The Effects of Atrazine on Nitrogen Cycling in a Freshwater Wetland Microcosm

by

Rhonda E. Wilhite

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APPROVED:

Arthur L. Buikema, Jr., Chairman

George M. Simmons

Robert E. Benoit

Albert C. Hendricks

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(Abstract)

Atrazine and other pesticides may damage non-target ecosystems, such as wetlands, by destroying vegetation or by disrupting microbial communities and nutrient cycling. The addition of atrazine to a wetland microcosm will disrupt the nitrogen cycle by inhibiting nitrifying and denitrifying bacteria. The inhibition or absence of functional groups of bacteria will limit the ability of the wetland to remove nutrients and herbicides, thus increasing nitrogen levels in non-point source pollution.

All tests were conducted in the laboratory using microcosms established from intact plant/sediment core subsamples collected from a natural freshwater wetland. Each microcosm recieved distilled water, atrazine, or acidified distilled water (pH 2) by groundwater seepage. Microcosms were dosed with 1.5 mg/L and 4.5 mg/L atrazine. Acid treatment of microcosms was done to provide a perturbation reference to the microcosm. Nitrite, nitrate, and ammonia levels were determined, as was dissolved oxygen, pH, and conductivity. The nitrifying bacteria and denitrifying bacteria were enumerated. Data were analyzed with one way

analysis of variance (ANOVA) with block and treatment interaction and Tukey's Studentized Range test.

Acid treatment of microcosms provided a positive control of microcosm disturbance. Acidification of the sediment disturbed the nitrogen cycle by suppressing ammonia dependent microbial processes. Atrazine (at 1.5 and 4.5 mg/L atrazine) did not significantly affect the number of nitrifying bacteria. The denitrifying or nitrate reducing bacteria were stimulated by exposure to atrazine and nitrate reduction became the dominant microbial process following exposure to atrazine. This shift toward denitrification and nitrate reduction processes caused increased levels of nitrite in the overflow waters. The continued accumulation of atrazine may cause nitrogen losses from an ecosystem which is already nitrogen limiting. Over time, predominant plant species may change, becoming primarily nitrogen fixing species. Changes in plant species would bring about changes in associated microbial populations. Excess nitrogen present in wetland runoff may cause algal blooms in recieving waters and pose health risks in contaminated drinking water. Accumulation of atrazine in wetlands may, ultimately, result in the loss of buffer zones.

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INTRODUCTION

In the last 30 years, herbicide use in the United States has increased fourfold, with atrazine being the most commonly used herbicide (Baker 1993). Atrazine (2-chloro-4-ethylamino-6-isopropyl-amino-1,3,5-triazine) is a broad-leaf control agent typically used in agricultural areas, specifically in corn fields. It is usually applied at an average rate of 1.12 pounds per acre and may be applied at pre-plant, pre-emergent, and post-emergent stages (Ribaudo and Bouzaher 1994). Much of this atrazine is lost in agricultural runoff (Clark et al. 1993; Gu et al. 1992; Jones et al. 1982; Mercurio 1995; Wolf and Jackson 1982).

Agricultural runoff accounts for approximately 60% of non-point source pollution entering surface waters (Osmond et al. 1995). Atrazine and other pesticides present may damage wetland ecosystems by destroying vegetation or by indirectly disrupting microbial communities and nutrient cycling activities associated with the plant rhizosphere.

Very little research has been done on the effects of atrazine on wetland ecosystems. Freshwater wetlands and constructed wetlands often act as buffer zones for the removal of nutrients and herbicides from agricultural runoff (Clark et al. 1993), pesticides (Abtew et al. 1995), and landfill leachates (Schwartz 1995). A wetland's ability to

TABLE 1. A summary of microbial reactions involving nitrogen in wetland sediments.

PROCESS	REACTION	OCCURRENCE	BACTERIA
Nitrogen Fixation	$N_2 \rightarrow NH_3$	Roots of Alnus trees	Frankia
Deamination (Mineralization)	organic matter $\rightarrow NH_4^+$		bacteria fungi
Chemoautotrophic Nitrification	$NH_4^+ \rightarrow NO_2^-$ $NO_2^- \rightarrow NO_3^-$	Aerobic rhizosphere	Nitrosomonas Nitrobacter
Heterotrophic Nitrification	$NH_4^+ \rightarrow NO_2^-$ $NO_2^- \rightarrow NO_3^-$	Aerobic regions	fungi Actinomycetes Pseudomonas
Denitrification	$NO_3^- \rightarrow N_2$	Anaerobic regions	Pseudomonas Acinetobacter Klebsiella
Dissimilatory Nitrate Reduction	$NO_3^- \rightarrow NO_2^-$ $NO_2^- \rightarrow NH_4^+$	Anaerobic regions	Pseudomonas Bacillia
Assimilatory Nitrate Reduction	$NO_3^- \rightarrow$ organic matter		fungi bacteria green plants

remediate pollutants and excess nutrients is dependent upon its dense vegetation and diverse microbial populations.

Nitrogen is frequently a limiting nutrient for plant growth in wetland systems (Dennison and Berry 1993). One of the most important zones of nitrogen cycling in wetlands is the rhizosphere. Wetland plants are capable of transporting gases, specifically oxygen, down into the roots (Hammer 1993), and transporting other gases (nitric oxide, nitrous oxide, and nitrogen) up into the atmosphere (Reddy et al. 1989). As a result, the thin region around the roots is aerobic, surrounded by an anaerobic region. Here, the plant roots are constantly exchanging nutrients and minerals with the sediment. This nutrient rich region is one of the most active zones of nitrification and denitrification (Reddy et al. 1989).

Nitrification, the oxidation of ammonia (NH^{4+}) to nitrite (NO_2^-) and nitrate (NO_3^-), is an aerobic process which occurs in the oxygenated rhizosphere and the very uppermost layers of sediment. Nitrification occurs in two steps: 1) the oxidation of ammonia to nitrite and 2) the oxidation of nitrite to nitrate. This formation of soluble nitrate provides the plant with a constant supply of nitrogen.

The ammonia and nitrite oxidizing bacteria are obligate chemolithotrophs which use ammonia or nitrite as an energy source and carbon dioxide as a carbon source. The ammonia

oxidizing bacteria include Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus, and Nitrosovibrio. Most of these are found in the soil environment (Bock et al. 1986). The nitrite oxidizing bacteria include Nitrobacter, Nitrococcus, Nitrospina, and Nitrospira. Only one of these, Nitrospira, is found in soils (Bock et al. 1986). The presence of only one genera of nitrite oxidizing bacteria in the sediment makes this process susceptible to inhibition or disruption.

Nitrification processes are quite sensitive and are often inhibited by a wide range of adverse conditions (Fenchel 1979). Because of its sensitivity to temperature, pH, nitrogen availability, and organic compounds, the process of nitrification is considered to be an excellent indicator of pollution (van Dijk 1987). Without adequate nitrification rates, there could be an increase in ammonia or nitrite compounds present in soils. Nitrogen compounds not used in the sediment will be lost to the overlying waters and eventually increase the levels of nitrogen coming from the wetland. This could result in algal blooms, and possibly, the formation of toxic compounds, such as nitrosamines.

Denitrification, the reduction of nitrate (NO_3^-) or nitrite (NO_2^-) to nitric oxide (NO), nitrous oxide (N_2O), or nitrogen gas (N_2), is an anaerobic process occuring primarily in anoxic zones of sediments, or in reduced microzones in the upper layers of sediments (Omerland et al. 1984; Sorensen

1978). Nitrate may also be reduced by dissimilatory nitrate reduction. This process reduces nitrate (NO_3^-) to nitrite (NO_2^-) to ammonia (NH_4^+). Nitrate is not usually adsorbed to soil particles making it readily available to denitrifying organisms in the sediment. Denitrifying bacteria have been shown to use other electron acceptors in the absence of nitrate (Sorenson 1982). The end products of nitrogen reduction are determined by the organic carbon:nitrate ratio and the types of nitrogen reducing bacteria present in the sediments (Herbert and Nedwell 1990; King and Nedwell 1987; Sorensen 1978).

Bacteria present in the sediment are usually divided into respiring or fermentative bacteria, which form different products; however, some bacteria are capable of forming either gaseous products or ammonia (King and Nedwell 1987). The most common denitrifying bacteria are Pseudomonas, Acinetobacter, Aeromonas, Klebsiella, and other members of the Enterobacteriaceae family (Herbert and Nedwell 1990). This is a large, diverse group of organisms which can adapt quickly to changes in the sediment environment.

Nitrification and denitrification processes occur in close proximity and feed off one another (Jenkins and Kemp 1984). It is the combination of these processes which removes excess nitrogen from wetland systems. Exposure to a pesticide may have two effects on these bacterial

populations: 1) the pesticide may have no effect on either population or 2) the pesticide may cause a temporary inhibition of one or both processes and 3) the pesticide may stimulate one or both processes (Camper 1991). An equilibrium is typically established when one bacterial group is inhibited or stimulated. For example, when chemoautotrophic nitrification is inhibited, ammonia may accumulate, and immobilization of ammonia by other microorganisms increases.

Atrazine is accumulating in ecosystems and we cannot predict its degradation by microbial populations if we do not know the effects of atrazine on those populations.

The objective of this study is to determine the effects of atrazine on nitrogen utilizing bacteria and the nitrogen cycle of a freshwater wetland. It is hypothesized that the addition of atrazine to a wetland microcosm will inhibit the nitrifying and denitrifying bacteria present in the sediment. The inhibition or absence of functional groups of bacteria will limit the ability of the wetland to remove nutrients and herbicides, thus increasing non-point source pollution.

LITERATURE REVIEW

ATRAZINE

Initially, most atrazine research focused on atrazine degradation in cropland soils and its toxicity to soil microbes. It soon became apparent that a great deal of atrazine was reaching waterways and the concern shifted toward the effects of atrazine on primary producers in aquatic ecosystems (Rocchio and Malanchuk 1986; Stay et al. 1985; Stay et al. 1989; Johnson 1986).

Toxicity

Atrazine inhibits several microbiological functions and processes, including fungal respiration (Bakalivanov 1972). Atrazine typically causes reduced algal density, decreased phytoplankton diversity, reduced biomass accumulation (100 ug/L) (Hamala and Kolliq 1985; Hamilton et al. 1988; Stay et al. 1985), and decreases in wetlands productivity (25 g/L) (Lee, Huggins, and Thurman 1995). Atrazine has been determined to be moderately toxic to fish (4.5 ppm for Rainbow Trout) and freshwater invertebrates (0.06 ppm to 1.25 ppm depending on the organism) (Ribaudo and Bouzaher 1994). It has been classified as a Class 2B or C human carcinogen

(Ribaudo and Bouzaher 1994).

Atrazine Contamination of Water Supplies

In loamy soils, atrazine has a half life of 60-150 days (Ribaudo and Bouzaher 1994). This rate is even slower in anaerobic sediments (660 days). Due to this persistence, and the frequent use of atrazine, it is accumulating in surface waters and groundwater. A recent study found that 94% of wetlands in the Central Nebraska Basins were contaminated with atrazine, regardless of surrounding land use (Frankforter 1995). Atrazine has been found in rivers throughout the year. The Mississippi, Missouri, Des Moines, Wabash, Maumee, and Sandusky Rivers have atrazine present at concentrations exceeding the Maximum Contaminant Level (MCL) year-round. Many of these rivers serve as public water supplies, and water treatment plants are struggling to remediate atrazine to safe levels (Ribaudo and Bouzaher 1994). Once atrazine reaches reservoirs, it's half life increase to 2 years (Ribaudo and Bouzaher 1994). Reservoirs frequently serve as community water supplies and are often contaminated with atrazine at levels exceeding the MCL. As many as six states have tainted supply reservoirs: Ohio, Illinois, Iowa, Nebraska, Kansas, and Missouri (Ribaudo and Bouzaher 1994).

Atrazine contamination is widespread and not limited to surface waters. Already, atrazine has been found in groundwater and in water supply wells at concentrations exceeding the MCL and the Health Advisory Limit (Baker 1993). The United States Geologic Survey reports that approximately 20.8% of wells are contaminated with atrazine at concentrations approaching the MCL (Ribaudo and Bouzaher 1994).

Atrazine Degradation

Degradation of atrazine is dependent on many physical and chemical parameters. Its persistence and disappearance in soils and aquatic sediments depends upon the rates of degradation, mineralization, migration (to subsurface levels), and sorption to soil organic matter and plants (Winkelmann and Klaine 1991a). The majority of sorption and breakdown occurs in the upper 2.5 centimeters of the sediment (Mudd et al. 1995).

The major pathways for atrazine degradation are N-dealkylation, dechlorination, and ring cleavage (Mandelbaum, Wackett, and Allan 1993). Several studies have proposed that dechlorination is a result of non-biological, chemical reactions (Skipper et al. 1967; Skipper and Volk 1972; Gu et al. 1992). Soil pH strongly influences this chemical

process, with neutral pH levels slowing dechlorination (Mandelbaum et al. 1993).

Degradation rates and pathways vary greatly depending on the physical, chemical, and microbial characteristics of the study site (Jones et al. 1982; Mirgain et al. 1993; Skipper et al. 1967; Skipper and Volk 1972; Winkelmann and Klaine 1991a; Winkelmann and Klaine 1991b; Wolf and Martin 1975). Microbial degradation was previously thought to account for little atrazine breakdown (Skipper et al. 1967; Geller 1980; Skipper and Volk 1972). A later study by Skipper and Volk (1972) determined that degradation of atrazine in soils was a combination of chemical hydrolysis and microbial degradation.

In his 1967 study, Skipper concluded that such a low rate of microbial use of atrazine was indicative of preferential use of other carbon sources. In 1980, Geller concluded that atrazine was bound by bacteria but not degraded. Winkelmann and Klaine (1991b) have since found that some bacteria and fungi are capable of metabolizing atrazine. Among these bacteria are several species of Pseudomonas.

This finding was supported when Mirgain et al. (1993) used subculturing of soil samples which had been previously exposed to atrazine, enrichment cultures, and carbon limitation to show bacterial co-metabolism of atrazine. They found that, although the number of bacterial species

decreased, atrazine co-metabolism was enhanced. Among those soil bacteria which degraded atrazine were Acinetobacter calcoaceticus, Pseudomonas alcaligenes in association with an Agrobacter sp., and a five member consortia of two Pseudomonas spp., Acinetobacter, Flavobacterium multivorum, and Enterobacter cloacea. In all cases, atrazine was degraded in 18 hours.

Microbial degradation may occur by hydrolysis at the number 2 carbon, N-dealkylation of the side chains, and ring cleavage (Skipper and Volk 1972). The major metabolic by-products of atrazine degradation are deethylatrazine, deisopropylatrazine, dealkylatrazine and hydroxyatrazine (Winkelmann and Klaine 1991b).

In anaerobic wetland sediments, microbial degradation of atrazine is extremely slow. Dehalogenation seems to be the slowest phase of degradation, leaving the central ring structure more susceptible to microbial attack by dominant anaerobic microorganisms which are capable of using organic compounds as electron acceptors (Gu et al. 1992). Microbial degradation plays a major role in the persistence of the metabolites. The dealkylated metabolites have a half-life of 17-26 days in soil. Ring cleavage, however, is slow, so hydroxyatrazine may persist for 121 days (Winkelmann and Klaine 1991b).

Wetland Remediation of Pollutants

Very little research has been attempted to evaluate the the effects of atrazine on wetland processes. Pesticide and nutrient removal in wetlands occurs by plant uptake, chemical precipitation, microbial uptake, and adsorption to the sediment (Hammer 1993; Corbitt and Bowen 1994). Many of these processes are dependent on the vegetation of the wetland.

Wetland vegetation is crucial to the microbial population of a wetland. Not only do plants retain sediments and increase surface area for adsorption and uptake of nutrients and pesticides, but also they provide additional environments for microbial populations (Hammer 1993). It has been determined that the type of vegetation present indirectly affects the rate of atrazine adsorption and breakdown by influencing the microbial community present (Mudd et al. 1995). Lee et al. (1995) further stated that the structure of the hydrophyte community was the determining factor in the breakdown of atrazine in wetlands.

MICROCOSM STUDIES

Due to the toxicity and seasonal use of atrazine, field

studies are rare. Most atrazine studies are conducted using microcosms. Several microcosm designs have been used to study the various effects of atrazine in soil, water, and wetlands. Few of these, however, take into account the entire ecology of a wetland system. Skipper (1967) used soil microcosms with greenhouse incubation. Wolf and Martin (1975) used a microcosm of sandy loam soil and pure cultures. Rocchio and Malanchuk (1986) used a continuous flow water microcosm. Johnson (1986) used a static 4-component wetland microcosm to simulate a northern prairie wetland. Pratt et al. (1988) used a polyurethane foam substrate to support a microbial community in a freshwater microcosm. Stay et al. (1989) used Leffler microcosms. Of these, the most applicable microcosm was used by Johnson (1986), which contained water, sediment, plants, and animals. However, it had no water flow.

The most informative microcosm study regarding nitrogen cycling was that of Rocchio and Malanchuk (1986). They assessed the effects of atrazine to nitrate and dissolved oxygen levels in an aqueous, continuous flow microcosm designed to monitor algal growth. They concluded that because nitrate and dissolved oxygen decreased, and nitrite did not accumulate, denitrification must have been the dominant process in the microcosm. Although this provided an indication of change in nutrient cycling, it addressed only

that small part of the cycle occurring in the water column.

Several of these microcosm studies report decreases in chemical parameters, such as dissolved oxygen, and nitrate levels (Rocchio and Malanchuk 1986), pH (Stay et al. 1989). Those studies which addressed the microbial component of the system reported no significant effects on microbial "activity", as measured by microbial respiratory ETS activity, metabolism of dissolved organic carbon, oxygen consumption, or alkaline phophatase activity (Johnson 1986; Pratt et al. 1988; Stay et al. 1989; Wolf and Martin 1975). Pratt et al. (1988) found that at the highest concentration of atrazine exposure, species number, and protein levels decreased. At lower levels of atrazine, species number, and protein increased. These unusual responses were attributed to shifting nutrient dynamics and stress responses.

Although many microcosms have been used to assess the effects of atrazine on various soil and aquatic microbial systems, none addressed the effects of atrazine to a specific group of bacteria. Also none used a continuous flow wetland microcosm which would be a more realistic representation of a wetland ecosystem. In a recent review of wetland models, Dixon and Florian (1993) concluded that the majority of previously used wetland microcosms were considered spatially homogenous with little or no contaminant flux. The use of an intact sediment core subsample containing wetland plants and

having a continuous flow of water should provide the most realistic of microcosms. In more recent studies, more complex or realistic wetland microcosms have been used, but these were designed to follow atrazine degradation (Lee et al. 1995).

MATERIALS AND METHODS

Study Site

All tests were conducted in the laboratory using sediment subsamples placed in plexiglass chambers. Each microcosm chamber was filled with an intact sediment core containing sediment, water, and cattails (Typha). Subsamples were collected from a natural freshwater non-tidal riverine wetland at Goose Creek, Montvale, Virginia. This wetland was populated primarily with cattails and rush. The Soil Conservation Service classifies the soil as Chewacla loam (Soil Conservation Service 1989). The accepted wetland classification is peaty-muck (hemist). The sediment remained water saturated throughout the year. Water flow in the wetland was primarily subsurface flow with two shallow surface channels through the center of the wetland.

Sediment core subsamples were collected using a rectangular plexiglass core sampler of the approximate dimensions of the microcosm chambers. The sampler was pushed into the sediment, a vacuum was established by plugging the sampler, and the sampler was pulled from the sediment. In this way, a large sediment core was extracted with minimal disturbance and compaction of the sediment. The sediment core was then placed inside the microcosm chamber, the

vacuum released, and the core sampler withdrawn, leaving the sediment in the chamber. To maintain water flow through the sediment, any gaps between the sediment core and the chamber walls were filled with sediment from the wetland. Subsamples were collected and dosed with atrazine in two trials (1.5 mg/L and 4.5 mg/L).

Each microcosm chamber was approximately 61.2 cm deep and 15.3 cm x 15.3 cm. Figure 1 shows a diagram of the plexiglass microcosm chamber used in the study. A perforated pipe in the bottom of the chamber was used to introduce water into the microcosm, thus simulating groundwater seepage. Runoff was collected in 600 ml acid washed glass beakers for analysis. Water flow is typically very slow or undetectable in wetlands (Chescheir et al. 1995; Sun et al. 1995), therefore the water flow through these microcosms was approximately 25 ml per hour.

1.5 mg/L Atrazine Exposure

Seven core subsamples of a freshwater wetland were collected and placed in the plexiglass chambers. The microcosms were allowed to develop in a controlled environmental chamber at 22°C under a 12L:12D photoperiod. Distilled water was continuously fed, at 25 ml per hour,

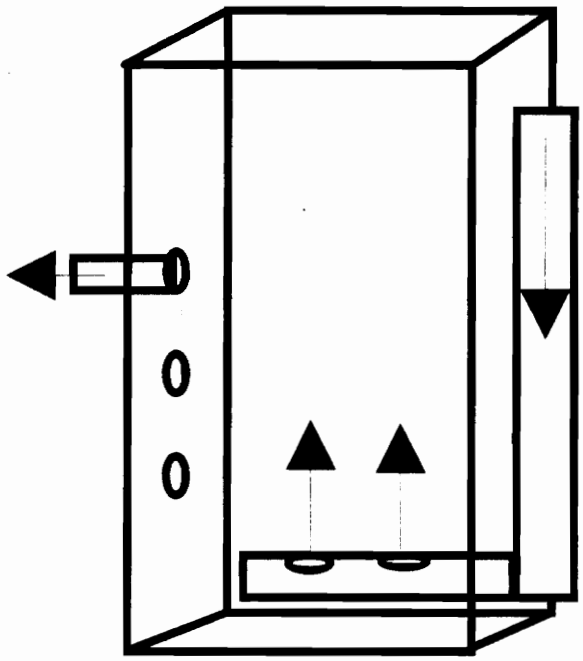


Figure 1. Wetland subsample chamber. Distilled water was pumped up through the sediment. Overflow water flowed through the side portal and into a glass beaker receptacle. Arrows show water flow through the chamber.

into the chambers to simulate groundwater seepage. The microcosms were allowed to equilibrate to test conditions for approximately 6 weeks. In an effort to increase the nitrifying bacterial population, all microcosms were fertilized with 10 mM ammonium phosphate mixed in distilled water and allowed to stabilize for an additional 4 weeks.

In this experiment, two microcosms were not exposed to atrazine (controls), three were exposed to atrazine, and three were exposed to acid. The study period was 33 days. Control systems recieved only distilled water throughout the study. Atrazine treated systems recieved 1.5 mg/L atrazine for 24 hr and distilled water for the duration of the experiment. During this 24 hr time frame, each chamber actually recieved 0.8 mg/L atrazine. Acid treatment provided a perturbation reference to the microcosm. Acid treatment of microcosms began at Day 0 and continued throughout the study. Distilled water was acidified with concentrated sulfuric acid to a pH of approximately 2 units.

During the 33 day study, sediment and water were monitored for various parameters. Sediment pH, ammonium, nitrite, and nitrate were monitored on log scale time intervals (-1,0,1,2,4,8,16, and 32 days). The nitrifying bacteria (ammonia oxidizers and nitrite oxidizers) and denitrifying bacteria populations were enumerated on the same

schedule. Overflow water parameters were monitored on the same schedule. The overflow water was monitored for ammonia, nitrite, nitrate, pH, dissolved oxygen, and conductivity. Dissolved oxygen, pH, and conductivity were determined daily.

4.5 mg/l Atrazine Exposure

Six core subsamples of a freshwater wetland were collected and placed in plexiglass chambers. Distilled water was continuously fed, at 25 ml/hr, into the chambers to simulate groundwater seepage. The microcosms were allowed to equilibrate for approximately 4 weeks to allow any recovery of the microbial nitrifier population. The microcosms were maintained at 22°C with a 12L:12D photoperiod.

In this trial, three microcosms were controls and three were exposed to atrazine. Since exposure to 1.5 mg/L atrazine had little effect on the microcosms, a much higher concentration was chosen, 4.5 mg/L atrazine. Atrazine treated systems recieved 4.5 mg/L atrazine for 24 hr and distilled water for the remaining time. During this 24 hr time frame, each chamber actually recieved 2.7 mg/L atrazine. Control systems recieved only distilled water.

The study period was 33 days. The sampling schedule was changed to document frequent changes in sediment chemistry. Sediment pH, ammonia, nitrite, and nitrate were monitored at

regular time intervals not exceeding five days (-7, -3, 0, 2, 4, 8, 12, 16, 20, 24, 29, and 33 days). The nitrifying bacteria (ammonia oxidizers and nitrite oxidizers) and denitrifying bacteria populations, were enumerated on the same schedule. Wetland overflow ammonia, nitrite, nitrate, pH, dissolved oxygen, and conductivity were also monitored according to this schedule.

Water Chemistry

Dissolved oxygen was measured using a YSI model 54A dissolved oxygen meter. Water and sediment pH were measured using a Fisher Accumet pH meter. Conductivity was measured with a YSI model 32 conductivity meter (APHA 1985). Nitrite concentrations were determined by an azo dye formation procedure (USEPA 1979). This procedure involves the addition of N-(1-napthyl)-ethylenediamine dihydrochloride and sulfanilimide to form a pink colored azo dye. Absorbance of standards and samples were read using a spectrophotometer at a wavelength of 543 nm. A standard curve was generated and sample nitrite concentrations were determined.

Nitrate levels were determined according to the cadmium reduction column method (APHA 1985). This method involves the reduction of nitrate to nitrite by cadmium and a subsequent azo-dye formation. Solutions were read on a

spectrophotometer and concentrations determined by a standard curve of nitrate concentration and absorbance.

Ammonia levels were determined according to the phenate method (APHA 1985), or the formation of a blue, indophenol compound. This indophenol is formed due to the reaction of ammonia, hypochlorous acid, and phenate, in the presence of a manganous salt. Absorbance values were read at 630 nm and ammonia concentrations determined from a standard curve of concentration and absorbance.

Sediment Chemistry

Sediment samples were collected from the microcosms using a scooped spatula to collect a small vertical core of sediment (approximately 15 g). Vertical cores were collected from the surface in order to obtain both aerobic and anaerobic regions of sediment. Sediment cores were collected close to the plants and around the root zone, since this is an active nitrification and denitrification zone.

Sediment inorganic nitrogen was extracted with potassium chloride (Keeney and Nelson, 1982). Sediment chemistry analyses of ammonia, nitrite, and nitrate were determined colorimetrically. Exchangeable ammonia was determined by an blue indolphenol method (Keeney and Nelson, 1982). This method uses sodium nitroprusside as a catalyst in the

ammonium/phenol reaction. Nitrate was determined by a cadmium reduction column method in which nitrate is reduced to nitrite (Keeney and Nelson, 1982). This process differs from the nitrate analysis of water in that it does not use ethylenediaminetetraacetic acid (EDTA) because it complexes with chloride ions used in the nitrogen extraction. Nitrite concentrations were determined using the same diazotizing and coupling reagents as used for water analysis (Keeney and Nelson, 1982).

Sediment Microbiology

Enumeration of nitrifying bacteria was determined by a Most Probable Number (MPN) method using a nitrifier medium and Griess-Ilovsay reagents (Schmidt and Belser 1982). Two selective media were used to assess both groups of the nitrifying bacteria. An ammonium carbonate broth was used to enumerate the ammonia oxidizing group, and a nitrite carbonate broth was used for the nitrite oxidizing group. Dilutions ranging from 10^{-1}- 10^{-6} were used, with 5 tubes per dilution, for each MPN series. Tubes were incubated at 25-30^{o}C in the dark for approximately 4 wks and nitrification was confirmed by Griess-Ilovsay diazotizing and coupling reagents (Schmidt and Belser 1982). Positive tubes produced a pink-red color upon addition of the Griess-Ilovsay reagents.

Denitrifying bacteria were enumerated by an MPN technique using nitrate nutrient broth, inverted tubes, and diphenylamine. Screw cap tubes were used to establish an anaerobic environment. The dilution range used for the MPN series was 10^{-2}-10^{-7}, with 5 tubes per dilution. Tubes were incubated at 25-30°C for 14 d. At that time, each tube was tested for the presence of nitrate or nitrite by the addition of diphenylamine. This reagent produces a blue coloration in the presence of either of these compounds and is considered evidence of nitrate reduction and denitrification (Tiedje 1982).

Statistics

Chemical and microbial data were analyzed with PC-SAS using one way analysis of variance (ANOVA) with block and treatment interaction and Tukey's Studentized Range test (Sokal and Rohlf 1995). Time was represented as blocks. There is frequently a great deal of background noise in sediment chemistry. Therefore, changes were determined to be significant at the 90% level ($p=0.10$). A parameter was considered significant if that parameter changed significantly over time and was significantly different from that of the control systems. Those p values shown represent significant changes over time.

RESULTS

Control Microcosms (1.5 mg/L Atrazine Trial)

In control microcosms, the pH of both sediment and water remained neutral throughout the study, and dissolved oxygen and conductivity of overflow water remained low. There was a great deal of fluctuation in the sediment nitrogen levels, shown in Figure 2, and microbial numbers due to microzones in the sediment. Ammonia oxidizing bacteria numbers remained stable throughout the study, until day 9, when microbial numbers decreased to 2.5×10^5. Sediment ammonia levels remained stable, but fluctuated around 350 ug/g dry soil. Overflow ammonia levels decreased slightly on Day 4, then returned to Day 0 levels (see Figure 3).

Nitrite oxidizing bacteria numbers remained low. However, on Day 2, there was a higher number of nitrite oxidizing bacteria. Sediment nitrite levels gradually decreased, with little fluctuation, until stabilizing on Day 4. Overflow nitrite levels steadily decreased until Day 9, when levels gradually increased. Overflow nitrite levels remained higher than those of treated microcosms throughout the study (see Figure 3).

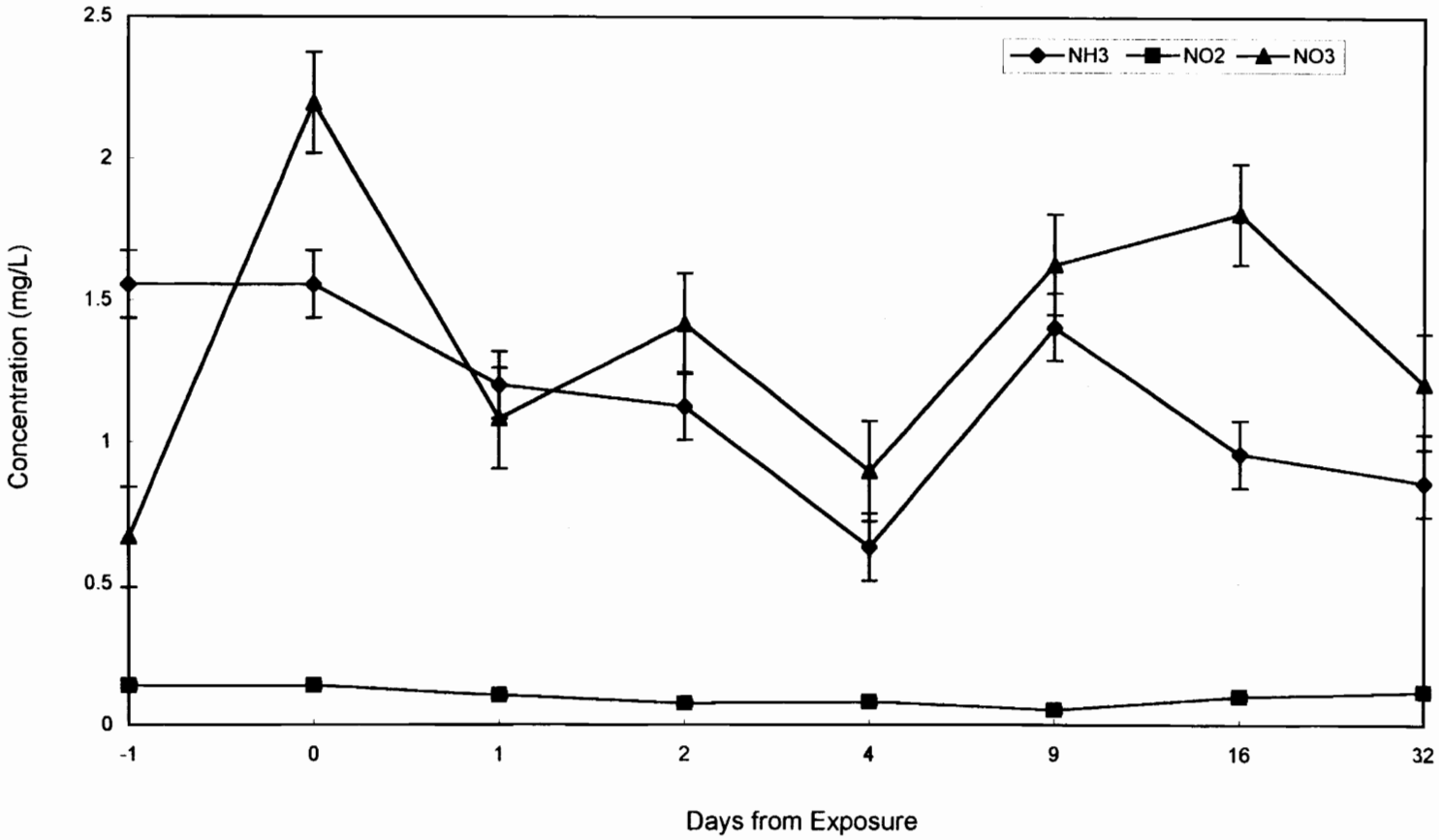


Figure 3. Overflow levels of ammonia, nitrite, and nitrate from control treated microcosms.

Denitrifying bacteria present in control microcosms decreased on Day 2 and increased on Day 16. Sediment nitrate levels remained stable, with the exception of a large increase on Day 4. Overflow nitrate levels fluctuated, but remained higher than those of treated microcosms throughout the study.

Acid Exposure

With acid treatment, overflow water pH remained around 2, the acidity of the flow-through water. The pH of the sediment did not change substantially during exposure. Prior to acidification, sediment pH was 6.7; after 32 days of continuous acidification, sediment pH had dropped to only 5.5. However, this may not be an accurate determination of the true sediment pH. It may be a reflection of the neutral distilled water used in the procedure. The conductivity of the overflow water fluctuated widely and gradually increased from 0.100 mmohs to 1.552 mmohs. Dissolved oxygen levels were unaffected by acidification.

Ammonia oxidizing bacteria were reduced following acidification ($p=0.0001$). Prior to acidification, ammonia oxidizing bacteria were enumerated to be 1.25×10^6 (bacteria per gram dry soil); after 32 days of acidification, the density had fallen to 2.5×10^5 (bacteria per gram dry soil)

bacteria. Sediment ammonia concentrations, shown in Figure 4, remained stable until decreasing drastically after Day 9 ($p=0.0003$). As a result of falling sediment ammonia, overflow ammonia levels increased after Day 4, reaching 2.417 mg/L NH_3 by Day 32 ($p=0.0030$) (see Figure 5).

Nitrite oxidizing bacteria decreased ($p=0.0042$) almost immediately after exposure, from 2.56×10^6 (bacteria per gram dry soil) at Day 0, to 1.29×10^4 (bacteria per gram dry soil) at Day 32. Overflow nitrite concentrations decreased from 0.014 mg/L nitrite to 0.002 mg/L nitrite. Nitrite levels steadily increased after Day 1 ($p=0.0061$) and returned to beginning concentrations by Day 4. Sediment nitrite levels were not affected by acidification.

Denitrifying bacteria increased ($p=0.0174$) from 3.4×10^5 (bacteria per gram dry soil) to 9.6×10^5 (bacteria per gram dry soil) at Day 32. Sediment nitrate concentrations decreased ($p=0.0441$) from 1656 ug/g nitrate to 105 ug/g nitrate at Day 32. Overflow nitrate levels also decreased ($p=0.0623$) from 1.194 mg/L nitrate at Day 0 to 0.193 mg/L nitrate at Day 32.

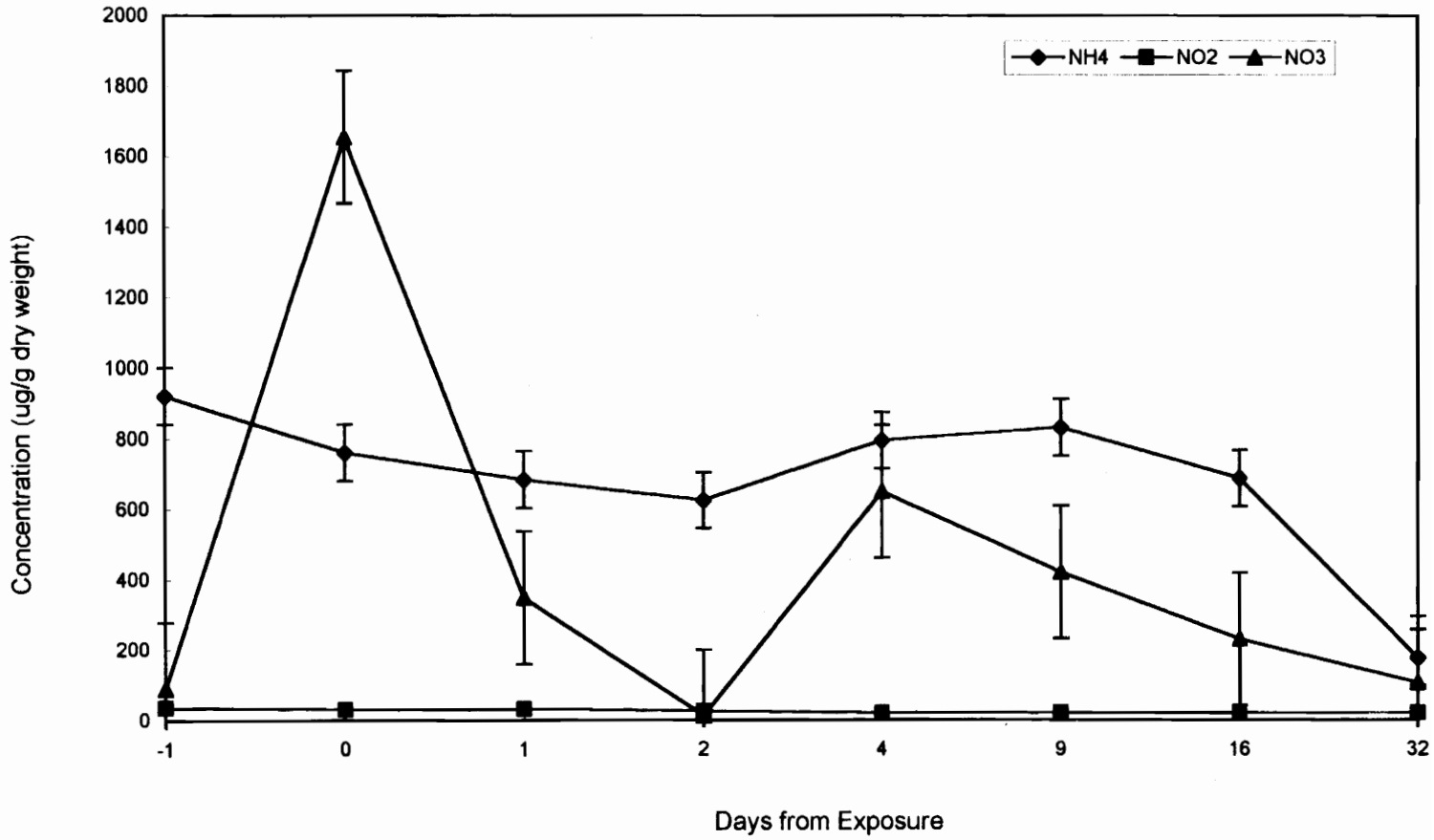


Figure 4. Sediment levels of ammonia, nitrite, and nitrate in acid treated microcosms.

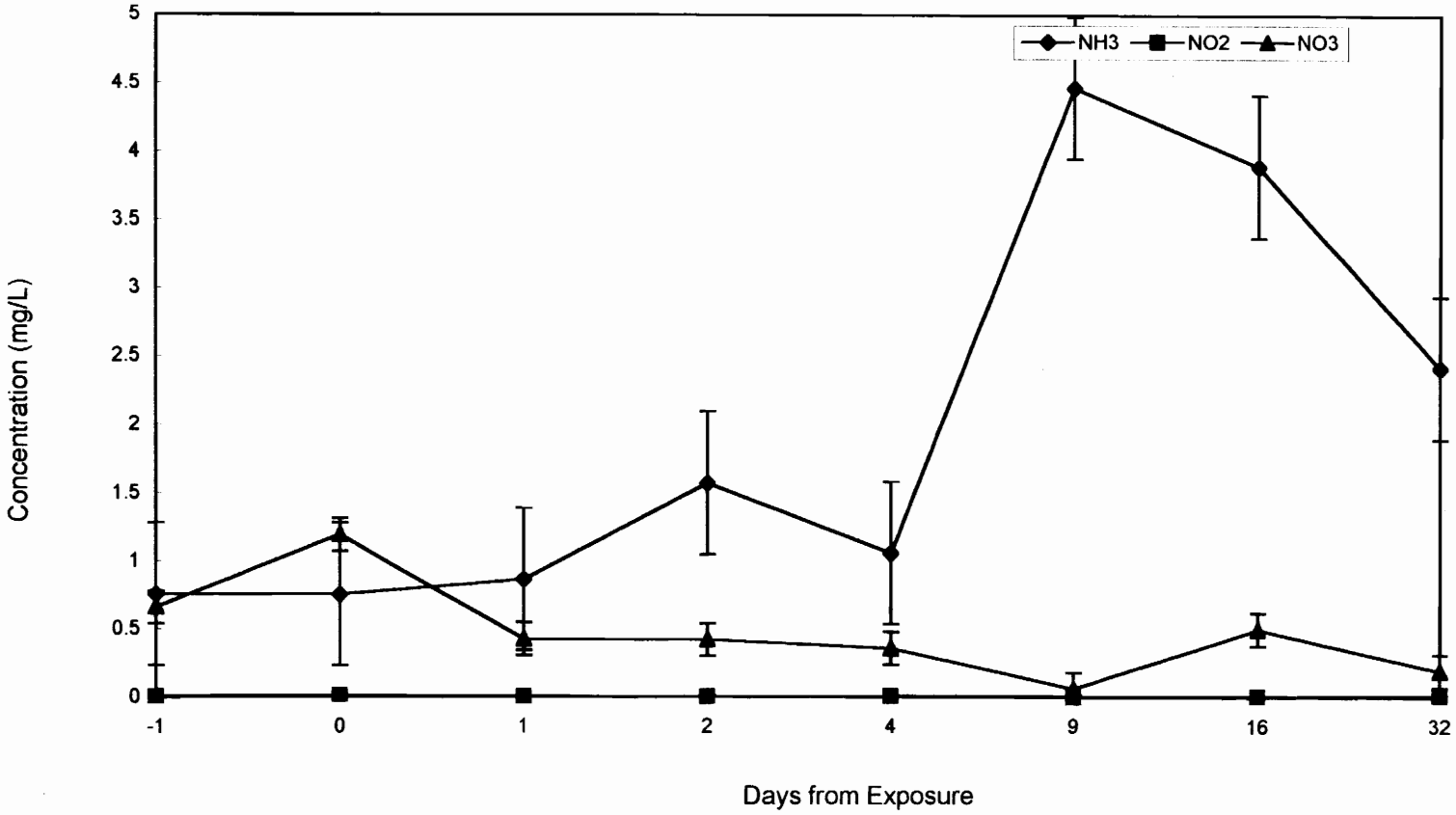


Figure 5. Overflow levels of ammonia, nitrite, and nitrate from acid treated microcosms.

1.5 mg/L Atrazine Exposure

There was no change in the pH of overflow water or sediment as a result of atrazine exposure. For the duration of the study, overflow pH and sediment pH remained neutral. The conductivity and dissolved oxygen of overflow water did not change as a result of atrazine exposure. Atrazine was being carried through the microcosms. Atrazine present in overflow waters from the three atrazine treated microcosms was: 0.253 ppm, 0.152 ppm, and 0.125 ppm atrazine.

There were no apparent effects of atrazine exposure on sediment ammonia oxidizing bacteria, which fluctuated around 2.03×10^6 (bacteria per gram dry soil). Changes in overflow nitrogen chemistry are shown in Figure 6. Overflow ammonia levels gradually increased after Day 1. Overflow ammonia levels peaked at Day 9 and slowly decreased over the remaining time of the study ($p=0.0979$). However, overflow ammonia levels remained higher in atrazine treated microcosms than in control microcosms. Sediment ammonia levels were unchanged (see Figure 7).

The number of nitrite oxidizing bacteria were not affected by atrazine exposure. The average number of nitrite oxidizing bacteria present in the sediment was 2.14×10^5 (bacteria per gram dry soil). Sixteen days after atrazine exposure, there was a substantial increase in the

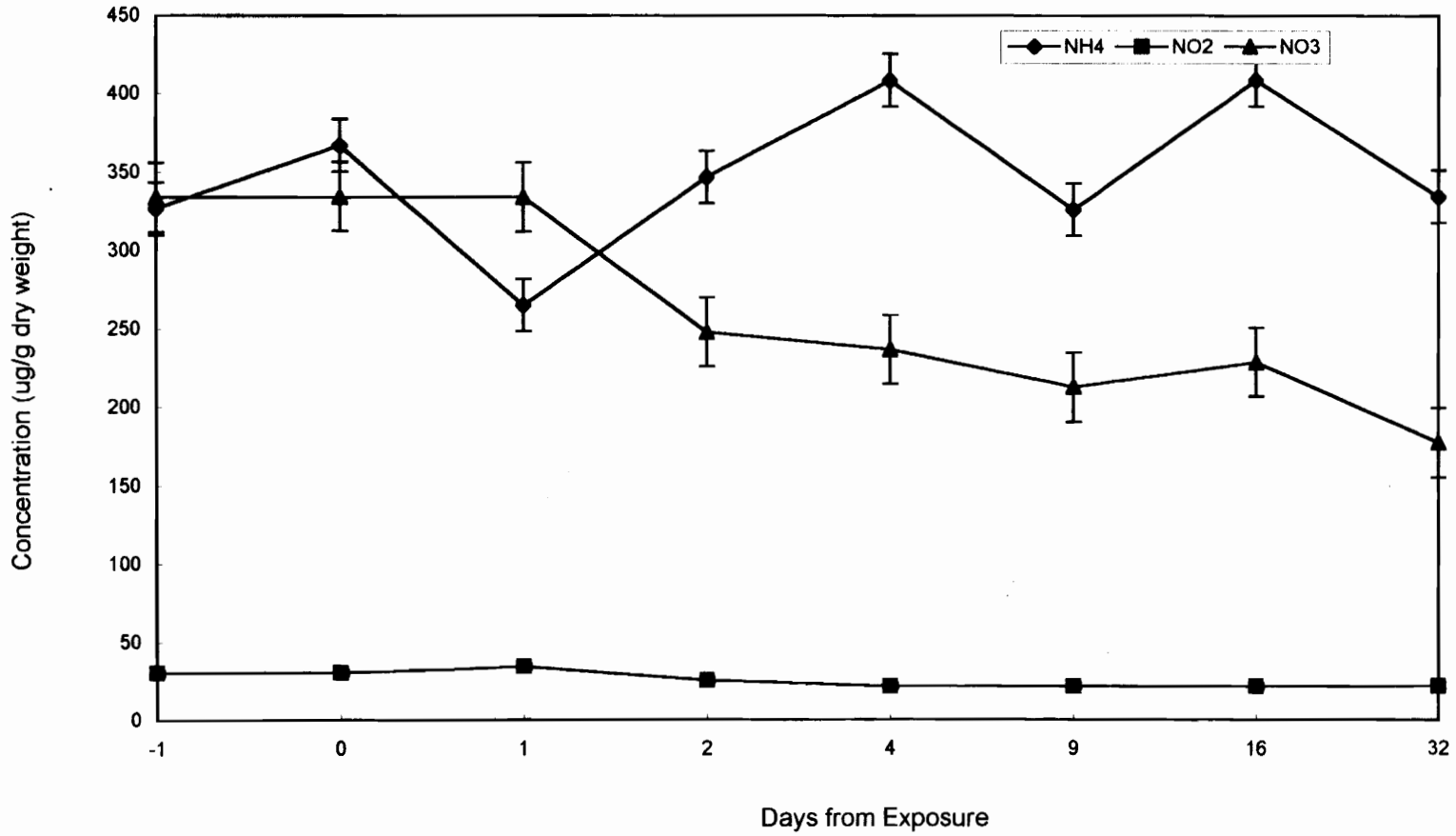


Figure 7. Sediment levels of ammonia, nitrite, and nitrate in atrazine (1.5 mg/L) treated microcosms.

amount of nitrite present in the overflow water ($p=0.0196$). Sediment nitrite levels were unchanged.

In atrazine exposed microcosms, denitrifying bacteria decreased from 1.0×10^6 (bacteria per gram dry soil) at Day 0 to 2.9×10^4 (bacteria per gram dry soil) on Day 16. After Day 16, the population increased to 9.3×10^5 (bacteria per gram dry soil). The concentration of nitrate in the overflow water did not change during the study. However, overflow nitrate levels remained suppressed after Day 4. Sediment nitrate decreased in atrazine treated microcosms ($p=0.0878$) (see Figure 7).

Control Microcosms (4.5 mg/L Atrazine Trial)

There was a great deal of fluctuation in sediment nitrogen and microbiology due to microzones in the sediment. The pH of both the sediment and the overflow water remained neutral. The conductivity and dissolved oxygen of overflow water slowly decreased throughout the study.

Ammonia oxidizing bacteria numbers widely fluctuated and decreased over the study period. Sediment ammonia levels, shown in Figure 8, steadily decreased until stabilizing on Day 12. Overflow water ammonia levels also fluctuated, yet, overall remained unchanged (see Figure 9).

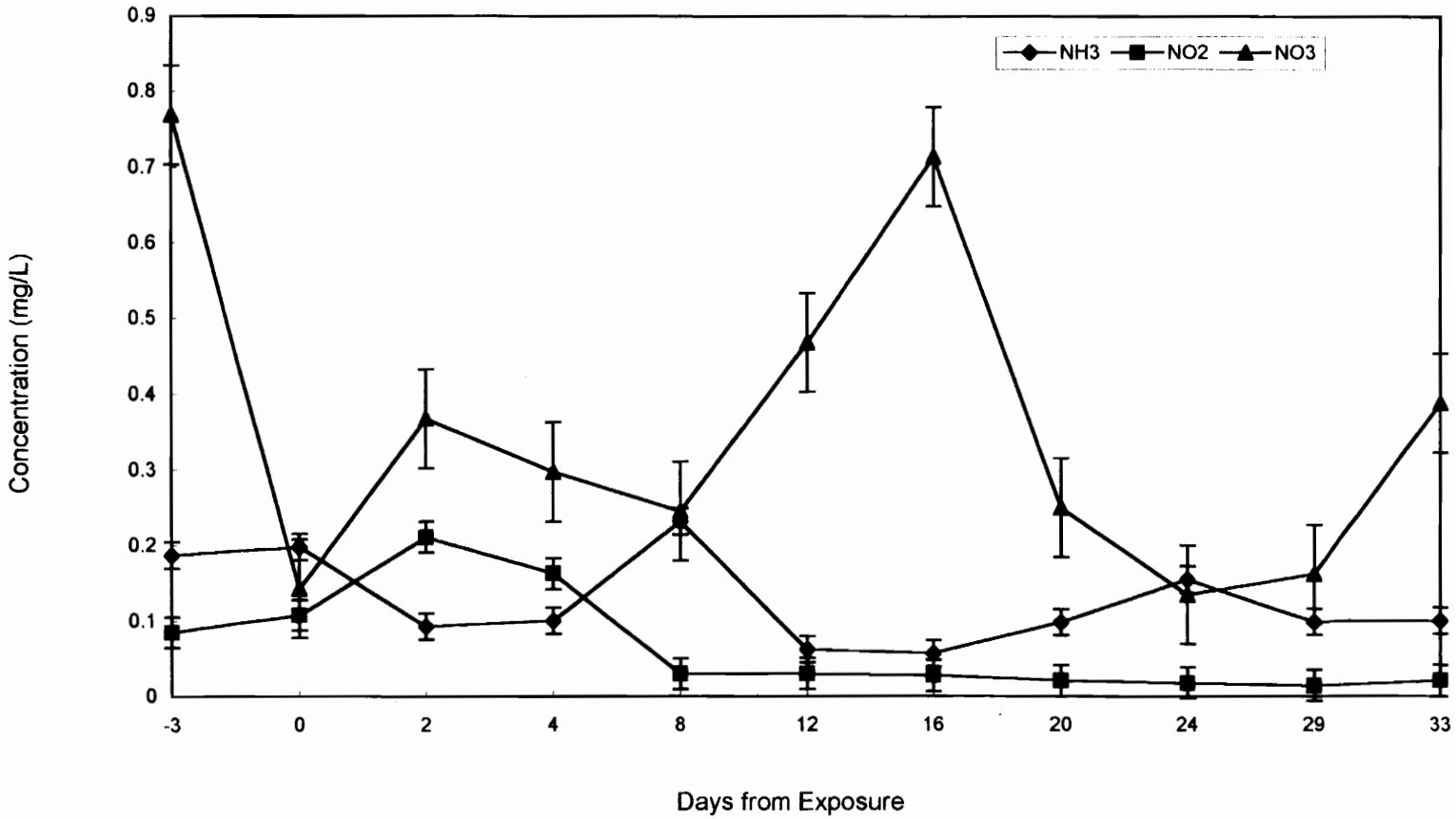


Figure 9. Overflow levels of ammonia, nitrite, and nitrate from control treated microcosms.

Nitrite oxidizing bacteria numbers fluctuated but did not change substantially. Sediment nitrite levels gradually increased over the study period. Nitrite present in overflow water was substantially higher than atrazine treated systems until Day 8, when nitrite levels decreased and stabilized. Denitrifying bacteria numbers fluctuated widely, yet were unchanged overall. Sediment nitrate levels decreased. Overflow levels of nitrate remained higher than those found in atrazine treated microcosms throughout the study.

4.5 mg/L Atrazine Exposure

There was no change in the pH of overflow water or sediment as a result of 4.5 mg/L atrazine exposure. Conductivity of overflow water remained low throughout the study. Dissolved oxygen of overflow water did not change. Atrazine present in overflow waters from the three atrazine treated microcosms was: 0.242 ppm, 1.050 ppm, and 0.481 ppm atrazine.

Changes in sediment chemistry are shown in Figure 10. Sediment ammonia did not change as a result of atrazine exposure. Ammonia oxidizing bacteria were not affected by atrazine exposure. The average number of ammonia oxidizing bacteria present in the sediment of atrazine treated

microcosms was 8.26 x 10^4 (bacteria per gram dry soil). Overflow ammonia levels began increasing at Day 29 (p=0.0955)(see Figure 11).

Sediment nitrite levels did not change as a result of atrazine exposure. The average number of nitrite oxidizing bacteria was 6.71 x 10^4 (bacteria per gram dry soil) and was not affected by atrazine exposure. Overflow nitrite levels did not change as a result of atrazine exposure.

Sediment nitrate levels remained higher than those of the control systems due to atrazine exposure (p=0.0487). Denitrifying bacteria were not significantly affected by atrazine exposure. The average number of denitrifying bacteria was 2.12 x 10^5 (bacteria per gram dry soil). Overflow nitrate concentrations steadily decreased until Day 20, when concentrations increased substantially (p=0.0001).

DISCUSSION

Acid Exposure

Acidification can occur naturally due to nitrification and the production of nitric acid. Nitrification processes form nitric acid which acidifies the soil around the plant roots (Hutterman and Ulrich 1984). Acidification also may reduce the solubility of organic matter, impeding microbial decomposition (Killham, 1994; Kinsman 1984). However, acidification does not naturally occur at the levels simulated in this study.

pH and Conductivity. Sediment pH was not affected by daily acidification efforts until the close of the study. This is due to the buffering capacity of the sediment. Drastic changes in soil pH are prevented by the release of buffering calcium ions (Hutterman and Ulrich 1984). Any decrease in pH results in calcium being released from the sediment into the overlying water, causing an increase in conductivity (Johnson 1986). Also, sulfuric acid may be neutralized by adsorption onto oxides of iron and aluminum. This results in the release of hydroxide ions (OH^-) from the soils. These mechanisms buffer increasing acidity of soil by neutralizing hydrogen (H^+) ions. Excess sulfur may also be immobilized by incorporation into organic matter (Johnson and

Reuss 1984).

Ammonia. Sediment ammonia levels decreased in acid treated systems. Acidification often causes a decrease in the solubility of organic matter, slowing deamination and dependent microbial processes (Killam 1994), thus affecting nutrient cycling (Abrahamsen 1984). This was evident in a decrease in the number of ammonia oxidizing bacteria.

The uptake of ions by roots acidifies the rhizosphere, one of the most important areas of ammonia oxidation. The reduction or inhibition of the ammonia oxidizing bacteria in the sediment was most likely an indirect effect of acidification via increased acidity of the rhizosphere or leaching of ammonia due to the high amounts of protons added to the sediment.

Overflow ammonia levels decreased in acid treated systems corresponding with decreased sediment ammonia and ammonia oxidizing bacteria. Taken together, these results support the conclusion that ammonia dependent microbial processes were suppressed as a result of acidification.

Nitrite. Sediment nitrite levels did not significantly change during the study nor did the number of nitrite oxidizing bacteria. Nitrite rarely accumulates in sediment. It is known that the accumulation of nitrite in sediments can lead to the formation of nitrosamines which are both carcinogenic and phytotoxic (Killham 1994).

Nitrite oxidizing bacteria numbers decreased. It is now known that nitrifying bacteria may withstand lower pH values than previously thought, due to their association with soil colloids (Killham 1994). This decrease in numbers is most likely a result of the disappearance of ammonia from the sediment and the subsequent decline in ammonia oxidizing bacteria. However, overflow nitrite levels increased. Although nitrite is a product of ammonia oxidation, in this case, it is a result of increased denitrification processes.

Nitrate. Sediment nitrate levels decreased as a result of acidification. Acidification is typically accompanied by the leaching of nutrients, particularly nitrate (Hutterman and Ulrich 1984). Acidification studies of forest ecosystems have found that nitrate production and utilization can respectively decrease and increase the soil pH (Hutterman and Ulrich 1984).

Nitrate is not usually adsorbed to soil particles making it readily available to denitrifying organisms in the sediment. That nitrate which is not utilized is easily leached from the sediment (Killham 1994). However, nitrate was not leaching from the sediments; overflow levels of nitrate decreased. This would indicate that nitrate production was inhibited or nitrate use was accelerated.

Although the ammonia and nitrite oxidizing bacteria decreased, denitrifying bacteria increased. Sediment ammonia

and nitrite levels decreased, showing that ammonia was being leached from the sediment. These results combined show a decrease in ammonia dependent microbial processes. The denitrifying bacteria, however, are not restricted to the use of nitrate as an electron acceptor.

Denitrifying bacteria may use other electron acceptors. Typically denitrification is by dissimilatory nitrate reduction producing nitrogen gases. A shift in soil chemistry may cause a shift towards fermentative bacterial groups. This would cause a change in the denitrification process to dissimilatory nitrate reduction producing ammonia (Killham 1994). Denitrification processes were actually stimulated by the acidification of the sediment.

The acidification of the sediment did provide a perturbation reference to the microcosm by affecting the nitrifying bacteria as was hypothesized. The denitrifying bacteria, however, seem to have been stimulated by the acidification of the sediment. It is obvious that the effects of sediment acidification may be evident before any change in sediment pH values.

Atrazine Exposure

There are two plausible conclusions to this study. First, atrazine exposure resulted in increased nitrate reduction processes. Secondly, atrazine exposure resulted in decreased nitrification processes. Both of these shifts result in increased ammonia and nitrite.

pH/Conductivity. There were no obvious changes in sediment pH, overflow pH, dissolved oxygen and conductivity as a result of exposure to 1.5 mg/L and 4.5 mg/L atrazine. These parameters are often indicators of ecosystem stress. In stressed ecosystems, when respiration exceeds productivity, there is a net increase in carbon dioxide concentration and a decrease in pH. This results in calcium being released from the sediment into the overlying water, causing an increase in conductivity (Johnson 1986). Pratt et al. (1988) found that oxygen production and the ability of microbial communities to sequester calcium and magnesium were decreased as a result of atrazine exposure. Changes such as these would have been evident in the conductivity of overflow water. Therefore, atrazine exposure, even at 4.5 mg/L, had little effect on the overall sediment chemistry.

Ammonia. There was no change in sediment ammonia levels as a result of exposure to 1.5 mg/L and 4.5 mg/L atrazine. The number of ammonia oxidizing bacteria present in the

sediment did not change due to atrazine exposure. Although nitrifying bacteria have been found to be sensitive to atrazine in slurries (at extremely high concentrations), in a natural setting, the sediment and plant roots provide additional protection from toxic compounds such as herbicides. Also, ammonia oxidizing bacteria first use extractable ammonia. After that supply is exhausted, the bacteria may then use strongly adsorbed, fixed ammonia (Lal and Lal 1988).

Overflow ammonia levels changed as a result of exposure to 1.5 mg/L atrazine. There was an initial increase then a steady decline after Day 9. However, overflow ammonia levels were constantly higher than those of the control groups. Exposure to 4.5 mg/L atrazine caused overflow ammonia levels to increase 29 days after atrazine exposure. Excess ammonia present in the overflow water may be due to increased ammonia production via dissimilatory nitrate reduction, decreased nitrification by the ammonia oxidizing bacteria, or a decrease in plant uptake of ammonia. Excess ammonia would have stimulated the ammonia oxidizing bacteria in the sediment, causing an increase in sediment nitrite levels. The number of ammonia oxidizing bacteria did not increase. Ammonia oxidizing bacteria may remain dormant for periods of time. Again, this would have resulted in an increase in sediment ammonia and nitrite. These parameters did not

change, therefore, this increase in overflow ammonia is likely a result of a shift in predominant bacterial processes, specifically towards denitrification and/or nitrate reduction.

Nitrite. There was no change in sediment nitrite levels as a result of 1.5 mg/L or 4.5 mg/L atrazine exposure. Accumulation of nitrite in the sediments is often a symptom of inhibition of nitrification by pesticides (Camper 1991). However, nitrite accumulation is rare. The number of nitrite oxidizing bacteria were not affected by atrazine exposure.

Atrazine exposure (1.5 mg/L and 4.5 mg/L) caused overflow nitrite levels to increase. Excess nitrite may be due to increased production by nitrification or denitrification or decreased nitrite oxidation. Inhibition of nitrite oxidizers is evidenced by an immediate increase in sediment nitrite levels. Sediment nitrite levels, however, were unaffected. Enumeration of the nitrite oxidizing bacteria does not show any inhibition due to atrazine. Inhibition effects on nitrite oxidation are often completely reversible (Lal and Lal 1988), so any temporary sensitivity may not be evident by enumeration.

Autotrophic nitrifying bacteria are typically associated with the plant root zone. The exchange of nutrients and uptake of chemicals by the roots protects the microbial population associated with the root zone. Increases in

overflow nitrite levels may be a result of processes not directly associated with the root zone, such as denitrification or nitrate reduction. The increases in overflow nitrite and ammonia are likely a result of increased denitrification or nitrate reduction processes in the sediment. The contribution of nitrification by fungi is unclear, since only the nitrifying bacteria were considered in this study.

Nitrate. Sediment nitrate levels are suppressed immediately following 1.5 mg/L atrazine exposure. Although there is no discernible decrease in nitrate concentration, this is conspicuous due to its consistency, or lack of fluctuations. This would suggest that sediment nitrate was steadily used by plants or microbes. There was an increase in the denitrifying or nitrate reducing bacteria in 1.5 mg/L and 4.5 mg/L atrazine treated microcosms following atrazine exposure.

At a higher concentration of atrazine (4.5 mg/L), sediment nitrate levels increased. This excess nitrate began accumulating almost immediately after atrazine exposure. It is unlikely that it was a result of an increase in nitrite oxidizing bacteria, and the number of nitrite oxidizers did not increase. The pooling of nitrate in the sediment was likely a more immediate impact on the plant community. Plants use nitrate more than ammonia because of the high

demand for ammonia in the sediment. Changes in sediment chemistry show that ammonia was more accessible for plant use and was likely being used as a nitrogen source. It is known that the presence of ammonia in the sediment suppresses the uptake of nitrate by plants (Bengtsson and Annadotter 1989). This made nitrate more available to the microbial population, specifically denitrifiers.

Nitrate Reduction vs. Nitrification

Accelerated nitrate reduction

Exposure to atrazine resulted in increased levels of ammonia and nitrite in overflow waters and a decrease in sediment nitrate and/or overflow nitrate levels.

Nitrate reducing bacteria utilize nitrate to produce ammonia and nitrite. These products would either be used in the sediment or would appear in the overflow water from the wetland. Reddy et al. (1989) reports that ammonia produced in the sediment would either diffuse into the root zone or the overlying water. If ammonia is produced in the rhizosphere, it is immediately used by the plant or other microorganisms in this region. So it is unlikely that ammonia present near the roots would reach overlying water. Both ammonia and nitrite concentrations in overflow water from atrazine

treated microcosms increased as a result of atrazine exposure.

Increases in ammonia and nitrite in overflow waters may be due to accelerated nitrate reduction in sediment not associated with the rhizosphere. Nitrate reducing bacteria are more prevalent in sediments than are true denitrifying bacteria (Bengtsson and Annadotter 1989). Also enumeration of denitrifying or nitrate reducing bacteria does not reflect actual rates of nitrate use. These bacteria preferentially use oxygen and only reduce nitrate under anaerobic conditions. So at any one time, it is difficult to determine how many "denitrifying" bacteria are actually reducing nitrate.

Very little inhibits the nitrate reducing bacteria because of their ability to utilize various substrates and their facultative aerobic metabolism. Because of their diversity and metabolism, they are not restricted in their association with plant roots and may exist in other areas of the sediment. This leaves them more susceptible to chemical influence. Exposure to atrazine accelerated the rate of nitrate reduction resulting in increased levels of ammonia and nitrite in overflow waters.

Inhibition of nitrification

The numbers of nitrifying bacteria were not affected by atrazine exposure (at 1.5 mg/L or 4.5 mg/L). These bacteria can remain dormant for periods of time, so it is difficult to determine nitrification processes based on enumeration. Ammonia and nitrite levels in the overflow water increased and sediment nitrate levels decreased. These changes could indicate the inhibition of nitrification processes. The characteristic decrease in nitrification processes is shown by an immediate increase in ammonia and nitrite (Keeney). Changes brought about by atrazine exposure did not appear for several weeks. Keeney also reports that nitrification inhibition causes an increase in the use of ammonia for immobilization processes. This was not evident because ammonia levels present in the overflow water increased. Tandon (1972) explains that nitrification processes may be inhibited or stopped for a period of time. When these processes resume, there is a decrease in levels of ammonia and nitrite. Again, this was not evident because levels of ammonia and nitrite increased and showed no signs of decreasing 32 days after atrazine exposure.

As we learn more about nitrification processes, it becomes clear that they are not as sensitive as previously thought. It has been learned that Nitrosomonas, a common

ammonia oxidizer, is capable of reducing nitrite under conditions of oxygen stress (Reddy et al. 1989; Goreau et al. 1980). Roy and Knowles (1994) have found that nitrification processes are related to methane oxidation processes. Methanogenic bacteria are capable of oxidizing ammonia and nitrifying bacteria may oxidize methane. Also, other groups of bacteria are capable of nitrification.

Heterotrophic nitrification may be carried out by Pseudomonas, Arthrobacter, fungi, Aspergillus, and actinomycetes (Paul and Clark 1989). In soils, heterotrophic nitrification is minor compared to chemoautotrophic nitrification occurring in the rhizosphere. The majority of nitrogen in sediments is from mineralization of nitrogen (Bowden 1987). Nitrogen processes in sediments do not occur by the standard aerobic and anaerobic processes and are strongly associated with plant litter. Bowden (1987) explains that "microbes in the [plant] litter immobilize nitrogen under aerobic conditions and mineralize nitrogen under anaerobic conditions." Also, the characteristics of the sediment may determine much of the impact of atrazine. Lal and Lal (1988) summarized 27 different studies on the impact of atrazine on nitrification. Twelve of these studies reported "no effect" and eight studies reported "increased activity." So, in sediments, it is unlikely that atrazine would inhibit nitrification processes.

Changes noted in sediment chemistry and overflow water chemistry due to atrazine exposure were: 1) increases in ammonia and/or nitrite levels in overflow water and 2) decreases in sediment nitrate levels. These changes were likely a result of a shift toward denitrification or nitrate reduction processes. Camper (1991) reports that an inhibition of one species of the microbial community is typically compensated for by increased activity of another. In the case of atrazine exposure, denitrifying bacteria seem to dominate the cycling of nitrogen. This same shift toward denitrification was reported by Rocchio and Malanchuk (1986). In a 1986 study, Johnson concluded there was no microbial inhibition in the water column or the sediment. Johnson (1986) also found that there was no change in total nitrogen in the atrazine treated microcosms. Changes in total nitrogen were attributed to translocation by the growing plants (Johnson 1986).

As hypothesized, there was a disruption in the nitrogen cycle resulting in increased levels of ammonia and nitrite present in wetland runoff. In summary, application of atrazine at 1.5 mg/L or 4.5 mg/L resulted in increased denitrification processes.

SUMMARY

Wetland microcosms were established from intact sediment core subsamples. Each microcosm contained cattail plants and recieved water, atrazine, or acid through simulated groundwater seepage. The conclusions of this study are as follows:

1) The number of nitrifying bacteria (ammonia oxidizing and nitrite oxidizing bacteria) were not affected by atrazine (at 1.5 and 4.5 mg/L atrazine) in wetland microcosms.

2) The number of denitrifying bacteria were not affected by atrazine. However, denitrification processes were stimulated by the application of atrazine, as evidenced by an increase in nitrite and ammonia in overflow water.

3) There was a disruption of the nitrogen cycle due to the addition of atrazine to the wetland microcosms. Prior to atrazine exposure, there was a balance in the nitrogen cycle, shown by the absence of nitrite in the sediment and overflow water. After exposure to atrazine, nitrate reduction became the dominant microbial process in the wetland system. This shift toward denitrification processes caused increased levels of nitrite and/or ammonia in the overflow

waters.

4) High amounts of atrazine present in wetlands may cause an increase in denitrification processes. This increase results in increased losses of nitrogen from the wetland ecosystem. The further limitation of nitrogen may cause shifts in plant species toward nitrogen fixing plants. Excess nitrogen in wetland runoff may cause algal blooms in recieving water systems and may accumulate in drinking water, posing health risks.

LITERATURE CITED

Abrahamsen, G. (1984). Effects of Acidic Deposition on Forest Soil and Vegetation. *In* J. Beament, A. D. Bradshaw, P. F. Chester, M. W. Holdgate, M. Sugden, and B. A. Thrush (Eds.) Ecological Effects of Deposited Sulphur and Nitrogen Compounds (pp. 111-124). London: The Royal Society.

Abtew, W., Chimney, M. J., Kosier, T., Guardo, M., Newman, S., and J. Obeysekera. (1995). The Everglades Nutrient Removal Project: A Constructed Wetland Designed to Treat Agricultural Runoff/Drainage. *In* K. E. Campbell (Ed.) Versatility of Wetlands in the Agricultural Landscape (pp. 45-56). Tampa, Florida: American Society of Agricultural Engineers.

American Public Health Association (APHA). (1985). Standard Methods for the Examination of Water and Wastewater (16th ed.) (pp. 394-396). Washington D. C.: APHA.

Bakalivanov, D. 1972. Biological Activity of Certain Herbicides on Microscopic Soil Fungi. Symposium of Biology Hungary 11: 373-377.

Baker, L. A. (1993). Introduction to nonpoint source pollution and wetland mitigation. *In* R. K. Olson (Ed.) Created and Natural Wetlands for Controlling Nonpoint Source Pollution (pp. 7-28). Washington D. C.: USEPA.

Bengtsson, G., and H. Annadotter. (1989). Nitrate Reduction in a Groundwater Microcosm Determined by ^{15}N Gas Chromatography-Mass Spectrometry. Applied and Environmental Microbiology 55(11): 2861-2870.

Bock, E., Koops, H., and H. Harms. (1986). Cell Biology of Nitrifying Bacteria. *In* J. I. Prosser (Ed.) Nitrification. Oxford: IRL Press.

Bowden, W. B. (1987). The Biogeochemistry of Nitrogen in Freshwater Wetlands. Biogeochemistry 4: 313-348.

Camper, N. D. (1991). Effects of Pesticide Degradation Products on Soil Microflora. *In* L. Somasundaram and J. R. Coats (Eds.) Pesticide Transformation Products: Fate and Significance in the Environment (pp. 205-216). Washington D. C.: American Chemical Society.

Chescheir, G. M., Amatya, D. M., and R. W. Skaggs. (1995). Monitoring the Water Balance of a Natural Forested Wetland. *In* K. E. Campbell (Ed.) Versatility of Wetlands in the Agricultural Landscape (pp. 451-462). Tampa, Florida: American Society of Agricultural Engineers.

Clark, J. R., M. A. Lewis, and A. S. Pait. 1993. Pesticide inputs and risks in coastal wetlands. Environmental Toxicology and Chemistry 12: 2225-2233.

Corbitt, R. A., and P. T. Bowen. (1994). Constructed Wetlands for Wastewater Treatment. *In* D. M. Kent (Ed.) Applied Wetlands Science and Technology (pp. 221-241). Boca Raton: Lewis Publishers.

Dennison, M. S., and J. F. Berry. (1993) Wetlands: Guide to Science, Law, and Technology. Park Ridge, New Jersey: Noyes Publications.

Dixon, K. R., and J. D. Florian, Jr. 1993. Modeling Mobility and Effects of Contaminants in Wetlands. Environmental Toxicology and Chemistry 12: 2281-2292.

Fenchel, T., and T. H. Blackburn. (1979). Bacteria and Mineral Cycling. London: Academic Press.

Frankforter, J. D. (1995). Association Between Local Land Use and Herbicide Concentrations in Wetlands of the Platte River Basin, Nebraska. *In* K. E. Campbell (Ed.) Versatility of Wetlands in the Agricultural Landscape (pp. 539-547). American Society of Agricultural Engineers: Tampa, Florida.

Geller, A. 1980. Studies on the Degradation of Atrazine by Bacterial Communities Enriched From Various Biotopes. Archives of Environemntal Contamination and Toxicology 9: 289-305.

Goreau, T. J., Kaplan, W. A., Wofsy, S. C., McElroy, M. B., Valois, F. W., and S. W. Watson. (1980). Production of NO_2^- and N_2O by Nitrifying Bacteria at Reduced Concentrations of Oxygen. Applied and Environmental Microbiology 40(3): 526-532.

Gu, J. D., Berry, D. F., Taraban, R. H., Martens, D. C., Walker, H. L., Jr., and W. J. Edmonds. (1992). Biodegradability of Atrazine, Cyanizine, and Dicamba in Wetland Soils. Blacksburg, Virginia: Virginia Water Resources Research Center, Virginia Polytechnic Institute and State University.

Hamala, J. A., and H. P. Kolliq. 1985. The Effects of Atrazine on Periphyton Communities in Controlled Laboratory Ecosystems. Chemosphere 14 (9): 1391-1408.

Hamilton, P. B., Jackson, G. S., Kaushik, N. K., Solomon, K. R., and G. L. Stephenson. 1988. The Impact of Two Applications of Atrazine on the Plankton Communities of In Situ Enclosures. Aquatic Toxicology 13: 123-1140.

Hammer, D. A. 1993. Designing Constructed Wetlands Systems to Treat Agricultural Nonpoint Source Pollution. *In* R. K. Olson (Ed.) Created and Natural Wetlands for Controlling Nonpoint Source Pollution (pp. 71-102). Washington D. C.: USEPA.

Herbert, R. A., and D. B. Nedwell. (1990). Role of Environmental Factors in Regulating Nitrate Respiration in Intertidal Sediments. *In* Revsbech, N. P., & J. Sorensen (Eds.) Denitrification in soil and sediment (pp.77-90). New York: Plenum Press.

Hutterman, A., and B. Ulrich. (1984). Solid phase-Solution-root Interactions in Soils Subjected to Acid Deposition. *In* J. Beament, A. D. Bradshaw, P. F. Chester, M. W. Holdgate, M. Sugden, and B. A. Thrush (Eds.) The Ecological Effects of Deposited Sulphur and Nitrogen Compounds (pp. 95-105). London: The Royal Society.

Jacobson, J. S. (1984). Effects of Acidic Aerosol, Fog, Mist and Rain on Crops and Trees. *In* J. Beament, A. D. Bradshaw, P. F. Chester, M. W. Holdgate, M. Sugden, and B. A. Thrush (Eds.) The Ecological Effects of Deposited Sulphur and Nitrogen Compounds (pp. 69-78). London: The Royal Society.

Jenkins, M. C., & W. M. Kemp. 1984. The Coupling of Nitrification and Denitrification in Two Estuarine Sediments. Limnology and Oceanography 29: 609-619.

Johnson, B. T. 1986. Potential impact of selected agricultural chemical contaminants on a northern prairie wetland: a microcosm evaluation. Environmental Toxicology and Chemistry 5: 473-485.

Johnson, D. W., and J. O. Reuss. (1984). Soil-mediated Effects of Atmospherically Deposited Sulphur and Nitrogen. *In* J. Beament, A. D. Bradshaw, P. F. Chester, M. W. Holdgate, M. Sugden, and B. A. Thrush (Eds.) The Ecological Effects of Deposited Sulphur and Nitrogen Compounds (pp. 125-132). London: The Royal Society.

Jones, T. W., Kemp, W. M., Stevenson, J. C., and J. C. Means. 1982. Degradation of Atrazine in Estuarine Water/sediment Systems and Soils. Journal of Environmental Quality 11(4): 632-638.

Keeney, D. R. 1986. Inhibition of Nitrification in Soils. *In* J. I. Prosser (Ed.) Nitrification. Oxforde: IRL Press.

Keeney, D. R., and D. W. Nelson. (1982). Nitrogen--Inorganic Forms. *In* A. L. Page, R. H. Miller, and D. R. Keeney (Eds) Methods of Soil Analysis Part 2 (2nd Edition) (pp. 643-687). Madison, Wisconsin: American Society of Agronomy, Inc., Soil Science Society of America.

Killham, K. (1994). Soil Ecology. Cambridge: Cambridge University Press.

King, D., and D. B. Nedwell. 1987. The Adaptation of Nitrate-reducing Bacterial Communities in Estuarine Sediments in Response to Overlying Nitrate Load. FEMS Microbiology Ecology 45: 15-20.

Kinsman, D. J. (1984). Ecological Effects of Deposited S and N Compounds: Effects on Aquatic Biota. *In* J. Beament, A. D. Bradshaw, P. F. Chester, M. W. Holdgate, M. Sugden, and B. A. Thrush (Eds.) The Ecological Effects of Deposited Sulphur and Nitrogen Compounds (pp. 221-226). London: The Royal Society.

Lal, R., and S. Lal (Eds.) (1988). Pesticides and Nitrogen Cycle Volume I. CRC Press, Inc.

Lee, K. E., Huggins, D. G., and E. M. Thurman. (1995). Effects of Hydrophyte Community Structure on Atrazine and Alachlor Degradation in Wetlands. *In* K. E. Campbell's (Ed.) Versatility of Wetlands in the Agricultural Landscape (pp. 525-538). Tampa, Florida: American Society of Agricultural Engineers.

Mandelbaum, R. T., Wackett, L. P., and D. L. Allan. 1993. Rapid Hydrolysis of Atrazine to Hydroxyatrazine by Soil Bacteria. Environmental Science and Technology 27: 1943-1946.

Mercurio, S. D. 1995. Comparison of Field Analyses and Regulation of Atrazine Use: Key Factors Impacting Local Aquatic Systems Missed by Simulations. AAAS/EPA Environmental Science and Engineering Fellow.

Miller, H. G. (1984). Deposition-plant-soil Interactions. *In* J. Beament, A. D. Bradshaw, P. F. Chester, M. W. Holdgate, M. Sugden, and B. A. Thrush (Eds.) The Ecological Effects of Deposited Sulphur and Nitrogen Compounds (pp. 81-95). London: The Royal Society.

Mirgain, I., Green, G. A., and H. Monteil. 1993. Degradation of Atrazine in Laboratory Microcosms: Isolation and Identification of the Biodegrading Bacteria. Environmental Toxicology and Chemistry 12: 1627-1634.

Mudd, E., Entry, J., Lowrance, R., and H. Walker. (1995). The Influence of Management of Riparian Areas on Movement of [14C]-Atrazine Through Undisturbed Soil Cores. *In* K. E. Campbell (Ed.) Versatility of Wetlands in the Agricultural Landscape pp. 201-209. Tampa, Florida: American Society of Agricultural Engineers.

Oremland, R. S., C. Umberger, C. W. Culbertson, and R. L. Smith. 1984. Denitrification in San Francisco Bay intertidal sediments. Applied and Environmental Microbiology 47: 1106-1112.

Osmond, D. L., Line, D. E., Gannon, R. W., Gale, J. A., Spooner, J., Coffey, S. W., Bartenhagen, K. A., Walker, J. C., Foster, M. A., Robillard, P. D., and D. W. Lehning. (1995). WATERSHEDSS: The Integration and Use of Wetlands and Riparian Areas in a Decision Support System. *In* K. E. Campbell's (Ed.) Versatility of Wetlands in the Agricultural Landscape (pp. 127-138). Tampa, Florida: American Society of Agricultural Engineers.

Paul, E. A., and F. E. Clark. 1989. Soil Microbiology and Biochemistry. San Diego: Academic Press.

Pratt, J. R., Bowers, N. J., Neiderlehner, B. R., and J. Cairns, Jr. 1988. Effects of Atrazine on Freshwater Microbial Communities. Archives of Environmental Contamination and Toxicology 17: 449-457.

Reddy, K. R., W. H. Patrick, Jr., C. W. Lindau. 1989. Nitrification-denitrification at the plant-root sediment interface in wetlands. Limnology and Oceanography 34(6): 1004-1013.

Ribaudo, M. O., and A. Bouzaher. (1994). Atrazine: Environmental Characteristics and Economics of Management. Agricultural Economic Report No. 699. Resources and Technical Division, Economic Research Service, United States Department of Agriculture.

Rocchio, P. M., and J. L. Malanchuk. 1986. The Effects of Atrazine on Dissolved Oxygen and Nitrate Concentrations in Aquatic Systems. Environmental International 12: 597-601.

Roy, R., and R. Knowles. (1994). Effects of Methane Metabolism on Nitrification and Nitrous Oxide Production in Polluted Freshwater Sediment. Applied and Environmental Microbiology 60(9): 3307-3314.

Schmidt, E. L., and L. W. Belser. (1982). Nitrifying Bacteria. *In* Page, A. L., Miller, R. H., and D. R. Keeney (Eds.) Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties (pp. 1027-1042). Madison, Wisconsin: American Society of Agronomy, Soil Science Society of America.

Sorenson, J. 1982. Reduction of Ferric Iron in Anaerobic, Marine Sediment and Interaction with Reduction of Nitrate and Sulfate. Applied and Environmental Microbiology 43(2): 319-324.

Stay, F. S., Larsen, D. P., Katko, A., and C. M. Rohm. 1985. Effects of Atrazine on Community Level Responses in Taub Microcosms. *In* Special Technical Testing Publication 865. American Society for Testing and Materials.

Stay, F. S., Katko, A., Rohm, C. M., Fix, M. A., and D. P. Larsen. 1989. The Effects of Atrazine on Microcosms Developed from Four Natural Plankton Communities. Archives of Environmental Contamination and Toxicology 18: 866-875.

Soil Conservation Service. 1989. Soil Survey of Bedford County Virginia. United States Department of Agriculture, Soil Conservation Service.

Sun, G., Riekerk, H., and L. V. Korhnak. (1995). The Hydrology of Cypress Wetland/Pine Upland Ecosystems in Florida Flatwoods. *In* K. E. Campbell (Ed.) Versatility of Wetlands in the Agricultural Landscape (pp. 489-500). Tampa, Florida: American Society of Agricultural Engineers.

Tandon, S. P. (1972). Effect of Organic Substances on Nitrite Formation by Nitrosomonas. Syposium Biology Hungary 11: 283-288.

Tiedje, J. M. (1982). Denitrification. *In* Page, A. L., Miller, R. H., and D. R. Keeney (Eds.) Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties (pp. 1011-1026). Madison, Wisconsin: American Society of Agronomy, Soil Science Society of America.

van Dijk, H. (1987). Ammonification and Nitrification: Laboratory Versus Field. *In* L. Somerville and M. P. Greaves (Eds.) Pesticide Effects on Soil Microflora. London: Taylor and Francis.

Winkelmann, D. A., and S. J. Klaine. 1991a. Degradation and Bound Residue Formation of Atrazine in a Western Tennessee Soil. Environmental Toxicology and Chemistry 10: 335-345.

Winkelmann, D. A., and S. J. Klaine. 1991b. Degradation and Bound Residue Formation of Four Atrazine Metabolites, Deethylatrazine, Deisopropylatrazine, Dealkylatrazine and Hydroxyatrazine, in a Western Tennessee Soil. Environmental Toxicology and Chemistry 10: 347-354.

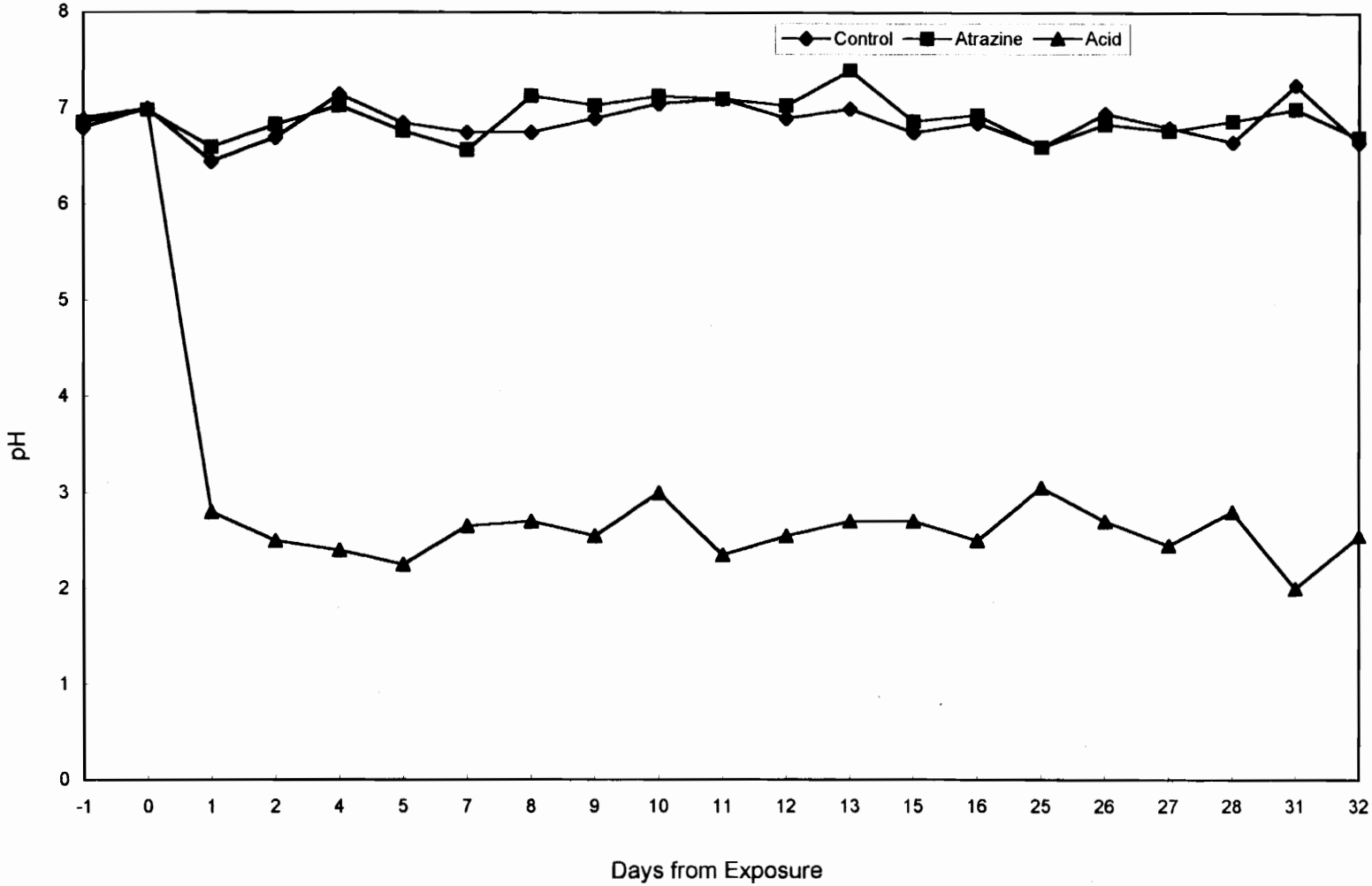
Wolf, D. C., and R. L. Jackson. August 1982. Atrazine Degradation, Sorption, and Bioconcentration in Water Systems: Project Completion Report. Technical Reports Department.

Wolf, D. C., and J. P. Martin. 1975. Microbial Decomposition of Ring-^{14}C Atrazine, Cyanuric Acid, and 2-chloro-4,6-diamino-s-triazine. Journal of Environmental Quality 4(1): 134-139.

USEPA. (1979). EPA Methods for Chemical Analysis of Water and Wastes (pp.354.1-1-354.1-2). Environmental Monitoring and Support Laboratory Office of Research and Development: Cincinnati, Ohio: USEPA.

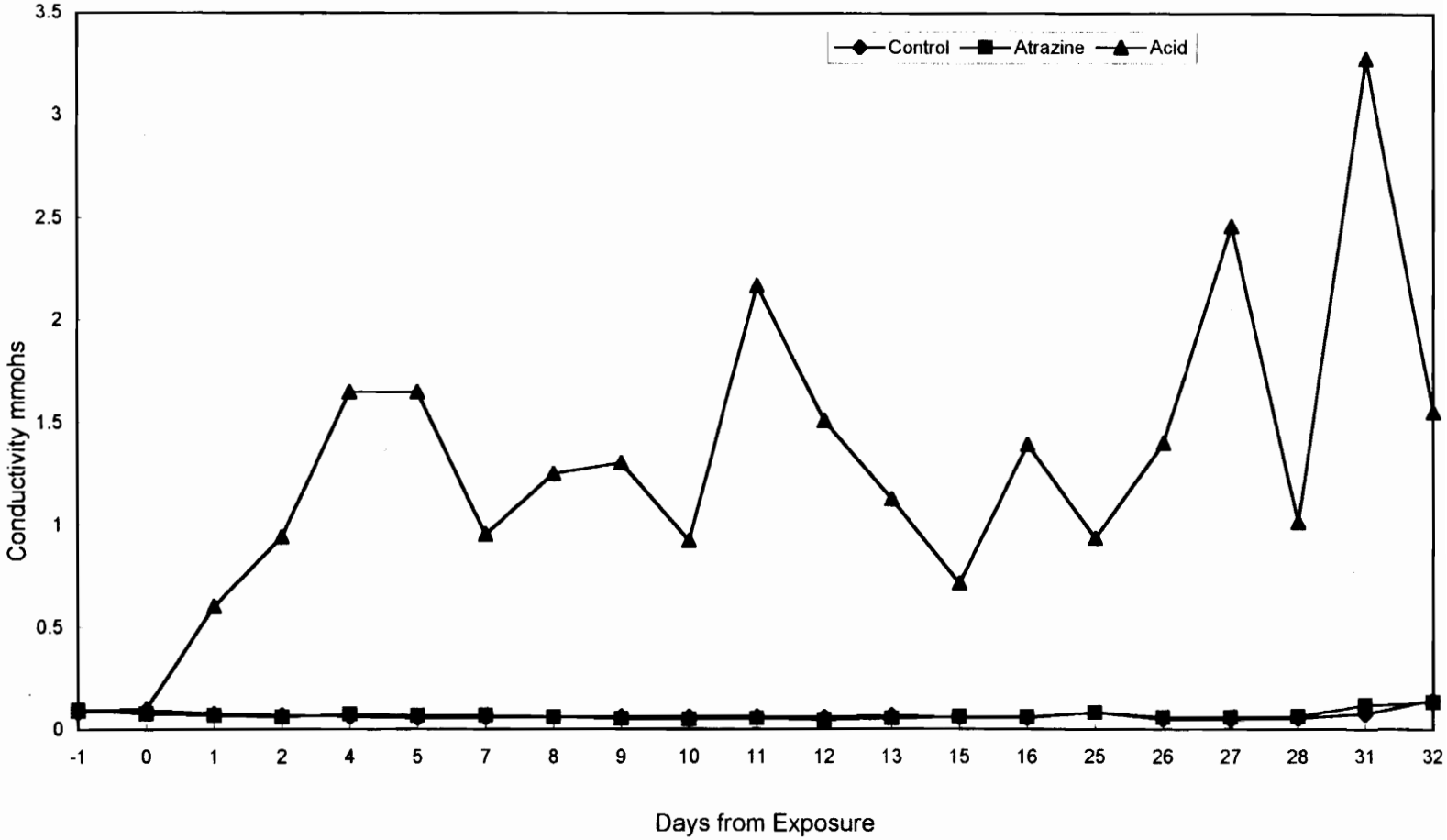
APPENDIX. Sediment and overflow water chemical and microbial parameters.

APPENDIX 1



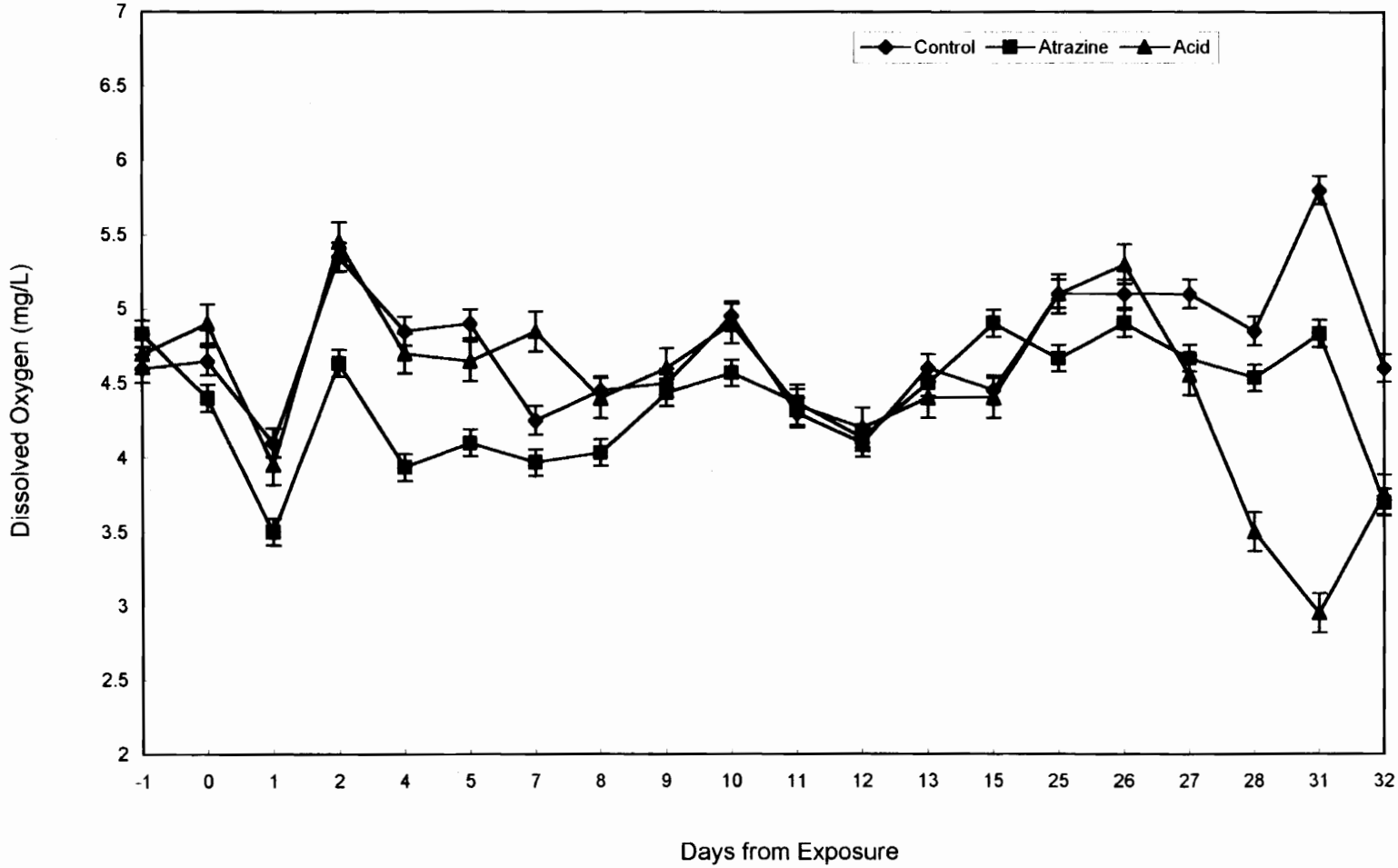
A-1. Overflow water pH from control, acid treated, and atrazine treated (1.5 mg/L) microcosms.

APPENDIX 2



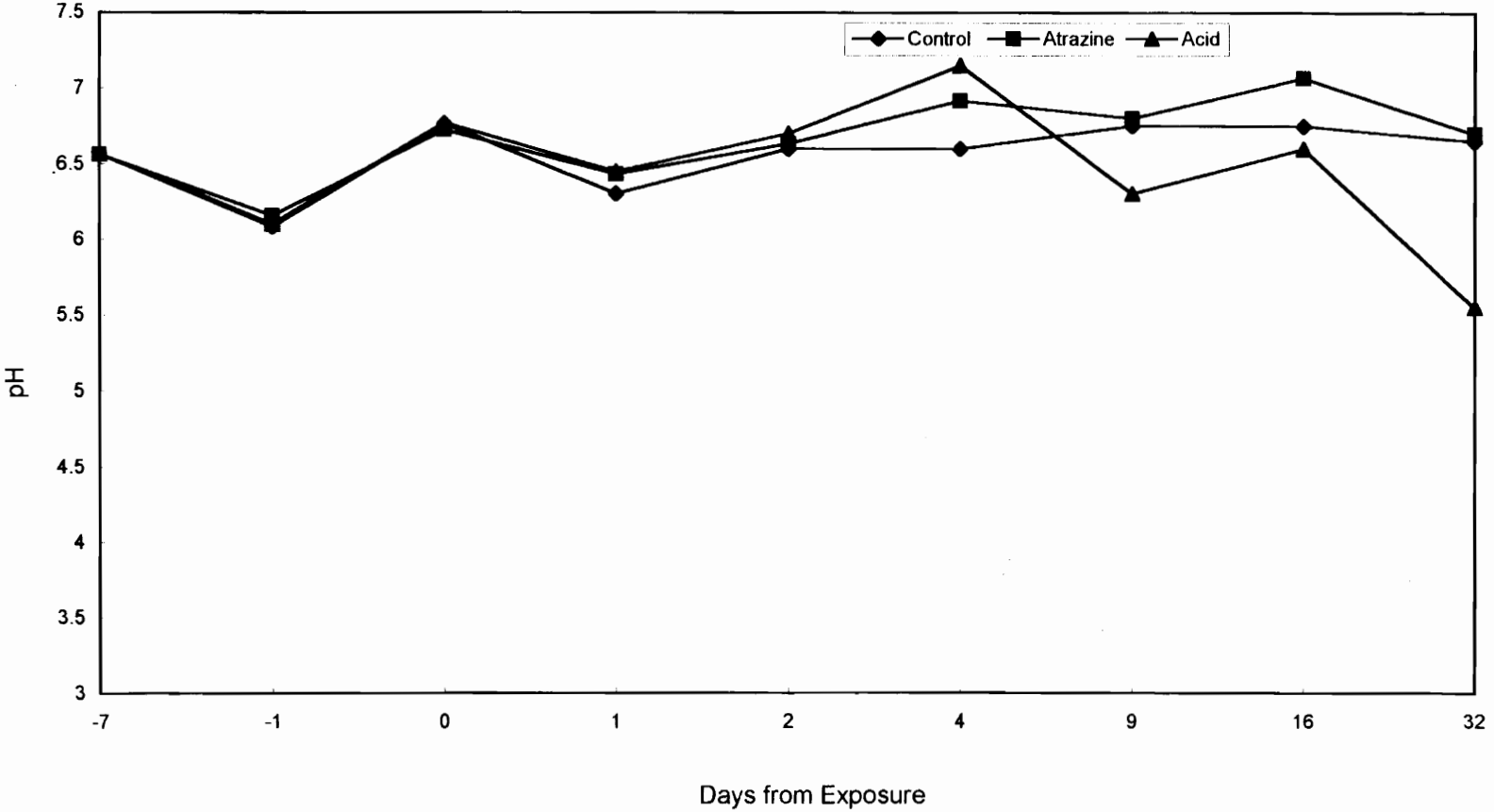
A-2. Conductivity of overflow water from control, acid treated, and atrazine treated (1.5 mg/L) microcosms.

APPENDIX 3



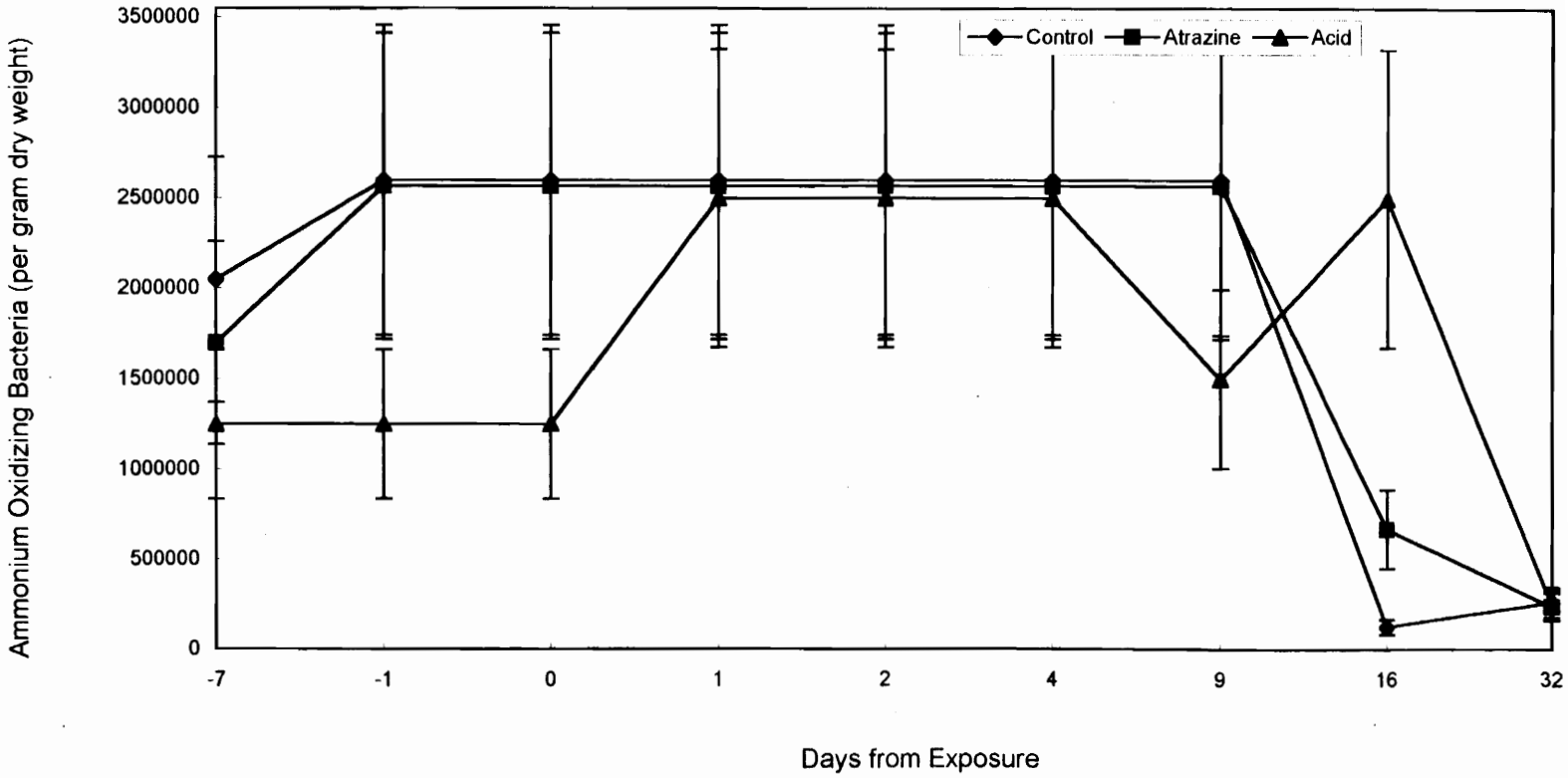
A-3. Overflow Dissolved Oxygen levels from control, atrazine (1.5 mg/L) treated, and acid treated microcosms.

APPENDIX 4



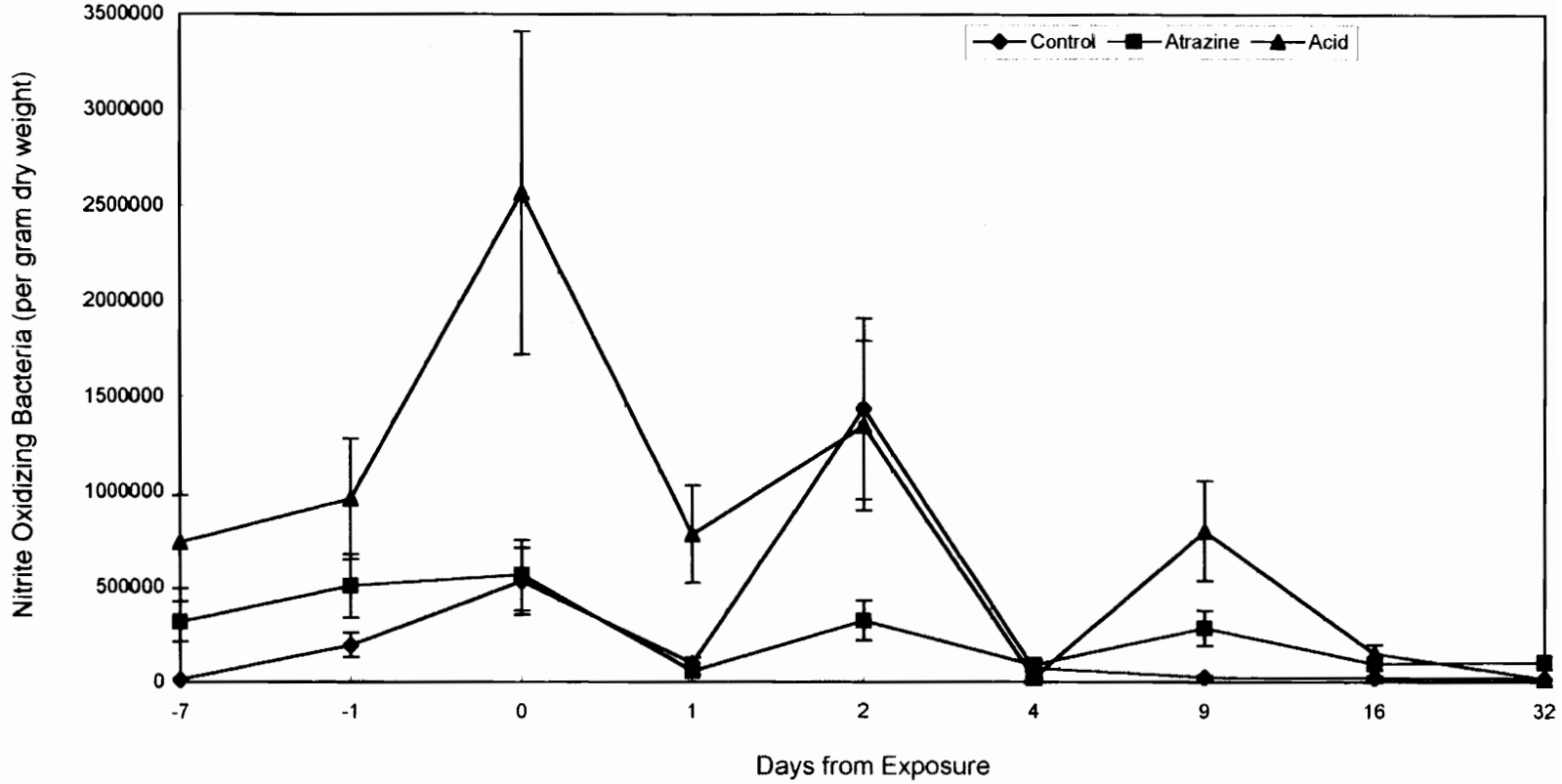
A-4. Sediment pH of control, acid treated, and atrazine treated (1.5 mg/l) microcosms.

APPENDIX 5



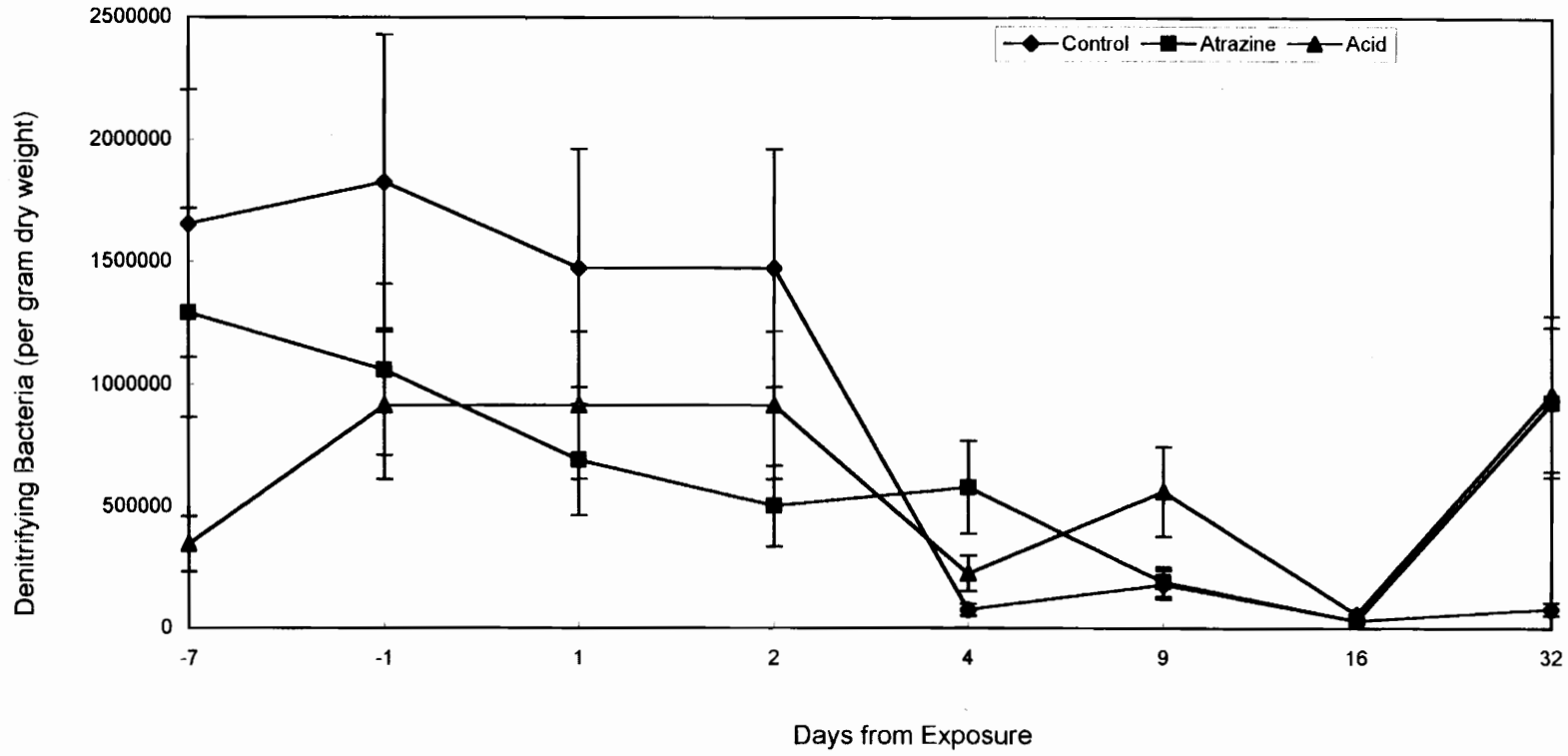
A-5. Ammonium oxidizing bacteria (per gram dry weight) present in control, acid treated, and atrazine treated (1.5 mg/L) microcosms.

APPENDIX 6



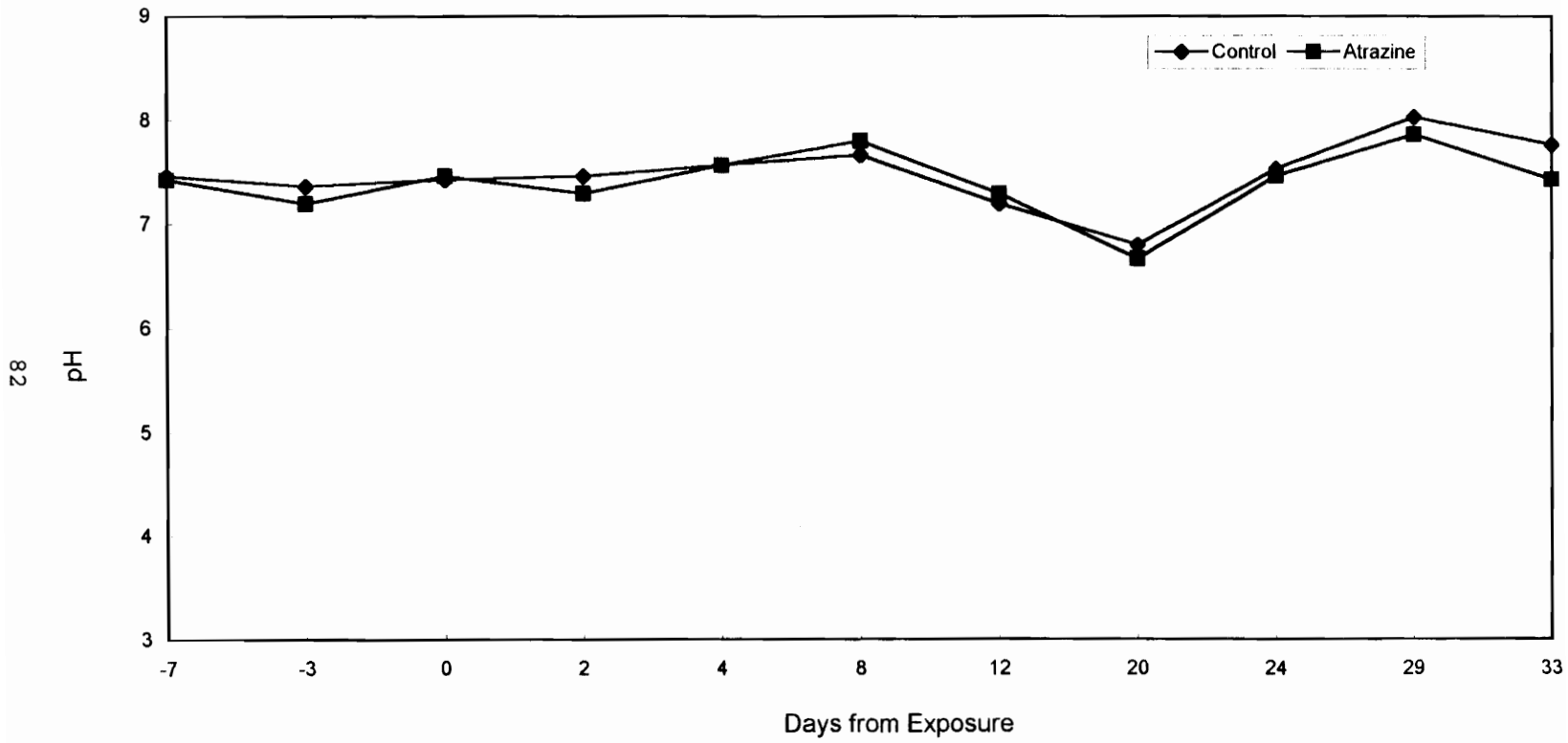
A-6. Nitrite oxidizing bacteria (per gram dry weight) present in control, acid treated, and atrazine treated (1.5 mg/L) microcosms.

APPENDIX 7



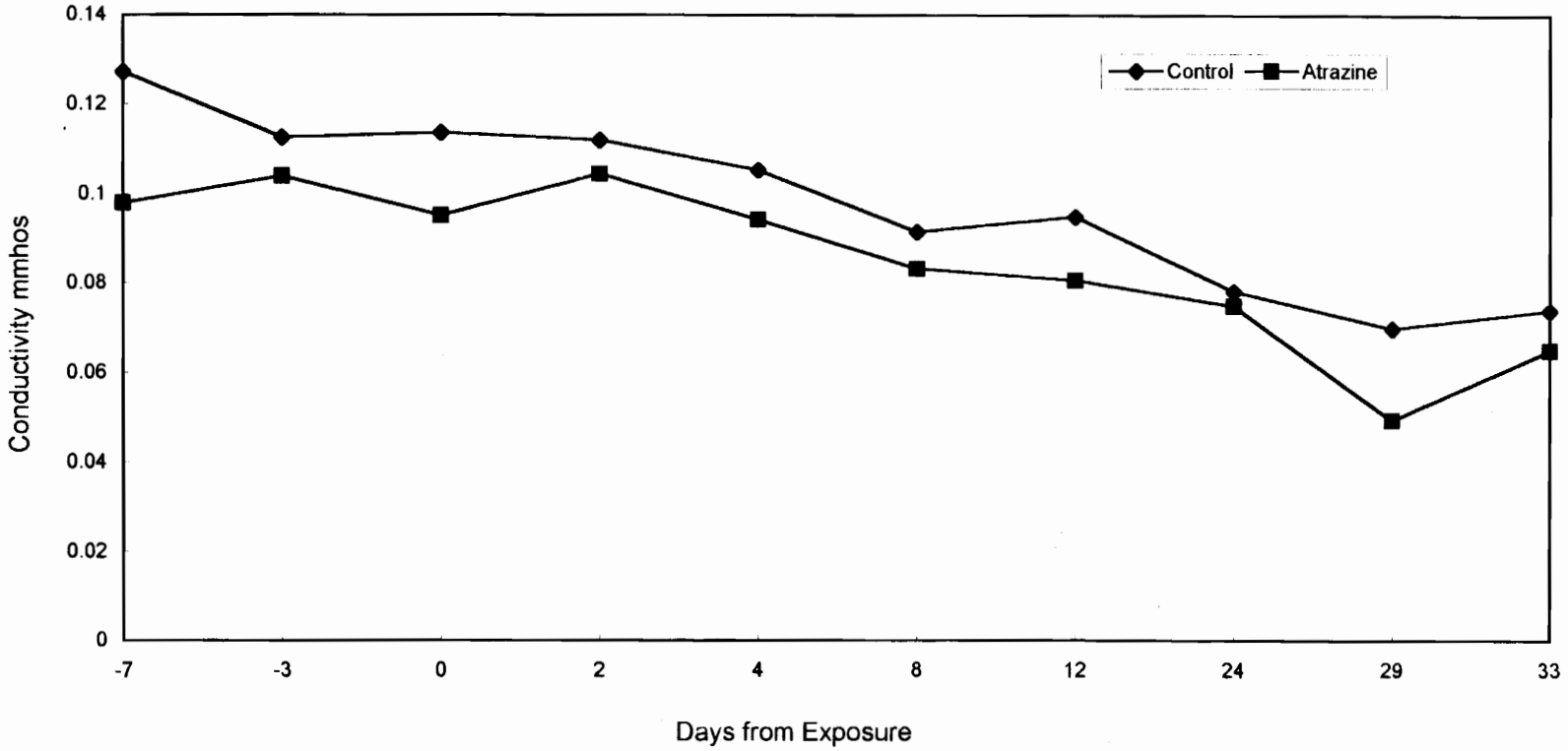
A-7. Denitrifying bacteria (per gram dry weight) present in control, acid treated, and atrazine treated (1.5 mg/l) microcosms.

APPENDIX 8



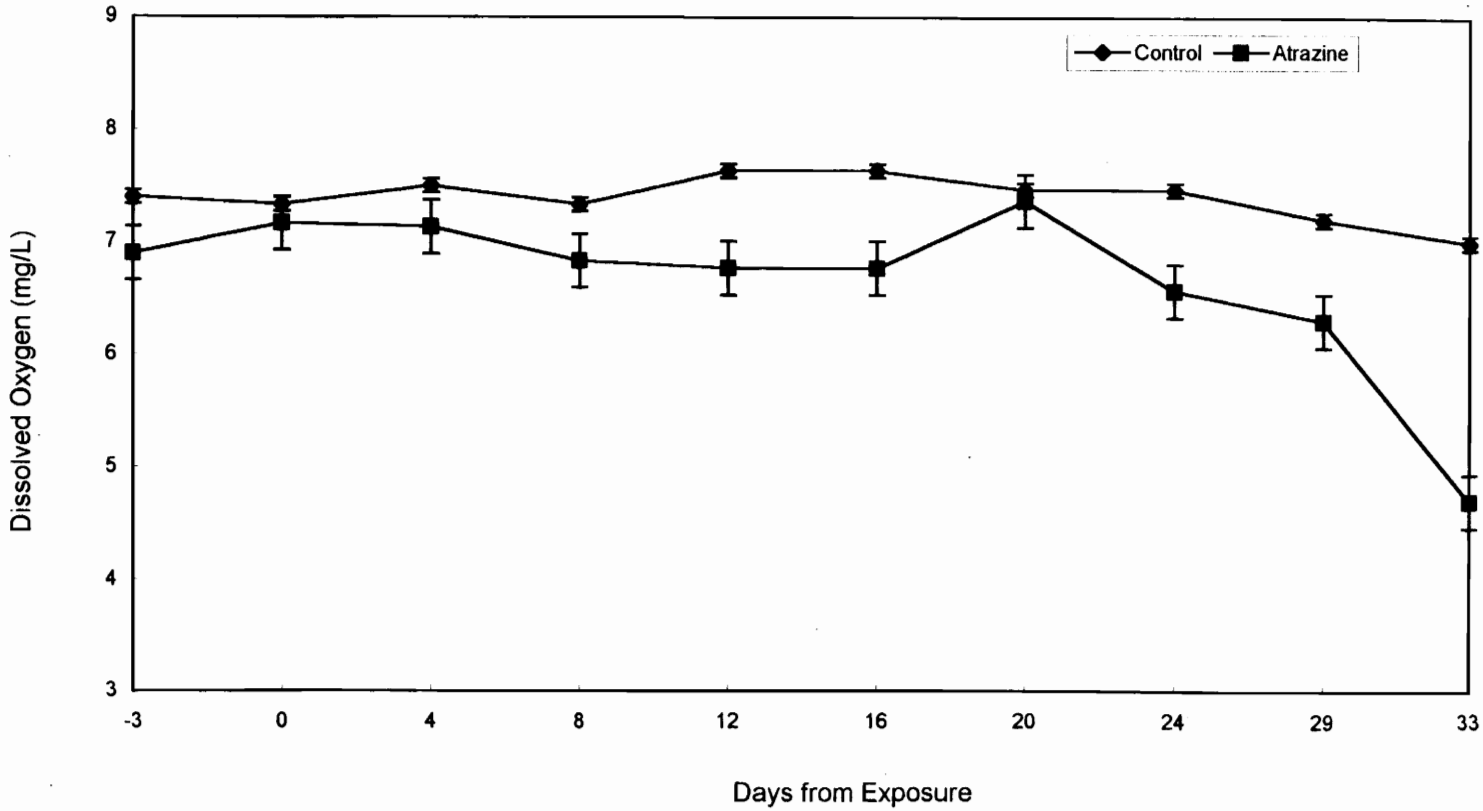
A-8. Overflow water pH of control and atrazine treated (4.5 mg/L) microcosms.

APPENDIX 9



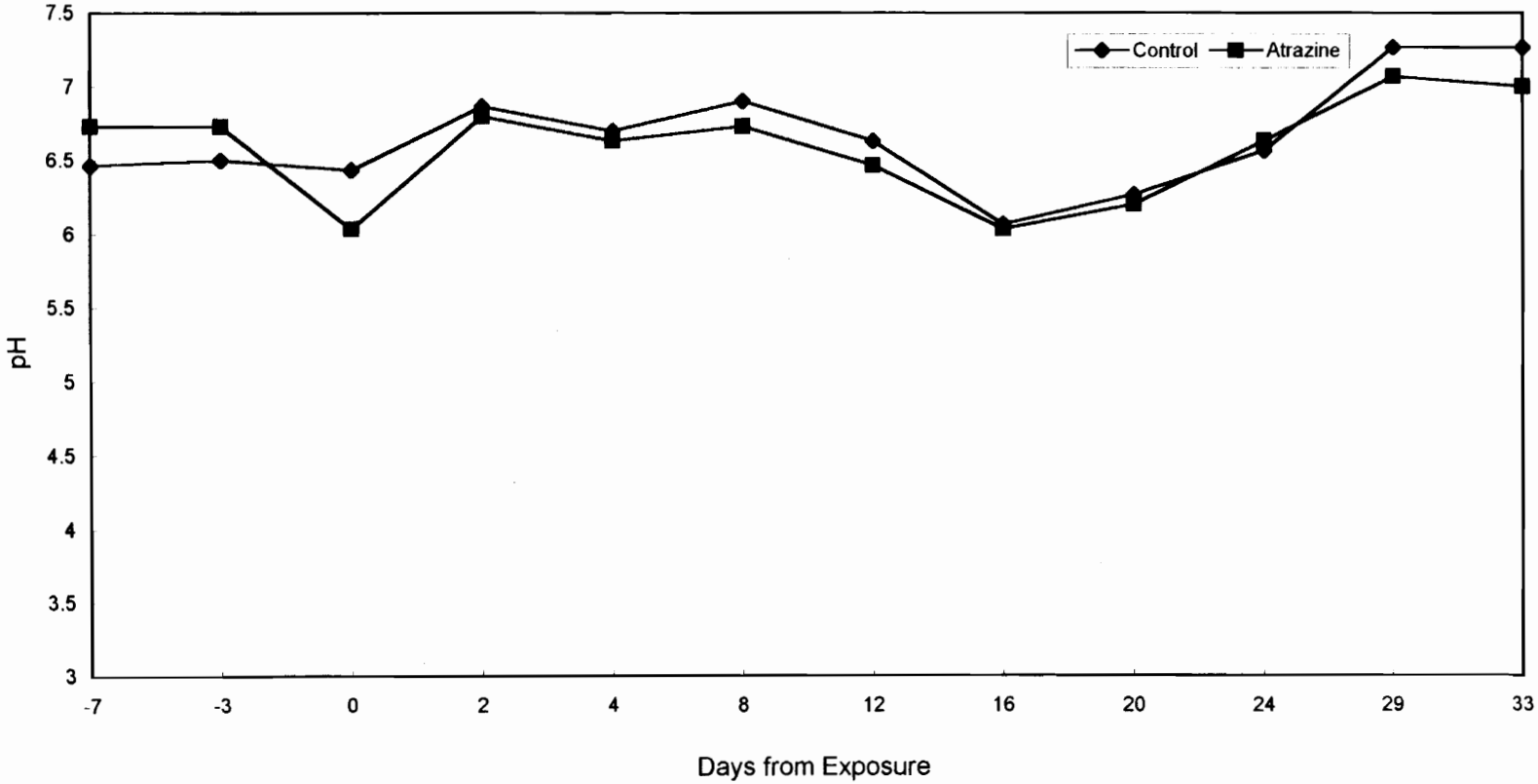
A-9. Conductivity of overflow water from control and atrazine treated (4.5 mg/L) microcosms.

APPENDIX 10



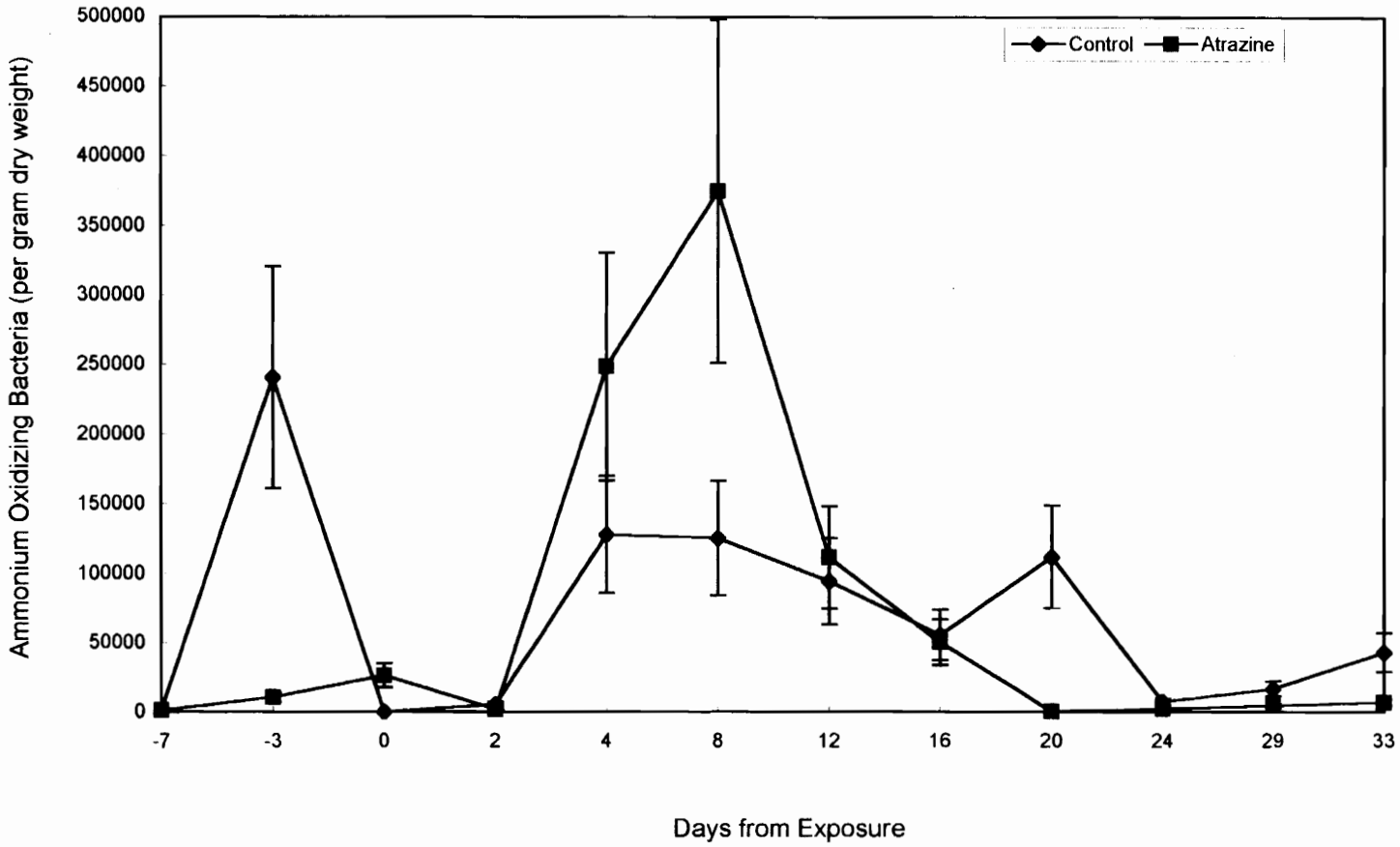
A-10. Overflow Dissolved Oxygen from control and atrazine (4.5 mg/L) treated microcosms.

APPENDIX 11



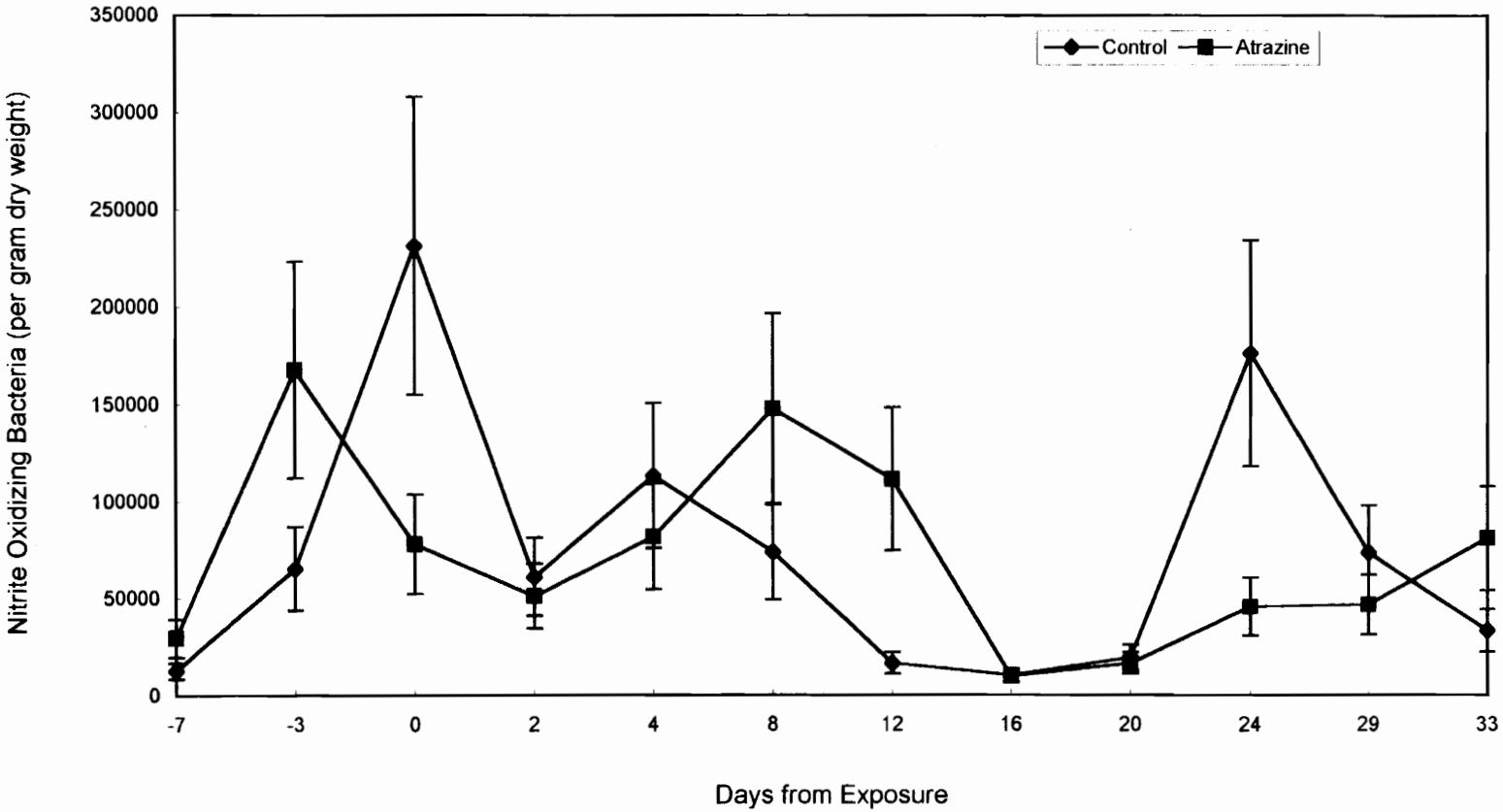
A-11. Sediment pH of control and atrazine treated (4.5 mg/l) microcosms.

APPENDIX 12



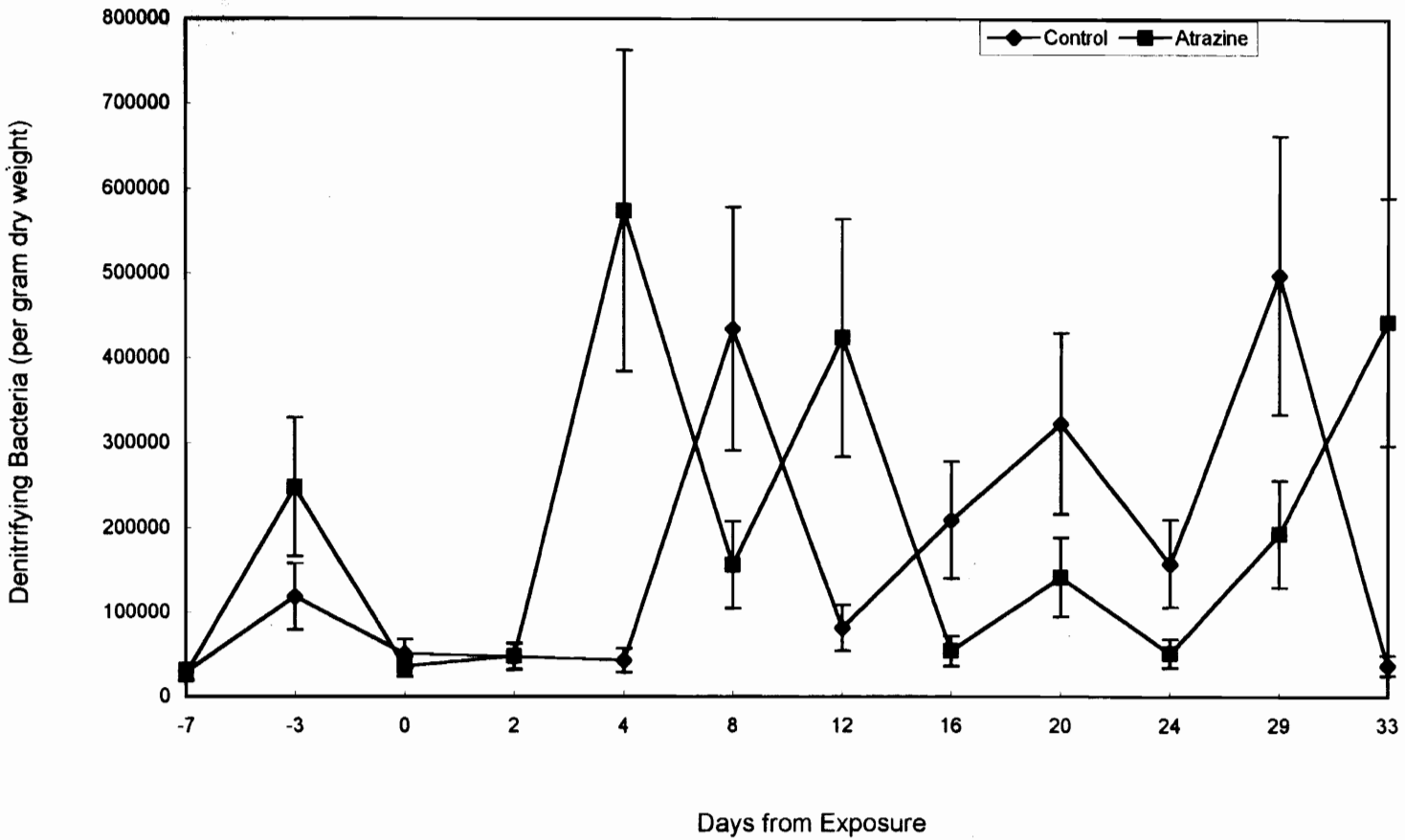
A-12. Ammonium oxidizing bacteria (per gram dry weight) present in control and atrazine treated (4.5 mg/l) microcosms.

APPENDIX 13



A-13. Nitrite oxidizing bacteria (per gram dry weight) present in control and atrazine treated (4.5 mg/L) microcosms.

APPENDIX 14



A-14. Denitrifying bacteria (per gram dry weight) present in control and atrazine treated (4.5 mg/L) microcosms

CURRICULUM VITAE

NAME: Rhonda E. Wilhite

SOCIAL SECURITY NUMBER: 406-92-6560

DATE: March 16, 1996

PERSONAL DATA: Born 7 August, 1968, Fairbanks, Alaska, USA

ADDRESS: 29 Quail Ridge Lane
Stafford, Virginia 22554
(540) 752-1099

EDUCATION:

1986-1988 University of Kentucky; Elizabethtown Community College, Elizabethtown, Kentucky. Associate of Arts Degree.

1988-1990 Western Kentucky University, Bowling Green, Kentucky. B. A. in Biology. Minor in Environmental Science.

1990 Summer program; Tennessee Technological University; Upper Cumberland Biological Station, Cookeville, Tennessee.

1993-present Virginia Polytechnic Institute & State University. M. S. in Ecology.

MAJOR PROFESSOR: Dr. A. L. Buikema

COMMITTEE: Dr. R. Benoit, Dr. G. M. Simpson, Jr., Dr. A. Hendricks

ADDITIONAL EDUCATION:

1994 V-QUEST Workshop on Gender and Minority Equity

1994 V-QUEST Workshop on the Implementation of Technology I in the Classroom

1994 V-QUEST Workshop on the Advancement of the V-QUEST Project

TEACHING EXPERIENCE:

1996 Graduate Teaching Assistant, Soil Microbiology Laboratory, Department of Biology, VPI & SU.

1995 Instructor for "Teaching Life Sciences" at the Graduate Teaching Assistant Training Program Fall Workshop, VPI & SU.

1995 Graduate Teaching Assistant, General Microbiology Laboratory, Department of Biology, VPI & SU.

1995 Graduate Teaching Assistant, Aquatic Microbiology Laboratory, Department of Biology, VPI & SU.

1994- Graduate Teaching Assistant, Implementation of new
1995 Biology laboratory exercises in Principles Biology Laboratory, Department of Biology, VPI & SU.

1993- Graduate Teaching Assistant, General and
1995 Principles Biology Laboratory, Department of Biology, VPI & SU.

RESEARCH EXPERIENCE:

1994- The effects of atrazine on nitrogenous bacteria and
1996 nitrogen cycling in a freshwater wetland microcosm. Master's thesis at VPI & SU.

1994 Use of a modular mesocosm to evaluate pesticide effects on estuarine ecology. John Lauth. Department of Biology, VPI & SU.

1993- The effects of Aldicarb on the nitrogen cycling
1994 bacteria in a simulated salt water marsh ecosystem. Previous Master's thesis at VPI & SU.

1990 Investigation of the River Continuum Concept. Undergraduate study at the Upper Cumberland Biological Station, Smithville, Tennessee.

WORK EXPERIENCE:

1990- 1993 Ogden Environmental Laboratory, Western Kentucky University, Bowling Green, Kentucky. Laboratory Technician and Laboratory Supervisor.

1990 Student Internship at the Kentucky Department for Environmental Protection, Bowling Green, Kentucky.