenty milliliter samples were treated with phenyl mercuric acetate (PMA) and stored at 4°C until pH, ammonium and nitrate were determined, usually within 24 hours.

Preliminary Denitrification Study

An initial laboratory study using test tubes of nitrate-amended solutions and marsh soil was designed to ascertain if denitrification would occur. Solutions of potassium nitrate in deionized water, in marsh water, and in oxidation pond water and of calcium nitrate in deionized water were prepared to a concentration of about 45 ppm NO_3-N. A 10 ml aliquot of each solution was added to a test tube (eight replicates) containing 6.0 (±0.2) grams of marsh soil and incubated at 25°C for 11 days. The same design without the soil was employed to ascertain if denitrification would occur in the water alone. Sampling involved pouring off the solution and analyzing it for ammonium and nitrate.

Nitrification and Denitrification Studies Using Soil:Water Columns

In order to better simulate actual marsh conditions, additional experiments for both nitrification and denitrification were conducted using intact soil:water columns, half with and half without Sagittaria lancifolia (arrowhead). Each column consisted of 45 cm of soil and either 15 or 30 cm of overlying effluent. Treatments in both studies

were the same (Figure 2). Four replications, two with and two without plants, of each of the following treatments were used:

1. Control - effluent to 15-cm depth.
2. N amended effluent to a 15-cm depth.
3. N amended effluent to a 30-cm depth.
4. N amended effluent to a 15-cm depth with 10 grams $CaCO_3$ added to adjust pH.

The amount of calcium carbonate was calculated using Yuan's (1974) double buffer method to bring the top 15 cm of soil to a pH of 7.0. It was applied on the surface of the soil, to the appropriate columns, several days before a study began. A second and third application of the appropriate form of N was pipetted into the individual soil:water columns as a more concentrated solution.

In all cases, evapotranspiration was accounted for (two to four times weekly) by adding deionized water to maintain the appropriate depth. Columns in both studies were aerated to maintain dissolved oxygen near saturation values. The overlying water in the columns was sampled (about 15 ml) at about half the water depth, i.e., 7.5 or 15 cm. Phenyl mercuric acetate was added and the sample was stored at $4^{o}C$ until ammonium and nitrate were determined, usually within 24 hours.

In Situ Nitrification and Denitrification Studies

For the purpose of obtaining natural nitrification and denitrification rates, an in situ study was conducted. Eno (1960) and more recently Struble (1977) found polyethylene bags acceptable for this type of study due to their permeability to gases (specifically oxygen and carbon dioxide) and impermeability to ions such as nitrate and ammonium. Polyethylene bags were filled with 100 grams marsh soil and subjected to the following treatments:

1. Control, shallow - soil in bags placed at 8 cm.
2. Control, deep, lime - soil plus lime in bags buried at 30 cm.
3. Nitrification, shallow - soil amended with ammonium in bags buried at 8 cm.
4. Nitrification, deep - soil amended with ammonium in bags buried at 30 cm.
5. Denitrification, shallow - soil amended with nitrate in bags buried at 8 cm.
6. Denitrification, deep - soil amended with nitrate in bags buried at 30 cm.
7. Denitrification, deep, lime - soil amended with nitrate plus lime in bags buried at 30 cm.

Bags were flattened to maximize surface area and heat sealed. For the nitrification study, soil was amended with ammonium (as ammonium chloride) to bring the final concentration to about 35 ppm NH_4-N.

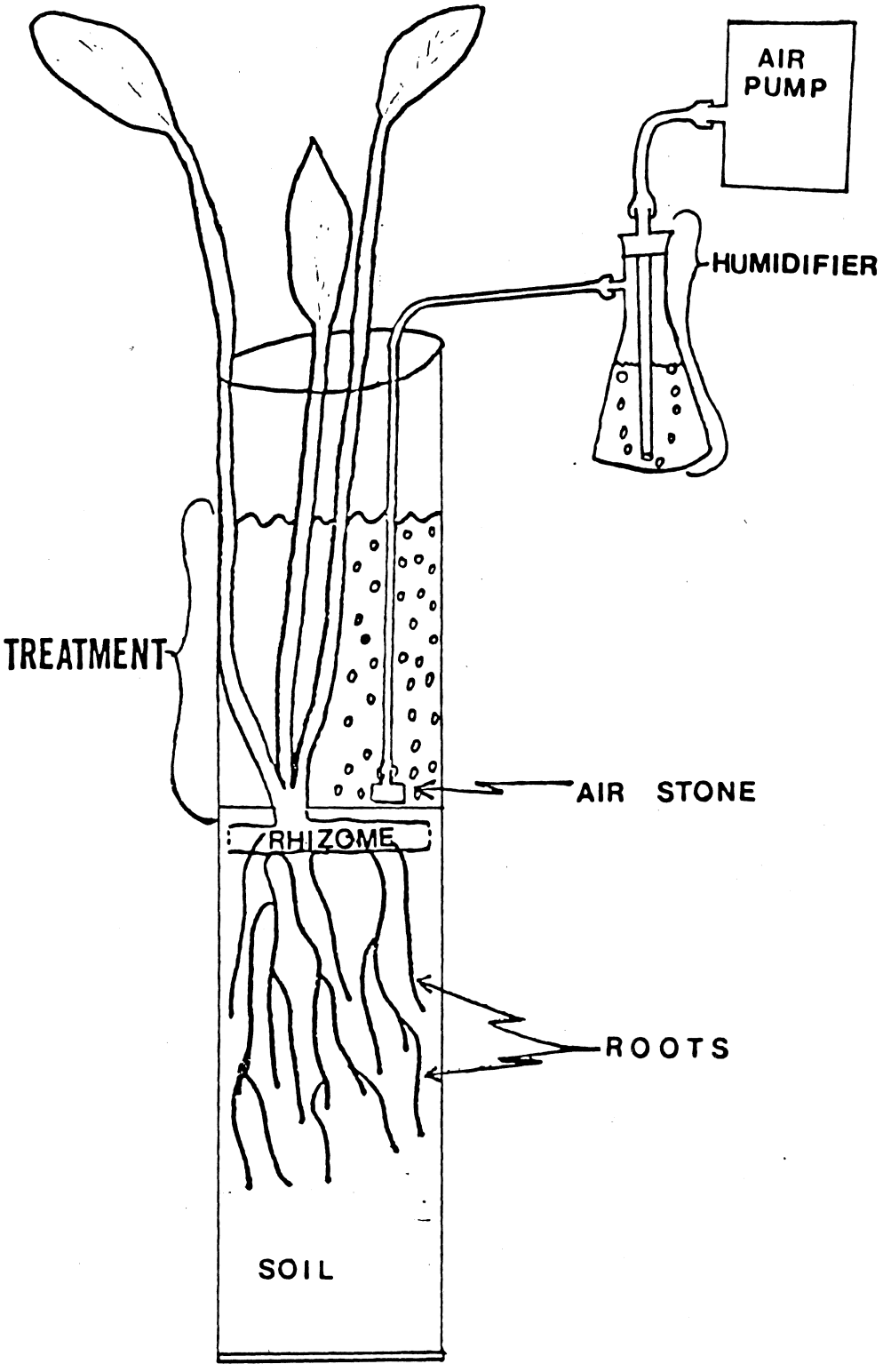

Figure 2. Experimental soil:water columns for nitrification and denitrification studies.

For the denitrification study, soil was similarly amended with nitrate (as potassium nitrate). Control soils received no nitrogen. Lime used in all cases was 0.85 gram of finely powdered calcium carbonate per bag which was the amount extrapolated from an earlier application of Yuan's double buffer method and was intended to bring the pH of the soil to 7.0.

Bags were retrieved at selected intervals. Temperature, pH, and dissolved oxygen readings were taken at each site. After bags were removed, sample pH was determined, and the contents of each bag were extracted with 2N KCl. The extractant was analyzed for ammonium and nitrate.

Analytical Procedures

Extraction Procedure

Ammonium, nitrite, and nitrate were extracted from the soil by transferring the 100 grams of wet organic soil into a polyethylene sample bottle, adding 100 ml of 2N KCl solution, shaking briefly and then allowing the suspension to stand for two hours. The mixture was shaken again before filtration (#40 Whatman filter paper) and finally stored, with PMA, at 4°C until inorganic nitrogen was determined (Bremner, 1965).

Determination of Ammonium and Nitrate

Ammonium and nitrite plus nitrate values were obtained through steam distillation of water samples or potassium chloride extractants (Bremner, 1965).

Determination of pH

An Orion 701 or 401 meter was used in conjunction with a combination pH electrode.

Dissolved Oxygen Determination

A YSI Model 54 oxygen meter with a YSI Model 5419 probe was used to determine concentrations of dissolved oxygen.

RESULTS AND DISCUSSION

Nitrification

Laboratory Investigations

Since nitrification has been reported to be predominently an autotrophic process (Alexander, 1977) and therefore not in need of organic carbon compounds, initial studies were designed to determine if nitrification would occur in the marsh water alone, i.e., without any soil. Preliminary results indicated that nitrification did not occur in the marsh water even though dissolved oxygen and pH were at acceptable levels. Thus, further studies were conducted to identify the reasons for the lack of nitrification. Since nitrification is reportedly inhibited by certain types of dissolved organic compounds (Rice and Pancholy, 1973; EPA, 1973; EPA, 1975), marsh water was filtered through charcoal to remove dissolved organics. Since it was also possible that nitrifying organisms simply were not present in the marsh water, an inoculant containing nitrifiers was added as a second treatment. Water from a eutrophic lake on the University of Florida campus (Lake Alice) was included for comparison purposes. All samples were aerated, amended with ammonium, and incubated in polyethylene containers for 30 days.

Results of charcoal filtration and nitrifier inoculation are given in Table 1. Evaporation caused an increase in ammonium concentration so the effects of the two treatments can best be compared using the nitrate data. Charcoal filtration had little effect on nitrification rate, however, inoculation increased the rate in both the marsh and lake water. These results indicate that nitrification will occur in overlying marsh water if nitrifiers are present, but at least in this marsh, nitrification is minimal due to a lack of nitrifiers. The nitrification that occurred in the uninoculated lake water indicated that some nitrifiers were already present which is a likely consequence of Lake Alice receiving sewage effluent. Having this nitrifying population already present would also explain why nitrification started sooner in the lake water than in the marsh water.

Since the nitrification process releases hydrogen ions and water has a low buffering capacity, the pH dropped substantially during the course of the experiment. After 25 days, the pH had dropped approximately two units and was below that normally acceptable for nitrification which necessitated artificially adjusting it upward. Five days after adjustment, it had again dropped two to three pH units in actively nitrifying samples. In a natural aquatic system, this could rapidly limit nitrification unless the system was adequately buffered to maintain an acceptable pH range. Dissolved oxygen was not limiting throughout the study period.

The above experiment suggested that nitrification did not occur in the overlying natural water of this marsh, but that nitrification would occur in the water if sewage effluent were added. Published work has indicated that nitrification will also occur in the thin, aerobic surface zone of a submerged soil. Thus, the next phase of the present study was designed using soil:water columns to simulate marsh conditions.

Table 1. Effect of charcoal filtration and inoculation with soil water extract on nitrification in marsh and lake waters without soil.

Water source	Treatments: Char. filt.	Treatments: Inoc.	Days: 0	3	7	11	18	25	25'†	30
			NH_4-N, mg liter^{-1}							
Marsh	yes	yes	52.4	49.0	51.5	51.3	52.2	59.7	---	54.7
Marsh	no	yes	50.1	46.5	49.6	47.1	50.8	50.1	---	52.9
Marsh	yes	no	52.4	51.9	52.1	54.1	57.6	61.6	---	65.6
Marsh	no	no	50.1	51.1	48.7	52.2	54.0	60.1	---	63.8
Lake	no	yes	52.3	45.9	38.6	33.0	32.3	33.0	---	27.0
Lake	no	no	52.3	46.5	40.6	35.5	36.8	39.1	---	40.8
			NO_3-N, mg liter^{-1}							
Marsh	yes	yes	0	0.58	1.0	3.6	4.9	7.9	---	17.0
Marsh	no	yes	0	0.70	1.0	2.7	4.9	11.9	---	13.4
Marsh	yes	no	0	0.10	1.0	0.18	0.46	0.10	---	0.8
Marsh	no	no	0	0	1.0	0.50	0.28	1.2	---	0.8
Lake	no	yes	0.35	0	6.2	12.3	12.8	---	---	23.2
Lake	no	no	0.35	1.1	3.4	9.5	10.3	11.5	---	11.5
			Dissolved oxygen, mg liter^{-1}							
Marsh	yes	yes	8.0	8.0	8.1	8.1	8.2	7.4	---	7.3
Marsh	no	yes	7.8	8.1	8.1	8.2	8.0	7.3	---	7.2
Marsh	yes	no	8.0	8.2	8.2	8.2	8.2	7.2	---	7.2
Marsh	no	no	7.8	7.8	8.1	8.0	8.3	7.5	---	7.3
Lake	no	yes	8.3	7.7	7.3	8.0	8.0	7.2	---	7.2
Lake	no	no	8.3	7.9	7.4	8.1	8.1	7.2	---	7.0
			pH							
Marsh	yes	yes	6.6	6.6	6.2	5.1	4.2	4.1	7.2	4.4
Marsh	no	yes	6.5	6.6	6.1	4.9	4.0	3.9	7.1	4.2
Marsh	yes	no	6.6	6.7	6.6	6.5	5.9	5.6	7.2	6.0
Marsh	no	no	6.5	6.5	6.5	6.1	5.8	4.5	7.2	5.5
Lake	no	yes	7.9	7.9	7.4	5.0	4.4	4.3	7.1	4.2
Lake	no	no	7.9	8.0	7.7	5.4	5.6	5.3	7.1	5.2

† pH adjusted to 7.2 (±0.1) with 0.5 N NaOH after 25 day samples taken.

Plants play an important role in controlling nitrogen forms and concentrations in marsh systems through plant uptake and decomposition as well as the introduction of root exudates and oxygen into the rhizosphere soil. For this reason, they were included in this study with water depth and pH as variables. Soil:water columns (10 cm I.D.) containing 45 cm of soil and either 15 or 30 cm of overlying secondarily treated sewage effluent were used. Treatments consisted of a control with sewage effluent to a depth of 15 cm, ammonium-amended effluent to a depth of 15 cm, ammonium-amended effluent to a depth of 30 cm, and ammonium-amended effluent to a depth of 15 cm and pH adjusted with $CaCO_3$. Four replications, two with and two without plants, of the above treatments were used.

Concentrations of ammonium and nitrate observed over a 52 day period in the overlying water are depicted graphically in Figures 3 through 5. Overlying water in the columns was not aerated initially and oxygen diffusion was not sufficient to provide adequate aeration. Consequently oxygen became depleted within the first few days of the study, curtailing nitrification. This resulted in the leveling off or increase of ammonium concentrations in columns without plants. The increase in ammonium concentration was probably due to ammonification, which does not require oxygen. This increase was especially evident in the limed columns as would be expected since ammonification is favored at neutral pH levels (Hubbell, 1971). The effect was not observed in columns with plants because the plants continued to assimilate ammonium. Subsequently, (day 9), all columns were artificially aerated.

Nitrification rate appears to be related to ammonium concentration; however, due to the limited amount of data, first-order kinetics cannot be assumed. No attempt will be made to quantitatively evaluate the kinetics of nitrification in flooded systems. It is likely that nitrification kinetics under the present experimental conditions are quite complex, since a portion of the nitrification is occurring in the water column or at the soil:water interface, while a portion occurs at some small depth within the soil. The former would be expected to be a zero-order process, while the latter is likely non-zero-order because of diffusion dependence (as discussed for denitrification in Chapter 1). Ammonium removal rates given in Table 2 are derived from the slopes of removal curves at approximately 15 to 25 mg $liter^{-1}$ ammonium-N concentration.

In the 15 cm water depth columns with plants, all added ammonium was depleted in approximately 15 days despite the above mentioned aeration problem (Figure 3). At 18 days, additional ammonium was added to both the treatments with and without plants. Ammonium removal continued to be rapid with plants and, in contrast to the earlier days of the study, ammonium removal was also relatively rapid in the columns without plants. Only small amounts of nitrate were detected in either column (Figure 3). This can be explained partly by the fact that some nitrate was removed by the plants and, more importantly, by nitrate diffusion into the anaerobic sediment and subsequent denitrification. Ammonium removal rate (Table 2) was greater with plants (2.9 kg N ha^{-1} day^{-1}) than without plants (1.5 kg N $ha^{-1}day^{-1}$).

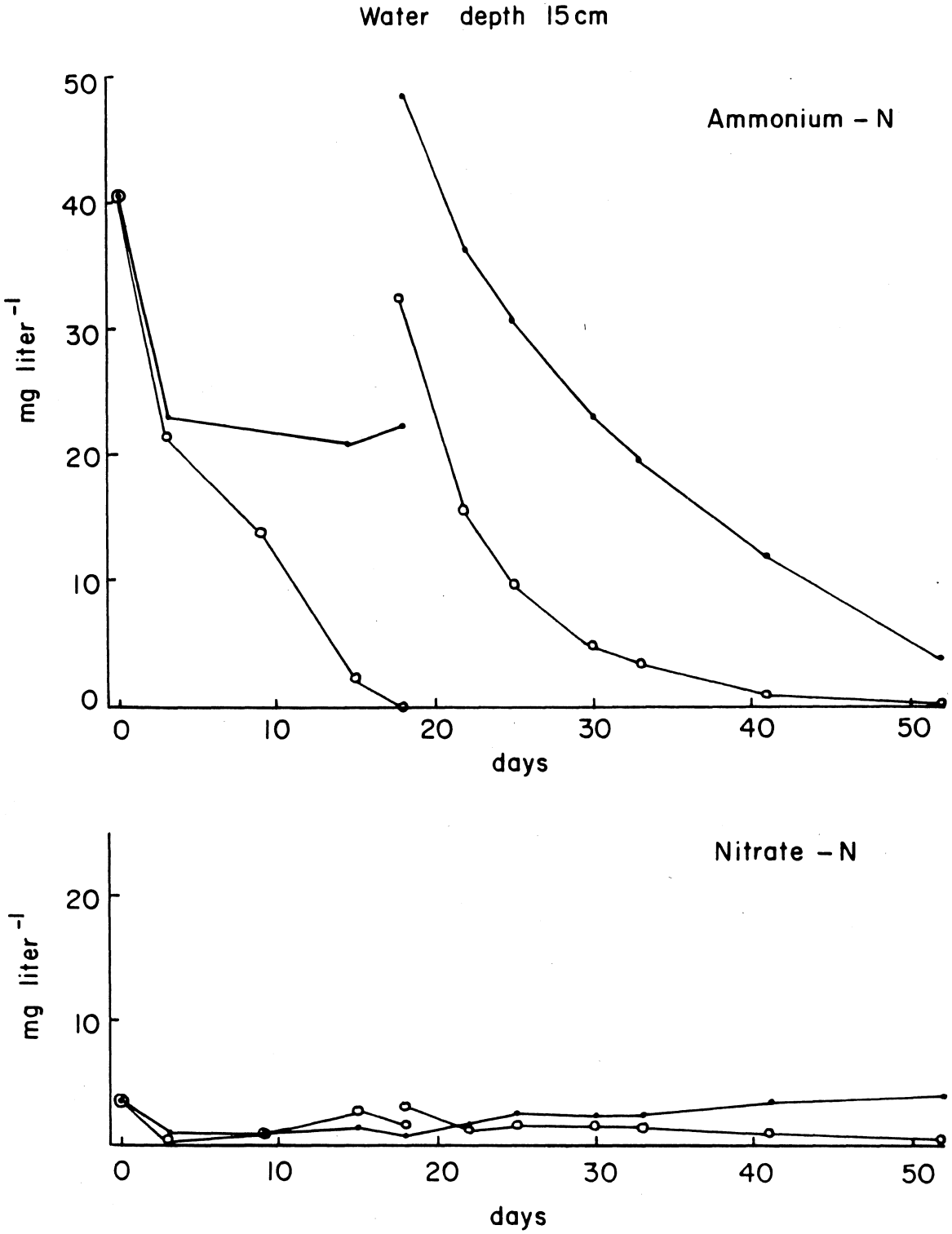

Figure 3. Nitrification in soil:water columns flooded with 15 cm of ammonium amended sewage effluent, with [o] and without [•] plants.

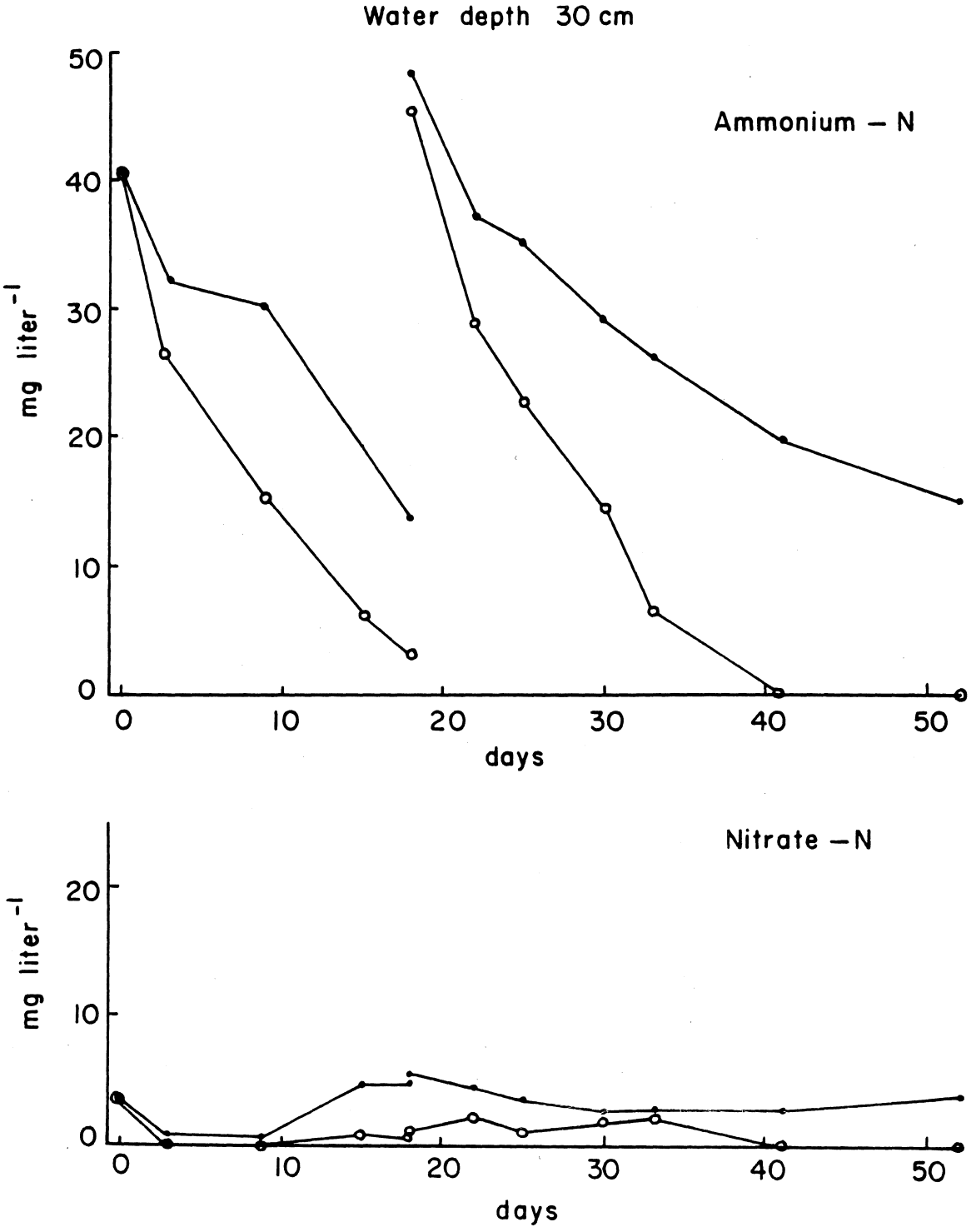

Figure 4. Nitrification in soil:water columns flooded with 30 cm of ammonium amended sewage effluent, with [o] and without [•] plants.

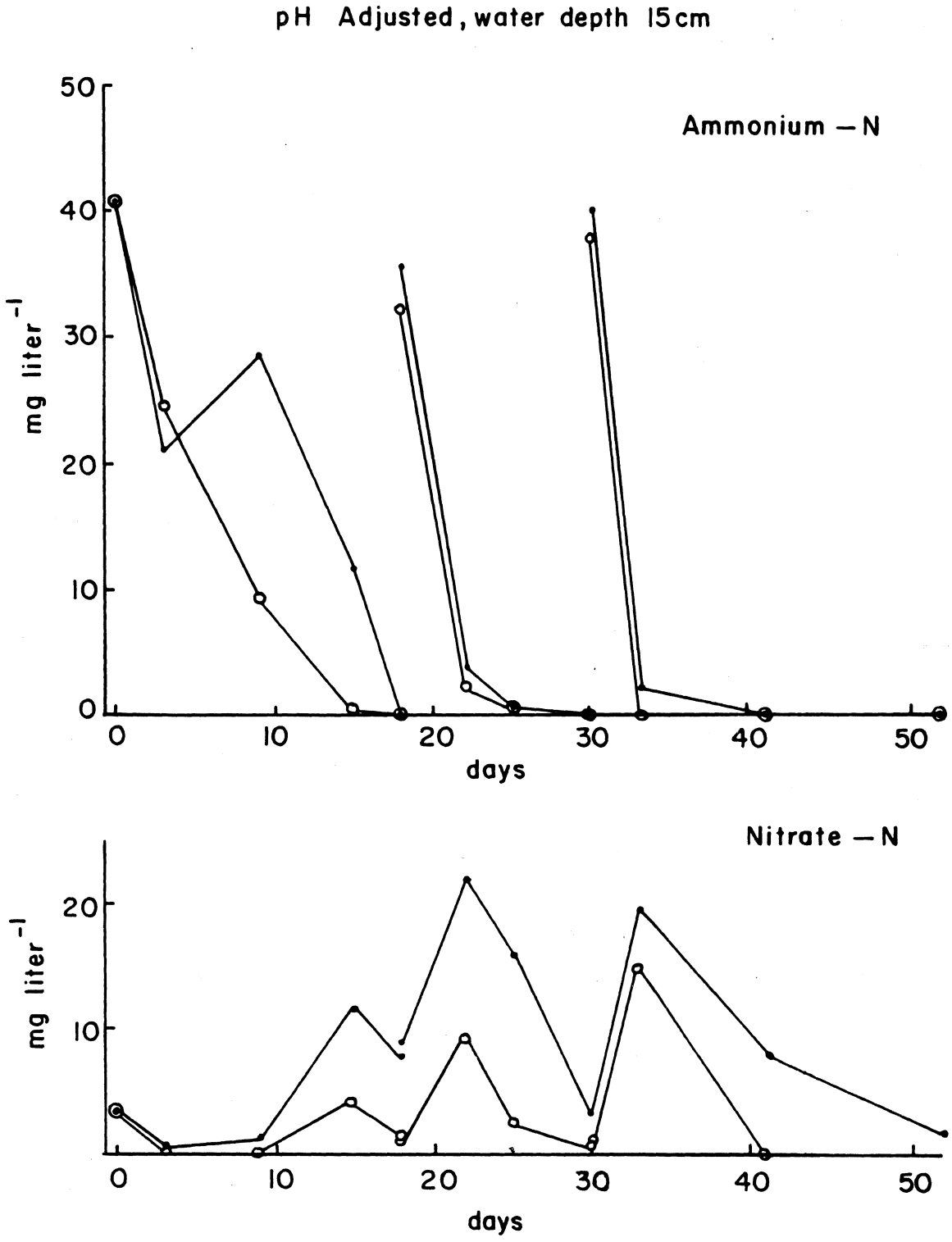

Figure 5. Nitrification in limed soil:water columns flooded with 15 cm of ammonium amended sewage effluent, with [o] and without [•] plants.

Table 2. Ammonium removal rates from overlying water of soil:water columns containing ammonium amended sewage effluent.

Treatment	Without Plant	With Plant
	---------kg N $ha^{-1}day^{-1}$---------	
15 cm water depth	1.5[†]	2.9
30 cm water depth	1.2	5.3
15 cm water depth, pH adj.	11.9	11.2

[†] Rates assume an ammonium-N concentration of 20 mg $liter^{-1}$.

Increasing the water depth to 30 cm did not greatly affect the ammonium removal rate (Table 2) but because the total amount of added ammonium was greater, the removal time was somewhat longer (Figure 4). As with the 15 cm water depths, the ammonium removal rate was greater with plants (5.3 kg N $ha^{-1}day^{-1}$) than without plants (1.2 kg N ha^{-1} day^{-1}). Nitrate concentrations (Figure 4) were low throughout the study period for the same reasons noted for the 15 cm water depth columns.

Increasing the pH significantly increased ammonium removal rate (Figure 5 and Table 2). Ammonium concentrations decreased from about 40 ppm N to less than 5 ppm within 3 days. The fact that nitrification was responsible for much of this decrease is indicated by the high (20 ppm N) nitrate concentrations observed in the overlying water. Nitrifying bacteria require a pH near neutrality to thrive and are considerably inhibited by pH values below 5. There was little difference in ammonium removal rates in pH adjusted treatments with or without plants possibly because the bacteria were able to effectively compete with the plants for the ammonium. The effect of the plants is, however, very evident from the nitrate concentrations. Nitrate concentrations were consistently lower in the presence of plants.

Control columns consisted of secondarily treated effluent to a 15-cm depth overlying marsh soil with no treatment except aeration. Concentrations of ammonium and nitrate in the controls with and without plants remained low throughout the study period.

When columns were dismantled at the end of the 52 days, pH in the top 15 cm of soil for all except the limed columns averaged around 5.5, dropping down to about 4.5 over the next 30 cm. Surface pH of the limed columns approached neutrality but decreased with depth (within 10 cm) to values similar to the other treatments since the lime had not been incorporated into the soil.

In Situ Investigations

Since nitrification did not appear to occur naturally in the water alone but did occur when in contact with soil in the column experiments, an *in situ* study was performed to determine location of and rates at which this oxidative process would occur in the soil profile. Polyethylene bags containing marsh soil amended with ammonium were placed at shallow (8 cm) and deep (30 cm) positions in the marsh soil.

Ammonium and nitrate concentrations in the sample bags are shown in Figure 6. Ammonium values declined with time at the 8 cm depth but remained relatively constant at the 30 cm depth. This indicates that nitrification was occurring at the 8 cm level but not at 30 cm. During the time of this *in situ* study, the water table flucuated at and slightly below the soil surface. Under these conditions, the 8 cm depth may have been under aerated conditions periodically due to the depth of the water table. However, oxygen has been reported to diffuse from the roots of a number of marsh plants into the surrounding waterlogged soil (Armstong, 1967) and this mechanism is likely responsible for providing much of the oxygen required by nitrification. Roots were largely absent at the

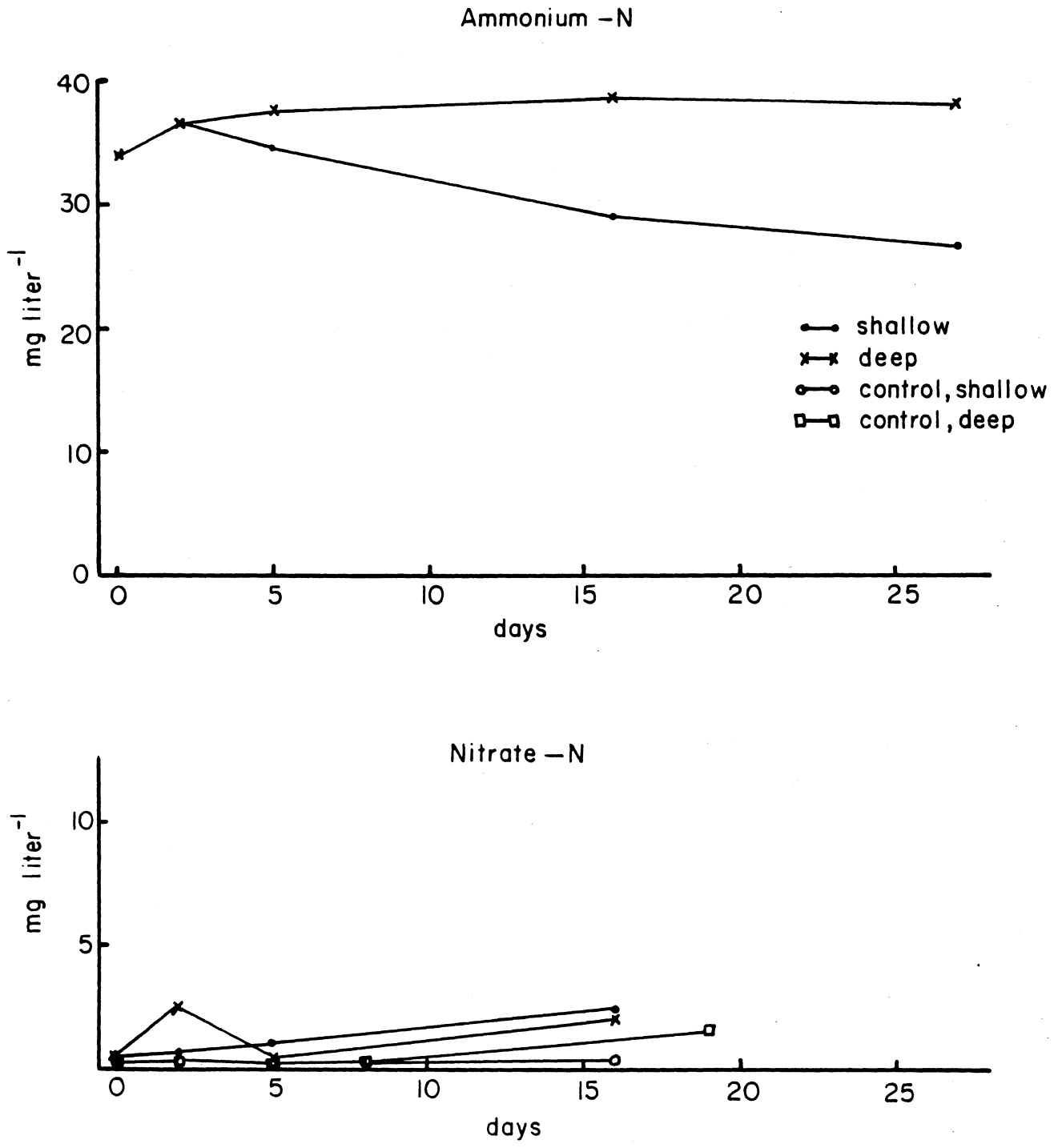

Figure 6. Ammonium and nitrate concentrations in ammonium amended marsh soil during in situ incubation in polyethylene bags.

30 cm depth of the deep samples. Small increases in nitrate concentration were observed at both depths. At the 8 cm depth the increase in nitrate concentration did not correspond in quantity to the decrease in ammonium concentration, indicating that some denitrification and/or plant assimilation was also occurring at this depth. The presence of nitrate at the 30 cm depth is likely an artifact of the sample extraction technique after removed from the soil.

Denitrification

Laboratory Investigations

Preliminary laboratory denitrification studies were conducted to obtain approximate denitrification rates and ascertain how these rates would be affected by the presence or absence of soil, associating cation, and water source. This entailed a "batch" type study which excluded plants. Water samples from several sources were amended with potassium or calcium nitrate salts and incubated with and without soil. The data (Table 3) shows a steady decline in nitrate concentrations with time, for all soil-water treatments. No clear effect of water source nor corresponding cation on nitrate removal rate was observed. The most likely nitrate removal mechanisms are denitrification and microbial immobilization, with the former being the dominant reaction. Solutions not in contact with soil showed no appreciable change (slight increase due to evaporation) which indicates that denitrification did not occur in these waters in the absence of soil. This is in agreement with similar work done by Engler et al. (1976) and likely was due to insufficient readily available organic carbon, even in oxidation pond water, to support denitrification.

After finding that water source and associating cation had little effect on the denitrification rate, an investigation was initiated using soil:water columns to simulate the marsh environment. Potassium nitrate and secondary sewage effluent were used as the nitrate and water source, respectively. Water depth, pH, and plants were used as variables in a manner similar to that used in the nitrification study.

Nitrate concentrations in the overlying water of each treatment are presented graphically in Figures 7 through 9. Nitrate removal appeared to follow first-order kinetics. (Denitrification kinetics, including methods for determining reaction order, are discussed in Chapter 3.) Both first-order rate constants (day^{-1}) and nitrate removal rates (kg N $ha^{-1}day^{-1}$, assuming a floodwater nitrate concentration of 10 mg N $liter^{-1}$) are given in Table 4. Although useful for theoretical and data manipulation purposes, first-order rate constants can be misleading because they express nitrate removal as a function of the amount of nitrate present. That is, if floodwater depth (and therefore the amount of nitrate in the system) is doubled while removal rate remains the same, the first-order constant will be reduced by one half. For this reason, when floodwater depth is a variable, nitrate removal rates are of more value for comparison purposes than are rate constants.

Table 3. Nitrate removal as affected by water source, associated cation and presence of soil.

Treatment	Days			
	0	2	5	11
	------NO_3-N, mg $liter^{-1}$------			
$Ca(NO_3)_2$ in deionized water (no soil)	40.5	41.8	41.3	42.3
KNO_3 in deionized water (no soil)	45.0	45.2	46.6	47.3
KNO_3 in oxid pond water (no soil)	51.6	53.0	54.7	58.0
KNO_3 in marsh water (no soil)	43.2	44.9	44.8	49.2
$Ca(NO_3)_2$ in deionized water (over soil)	40.5	30.9	25.5	12.2
KNO_3 in deionized water (over soil)	45.0	33.9	26.3	15.0
KNO_3 in oxid pond water (over soil)	51.6	43.0	30.0	21.5
KNO_3 in marsh water (over soil)	43.2	35.0	26.5	14.3
	------% NO_3-N remaining------			
$Ca(NO_3)_2$ in deionized water (over soil)	100	76.3	63.0	30.1
KNO_3 in deionized water (over soil)	100	75.2	58.4	33.4
KNO_3 in oxid pond water (over soil)	100	83.3	58.2	41.6
KNO_3 in marsh water (over soil)	100	81.0	61.3	34.3

Table 4. Nitrate removal rates from overlying water of soil:water columns containing nitrate amended sewage effluent.

Treatment	Nitrate removal rate†	Denitrification rate constant
	---kg N $ha^{-1}day^{-1}$---	----------day^{-1}----------
15 cm water depth		
without plant	1.9	0.127
with plant	1.5	0.102
30 cm water depth		
without plant	0.8	0.026
with plant	2.9	0.098
15 cm water depth, pH adj.		
without plant	1.0	0.067
with plant	1.6	0.105

† Assuming floodwater nitrate-N concentration of 10 mg $liter^{-1}$.

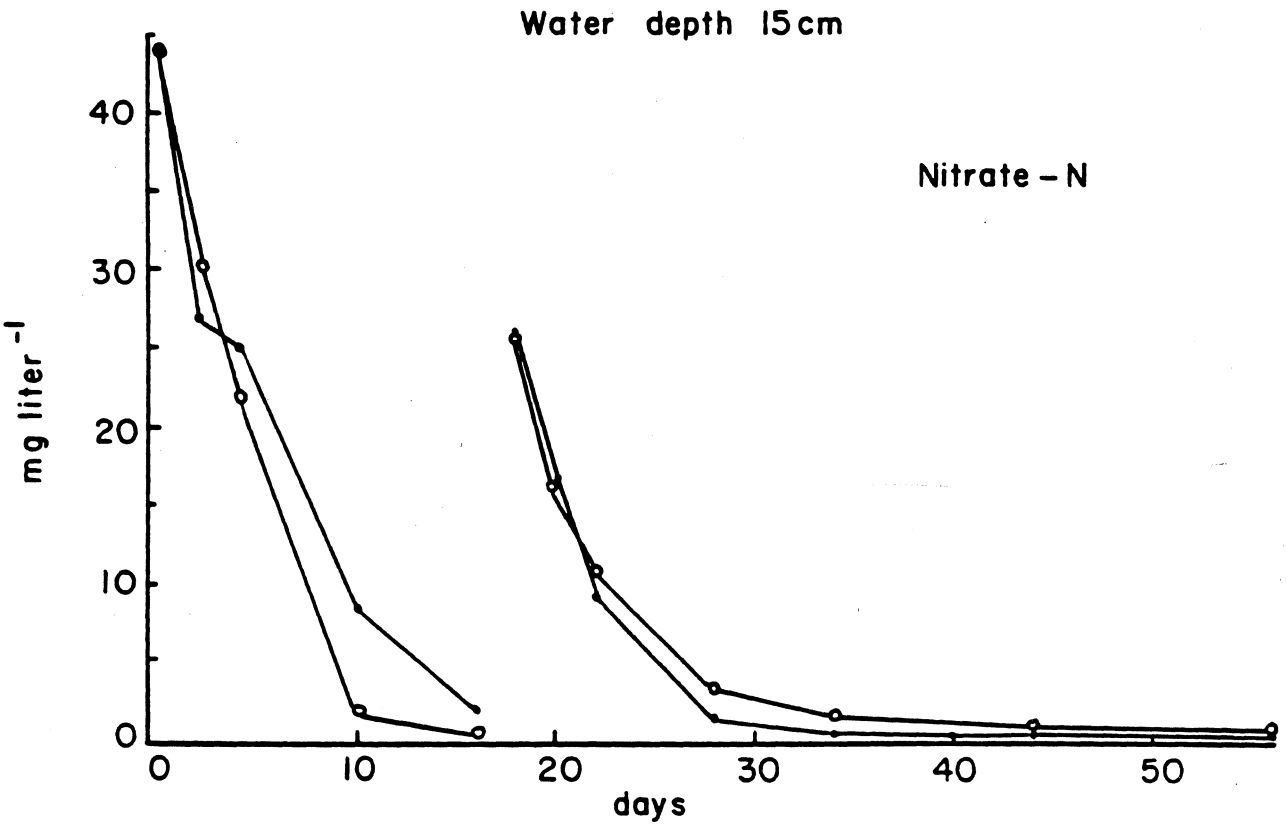

Figure 7. Nitrate removal from overlying water of soil:water columns (with [o] and without [•] plants) containing nitrate amended sewage effluent to a depth of 15 cm. Nitrate was added on day 0 and day 18.

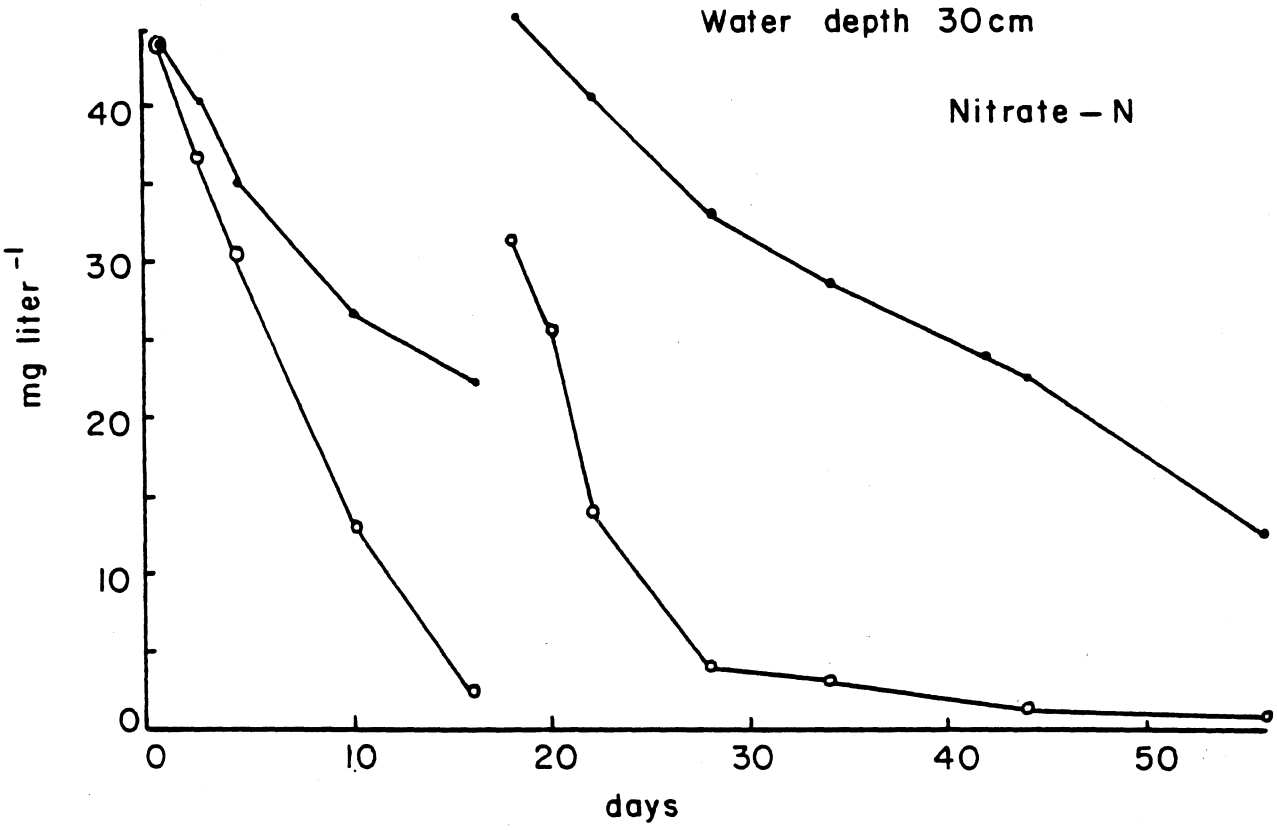

Figure 8. Nitrate removal from overlying water of soil:water columns (with [o] and without [•] plants) containing nitrate amended sewage effluent to a depth of 30 cm. Nitrate was added on day 0 and day 18.

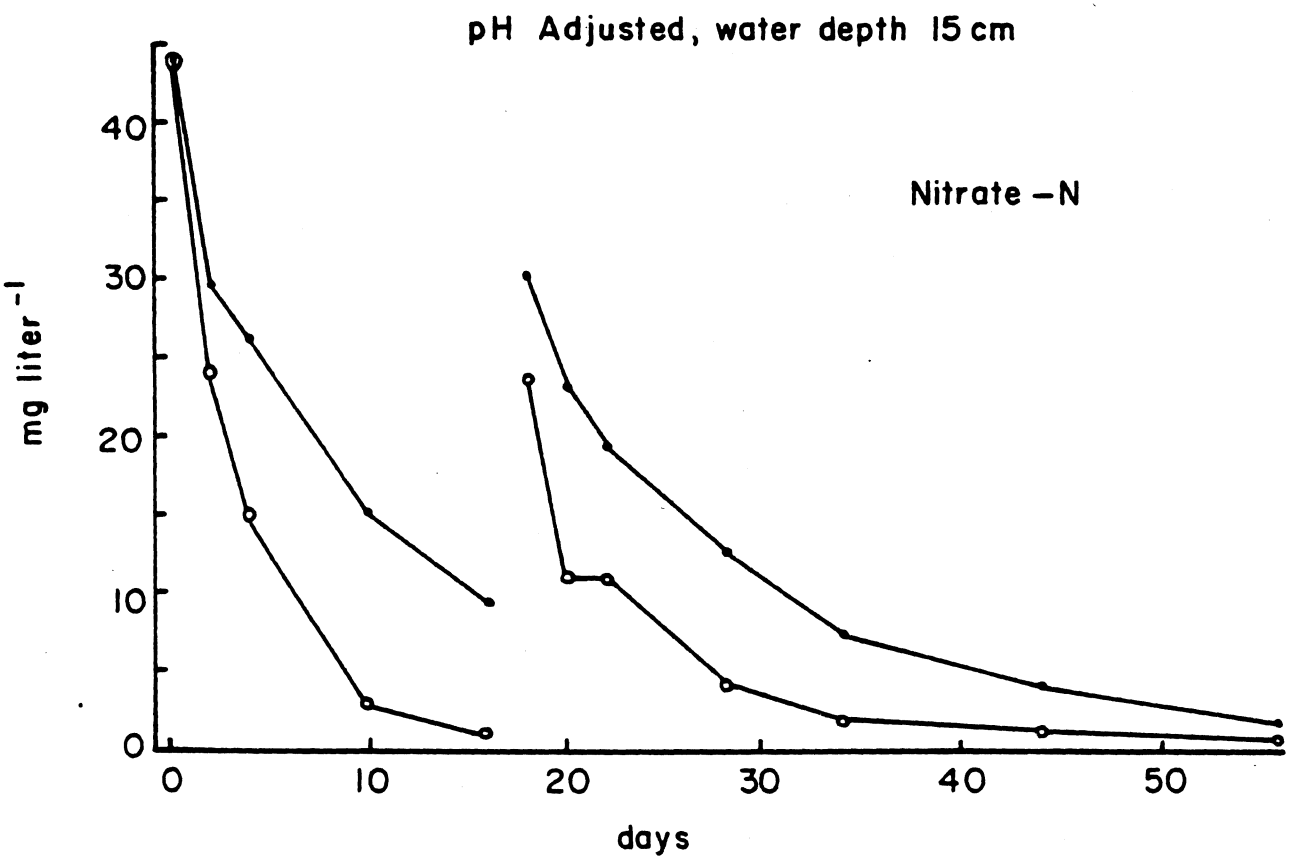

Figure 9. Nitrate removal from overlying water of limed soil:water columns (with [o] and without [•] plants) containing nitrate amended sewage effluent to a depth of 15 cm. Nitrate was added on day 0 and day 18.

Removal rates for the treatments without plants ranged from 0.8 to 1.9 kg N $ha^{-1}day^{-1}$. Results of the three treatments are difficult to compare because of the low number of replications and the high spacial variation found in wetland soils. (Chapter 3 contains further discussion of spacial variation found in a number of wetland soils.) The two water levels were originally utilized because it was felt that water depth would influence the aeration status of the system, with possibly less oxygen reaching the soil with the deeper water depth, thereby increasing the rate of denitrification. However, the need to artificially aerate the water column negated the water depth effect. It was also anticipated that pH adjustment would increase denitrification rate. Since the pH adjusted treatment had the lowest rate, it was assumed that this value reflected natural variation rather than a true pH effect. (Lack of pH effect is probably the result of lime having been applied to the soil surface while denitrification is a subsurface process. Incorporation of lime into the soil would be impractical in the field due to plant destruction, etc.) Thus, the overall denitrification rate in these systems was taken as the average of the three treatments. Therefore, a removal rate of 1.2 kg N $ha^{-1}day^{-1}$ (at 10 mg nitrate-N $liter^{-1}$) would be expected for this soil. Removal rates at other nitrate concentrations would vary in direct proportion to the concentration, since the reaction is first-order.

Inclusion of plants in the system substantially increased nitrate removal rates. A number of factors may have been responsible for this. Plant roots may have provided more favorable conditions for denitrification via root exudates or other influences. Downward movement of floodwater into the soil due to transpirational pumping may have increased denitrification rates by accelerating nitrate transport to denitrification sites. Plants may also have assimilated nitrate, augmenting denitrification as a removal mechanism. Because plants in different columns varied both in size and vigor, as well as for reasons noted for the columns without plants, removal rates for columns with plants, averaged over the three treatments, were used as an indication of typical removal rates. Values (again assuming 10 mg nitrate-N $liter^{-1}$) ranged from 1.5 to 2.9 kg N $ha^{-1}day^{-1}$ with an average removal rate of 2.0 kg N $ha^{-1}day^{-1}$.

Ammonium levels in the overlying water of all treatments were less than 1 mg N $liter^{-1}$ at all except the initial sampling date. The control treatment also had low nitrate values. This indicates that either very little ammonium was being released from the soil, or that it was being nitrified and then rapidly denitrified.

In Situ Investigations

Nitrate removal as a function of depth and incorporated lime was investigated by *in situ* incubation of nitrate and lime amended marsh soil in polyethylene bags. Soils amended with nitrate showed rapid nitrate removal, with 25 mg $liter^{-1}$ nitrate removed in 2 to 8 days, depending on treatment (Figure 10). The effect of depth on nitrate removal is shown by comparing data for unlimed, shallow site (3.1 µg N per gram wet soil per day) with unlimed, deep samples (5.1 µg N $g^{-1}day^{-1}$).

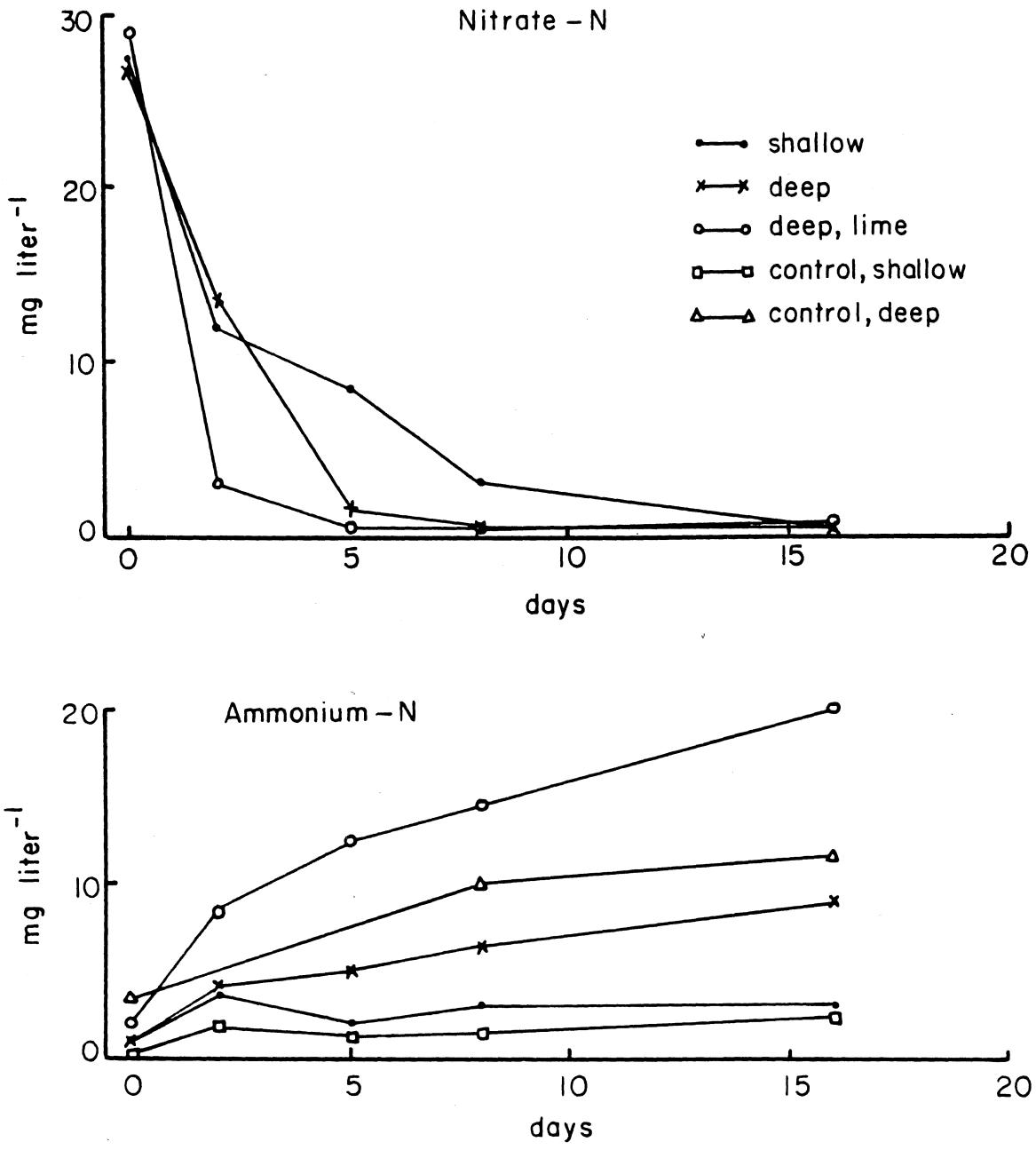

Figure 10. Nitrate and ammonium concentrations in nitrate amended marsh soil during in situ incubation in polyethylene bags.

It appears that at the shallow depth there are both aerobic (likely in close proximity to plant roots) and anaerobic sites. Thus, nitrification and denitrification were occurring simultaneously at this depth. This is also indicated by the small amount of ammonium accumulation and slower nitrate removal compared to the 30 cm depth.

Limed soils amended with nitrate and placed deep in the profile removed 12.8 μg N $g^{-1}day^{-1}$. When this is compared to a similarly placed, unlimed sample in which 5.1 μg N $g^{-1}day^{-1}$ was removed, it is obvious that liming enhanced the removal rate. Apparently, denitrification is limited by the natural soil pH of 4.7. The immediate stimulation of denitrification by raising the pH is indirect evidence that most nitrate removal occurs biologically rather than chemically, since the latter is inhibited at higher pH values. Another interesting aspect of liming is the increased ammonification shown by limed treatments (Figure 10). This ammonium accumulation did not occur in shallow samples, probably due to the presence of small amounts of oxygen, but occurred in the deep, more anaerobic samples.

SUMMARY AND CONCLUSIONS

Nitrification and denitrification rates in a freshwater marsh receiving secondarily treated sewage effluent were investigated in the laboratory and *in situ*. Laboratory experiments measured these processes both in water alone and in soil:water columns. *In situ* work consisted of incubation of buried polyethylene bags containing ammonium or nitrate amended soil.

Nitrification did not occur in the natural marsh water, but did occur in marsh water receiving sewage effluent. Ammonium in the overlying water of soil:water columns was nitrified at a rate considerably greater than in the absence of soil (Table 2, page 24). Water depth (15 cm vs. 30 cm) had little effect on nitrification rates, although the water depth effect may have been negated by artificial aeration. Inclusion of plants increased ammonium removal rates, likely due to plant assimilation of ammonium in addition to nitrification. Addition of calcium carbonate (agricultural limestone) increased the nitrification rate to the point where nitrate accumulated in the overlying water. *In situ* incubation of ammonium amended marsh soil indicated that some nitrification would occur in the plant root zone, probably due to oxygen diffusing from the roots. Nitrification was not observed below the root zone.

Denitrification did not occur in marsh water or in oxidation pond water in the absence of soil. Apparently there was not sufficient available organic carbon in these waters to deplete oxygen supply and allow denitrification to occur. Rapid, first-order denitrification was observed in soil:water columns (Table 4, page 28). Average nitrate removal rates, assuming a floodwater nitrate concentration of 10 mg N $liter^{-1}$, were 1.2 kg N $ha^{-1}day^{-1}$ for columns without plants and 2.0 kg N $ha^{-1}day^{-1}$ with plants. As with nitrification, water depth did not affect denitrification rate, again probably due to artificial aeration. In contrast to the results for nitrification, liming had no effect on denitrification rate. This may have been due to the fact that the lime was applied to the soil surface while denitrification occurs below the soil surface. Given sufficient time, it is anticipated that the lime would move downward in the soil and increase the pH in the zone of denitrification. Denitrification was rapid in nitrate amended soil samples incubated *in situ* (at both 8 and 30 cm depths) and was accelerated by increasing the soil pH with lime.

The above nitrification and denitrification rates represent our best estimates based on primarily laboratory investigations. Further field investigations are needed to ascertain the effects of natural variations in water depth, dissolved oxygen concentration, plant density, and temperature.

CHAPTER III

DENITRIFICATION IN FIFTEEN SELECTED FLORIDA WETLAND SOILS

Denitrification can be an important mechanism for the removal of nitrate from wastewater applied to wetlands, as indicated in the previous chapter. The objective of this investigation was to measure denitrification rates in a variety of flooded wetland soils and to determine quantitative relationships between denitrification rate and selected soil characteristics in order to develop a predictive equation for denitrification rates in specific soils. Since nitrous oxide, one of the gaseous end-products of denitrification, is known to deplete ozone in the stratosphere, a quantitative evaluation of nitrous oxide evolution from wetland soils was also included in this study.

METHODS AND MATERIALS

Soils and Soil Sampling

Soils

A total of 15 soils of widely varying organic matter content, reaction (pH), and texture were selected for study. All are classified as very poorly drained or poorly drained, and have water standing on the surface for one to nine months per year. Geographic distribution, ranging from Palm Beach and Lee Counties in the south to Walton County in the northwest, is shown in Figure 11. A list of soil types, classifications, and descriptions* is given below. These are general descriptions of the soil series, not of the actual pedons at sampling sites. Soils are listed in declining order of denitrification rate, as determined in the present study.

Everglades muck--typic medihemist--very poorly drained organic soils that formed in thick deposits of hydrophytic plant remains. They occur in freshwater swamps and marshes. In a representative profile, the surface layer is a black muck, about 12 inches thick. Below it, to a depth of 46 inches, is a reddish-brown mucky peat. Below a depth of 46 inches, and extending to below 78 inches, is dark brown peat.

Floridana sand--arenic argiaquoll--a very poorly drained soil that occurs on broad low flats and in depressions. A representative profile has a black sand surface soil about 12 inches thick and a grayish-brown sand subsurface layer over a dark gray sandy clay loam subsoil that begins within a depth of 20 to 40 inches below the soil surface. These soils are formed in sandy and loamy marine sediments.

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* Adapted from U.S.D.A. Soil Conservation Service soil interpretation sheets (Soil Form #5) for the soils series described.

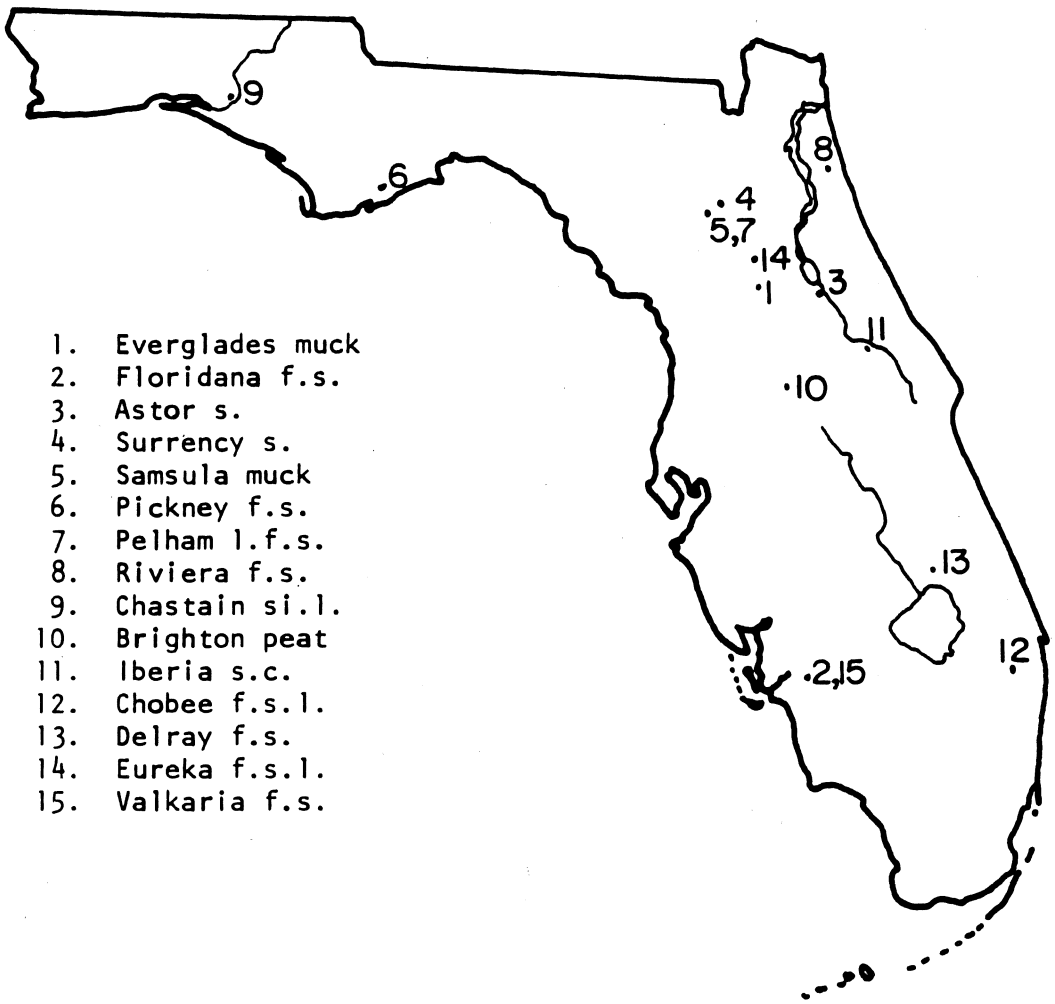


Figure 11. Location of sampling sites.

Astor sand--cumulic haplaquoll--very poorly drained soils that occur in low flat areas, poorly defined drainageways, and floodplains in the lower coastal plain. A typical profile has a black to very dark grayish-brown sand surface layer about 32 inches thick. Below this to a depth of 80 inches is dark grayish-brown sand.

Surrency sand--arenic paleaquult--very poorly drained nearly level soils in Central and North Florida. In a representative profile, the surface layer is sand about 22 inches thick. The upper 16 inches is black and the lower 6 inches is dark gray. The subsurface layer is light brownish-gray sand about 9 inches thick. Between depths of 31 and 65 inches, the subsoil is gray sandy clay loam.

Samsula muck--terric medisaprist--nearly level very poorly drained organic soils. They occur in fresh water swamps and marshes. In a representative profile, the surface 9 inches is a black muck. The subsurface layer is a dark reddish-brown muck to a depth of 36 inches. Next is 10 inches of dark grayish-brown sand and then gray sand to below a depth of 55 inches. These soils formed in hydrophytic plant remains.

Pickney fine sand--cumulic humaquept--very poorly drained soils in depressions and drainageways of the lower coastal plain. The typifying pedon has black loamy fine sand A horizons over dark gray fine sand C horizons.

Pelham loamy fine sand--arenic paleaquult--poorly drained nearly level soils on the coastal plain. Typically the surface layer is very dark gray loamy fine sand about 5 inches thick. The subsurface is gray loamy sand about 21 inches thick. The subsoil extends below a depth of 68 inches. It is mostly light gray sandy clay loam mottled with yellow and red.

Riviera fine sand--arenic glassaqualf--very poorly drained soils in depressed areas of the coastal plain. In a representative profile, the surface layer is dark grayish-brown sand about 6 inches thick. The subsurface layer is white sand about 22 inches thick and it extends into the underlying grayish-brown sandy clay loam subsoil. They formed in unconsolidated marine sands and loams.

Chastain silt loam--typic haplaquept--poorly drained, slowly permeable soils on flood plains of rivers on the coastal plain. Typically these soils have yellowish-brown surface layers over gray clayey subsoils.

Brighton peat--typic medifibrist--very poorly drained organic soils that occur in depressions and freshwater marshes and swamps in peninsular Florida. Typically they have a black peat surface layer 9 inches thick. Beneath the surface layer dark yellowish-brown and dark brown peat extends to 63 inches or more deep.

Iberia sandy clay--vertic haplaquoll--poorly drained, very slowly permeable soils. They have a black clay surface and a dark gray clay subsurface. These soils formed in alkaline clayey alluvium. They occur on the lower part of the natural levees, often in depressions.

Chobee fine sandy loam--typic argiaquoll--very poorly drained soils that occur in small to large depressions, low areas, or river flood plains. A representative profile has a black fine sandy loam surface layer about 7 inches thick and a black to very dark gray sandy loam subsoil that contains calcium carbonate nodules.

Delray fine sand--grossarenic argiaquoll--deep, very poorly drained soils that occur on low, broad flats or depressions in South and Central Florida. A representative profile has a black fine sandy surface layer. Between a depth of 46 to 60 inches is a dark grayish-brown fine sandy loam subsoil. These soils formed in thick deposits of sandy and loamy marine sediments.

Eureka fine sandy loam--typic albaqualf--poorly drained soils that occur in South and Central Florida. In a representative profile the surface layer is black loamy fine sand about 4 inches thick. The subsurface layer is grayish-brown loamy fine sand about 7 inches thick. The subsoil begins at depths of 11 inches and extends to 72 inches and deeper. It is gray sandy clay.

Valkaria fine sand--spodic psammaquent--poorly drained soils that occur on low, poorly defined sloughs and areas adjoining swamps in the lower coastal plain. In a representative profile, the surface layer is sand about 9 inches thick; the upper 5 inches is black and the lower 4 inches is dark brownish-gray. Between depths of 9 to 15 inches is light gray sand, with brownish or grayish sand to depths of 80 inches.

Collection of Samples

Samples for column studies were collected in 5 cm i.d. by 60 cm long plexiglass columns. Where possible, sharpened columns were simply driven into the soil to a depth of 25 cm with a hammer. In cases where this resulted in excessive soil compaction, a more involved method was used. A rubber stopper exactly matching the inner diameter of the column was inserted in the bottom of the column and suspended at the soil surface from a tripod by a cord running down the center of the column (Figure 12(a)). A small amount of water was poured into the column to help maintain a seal between column and stopper. The column was then driven into the soil while the stopper remained stationary, creating a suction in the column to prevent the soil surface from receding as the column moved downward (Figure 12(b)). To remove the stopper, the cord was pulled upward at an angle, thereby tilting the stopper and breaking the seal (Figure 12(c)). Columns were extracted with a rocking, twisting motion after stoppering the top to prevent soil loss out of the bottom. This sampling method was adapted from that of Coleman (1979).

Bulk samples of the top 10 cm of soil were stored in polyethylene bags at $4^{o}C$ for a maximum of 5 days before determination of mineralizable organic carbon and pH. Subsequently, samples were oven dried at $104^{o}C$ for total organic carbon determination.

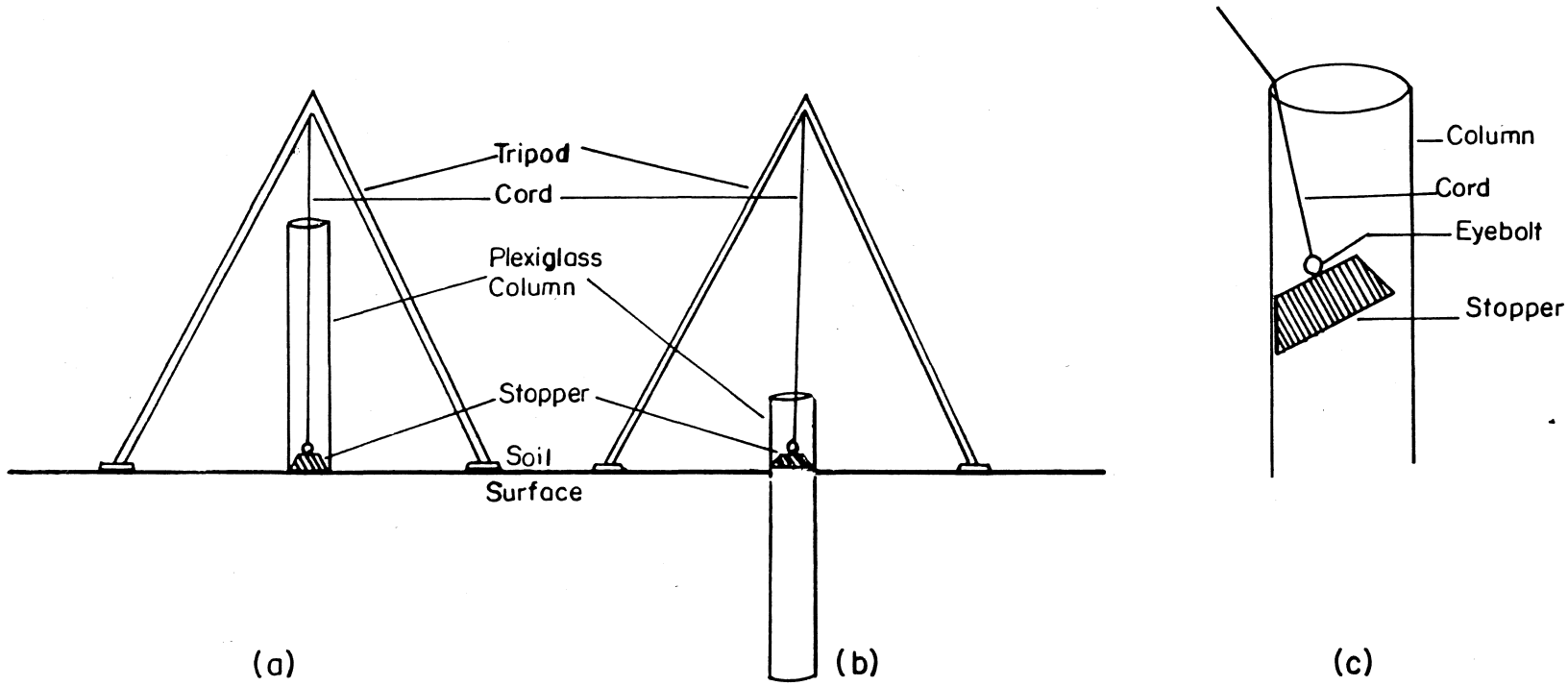


Figure 12. Apparatus for obtaining soil cores with minimum soil compaction.

Description of Experiments

Denitrification and Nitrous Oxide Evolution

A laboratory study was conducted to determine denitrification rates and nitrous oxide (N_2O) evolution rates for 14 of the soil types collected. Three columns of each soil type were flooded with deionized water to a depth of 15 cm. Aquarium airstones (previously determined to be carbonate and ammonia free) were used to aerate and mix floodwater. Air was prehumidified and stripped of ammonia by use of an acidified (H_2SO_4) water trap. Air flow rate in each column was approximately 30 to 40 ml min^{-1}. Water levels were maintained throughout the study by periodic additions of deionized water. This and all other studies were conducted within a temperature range of $26^{\circ}C \pm 2^{\circ}C$.

Columns, prepared as described above, were allowed to stand for 7 days before initial nitrate application. Background floodwater nitrate concentrations were measured during this time. Water samples for nitrate determination were pipetted from the floodwater surface. Nitrate-N levels in the floodwater were then raised to 20 mg $liter^{-1}$ by addition of potassium nitrate solution. A 2 week incubation period followed, for the purpose of allowing microbial adaptation to nitrate and establishment of a relatively stable nitrate diffusion front in the soil. During this period, concentrations of nitrate were monitored every 3 to 4 days and additions made if nitrate-N dropped below 10 to 15 mg $liter^{-1}$. No additions of nitrate were made within 3 days of the end of the adaptation period. Floodwater nitrate concentrations were measured at 0, 4, 8, and 12 days after the end of this period. Nitrate in two arbitrarily selected extra colums (Surrency soil) was measured more frequently and for a longer period of time for the purpose of obtaining detailed nitrate disappearance curves. One column per soil type was selected for determination of N_2O evolution rate by the method described below. This was done approximately 3 weeks after initial nitrate application.

Long Term Denitrification and Nitrous Oxide Evolution

Two soils, Samsula and Pelham, were selected for a more detailed study to determine changes in denitrification and N_2O evolution rates over a 10 week period. Eight columns of each of the soils were treated similarly to those in the previous experiment. Denitrification rates (calculated as described in results and discussion) were determined at 2, 5, and 8 weeks, with nitrate-N maintained between 5 and 20 mg $liter^{-1}$ throughout the study. Each denitrification rate determination consisted of three nitrate concentration measurements made at 4 day intervals. Nitrous oxide evolution from each of two Samsula and three Pelham columns was determined at least three times during the study period by the method described below.

Effect of Floodwater Nitrate Concentration on N_2O Evolution

A short experiment was conducted to determine if N_2O evolution was proportional to nitrate concentration. Two Samsula and two Pelham columns were treated as in previous experiments. Nitrous oxide

evolution was measured at least three times per column over a period of several days as floodwater nitrate-N concentrations fell from 15 mg liter^{-1} to below 5 mg liter^{-1}. Background evolution rates for these columns were determined prior to initial addition of nitrate to the floodwater.

Method for Measuring N_2O Evolution

Nitrous oxide evolution from the columns was determined in a semi-enclosed, continuous flow collection chamber. This involved sealing the top of the column, except for small air entry and exit tubes. Aeration continued, but at a reduced, controlled rate of 7 to 20 ml min^{-1}. Floodwater oxygen concentration was periodically measured to insure sufficient aeration. Air flow was measured with a soap-film flow meter connected to the air exit tube (Figure 13).

In this semi-enclosed chamber, N_2O concentration in the headspace will rise slightly due to N_2O production in the soil. In response to this, N_2O dissolved in the floodwater also increases. Eventually an equilibrium is established, and N_2O no longer accumulates in the floodwater or headspace. At this time N_2O entering the chamber plus N_2O produced in the column will equal N_2O exiting the chamber. If flow rate, influent N_2O concentration, and effluent N_2O concentration are known, N_2O production can be calculated as follows:

$$R = (4.6\times10^{-14})(E - A)(f)(4.9\times10^{6})(28) \qquad [2]$$

where R is N_2O-N evolution rate, ha^{-1}day^{-1}; 4.6×10^{-14} is the number of moles of gas in 1 ml at a concentration of 1 ppb (v:v) at standard temperature and pressure; E and A are effluent and ambient (incoming air) N_2O concentrations, ppb (v:v); f is air flow rate, ml day^{-1}; 4.9×10^{6} converts the column surface area to hectares; and, 28 is the mole to gram conversion factor for N_2O-N. In practice, a 12 hour equilibration time was found to be more than sufficient to establish constant N_2O concentration in effluent air. Five, 1 ml samples of effluent air and five samples of influent (ambient) air were collected for each N_2O evolution determination. A similar method was described by Kaspar and Tiedje (1979) for use with unflooded soils.

In the long term study described above, N_2O samples were stored in plastic syringes for up to 3 days. It was found that concentrations decreased with storage; i.e., the difference between sample and normal ambient (300 ppb) N_2O concentrations declined by approximately 24, 45, and 65% after 1, 2, and 3 days, respectively. Measured N_2O concentrations of stored samples were corrected by the appropriate loss factor, and corrected values were used to determine evolution rates.

Analytical Methods

Nitrate

Nitrate concentration was measured with an Orion model 93-07 nitrate electrode in conjunction with an Orion 90-01 double junction reference electrode and Orion 701 digital meter. Ionic strength of sample and standard solutions were adjusted with 2N ammonium sulfate.

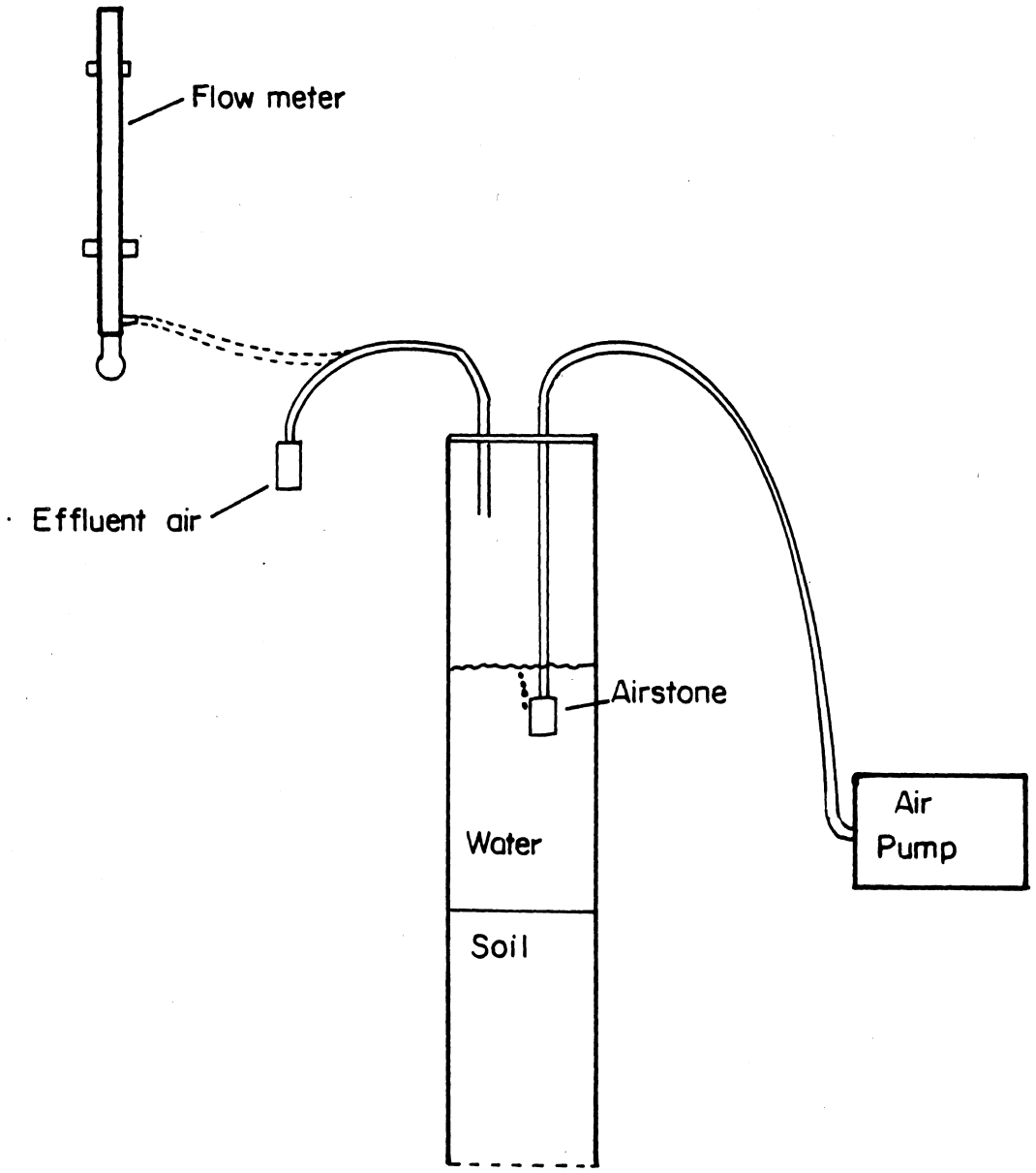


Figure 13. System for determining nitrous oxide evolution from flooded soil cores.

Nitrous Oxide

A Tracor model 222 gas chromatograph with a Ni-63 electron capture detector were used for N_2O determinations. Carrier gas was 5% methane in argon, with a flow rate of 28 cm^3 min^{-1}. Porapak Q was used in the separation column. Column temperature was 50°C and detector temperature was 350°C. Sample volume was 1.0 ml.

Bulk Density and Porosity

Bulk density and porosity of the upper 10 cm of soil in each column were determined gravimetrically. Saturated soil sections were weighed, dried overnight at 104°C, and reweighed. Bulk density was calculated as the dry weight per original soil volume, and porosity was calculated as the volume of water lost per original soil volume.

Other Methods

Fresh soil samples (not dried) were used for pH determination. Measurements were made in deionized water with a soil:liquid ratio of 1:1 (v:v). A combination pH electrode and Orion 701 meter were used. Total N was determined by the micro-Kjeldahl procedure. Total organic carbon was determined by the Walkley-Black method (Allison, 1965). Efficiency of organic matter oxidation by dichromate was assumed to be 77%.

RESULTS AND DISCUSSION

Comparative Denitrification Rates

Denitrification rates were compared for 14 soil types encompassing a wide range of organic matter contents and pH values. Values represent an average of three replications. Measurements of nitrate concentration in the overlying water were taken three or four times over a 12 day period following a 2 week preincubation period. In addition, two columns of Surrency soil were sampled ten times each in order to obtain more detailed nitrate disappearance curves. Prior to rate comparison, the data were analyzed to determine the rate-order of the denitrification reaction.

Floodwater nitrate disappearance data are presented in Figures 14 and 15. Simple visual examination of the graphs indicates that the process is not zero-order. If the reaction is first-order (as in the 1974 model of Bouldin et al.), the following relationship between concentration and time exists:

$$\ln \frac{C_0}{C} = k_1 t \qquad [3]$$

where C_0 is initial nitrate concentration, mg liter^{-1}; C is nitrate concentration at time t, mg liter^{-1}; k_1 is first-order rate constant, day^{-1}; and t is time, days. If the reaction is half-order (i.e., rate is proportional to the square root of concentration, as in Bouldin's 1968 model), the concentration versus time relationship is as follows (Gucker and Siefert, 1966):

$$C^{\frac{1}{2}} - C_0^{\frac{1}{2}} = -\tfrac{1}{2} k_{\frac{1}{2}} t \qquad [4]$$

where $k_{\frac{1}{2}}$ is half-order rate constant, day$^{-\frac{1}{2}}$, and other variables are as in Equation 3.

The data in Figure 14 were fitted to Equations 3 and 4 by least-squares linear regression. Due to differing initial floodwater nitrate concentrations (made unavoidable by the adoption of the preincubation period) and differing denitrification rates in each column, each was treated separately, with separately calculated k values and regression coefficients. Since a total of 38 columns were studied, 38 separate regression procedures were performed for each equation, each with 1 or 2 degrees of freedom. Mean r^2 values were 0.981 for Equation 3 and 0.978 for Equation 4. Data from 21 of the columns fit Equation 3 better, 12 columns fit Equation 4 better, and 7 columns fit both equations equally well. Thus, the data weakly support Equation 3, or first-order kinetics, over Equation 4, or half-order kinetics.

Results for the two more intensively sampled columns of Surrency soil (Figure 15) were ambiguous. Column A yielded r^2 values of 0.988 and 0.986 for Equations 3 and 4, respectively, while column B yielded

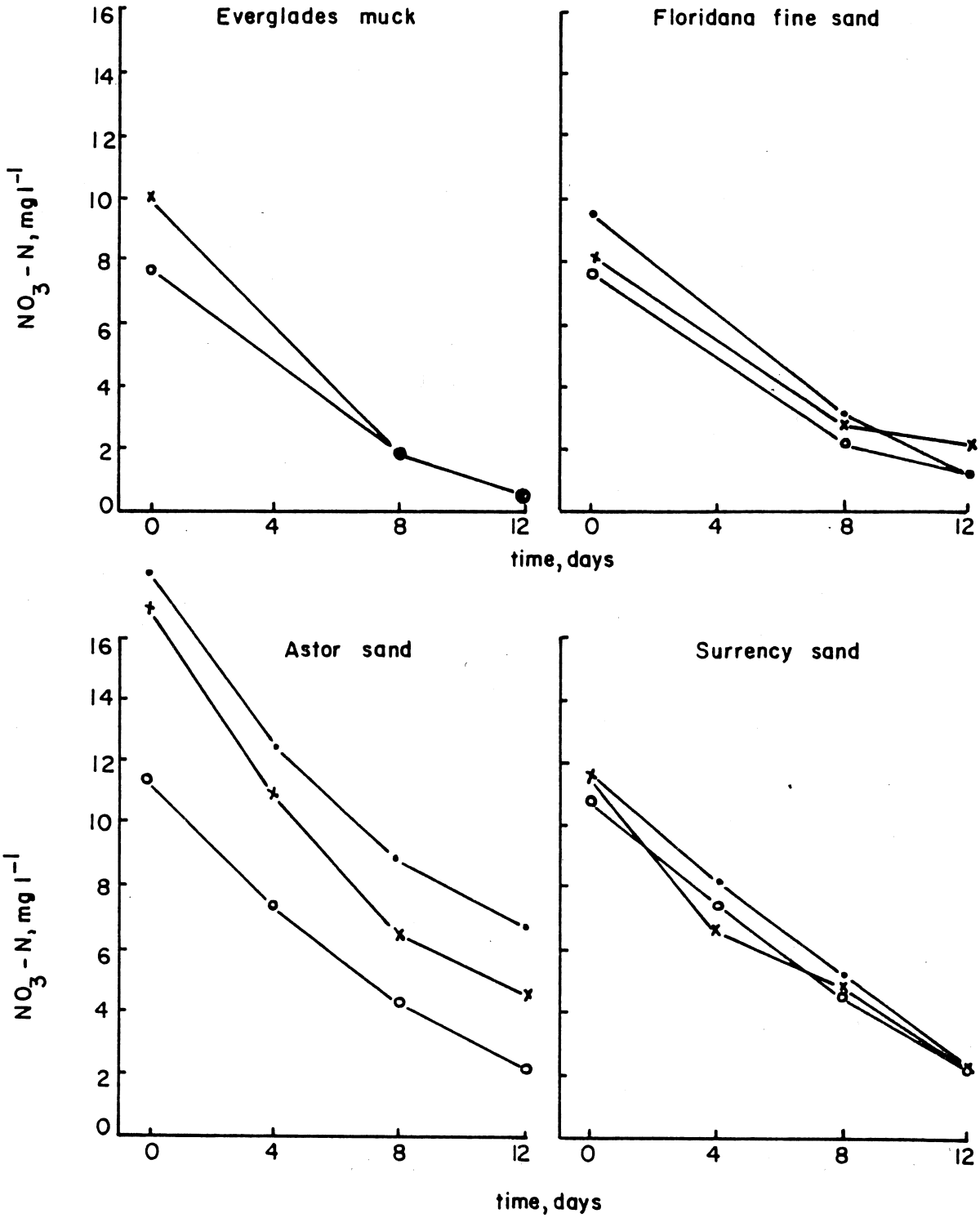


Figure 14. Changes in floodwater nitrate concentrations with time in soil:water columns of 14 soil types.

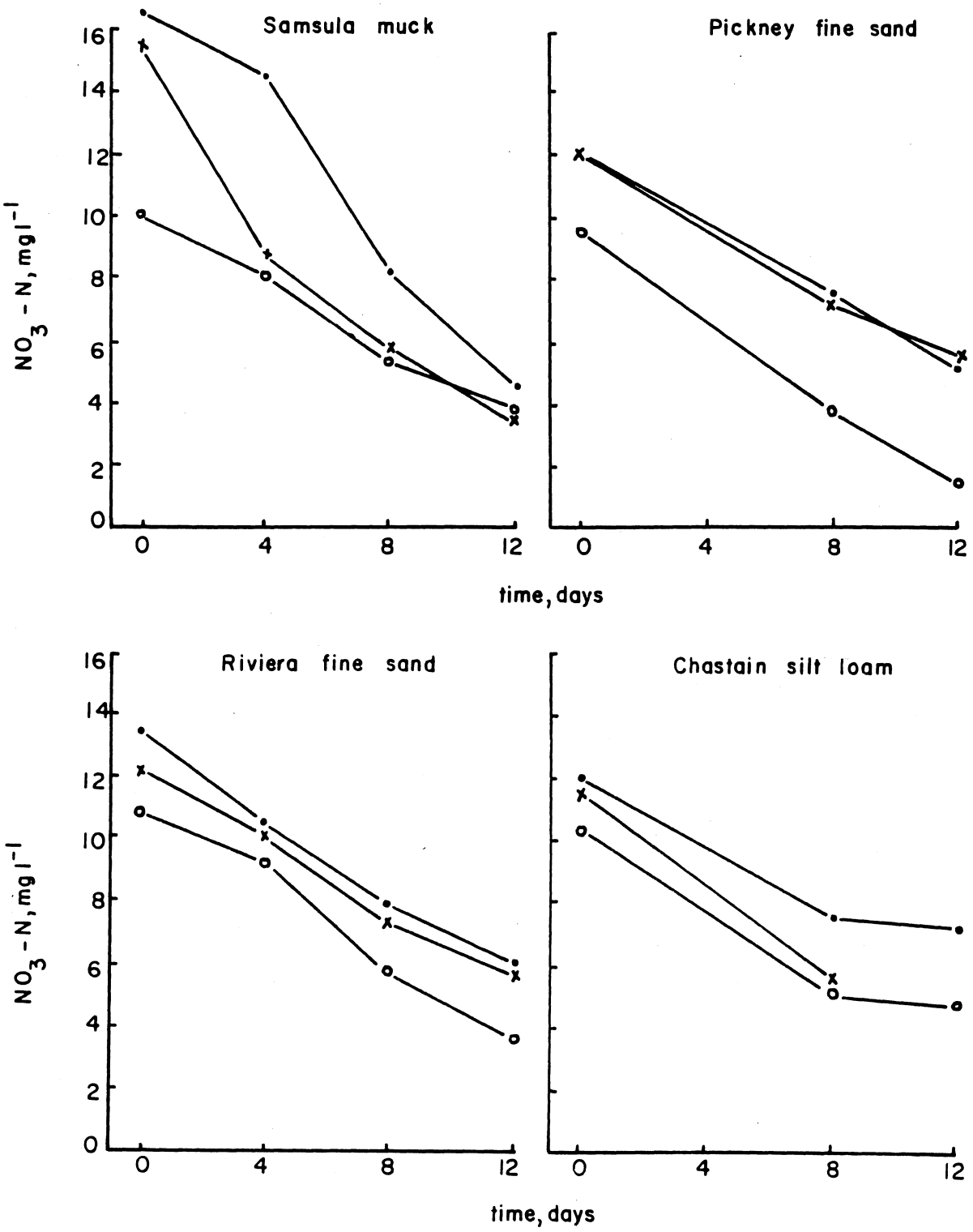
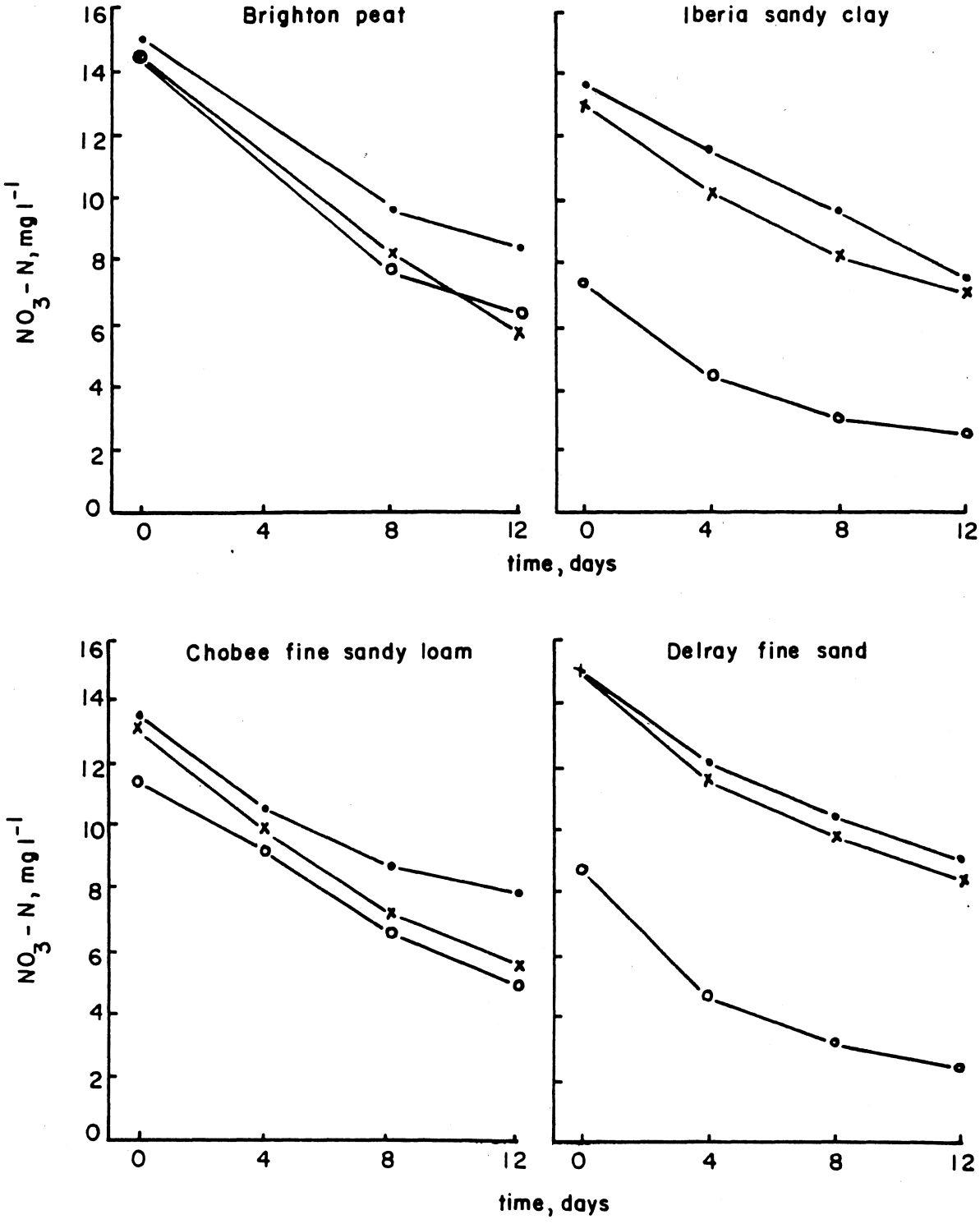


Figure 14. (Continued.)



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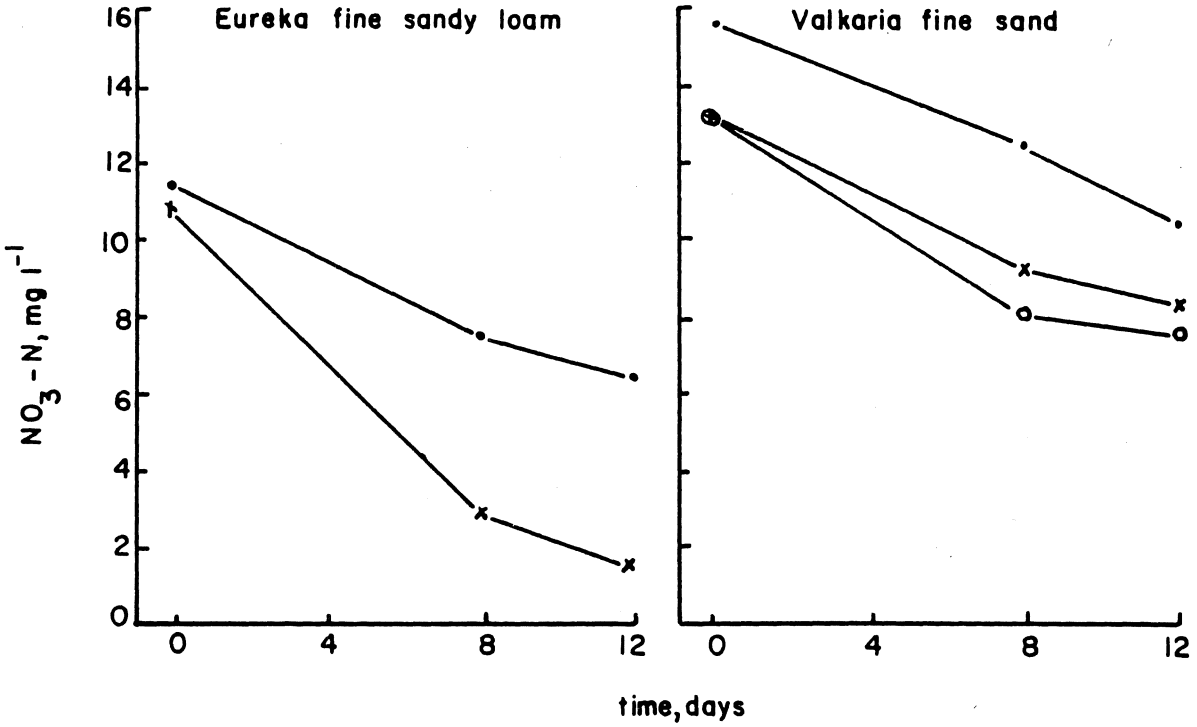

Figure 14. (Continued.)

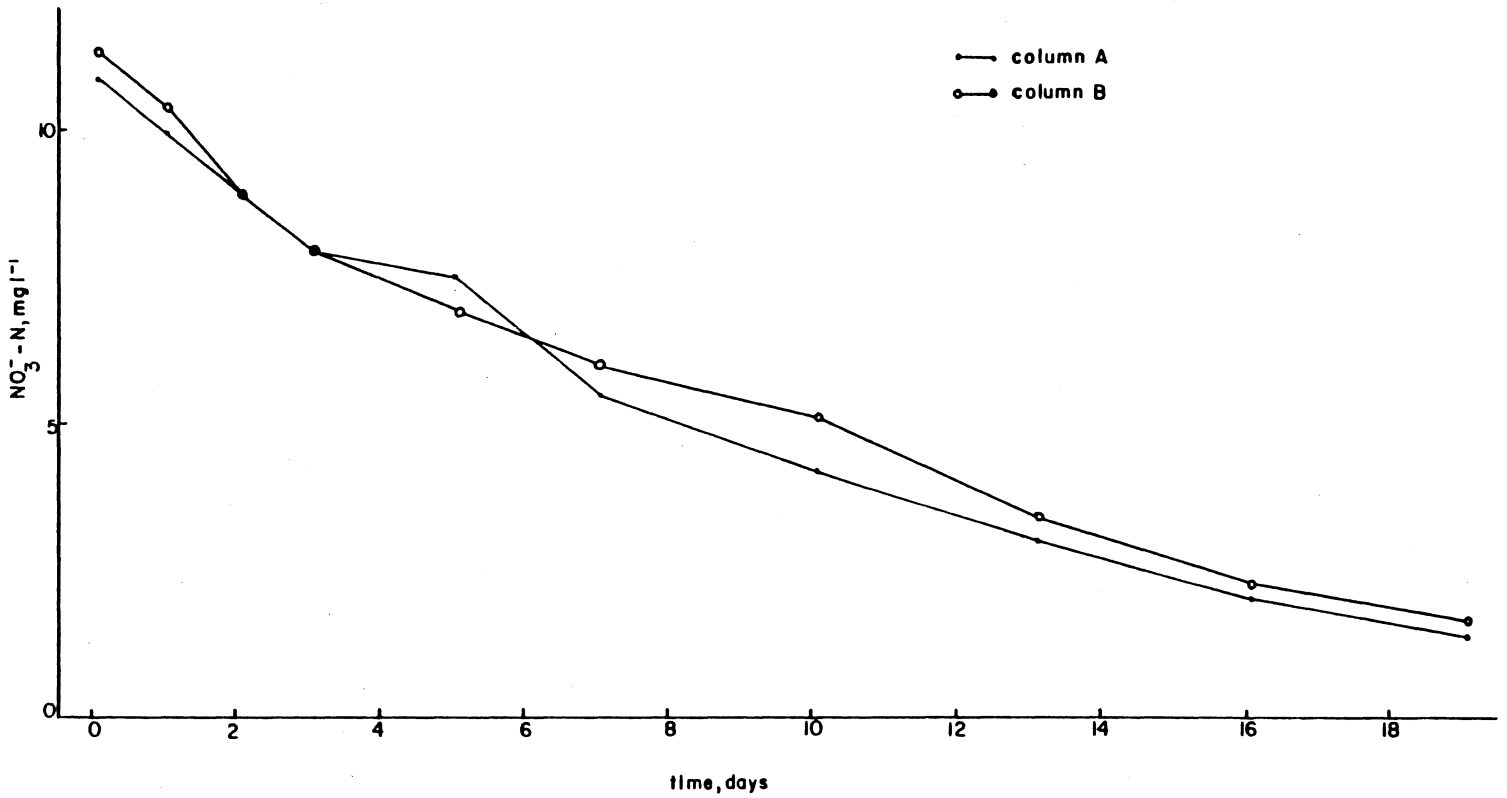

Figure 15. Changes in floodwater nitrate concentrations with time in two soil:water columns of Surrency soil.

r^2 values of 0.993 and 0.994 for the respective equations. Statistical analysis of the data from these columns used ten observations, or eight degrees of freedom per column.

Although the data appear to fit both first-order and half-order apparent kinetics, first-order kinetics are assumed for the remainder of this work, and only first-order rate constants are reported. This is partly in order to be consistent with published work and partly because the concept of first-order rate constant is more meaningful to most readers than that of half-order rate constant. The idea of half-order kinetics has been raised here to illustrate that a good fit of data to first-order kinetics does not preclude other possibilities. It is likely that the apparent kinetics of denitrification in flooded systems are complex and lie somewhere between first-order and half-order reaction rates, as suggested in Chapter 1.

Mean first-order denitrification rate constants for the 14 soil types and results of Duncan's New Multiple Range Test are presented in Table 5. Rate constants range from 0.192 day^{-1} for the Everglades soil to 0.040 day^{-1} for the Valkaria soil. Significant differences exist among the soils, but groups of non-significantly different soils are broad and overlapping. This is due primarily to the high degree of variation within soil types. Spacial variation in soil organic matter content and availability may account for this. Factors such as the presence of decomposing soil fauna may increase organic matter availability and, consequently, denitrification rates. In cases where decomposing animals were visually apparent, columns were discarded.

The last column in Table 5 gives nitrate-N removal rates in g ha^{-1} day^{-1}, assuming maintenance of 10 mg $liter^{-1}$ nitrate-N in the floodwater. The Everglades soil will remove 2,900 g $ha^{-1}day^{-1}$, while only 600 g $ha^{-1}day^{-1}$ is removed by the Valkaria soil. All denitrification data given here assume a 15 cm floodwater depth. As noted in Chapter 2, first-order rate constants are dependent on floodwater depth, but equivalent nitrate-N removal rates are not.

The experimental conditions of this study include well-aerated floodwater and no downward flow of water into the soil. As a result, floodwater nitrate must diffuse through a relatively thick aerobic soil zone into the zone of denitrification, and denitrification is relatively slow. Downward flow of water, which often occurs in the field, can be expected to increase denitrification rate by accelerating nitrate transport. Reduced floodwater oxygen content would also increase denitrification rate by narrowing the aerobic soil zone. For these reasons, denitrification rates presented here should be regarded as minimum expected *in situ* rates.

Selected characteristics of 15 soil types (Table 6) were examined for possible correlation with denitrification rate. The 14 soil types discussed above, plus Pelham soil, discussed later, were studied. Simple and multiple linear regression models were constructed using various combinations of characteristics as independent variables and denitrification rate as the dependent variable. Complex models were analyzed by the General Linear Models procedure of the Statistical

Table 5. Comparative denitrification rates of 14 representative Florida wetland soils.

Soil type	Denitrification rate constant	Standard error of mean		Equivalent nitrate-N removal rate†
	day^{-1}			g ha^{-1} day^{-1}
Everglades muck	0.192	0.017	--‡	2,900
Floridana f.s.	0.149	0.020	ab§	2,200
Astor s.	0.108	0.018	bc	1,600
Surrency s.	0.105	0.001	c	1,600
Samsula muck	0.102	0.009	c	1,500
Pickney f.s.	0.087	0.025	cd	1,300
Riviera f.s.	0.076	0.009	cd	1,100
Chastain si.l.	0.067	0.014	cd	1,100
Brighton peat	0.066	0.010	cd	990
Iberia s.c.	0.063	0.012	cd	950
Chobee f.s.l.	0.062	0.009	cd	930
Delray f.s.	0.061	0.021	cd	920
Eureka f.s.l.	0.049	-- ‡	--‡	740
Valkaria f.s.	0.040	0.005	d	600

† Assuming a nitrate-N concentration of 10 mg liter^{-1}.

‡ Omitted from statistical analysis due to missing data.

§ Rates followed by the same letter are not significantly different; $\alpha = 0.05$.

Table 6. Selected chemical and physical characteristics of surface horizons (top 10 cm) of the soils studied.

Soil type	Denitrification rate constant	Total organic carbon		pH	Bulk density	C:N ratio
		By vol.	By wt.			
	----day^{-1}-----	mg cm^{-3}	--%--		g cm^{-3}	---w:w---
Everglades muck	0.191	59	46.0	6.2	0.13	25:1
Floridana f.s.	0.149	127	12.6	6.5	0.97	8.8:1
Astor s.	0.108	60	7.4	6.7	0.81	6.3:1
Surrency s.	0.105	80	42.0	4.9	0.19	27:1
Samsula muck	0.102	38	54.0	4.6	0.08	16:1
Pickney f.s.	0.087	149	27.0	5.0	0.55	33:1
Pelham l.f.s.	0.080†	N.D.‡	6.5	5.6	N.D.	N.D.
Riviera f.s.	0.076	52	5.0	4.5	1.04	13:1
Chastain si.l.	0.067	24	5.2	5.6	1.37	N.D.
Brighton peat	0.066	44	53.0	4.2	0.09	21:1
Iberia s.c.	0.063	40	4.0	6.1	1.00	N.D.
Chobee f.s.l.	0.062	59	7.4	6.8	0.79	7.5:1
Delray f.s.	0.061	65	6.4	6.9	1.01	6.0:1
Eureka f.s.l.	0.049	48	5.7	5.5	0.84	6.6:1
Valkaria f.s.	0.040	6.9	0.43	6.6	1.60	19:1

† Rate determined in long term study, discussed in later section.

‡ N.D. = not determined.

Analysis System (Barr et al., 1976). Since denitrification is dependent on an available carbon source, total organic carbon content on a weight per soil volume basis and on a weight per soil weight basis were tested as independent variables. The literature suggests that soil pH has a significant effect on denitrification rate only at values somewhat below neutrality. For this reason, a pH-deficit variable was introduced. The new variable was defined as follows:

if observed pH$\geq$X, pH-deficit = 0

if observed ph<X, pH-deficit = X - observed pH

where X is an arbitrary pH threshold. A number of values for X were tested, ranging from 5.5 to 7.0; pH 6.5 was found to perform best in regression equations.

Organic carbon content on a weight per volume basis showed a rather poor correlation with denitrification rate. Theoretically, this parameter would be expected to perform as well as or better than organic carbon content by weight, since microbial activities tend to depend on volume-based, rather than weight-based substrate concentrations. A possible explanation for the poor performance of this variable lies in the fact that volume-based organic carbon values were calculated by multiplying weight-based values by bulk density. Bulk densities were determined for the top 10 cm of each soil column. Substantial vertical variation in bulk density within individual columns was visually apparent, with very low densities existing in the top few centimeters of some columns. Average bulk densities (and therefore volumetric carbon contents) for 10 cm sections might thus not reflect those very close to the surface, where much or all of the denitrification process occurs.

As single independent variables, neither pH-deficit nor organic carbon by weight correlated well with denitrification rate, but models combining these variables fit the data quite well. The best performing model (r^2 = 0.864, d.f. = 13) is given in Equation 5:

$$k_1 = \sqrt{(8.9\times10^{-4})(OCW) - (3.9\times10^{-4})(OCW)(pH_{6.5}) + 0.002} \qquad [5]$$

where k_1 is apparent first-order denitrification rate constant, day^{-1}; OCW is organic carbon content by weight, %; and $pH_{6.5}$ is pH-deficit, as described above. Although Equation 5 is a somewhat empirical relationship, it complies with certain theoretical considerations. Introduction of the square root factor is appropriate, since both the 1968 model of Bouldin and the 1974 model of Bouldin et al. indicate that denitrification rate is proportional to the square root of biochemical electron acceptor demand. The above combination of organic carbon content by weight, a pH correction factor for acid soils, and a small constant appears to be a fairly good estimation of electron acceptor demand. The constant (0.002) in the equation presents a problem by causing the predicted k_1 value of a soil with no organic matter to be 0.04 when such a soil would probably have a k_1 value of zero. It is worth noting

that because of the square root factor, a 50% reduction in electron acceptor demand only reduces denitrification rate by 29%. This helps explain the fairly narrow range of denitrification rates among the soils studied, in spite of the wide range of organic carbon contents and expected electron acceptor demands.

It is felt that Equation 5 could act as a simple predictor of denitrification capacities of wetland soils in Florida. Only two easily determined soil characteristics need be measured in order to make a prediction. Table 7 gives observed denitrification rates for each soil type, rates predicted by Equation 5, and percent error of predictions. Some errors are relatively high, particularly for some of the more slowly denitrifying soils. In view of the wide variation in denitrification rates within soil types (Tables 5 and 9), broad confidence intervals should be assumed for any predictive equation. Due to the considerations noted above, the equation should be regarded as invalid for soils with organic carbon contents below 0.5%. Fortunately, most wetland soils in Florida are fairly high in organic carbon content.

Nitrous Oxide Evolution

Nitrous oxide evolution rates were determined for one column of each of 14 of the soil types discussed above. Observed values were adjusted to account for variations in floodwater nitrate concentration at time of measurement. Adjusted evolution rates were correlated with denitrification rates of the columns on which the N_2O evolution measurements were performed.

The continuous flow, semi-enclosed system used to determine N_2O evolution has certain advantages and disadvantages as compared to the more commonly used totally enclosed system. Perhaps the most important advantage is that N_2O levels in the soil:water:air system remain fairly close to those which would occur in the field, where evolved N_2O can freely escape into the atmosphere. The highest observed N_2O concentration in effluent air from the semi-enclosed system was approximately 2.5 times the normal ambient concentration. In contrast, completely closed systems often allow N_2O concentrations to increase to more than 10 times the ambient concentration (Denmead, 1979). Thus the microorganisms in closed systems are exposed to a highly N_2O enriched environment which might have an effect on further production and reduction of N_2O. A major disadvantage of the semi-enclosed system is that low N_2O concentrations result in diminished measurement accuracy. In some cases, effluent concentrations were only 10% greater than ambient levels, and therefore accuracy suffered. Five replicate samples per evolution determination were used in an attempt to offset this problem, resulting in a ± 15 ppb (v:v) confidence interval about the mean ($\alpha = 0.05$). Use of airstones in the collection system offered the advantage of maintaining aerobic floodwater such as would often occur in the field, but also resulted in some difficulties. Pores of airstones tended to clog, sometimes reducing airflow to nearly zero. This caused many delays in data collection, since a 12 hour equilibration period at a relative constant flow rate was required before sampling.

Table 7. Denitrification rate constants as predicted by Equation 5.

Soil type	pH	Total organic carbon†	Rate constant		Error‡
			Observed	Predicted	
		---%---	--------day^{-1}---------		--%--
Everglades muck	6.2	46.0	0.192	0.193	+ 1
Floridana f.s.	6.5	12.6	0.149	0.115	-23
Astor s.	6.7	7.4	0.108	0.093	-14
Surrency s.	4.9	42.0	0.105	0.113	+ 8
Samsula muck	4.6	54.0	0.102	0.098	- 4
Pickney f.s.	5.0	27.0	0.087	0.108	+24
Riviera f.s.	4.5	5.0	0.076	0.050	-34
Chastain si.l.	5.6	5.2	0.067	0.069	+ 3
Brighton peat	4.2	53.0	0.066	0.057	-14
Iberia s.c.	6.1	4.0	0.063	0.070	+11
Chobee f.s.l.	6.8	7.4	0.062	0.093	+50
Delray f.s.	6.9	6.4	0.061	0.088	+44
Eureka f.s.l.	5.5	5.7	0.049	0.069	+41
Valkaria f.s.	6.6	0.43	0.040	0.049	+23

† By volume.

‡ [(predicted-observed)/observed] x 100.

Sampling delays made it necessary to make N_2O evolution determinations at different floodwater nitrate-N concentrations for each column. Concentrations ranged from 4 to 9 mg liter^{-1}. In order to allow comparison of evolution values, observed values were adjusted to reflect a 10 mg liter^{-1} nitrate-N concentration in all columns. This was done on the assumption that N_2O evolution is directly proportional to floodwater nitrate concentration. In an experiment designed to verify this assumption, evolution rates from two Samsula and two Pelham columns were measured as floodwater nitrate-N concentrations declined from 15 mg liter^{-1} to less than 5 mg liter^{-1}. Results are given in Figure 16 as graphs of nitrate-N concentration versus N_2O evolution. Plots are reasonably linear, indicating that within the concentration range in question, the above assumption is approximately valid. There is some indication, particularly in the case of Samsula, that the relationship deviates from linearity, but this deviation does not appear to be extreme. It has been reported that N_2O evolution relative to nitrate concentration can increase as nitrate concentration increases, due to nitrate inhibition of N_2O reduction (Blackmer and Bremner, 1979; Terry and Tate, 1980). Such inhibition does not appear to have occurred to any significant degree in this experiment. Since nitrate must diffuse from floodwater through an aerobic soil zone, it is likely that nitrate concentrations in the zone of active denitrification were somewhat lower than those in the floodwater. Such extremely low concentrations may not have been sufficient to cause significant inhibition of N_2O reduction.

Nitrous oxide evolution values for the 14 soil types are presented in Table 8. Since evolution was determined for only one column per soil type, values presented here may vary from the mean evolution rates of the individual soils. It is likely, however, that the data accurately represent the range of N_2O evolution rates to be expected in Florida wetland soils. The data also allow examination of the correlation between N_2O evolution and various soil characteristics. Evolution rates, based on a 10 mg liter^{-1} floodwater nitrate-N concentration, range from less than 5 g N ha^{-1}day^{-1} for the Floridana soil to 42 g N ha^{-1}day^{-1} for the Riviera soil, with a mean of 20.9 g N ha^{-1}day^{-1}. The percentage of consumed nitrate-N accountable for as N_2O-N ranges from less than 0.2% for Floridana to 6.5% for the Valkaria, with a mean of 2.1%. Mean values for organic soils are 18.5 g ha^{-1}day^{-1} and 1.1%, while mineral soils have mean values of 22.6 g ha^{-1}day^{-1} and 2.3%. The lower means for organic soils may reflect a higher electron acceptor demand, as discussed below. Percentage figures are in fairly close agreement with the 0.8 to 1.4% values reported for flooded soils by Denmead et al. (1979).

Nitrous oxide evolution and percent of consumed nitrate-N accountable for as N_2O-N were tested for possible correlation with nitrate consumption rate (apparent first-order denitrification rate constant) and soil pH. Neither of the N_2O parameters showed any correlation with soil pH. Nitrous oxide evolution as g N ha^{-1}day^{-1} was weakly, negatively correlated with nitrate consumption rate ($r^2 = 0.164$). Percent N_2O showed fairly strong correlation with nitrate consumption rate,

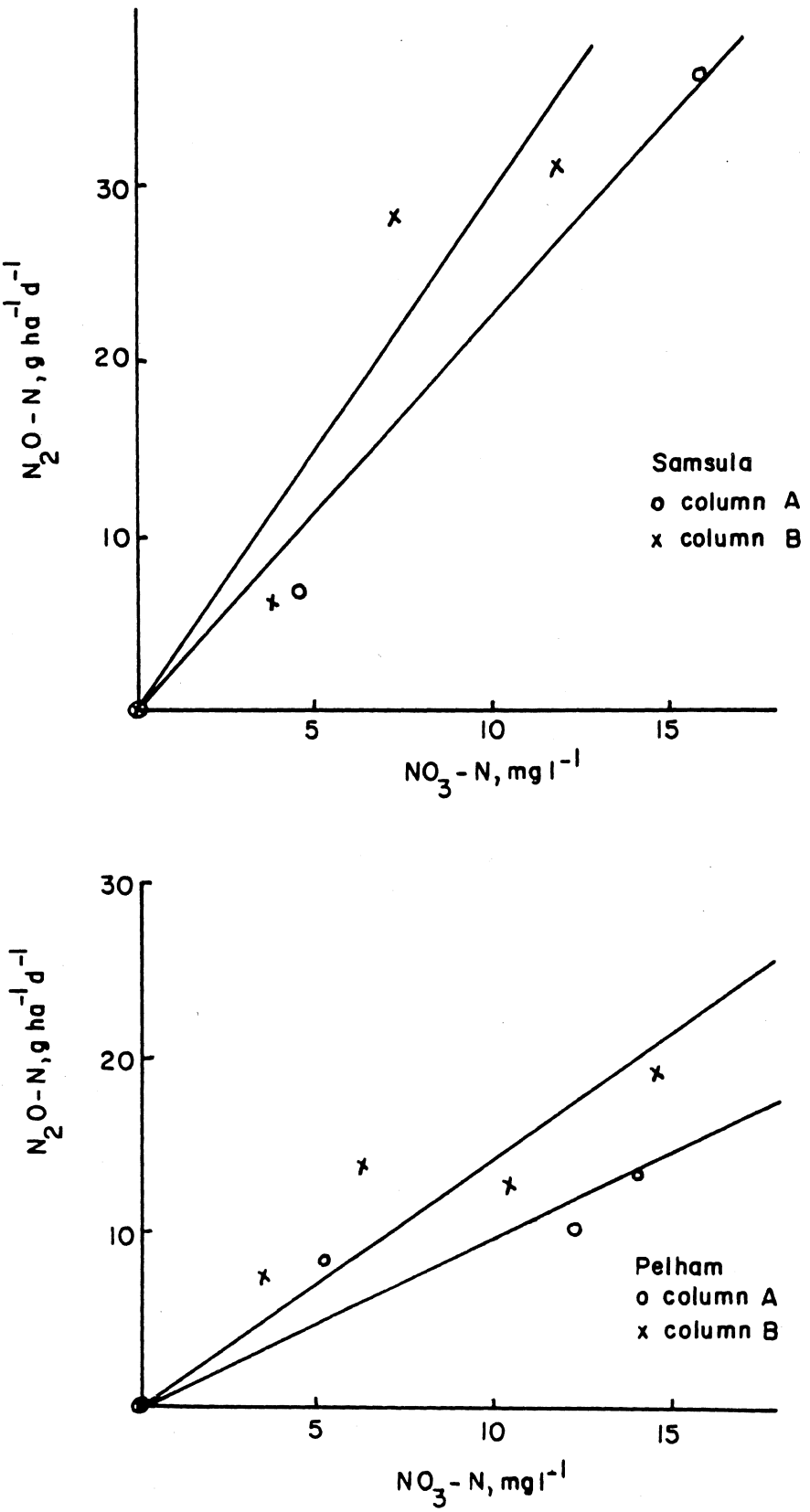

Figure 16. Nitrous oxide evolution from flooded soil cores in relation to floodwater nitrate concentrations.

Table 8. Nitrous oxide evolution rates from 14 representative Florida wetland soils.

Soil type	Denitrification rate constant†	Floodwater NO_3-N conc.	N_2O-N evolution rate		
			Observed	Adjusted‡	% N_2O§
	----day^{-1}-----	mg $liter^{-1}$	--------g ha^{-1} day^{-1}---------		
Everglades muck	0.175	4.0	7.2	18.0	0.7
Floridana f.s.	0.112	4.0	t	t	0.2
Astor s.	0.077	7.0	7.0	10.0	0.9
Surrency s.	0.105	5.0	13.4	26.8	1.7
Samsula muck	0.110	8.0	15.4	19.3	1.2
Pickney f.s.	0.065	6.5	13.1	20.2	2.1
Riviera f.s.	0.065	5.0	21.0	42.0	4.4
Chastain si.l.	0.092	6.0	15.5	25.8	1.4
Brighton peat	0.081	7.5	7.9	10.7	0.9
Iberia s.c.	0.050	9.0	24.1	26.8	3.6
Chobee f.s.l.	0.071	7.0	10.9	15.6	1.5
Delray f.s.	0.043	9.0	18.5	20.6	3.2
Eureka f.s.l.	0.161	3.5	5.0	14.3	0.6
Valkaria f.s.	0.040	8.5	32.5	38.2	6.5
mean				20.9	2.1

† Rate for individual column, not mean rate for soil type.

‡ Adjusted to 10 mg $liter^{-1}$ floodwater NO_3-N.

§ (N_2O-N evolved/NO_3-N consumed) x 100.

as indicated in Figure 17, and in the equation below ($r^2 = 0.706$, d.f. = 12):

$$\%N_2O = 0.25(1/k_1) - 1.26 \qquad [6]$$

where k_1 is the first-order denitrification rate constant, day^{-1}, and $\%N_2O$ is (N_2O-N evolution rate/nitrate-N consumption rate) x 100. Equation 6 agrees with the relationship between N_2O release and electron acceptor demand reported by Focht and Vertraete (1977). In this case, denitrification rate can be considered an indication of electron acceptor demand. Apparently, in soils with high electron acceptor demand, N_2O produced by denitrification tends to be further reduced to N_2, thus acting as an additional sink for electrons. In soils with lower electron acceptor demands, less of the N_2O produced is further reduced and thus more is evolved.

Long Term Denitrification and Nitrous Oxide Evolution

Columns of Samsula and Pelham soils (eight per soil type) were incubated for 10 weeks with floodwater nitrate-N concentrations maintained between 5 and 20 mg $liter^{-1}$. Denitrification rates were determined at 2, 5, and 8 weeks. Nitrous oxide evolution from two Samsula and three Pelham columns was also measured periodically during this time.

Denitrification rates are given in Table 9. Mean denitrification rates for the two soil types remained relatively constant throughout the study period. Individual denitrification rates fluctuated somewhat, however. In most columns, the change in rate appeared to be random. This may reflect irregular transport of nitrate into the soil. On several occasions, relatively large volumes of gases (presumably biogenic) were observed to be rapidly released from the soil. Release of gases had a stirring effect on the upper layer of soil, resulting in a loose, flocculent soil mass. In one case, a layer of soil material approximately 1 cm thick was observed floating on top of the floodwater, having been lifted by trapped gas. Such disruptions of the soil surface could result in increased transport of floodwater nitrate into the soil and thus increase the denitrification rate for short periods following disruptions. It may be assumed that this effect also occurs in nature. Temporal variation in decomposition rates of soil, plant and animal matter may also have contributed to variations in individual column denitrification rates.

In the case of Samsula columns 5 and 6, denitrification rates rose drastically during the study period. The rise was accompanied by darkening of the floodwater, probably due to accumulation of dissolved organic substances. Darkening of floodwater occurred to a lesser degree in all Samsula columns. Increased organic substrate availability is a likely cause for the accelerated denitrification. It is possible that columns 5 and 6 contained plant rhizomes or other organic materials which decomposed only after a lag period. Such lag periods are not uncommon in soil microbiological systems, and may be the result of such factors as substrate recalcitrance and microbial succession (Stotzky, 1973).

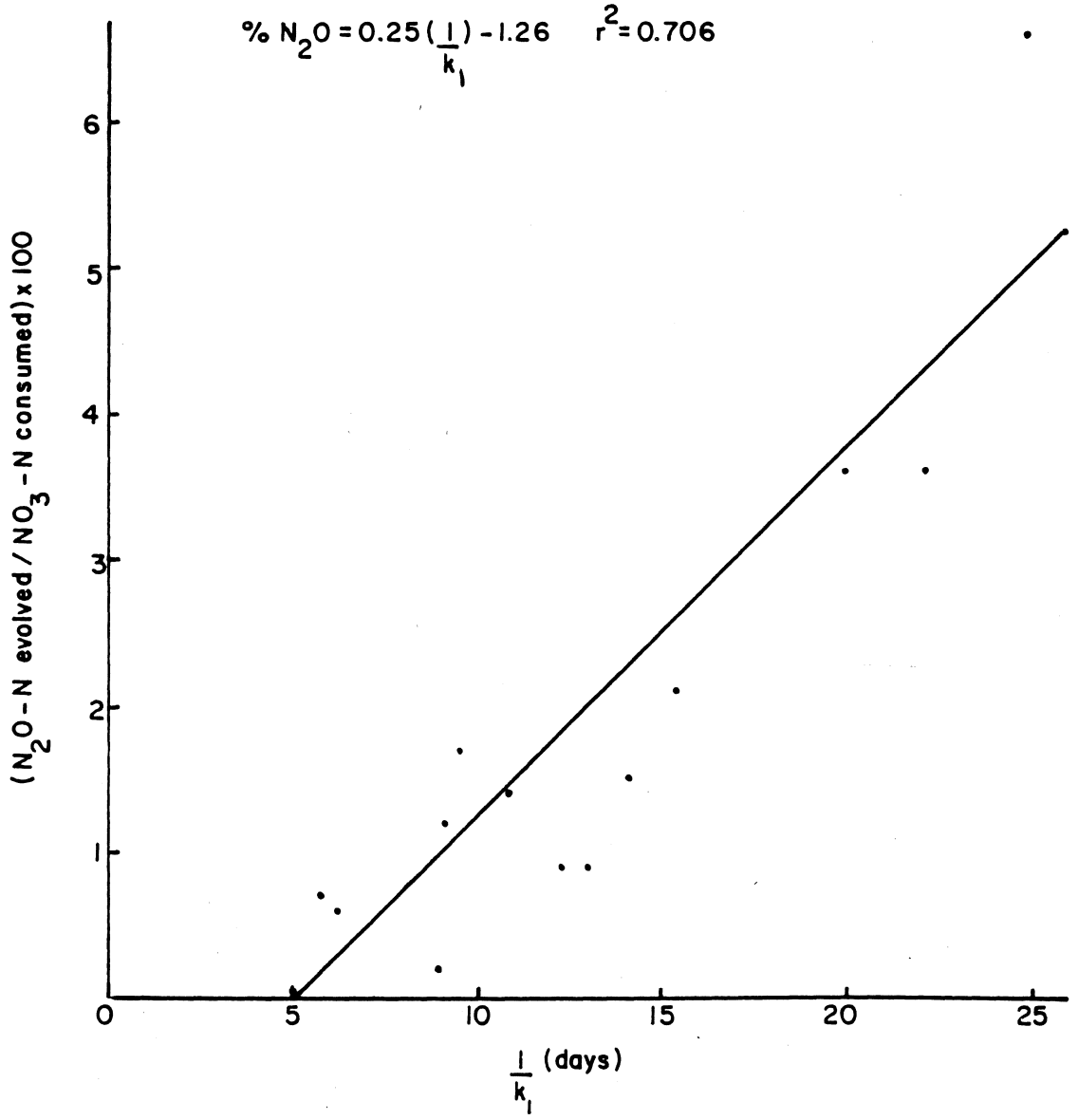

Figure 17. Relationship between reciprocal of denitrification rate constant and percent nitrous oxide evolution for 14 soil types.

Table 9. Long term (8 week) denitrification rates in Samsula and Pelham soil columns.

Soil type	Replicate	Denitrification rate constant		
		Week 2	Week 5	Week 8
		----------day^{-1}----------		
Samsula muck	1	0.093	0.080	0.073
	2	0.109	0.062	0.074
	3	0.067	0.077	0.054
	4	0.049	0.080	0.105
	5	0.112	0.091	0.208
	6	0.105	0.209	0.237
	7	0.060	0.062	0.059
	8	0.101	0.064	0.074
	avg.	0.087±0.021	0.091±0.041	0.095±0.050
Pelham l.f.s.	1	0.090	0.097	0.094
	2	0.064	0.081	0.065
	3	0.101	0.062	0.100
	4	0.078	0.100	0.074
	5	0.053	0.037	0.054
	6	0.091	0.061	0.064
	7	0.092	0.072	0.048
	8	0.051	0.071	0.054
	avg.	0.078±0.016	0.073±0.017	0.069±0.016

Nitrous oxide evolution from Samsula (columns 3 and 8) and Pelham (columns 5, 6, and 7) soils are presented in Figure 18. Evolution is shown as percent of consumed nitrate-N accountable for as N_2O-N. Nitrate consumption is based on the denitrification rate occurring in each column at the time N_2O evolution was determined. In three of the five columns, evolution decreased during the study period. This agrees with the observation of Guthrie and Duxbury (1979) that N_2O evolution decreases with increased time of soil exposure to nitrate. This decrease would be expected to result from microbial adaptation and selection. With time, the microbial community is likely to maximize use of N_2O as an electron acceptor, since electron acceptors are in short supply in anoxic environments. Nitrous oxide evolution increased in the case of column 7 of Pelham soil. The first value for this column is extremely low, and may be in error. Unusual population dynamics or soil conditions may be responsible for the upward trend in evolution.

An interesting phenomenon was accidently observed in column 8 of the Samsula soil (Figure 18). Air flow in the column was inadvertently reduced to less than 3 ml min^{-1}, and floodwater dissolved oxygen concentration dropped to approximately 1 mg $liter^{-1}$. Coinciding with this was a peak in percent N_2O evolution of 47 g $ha^{-1}day^{-1}$. Evolution subsided to 35 g $ha^{-1}day^{-1}$ one day later, after flow was increased to 7 ml min^{-1} and dissolved oxygen content increased to about 7 mg $liter^{-1}$. A similar effect occurred in column 4 of Samsula soil during an earlier, unreported phase of this study. Aeration slowed and dissolved oxygen concentration dropped to less than 1 mg $liter^{-1}$, resulting in an evolution rate of 40 g N $ha^{-1}day^{-1}$. Five days earlier, evolution from this column had been 23 g N $ha^{-1}day^{-1}$ and 9 days later the value was 16 g N $ha^{-1}day^{-1}$, with more adequate aeration in both cases. The observed increases in N_2O evolution rate may be due to accelerated denitrification resulting from low floodwater dissolved oxygen, a phenomenon reported by Van Kessell (1977). No increase in denitrification rate could be detected in the present study because the periods of anoxia were very short compared to nitrate sampling intervals.

Regardless of the actual cause, variation in floodwater oxygen content appears to have a marked effect on N_2O evolution and perhaps on denitrification. The problem arises as to how closely results presented in this study reflect those occurring in nature. With the above exceptions, floodwater dissolved oxygen content remained relatively constant throughout the study. In nature, dissolved oxygen content often undergoes diurnal fluctuations due to daytime oxygen production during photosynthesis. This difference may have resulted in lower observed N_2O evolution and denitrification rates in this study than would occur in the field.

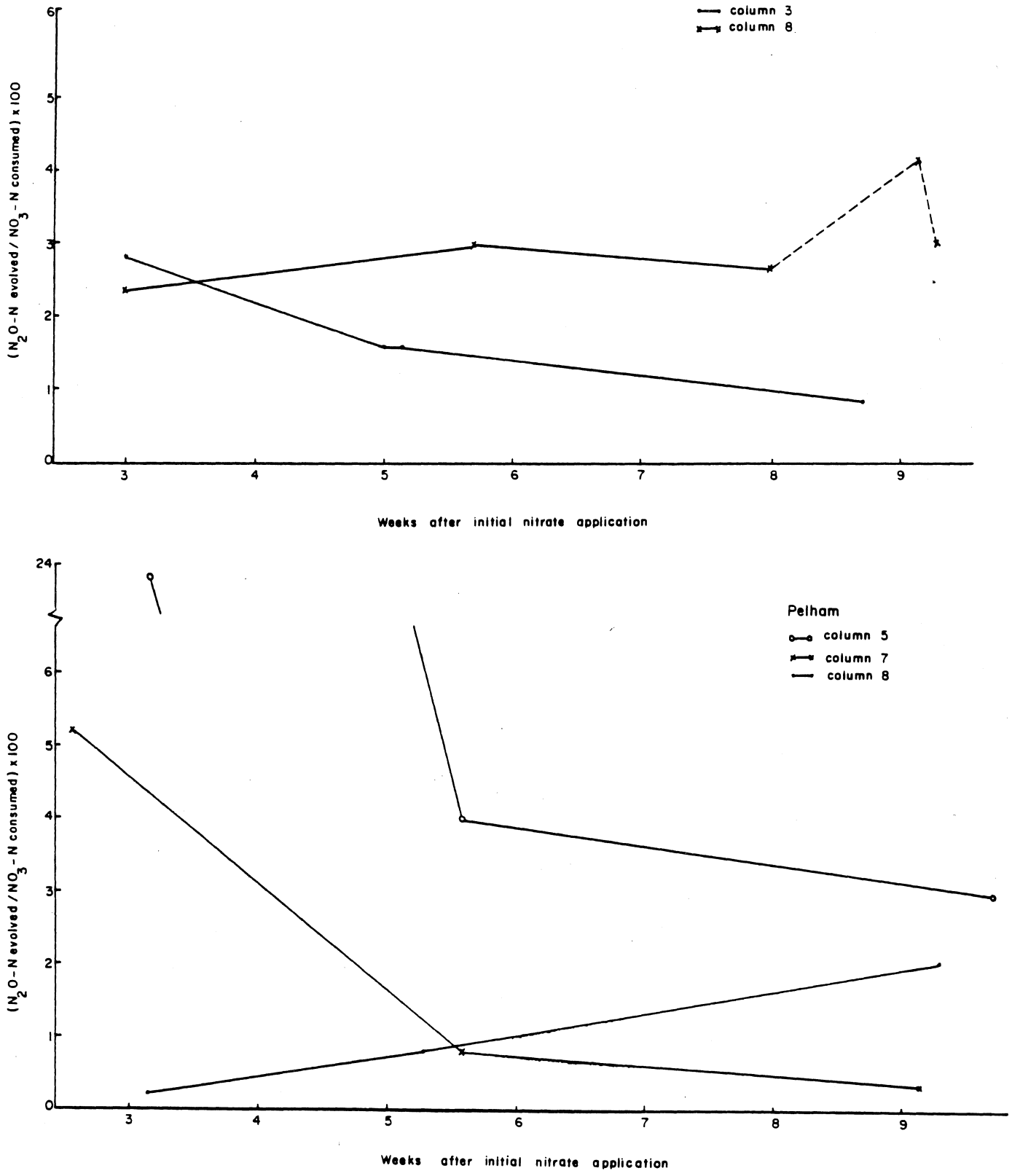

Figure 18. Long term (10 week) nitrous oxide evolution from flooded Samsula and Pelham soil columns.

SUMMARY AND CONCLUSIONS

Denitrification and N_2O evolution rates in a wide variety of Florida wetland soils were investigated in the laboratory using intact soil columns. It was determined that denitrification in the flooded soils followed apparent first-order kinetics, with rate constants ranging from 0.040 day^{-1} to 0.192 day^{-1}. Denitrification rate was related to soil organic carbon content and pH by the following formula:

$$k_1 = \sqrt{(8.9\times10^{-4})(OCW) - (3.9\times10^{-4})(OCW)(pH_{6.5}) + 0.002}$$

where k_1 is denitrification rate constant, day^{-1}; OCW is organic carbon content by weight, %; and $pH_{6.5}$ is pH deficit below pH 6.5. Residence times required for 50% removal of floodwater, assuming a floodwater depth of 15 cm and irrespective of nitrate concentration, ranged from 3.6 to 17 days. For 90% removal, 12 to 58 days would be required. These figures represent minimum removal rates. Either mass flow of floodwater into the soil or low floodwater dissolved oxygen content would be likely to result in higher removal rates. Assimilation of nitrate by vascular plants would also increase removal rates significantly. It is felt that many Florida wetland soils could function as efficient treatment systems for nitrate-bearing wastewater. Soil organic carbon content and pH, as well as hydrologic factors need to be considered in site selection.

A semi-enclosed chamber system was devised for determination of N_2O evolution rates. This system functioned satisfactorily without exposure of the soil:water environment to greatly elevated N_2O concentrations, as occurs in totally enclosed systems. Nitrous oxide accounted for from less than 0.2 to 6.5% of consumed nitrate. Wetland denitrification thus appears to be a relatively innocuous means of nitrate disposal, from the viewpoint of N_2O production and stratospheric ozone destruction. Soils which denitrified rapidly tended to evolve less N_2O than those with slower denitrification rates. Contrary to many reports, N_2O evolution in the soils studied was not related to soil pH. This may be due to the relatively low floodwater nitrate-N concentrations (less than 20 mg $liter^{-1}$) in the present study.

Two soil types were studied during a 10 week period of continuous nitrate exposure. Denitrification rates of individual columns tended to fluctuate randomly, but average rates remained relatively constant. Average N_2O evolution rate declined by approximately 60% between weeks 3 and 10, probably due to microbial adaptation and selection. Reduction in floodwater dissolved oxygen content appeared to result in increased N_2O evolution.

Further study is needed to determine more precisely the effect of naturally occurring fluctuations in floodwater oxygen content on denitrification and N_2O evolution rates. Application of high BOD wastewater could be studied in conjunction with this. The effect of

downward water flow on the above rates also warrants investigation. Denitrification and N_2O evolution should be investigated in other wetland ecosystems, including estuarine environments. *In situ* studies and refinement of *in situ* methods, particularly for N_2O evolution measurement, are needed in order to confirm the results of laboratory work.

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