Water Resources Research Institute of the University of North Carolina

Annual Technical Report

FY 1998

Introduction

Research Program

SUMMARY The North Carolina Water Resources Research Institute program for 1998-99 (federal fiscal Year 98) continued to focus on three broad areas of concern: surface waters, groundwater, and urban water management. The Institute program also emphasized technology transfer in the form of publications, conferences, workshops, forums, seminars and newsletters. Support from the U.S. Geological Survey through the State Water Resources Institute Program (SWRIP) was supplemented by state appropriations, North Carolina Board of Science and Technology, Urban Water Consortium, American Water Works Association Research Foundation, National Park Service and various divisions of the North Carolina Department of Environment and Natural Resources. More than forty active research projects were supported with these combined resources. A portion of the research program focused on five water quality projects. Funds for these projects were obtained through regional competition under the Section 104 research grant program. The technology transfer program for the Institute focused on disseminating the results of the Institute's research program; on gathering and disseminating information on emerging water issues, laws, regulations, and problems; and on transferring regulatory and technical information to facilitate changes in North Carolina's water quality related programs. The following strategies were used: (1) reviewed and published nine reports as part of the Institute report series; (2) evaluated all research projects with research investigators and users to assess progress; (3) developed separate brochures describing each of the current research projects and listing Institute publications; (4) published a bi-monthly newsletter summarizing research results, new state and federal water laws, regulations and program changes, and announcements of conferences, workshops, forums and seminars; and (5) organized and co-sponsored eight seminars, four forums, five workshops, and two conferences on key water issues. Nineteen ninety-eight continued to bring into focus the need for better scientific information to support and guide policy decisions and regulatory activities regarding water quality and water resource management in North Carolina. The problem is not just one of increasing magnitude. New technology spawns new kinds of potential threats to public health and the environment, and advances in science are making those threats more apparent. Moreover, the mix of agricultural, industrial, and urban activities are affecting the state's water resources in ways that have not been sufficiently explored. Just as the state faces unprecedented demand for water use, it also enjoys unprecedented public support for regulations, arrangements, and technologies to protect that resource. North Carolina's legislature as well as the press continue to devote much attention to water-related environmental issues. Among those issues that have drawn the most attention during the year are: 1) restoration of the quality of the state's coastal waters; 2) water quality implications of animal operations; and 3) soil erosion and sediment pollution.

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Microbial Impacts of Animal Wastes on Water Resources (70152)
Project Number	C-03
Start Date	06/15/1996
End Date	08/31/1998
Research Category	Biological Sciences
Focus Category #1	Non Point Pollution
Focus Category #2	Waste Water
Focus Category #3	Water Quality
Lead Institution	Water Resources Research Institute of The University of North Carolina

Principal Investigators

Principal Investigators			
Name	**Title During Project Period**	**Affiliated Organization**	**Order**
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Problem and Research Objectives

The microbial quality of water resources and the management of the microbially laden wastes generated by the burgeoning animal agriculture industry are critical local, regional and national problems. Animal wastes from cattle, hogs, sheep, horses, poultry and other livestock and commercial animals can contain high concentrations of microorganisms that are pathogenic to humans. In particular, the protozoans Cryptosporidium parvum and Giardia lamiblia can be present in cattle (Angus, 1990), hog, horse (Kim, 1990) and other animal wastes and both organisms have been responsible for many outbreaks of gastrointestinal illness caused by contaminated drinking water (MMWR, 1996). The massive, waterborne Cryptosporidiosis outbreak in Milwaukee, Wisconsin, with more than 400,000 cases, as well as the earlier outbreak in the southeastern community of Carrolton, GA, are suspected of being caused by fecal contamination from commercial cattle and possibly other animal operations in the watersheds (Hayes et al., 1988; MacKenzie et al., 1995). Cattle operations also were implicated in an outbreak in England (Lisle and Rose, 1995). Ground waters as well as surface waters can be contaminated by Cryptosporidium as indicated by two ground waterborne outbreaks in the United States (Texas and Pennsylvania) and the detection of the organisms in well water (Lisle and Rose, 1995). Although Cryptosporidium, Giardia and other human pathogens of animal origin are widespread in both animal and human wastes and in environmental waters, little is known about the relative contributions of agricultural animal wastes to such environmental contamination of water resources. The project intends to characterize and quantify the relative contributions of agricultural and commercial animal fecal wastes and human sewage effluents to microbial pathogens levels in surface and ground waters. Attention is focused on the protozoan parasites Cryptosporidium and Giardia. This is because their fecally excreted, environmentally stable forms (oocysts and cysts, respectively) persist for long periods (months) in water, and are very resistant to disinfection by chlorine and other water and

wastewater disinfectants. Both Giardia cysts and Cryptosporidium oocysts are small enough (about 5-15 m m and 4-6 m m in diameter, respectively) to pass through granular filtration media such as sand. However, agricultural animal wastes may contain other enteric pathogens of humans, including Salmonella bacteria. Therefore, the range of human pathogens potentially present in animal wastes must be considered. In North Carolina there have been dramatic incidents of river water contamination (spills) with agricultural animal wastes due to failures in the structural integrity of hog and poultry waste lagoons. Also,in other southeastern states and the nation as a whole, there is emerging evidence of ground water contamination from lagooned animal wastes, and there are growing concerns about widespread surface water contamination due to inadequate animal waste management practices. In order to better characterize the potential risks to human health and to identify the appropriate and effective animal waste management practices, information is needed on (i) the occurrence, survival and treatment of Cryptosporidium and other human pathogens in animal wastes; (ii) the extent to which animal wastes are causing surface and ground waters to be contaminated with these pathogens; and (iii) the effectiveness of current and candidate animal waste treatment processes to remove and destroy these pathogens in agricultural wastes before they are discharged to the environment. Because pathogen analysis is not yet practical for routine monitoring, reliable indicators are needed to detect and distinguish between animal and human fecal waste contamination. This information is needed by farmers, managers, scientists, engineers and regulators. This project is to determine the prevalence and concentrations of Cryptosporidium, Giardia, other enteric microbial pathogens and candidate indicators for these pathogens in the treated and untreated wastes of hogs, cattle and perhaps other agricultural animals, and the impacts of these wastes on surface and ground water resources.

Methodology

Cryptosporidium and Giardia Analyses Cryptosporidium and Giardia field samples of water are concentrated initially using previously reported membrane filtration and centrifugation methods (Aldom and Chagla, 1995; Ongerth and Stibbs, 1986; Nieminski et al., 1995). Cryptosporidium and Giardia recovered by the membrane filtration method and in field samples of wastes will be further concentrated and purified by standard centrifugation and density medium flotation methods (Ash and Orihel, 1991; Nieminski et al., 1995). These methods allow sufficient concentration and purification of cysts and oocysts for precise and accurate enumeration and determination of viability/infectivity. Cryptosporidium parvum oocysts seed waste samples for die-off studies will be obtained from a commercial source (Pat Mason, Pleasant Hill Farm, Troy, Idaho). Oocysts will be seeded into waste samples to achieve sufficiently high initial concentrations to follow at least 99.99% (4 log10) inactivation (die-off) without the need for extensive and laborious oocyst concentration from the samples. However, to allow their assay, oocysts will be recovered from seeded samples by centrifugation and density medium flotation. Cryptosporidium oocysts and Giardia cysts in field samples and Cryptosporidium parvum oocysts from the supplier and in seeded waste samples will be assayed for total concentration by microscopic counting in a hemacytometer after immunofluorescent staining. Oocysts will be assayed for viability/infectivity by in vitro excystation and dye exclusion methods (propidium iodide and DAPI) in order to determine inactivation or die-off rates (Campbell et al., 1992; Grimason et al., 1994). In laboratory die-off studies and in-situ die-off studies using microbial chambers, the infectivity of Cryptosporidium oocysts will be determined by newly developed methods based on infectivity in cell cultures (Arrowood et al. 1994; 1996). Processed samples are exposed to excystation medium, inoculated into slide chamber cultures of MDCK cells and incubated for 1-2 days. Then the infected cells are stained with fluorescent labeled monoclonal antibodies against the living stages of Cryptosporidium. Living stages (meronts and garnonts) are quantified microscopically to determine the concentration of viable oocysts in the initial samples. Analyses for F-specific RNA Coliphage Serotypes F-specific coliphages are enumerated on the host strains of Havelaar and colleagues (strain WG49 of

Salmonella typhimurium; Havelaar and Hogeboom, 1984; Havelaar and Nieuwstad, 1985; Havelaar et al., 1984) and Cabelli and colleagues (E. coli "F-AMP"; Debartolomeis and Cabelli, 1991); somatic coliphages are enumerated on E. coli CN-13 (Payment and Franco, 1993) and somatic Salmonella phages are enumerated on both S. typhimurium WG49 (the F-specific coliphage host) and its F-minus parent strain S. typhimurium WG45. Isolation and enumeration is by plaque assay using one of several methods: (i) soft agar overlay (Adams, 1959); (ii) single agar layer (Grabow and Coubrough, 1986); or (iii) adsorption to and elution from membrane filters (Sobsey et al., 1996). The latter method is very useful for enumerating low levels of phages in large volumes (100-1,000 ml) of water. Plates are incubated at 37 0C for 8-18 hours and then plaques are counted to compute phage concentration. Typing F-specific RNA Coliphages In order to demonstrate that it is possible to distinguish between human and various animal waste sources, representative numbers of F+ coliphage plaques from assay plates of water and waste samples are isolated to identify to which of the four groups they belong. F-specific coliphage plaques are picked into 0.5 ml of buffered water to determine type of viral nucleic acid (RNA or DNA) and F-specific RNA coliphage genotype using the methods of Hsu et al. (1995, 1997) for filter hybridization with non-radioactive oligonucleotide probes. We have developed oligoprobes for each of the four F+ RNA coliphage serotypes. The oligoprobes are 3' end-labeled with digoxigenin (Genius 5 Kit, Boehringer Mannheim) for use in nucleic acid hybridization tests with genomic F+ coliphage RNA. Phages are transferred from zones of lysis on host cell lawns to a nylon membrane and phage RNA is denatured and bound to the membrane for subsequent hybridization assay with immunoenzymatic calorimetric detection (Boehringer Mannheim kit). Analyses for Bacteria In water and waste samples that can be filtered, fecal coliforms and E. coli are analyzed by membrane filter methods using mFC medium (APHA, 1989; 1992), followed by membrane transfer to plates of nutrient agar-N4UG substrate, incubation for 4 hours, and long wavelength UV light exposure to score E. coli on the basis of glucuronidase activity (Mates and Schaefer, 1989). Membrane filter assay of enterococci uses modified mE medium and incubation at 41 0C (APHA, 1989; 1992; Dufour, 1980). Spores of C. perfringens are enumerated by heating samples to 65-70 0C for 15-20 minutes, membrane filtering, and incubating anaerobically on mCp agar (Gas-pak jars or bags) 3-5 hours at 35-37 0C followed by 20-48 hours at 45 0C (Bisson and Cabelli, 1979). Membranes with presumptive C. perfringens colonies are exposed to ammonium hydroxide fumes as an acid phosphatase test to confirm identity. For wastes that cannot be membrane filtered, assays are done by multiple tube tests using appropriate media and incubation conditions for each organism: fecal coliforms and E. coli in lauryl tryptose broth at 35 0C followed by EC-MUG broth at 44.5 0C; enterococci in azide dextrose broth at 35 0C, followed by spotting positive broth cultures on modified mE medium at 41 0C; and C. perfringens in iron milk medium at 44.5 0C. Concentrations of indicator bacteria are expressed as colony forming units (CFU) or Most Probable Number (MPN) per 100 ml (or grams). Salmonella spp. in water and waste samples are assayed by inoculating different replicate volumes (or weights) of water (or manure) into liquid selective enrichment medium or by membrane filtration and placement of membranes on selective agar. After incubating at 43 0C for 24 hours, Salmonella enrichment broth and presumptive Salmonella colonies on membrane filters are identified by filter hybridization using non-radioactive oligonucleotide probes (Lin and Tsen, 1995). Enrichment broth and membrane filter agar is lactose combined tetrathionate (CTET) medium. Cells are recovered directly from enrichments by centrifugation and washing for subsequent release of RNA and oligonucleotide probing by filter hybridization using digoxigenin-labeled probe. Colonies on membrane filters are lifted onto hybridization membranes, denatured, fixed and then probed by standard procedures. Enrichment tubes that are positive by oligonucleotide probing will be scored as confirmed positives and Salmonella concentrations will be computed as and expressed as MPN per 100 ml (or grams). Hybridized colonies are enumerated directly as CFU per unit volume. Chemical Analyses Water and waste samples will be analyzed for the following physical and chemical parameters by the analytical laboratory of the Department of Biological and Agricultural Engineering, North Carolina State University: pH, turbidity, conductivity, total solids, volatile solids, suspended solids, total organic carbon, TKN, NH3-N, N03 + N02-N, total P, ortho P

and Cl. These data will be used to determine if there are relationships between the prevalence and concentrations of any of these physical and chemical parameters and any of the microbiological parameters. Of particular interest will be any changes or reductions of specific physical and chemical parameters that may serve as practical markers of reductions in microbial pathogens and their microbial indicators by treatment or storage processes. Data Analysis and Interpretation The data on concentrations of pathogens and indicators in treated and stored wastes will be analyzed to determine the extent of reduction of these microbes. The frequency and concentrations of pathogens and indicators in raw, untreated wastes will be compared to that of the wastes subjected to specific treatment processes (lagooning, ponding, constructed wetlands, and overland flow). By constriction of and comparisons between or among frequency, the frequency distributions of pathogen concentrations in raw and treated wastes, quantitative reductions of the microbes by the specific treatment processes will be determined. From these analyses, it will be possible to determine if treated animal wastes still contain such high concentrations of pathogens that they may pose a risk to the environment and to public health. The interpretation of these risks will be made with the consideration of the various waste management alternatives. The data on concentrations of pathogens and indicators in field samples of surface and ground waters that receive specifically treated wastes will be analyzed to determine the impact of the treated waste on water quality. Concentrations of pathogens and indicators will be compared for different samples within the same watershed or aquifer to determine significant differences. Proximity to or impact from specific waste sources will be considered in these analyses. Statistical analyses also will be performed to determine which indicators are most reliable in predicting the presence and concentrations of pathogens and in distinguishing between types or sources of fecal contamination from humans and the different animal sources. Indicators also will be evaluated on the basis of sensitivity, specificity, accuracy, reliability, technical difficulty, speed and cost. One goal of the analyses is to determine if levels of indicators (F+ RNA coliphages types, somatic coliphages, somatic Salmonella phages, C. perfringen's spores, etc.) in water and waste samples can be used to predict and quantify the presence and levels of the various pathogens in water and wastes (Cryptosporidium, Giardia, and Salmonella). A 2x2 table is formed that cross tabulates the level of a specific pathogen (high versus zero or low) against levels of an indicator (high versus zero or low). Sensitivity, specificity and measures of agreement (e.g., kappa statistic) will be calculated. This analysis is repeated to compare each pathogen to each indicator, in turn. A next step in the analysis is to determine if each pathogen has an appropriate dose-response when compared with indicators for water samples impacted by a particular type of animal fecal contamination. Intraclass correlation coefficients will be compared, both on log-transformed and ranked data, in order to determine if relatively high levels of the indicators are associated with relatively high levels of the pathogens. If correlations are not found, stratified analyses based on contingency tables will be used. This is an elaboration of the 2x2 contingency analysis described above.

Principal Findings and Significance

In the Long Creek watershed (Gaston County) significant increases were observed in the levels of fecal coliform, E. coli, enterococci, C. perfringens and somatic coliphages between dairy farm stations and upstream (control) stations. Increases in microbial concentrations also were observed in stream samples adjacent to an area where municipal biosolids are land applied; increases were statistically significant only for somatic coliphages. In an urban section of the watershed the highest concentrations of all microbial indicators except enterococci were the highest at a station just below the treated effluent discharge of a municipal wastewater treatment plant (City of Gastonia). Enterococci were highest at a station immediately downstream from the city. Overall, the highest concentrations of fecal indicator microbes found in any watershed samples were those in the two stations at the dairy farm. Stream water samples at Lake Wheeler Farm (Wake County) were collected and analyzed quarterly over a two-year

period to investigate the microbial impacts of swine and dairy cattle waste management activities. Concentrations of all microbial indicators except C. perfringens increased above background levels as stream water traveled through a cattle pasture area (west section) and through the swine and dairy cattle operations (feed lot, milking, and additional pasture; north section). Microbial increases were statistically significant only for somatic coliphages (west area) and F+ coliphages (north area). Seasonal differences in indicator microbe concentrations of stream samples were not observed in the Long Creek watershed. However, there were significant seasonal differences in stream water concentrations of indicator bacteria at Lake Wheeler Farms. Waste runoff water from dairy cattle feedlot at Lake Wheeler Farms contained high concentrations of microbial indicators in untreated and treated swine and cattle wastewater and indicator reductions by alternative waste treatment process also were determined. Microbial indicator levels in raw swine and dairy cattle wastes exceeded those in raw domestic or municipal sewage. The results of these studies indicate that agricultural animal wastes have appreciable impacts on the microbial quality of adjacent surface waters, even current Best Management Practices (BMP).

Descriptors

Animal waste, Microbes, Pathogens, Public health, Wastewater, Wastewater treatment, Water quality monitoring

Articles in Refereed Scientific Journals

Book Chapters

Dissertations

Water Resources Research Institute Reports

Conference Proceedings

Other Publications

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Biologically Mediated Nitrogen Dynamics in Eutrophying Estuaries: Assessing Denitrification and N2 Fixation Responses to Proposed N Loading Reductions in the Neuse River Estuary (70166)
Project Number	C-05
Start Date	07/01/1997
End Date	12/31/1998
Research Category	Water Quality
Focus Category #1	Nutrients

Category #1	
Focus Category #2	Surface Water
Focus Category #3	Water Quality
Lead Institution	Water Resources Research Institute of The University of North Carolina

Principal Investigators

Principal Investigators			
Name	**Title During Project Period**	**Affiliated Organization**	**Order**
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Hans W. Paerl	Professor	University of North Carolina at Chapel Hill	05

Problem and Research Objectives

Troubling symptoms of nutrient-driven eutrophication are proliferating regionally and nationally. Most larger river systems and estuaries in the southeastern U.S. are showing signs of water quality degradation. The Neuse River-Estuary, NC, is an ecosystem experiencing sudden and rapid reduction in water quality and urgently requires the development of effective, attainable, and ecologically sound nutrient management strategies. Within the past two decades, the Neuse has undergone a transition from a balanced and productive system to one experiencing unprecedented biogeochemical change and trophic deterioration. Nuisance dinoflagellate, cryptomonad, and cyanobacterial blooms, accompanied by hypoxia/anoxia, toxicity, fin- and shellfish kills, are growing in frequency, duration, and areal extent (Paerl 1987, Paerl et al. 1995). The research examines the role of two biologically mediated processes (denitrification and phytoplanktonic N2 fixation) that are potentially major regulators of N-cycling characteristics and rates in riverine and estuarine habitats. Phytoplankton primary production in the lower Neuse River and estuary is controlled by N availability (i.e., N-limited) throughout much of the year (Paerl 1987, Paerl et al. 1995, Boyer et al. 1994). Excessive N-loading and expanding algal blooms have promoted eutrophic conditions in the lower reaches of the Neuse River (Boyer et al. 1993, Paerl et al. 1995). Riverine loading of dissolved inorganic nitrogen (DIN) is dominated by nitrate-nitrite (NOx) which declines non-conservatively down-estuary under most flow conditions (Christian et al. 1991). While much emphasis has been placed on biotic assimilation and recycling of nitrogen in the Neuse (Boyer et al. 1995, Christian et al. 1991), an alternative fate of DIN is microbially mediated denitrification in estuarine sediments. Denitrification is thought to be a substantial annual sink for nitrogen inputs to estuarine systems (Kemp et al. 1990, Seitzinger 1988, Smith et al. 1985), but no published data exist for the Neuse. Determining the role of microbially mediated denitrification in N cycling and N budgets of the Neuse River Estuary is critical for a complete understanding of the fate and effects of nutrient inputs to estuarine ecosystems. Also an assessment of the impact of N-loading reductions on phytoplankton communities is needed to determine if additional controls may be necessary to effectively manage nuisance cyanobacterial growth in the Neuse River Estuary.

Methodology

The nutrient dilution bioassay technique is a simple manipulative method for reducing the concentrations of ambient dissolved ions in natural water samples. In this assay, concentrations of

potential growth-limiting nutrients, chiefly N and P compounds, are reduced while maintaining non-growth-limiting ions at naturally occurring levels. Neuse River water will be obtained from an estuarine (25.9 km) and riverine site (7.6 km). Community composition will be characterized using standard microscopic (direct enumeration) and chemosystematic (photopigment) techniques. For microscopic enumeration, phytoplankton samples (100 ml) will be preserved with Lugol's iodine solution and later concentrated to a 5 ml slurry using settling chambers. For each sample, ca. 1.0 ml of slurry will be dispensed into a counting cell and the phytoplankton enumerated using a Zeiss (research grade) microscope (480x or 750x). Water samples (300 - 600 ml) for photopigment analyses will be collected from Cubitainers at specified time intervals, filtered onto Whatman GF/F filters (47 mm), and frozen (-80 0C). Photopigments will be extracted using a 90% aqueous acetone solvent and sonication. High performance liquid chromatography (HPLC) will be used to quantify the relative biomass of algal groups (i.e., cyanobacteria, diatoms, dinoflagellates, cryptomonads, chlorophytes, etc.) in the phytoplankton community based on biomarker photopigment (chemosystematic chlorophylls and carotenoids) concentrations. N2 fixation rates (nitrogenase activity) will be estimated using the acetylene reduction assay (Stewart et al. 1967). Water samples (50 ml) will be taken from Cubitainers following bioassays and transferred to serum vials for measurements of N2 fixation rates (nitrogenase activity). Sealed vials will be injected with acetylene and incubated under ambient light and temperature conditions. Nitrogenase activities will be measured for all experimental treatments (controls and nutrient manipulations) as well as under light and dark incubation conditions. Gas samples will be taken at the end of the assay (ca. 3 h) and analyzed using gas chromatography with flame ionization detection (GC FID) to quantify ethylene concentrations. Acetylene is converted to ethylene by the nitrogenase enzyme and is a relative measure of N2 fixation rates. Limitations of the acetylene inhibition technique for measuring denitrification in sediments have recently been documented (Thompson et al. 1995, Seitzinger et al. 1993). Acetylene inhibition of the reduction of N2O to N2 has been shown to be ineffective at low (<10 m M) NO3- concentrations (Kaspar 1982, Oremland et al. 1984, Slater and Capone 1989). Acetylene also inhibits nitrification (Hynes and Knowles 1981), yielding underestimates of denitrification in systems where nitrification is a significant source of NO3- for (i.e. coupled to) denitrification. However, the simplicity of the acetylene inhibition technique allows for greater spatial replication relative to other techniques and the short incubation time (minutes to hours) permits the measure of short-term changes in denitrification. The acetylene inhibition technique has been adapted for use in estuarine sediments by measuring denitrification rates at incremental NO3- additions to determine saturation kinetics, providing both potential (Vmax) and an estimate of in situ denitrification activity. This technique has been successfully utilized in Dr. Paerl's laboratory during the past several years for measuring denitrification in estuarine headwater creeks and salt marsh sediments. A relatively new technique for measuring rates of denitrification is the detection of short term (24 hour) changes in N2:Ar ratio in the water of in situ sediment (Kana et al. 1994). Dr. Samantha Joye of Texas A&M has successfully used the technique to measure denitrification in estuarine sediments. A method comparison (acetylene block and N2:Ar) will be conducted in sediment cores and replicate sediment chambers at a selected station on one occasion. Concurrent measures of nitrification and NO3- and NH4+ fluxes (Sloth et al., 1992) in 6 replicate intact cores will provide a third comparison (Denitrification=Nitrification-NO3-flux). Additionally, we are prepared to measure denitrification (N2:Ar) in sediment chambers at several stations on the Neuse in cooperation with NC Division of Water Quality, pending formalization of sampling plan. Water samples (surface and bottom) and porewater from surface sediments collected bi-weekly from Neuse transect stations will be analyzed for NOx and NH4+ and for CHN content. Water samples will be filtered through muffled glass fiber filters and analyzed with a Lachat QuickChem automated ion analyzer. Porewater NOx and NH4+ will be extracted from the sediments with 2N KCl, filtered and analyzed with the Lachat autoanalyzer. Filters and surface sediments will be analyzed for CHN with a Perkin Elmer 2400 Series II elemental CHN analyzer. Water samples will be analyzed for dissolved organic N (catalytic oxidation) and dissolved organic C (infrared analysis). Water column parameters including profiles of dissolved oxygen, salinity,

temperature, pH and depth will be obtained with a Hydrolab Surveyor 3 data logging system. Biological parameters, including primary productivity (14C Method), phytoplankton species (M3, gravity filtration/centrifugation), photopigments (HPLC-PDAS) and growth rates (Packard 525a Flow Scint Counter) are measured bi-weekly at the same stations as part of USDA project: Nitrogen-driven eutrophication of the Neuse River, NC. We will determine surface sediment O2 dynamics in parallel with work using a stirred core technique. Biological and chemical oxygen demand are determined by micro Winkler titration of water overlying sealed sediment cores incubated for 6-48 hrs. This analysis, which is supported by other research funds (USDA) will be of value to this project by helping to clarify temporal and spatial relationships between bottom water hypoxia/anoxia and denitrification dynamics (i.e. PO2 as an environmental regulator of denitrification potential in the Neuse Estuary)

Principal Findings and Significance

(Progress Report) Denitrification is thought to be an important component of the nitrogen cycle of coastal and estuarine waters, potentially alleviating the impacts of excessive loading. Denitrification was measured monthly over a 2.5 year period in sediments collected along a 41 km transect of the Neuse River Estuary. Rates were measured with an adaptation of the acetylene block technique, providing both potential (nitrate amended) and simulated in situ rates. Mean annual removal of nitrogen (N) via denitrification within the transect was calculated at 527.96 metric tons for potential rates and 47.82 for simulated in situ rates. Nitrogen removal in sediments of the assayed segment of the Neuse was found to be 21.81% (potential rates) or 1.06% (simulated in situ rates) of total dissolved N loaded to the uppermost transect station, calculated as the mean of monthly percentage removals for 25 months sampled (loading data provided by Dr. Marty Lebo, Weyerhaueser Inc.). Sampling was conducted in conjunction with bi-weekly monitoring of physical, chemical and biological parameters to assess the influence of substrate limitation (nitrate and organic carbon) and estuarine hydrology on spatial/temporal patterns of denitrification activity. Using linear regression model, location explained only 7% of the variability in potential denitrification. Potential rates peaked in the summer and were significantly greater than fall rates. No assayed parameters were significant to stepwise multiple linear regressions of potential denitrification at upstream locations for any season. At downstream stations, the regression model included dissolved oxygen (+) and carbon:nitrogen (-) in the summer and carbon:nitrogen (-) in the fall. For simulated in situ rates, 40% of the variability was explained by location with rates decreasing downstream. Differences between mean seasonal rates of in situ denitrification were only significant at downstream locations where rates were elevated in the spring and fall. Nitrate concentration appeared to regulate these rates most directly, explaining 57% of the variability for pooled location/season data. Rates for nitrification and denitrification (calculated by difference) determined in several nutrient flux experiments on sediments from Marker 15 were 0.75 to 1.2 and 0.73 to 0.88 m mol N*m-2*day-1 respectively, comparable to potential denitrification rates measured with the acetylene block technique at this site (0.54 m mol N*m-2*day-1). It is likely that nitrification and denitrification are closely coupled at downstream locations in the Neuse where ambient nitrate concentrations are low, in which case denitrification rates are underestimated by in situ rates determined in this study. To enhance water and habitat quality in the Neuse River Estuary, reduction in nitrogen (N) loading has been selected as means of controlling eutrophication. Dilution bioassays are manipulative experiments that dilute concentrations of limiting nutrients to assess effects on water column processes. Experiments are being conducted on samples from a riverine site (Streets Ferry Bridge) and an estuarine site (Marker 15) in the Neuse River Estuary. Thirty percent reductions in concentration of N and both N and P are being used to predict the effects of implementing the Neuse River Nutrient Sensitive Waters Management Strategy Rules on the native phytoplankton community. This study is examining the effects of reductions in nutrient concentrations on phytoplankton productivity and biomass and the potential for phytoplankton community composition alterations.

Reducing N concentrations by 30% decreased phytoplankton productivity. Mean assimilation numbers (productivity/Chl a) after 84 hour incubations were lower than the control (current river conditions) at both sites 11 out of 14 measurements throughout 1997 and 1998. However, reducing N loading unilaterally (i.e. without accompanying P reductions) may lead to decreased N:P ratios in the system and may create conditions conducive to increased dominance by N2 fixing cyanobacteria. Selection for N2 fixers could be problematic for several reasons including trophic perturbation, ineffectiveness of N controls in reducing phytoplankton productivity, and increased biological fixation of N. Results from this study show that native cyanobacteria fix more N when N alone is reduced as compared to reduction of both N and P and the current conditions in the river. Additionally, HPLC photopigment analysis indicated that the relative abundance of cyanobacteria increased in reduced N conditions. N2 fixation, however, was only observed in 2 of the 10 bioassays conducted to this point (both times in the late summer) and only at the estuarine site. Cyanobacteria were present in the river in significant numbers prior to the initiation of the bioassays in which N2 fixation was observed. We have also begun to direct significant effort toward analysis of the genetic potential for N2 fixation in the Neuse River after the enactment of the 30% reduction in N loading. Of the clones extracted and sequenced from the dilution bioassay samples, there are 5 distinct nifH sequences thus far. These sequences are all cyanobacterial, with the vast majority being heterocystous, suggesting heterocystous cyanobacteria play an important role in N2 fixation in the Neuse River. The majority of the nifH sequences cluster with Anabaena sp., which is among the most commonly observed cyanobacterium in the Neuse River.

Descriptors

Algae, Nitrogen, Water quality

Articles in Refereed Scientific Journals

Book Chapters

Dissertations

Water Resources Research Institute Reports

Conference Proceedings

Other Publications

Basic Project Information

Basic Project Information	
Category	**Data**
Title	An Improved Characterization of a Fractured-Rock Aquifer by the Transient Flowmeter and a New Single-Borehole Tracer Test for Aquifer Characterization (70165)
Project Number	C-06
Start Date	07/01/1997
End Date	12/31/1998

Research Category	Ground-water Flow and Transport
Focus Category #1	Groundwater
Focus Category #2	Hydrology
Focus Category #3	Water Quantity
Lead Institution	Water Resources Research Institute of The University of North Carolina

Principal Investigators

Principal Investigators			
Name	**Title During Project Period**	**Affiliated Organization**	**Order**
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Problem and Research Objectives

The lack of detailed information on the distribution of aquifer parameter sat scales compatible with model grid scales is a major barrier to successful deterministic or stochastic forecasting of the fate of groundwater contamination. Even intermediate-scale hydraulic conductivity estimates from multi-level single-borehole techniques are often insufficient for accurate contaminant transport modeling. There is a need for improved aquifer characterization methodologies. Two such methodologies are considered in this project, the transient flowmeter test (TFMT) and the dipole-flow test with a tracer (DFTT). The first one takes advantage of the fact that before every quasi-steady-state (traditional) flowmeter test is conducted, it has to go through the transient stage, which provides useful information about the formation. The second one, which combines the dipole-flow test with a tracer test, represents the first single-borehole tracer test ever conducted. During the period specified above we have addressed the following objectives of Part I (TFMT) of the project: 1) develop a theory of the TFMT and its interpretation; 2) evaluate the transient flowmeter test in synthetic numerical experiments; 3) evaluate the new test in the field at the Gate 11 Duke Forest Site; and 4) measure the downhole distributions of fracture transmissivity and storativity in a number of the monitoring wells. We have also addressed the following objectives of Part II (DFTT): 1) decide on how to model the local dispersion process; 2) develop a semi-analytic model for the DFTT performed in a homogeneous aquifer; 3) develop an associated inverse model; and 4) perform and interpret the dipole-flow test with a tracer (DFTT) on a number of wells at the Lizzie site.

Methodology

Part I: Transient Flowmeter Test (TFMT) The theory of the TFMT was developed by formulating two initial boundary value problems (IBVP) of different complexity, one neglecting the layer crossflow and one accounting for it. The IBVPs were then solved via integral transforms or other well-established techniques. The models were validated and evaluated by comparing their performance to that of the numerical "truth" model. The two forward models were then employed in the modified Levenberg-Marquard least-squares algorithm for parameter estimation, which allow one to interpret the field tests.

The state-of-the-art electromagnetic flowmeter (Tysco, Inc.) was calibrated in our laboratory and re-calibrated in the well before each field test. It was used along with a pressure transducer and (GrundFos RediFlow 2) pump to collect the transient flux and drawdown data at two sites, the Gate 11 Duke Forest Site and the Lizzie Intensive Study Area. The first site, located about one mile south of Route 70 and State Route 751 in Durham County, has a fractured rock aquifer, whereas the second site, located near Greenville, North Carolina, provides access to two coastal aquifers. Part II: Dipole-Flow Test with a Tracer (DFTT) We assumed that the tracer is released after the steady-state dipole-flow pattern is established. For this flow pattern, we used the streamtube modeling approach outlined in the literature to develop a semi-analytical model of the tracer transport in the DFTT. The model, which accounts for the longitudinal dispersion only, was used to develop type curves for parameter estimation and interpretation of field DFTTs. The downhole DFTT device consists of three (Aardvark Corporation) packers, cables, hoses, pressure transducers (0-5 psig transducer above the device, two 0-15 psig transducers for the chambers, and a 0-30 psig transducer below the device), and (Grundfos RediFlow 2) variable speed pump. The above ground equipment consists of a nitrogen cylinder (to inflate the packers), an associated regulator, a mechanical flowmeter, a tripod, (Scientific CR10X)Data Logger, a laptop computer, the dye injection port, the fluorometer isolation valve, and a (Turner Designs Model AU-10) Field Fluorometer with a continuous flow cell set up for optically measuring the concentration of Rhodamine WT. The fluorometer is calibrated before each field test. The preliminary tests was conducted at Lizzie Intensive Study Area.

Principal Findings and Significance

Part I: Transient Flowmeter Test (TFMT) After performing the flowmeter test in well MW-3 at the Duke Gate 11 site, we found that along the 20 ft-long screen there is only one fracture right below the top of the screen. This surprising conclusion was reached after the flowmeter was lowered from above the screen to the location 1-2 ft below the screen top. The recorded flowrate dropped then to zero (below the detection limit). Similarly sparse fractures are found in other wells at the site. This convinced us that the proposed porous media model for the fractured aquifer would not apply to the Duke Gate 11 site very well. To proceed further we could either describe the flow in each fracture, which entails mapping each fracture at a prohibitive cost, or change site and continue with the development of the proposed model. We selected the second alternative and decided to apply our models to the Lizzie site mentioned above and already used for Part II of this project. For 2-layer aquifers with hydraulic diffusivity contrasts of up to two orders of magnitude, the semi-analytic TFMT models compare favorably to the numerical "truth" model. The drawdown errors do not exceed 3%, whereas the layer flux errors do not exceed 8.0%. A number of synthetic tests performed with the numerical "truth" TFMT model were interpreted with the least-squares parameter estimation methodology and produced accurate estimates of the layer hydraulic conductivities and reasonable estimates of layer storativities. Sensitivity analysis demonstrates that the TFMT response is not sensitive to the skin zone storativities nor to layer vertical hydraulic conductivities. Therefore, they cannot be estimated from the test. The TFMT provides drawdown and layer flux data in a much shorter time (an order of magnitude) than the quasi-steady-state flowmeter test. The TFMT may thus be advantageous for aquifer characterization, especially at highly contaminated sites, where pumped water needs to be treated as a hazardous waste. Part II: Dipole-Flow Test with a Tracer (DFTT) Our preliminary numerical synthetic DFTTs are very encouraging. They show that the DFTT breakthrough curves obtained in homogeneous anisotropic aquifers possesses a single dispersed peak. The time to this peak is strongly affected by the hydraulic conductivity, anisotropy ratio and marginally by the longitudinal dispersivity. The radial and vertical hydraulic conductivities can be estimated from this measurement and based on the time to the peak breakthrough concentration and the steady-state drawdowns. Preliminary field tests conducted at the Lizzie site are also encouraging and our DFTT model is promising in interpreting the generated field

data. However, one of the DFTTs produced no breakthrough curve. This test was repeated at two different times with three different levels of Rhodamine WT (RWT) concentration. They all confirmed the first finding of RWT disappeared in the aquifer. This strongly suggests that a conservative tracer, such as bromide, should be used instead of RWT or, better, that a conservative and sorbing tracer be used to learn not only about the hydrodynamic properties of the aquifer but also about its chemical properties related to sorption. We have already applied for bromide injection permit for this site. We also carefully investigated sorption properties of RWT in a number of column experiments involving RWT transport through the porous media collected from the Lizzie site. We could not recover all the mass in a reasonable time (of about 1 week) - our recoveries hovered around 70-80%. It is now clear that a portion of RWT mass strongly sorbed to the medium. In collaboration with Dr. Dharni Vasudevan from Duke's School of Environment, we confirmed in spectroscopic analysis that the commercial RWT has two fluorecent isomers. We then fitted the column breakthrough curves to a number of models of increasing complexity. Our new two-isomer two-sorption-site (equilibrium and nonequilibrium) model simulates the experimental data very well. It turns out that one RWT isomer has a reasonably small retardation coefficient, whereas the other one has a retardation coefficient around 20. This explains why we could not recover all the mass.

Descriptors

Ground water, Hydrology, Aquifer characterization, Well hydraulics, Transient flowmeter test, Solute transport, Single-borehole tracer test

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Other Publications

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Eutrophication in the Tar-Pamlico and Neuse River Estuaries of North Carolina (70161, 70167, 70172)
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Focus Category #1	Water Quality
Focus Category #2	Nutrients
Focus Category #3	None
Lead Institution	Water Resources Research Institute of The University of North Carolina

Principal Investigators

Principal Investigators			
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James L. Pinckney	Assistant Professor	University of North Carolina at Chapel Hill	07
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Hans W. Paerl	Professor	University of North Carolina at Chapel Hill	07
Donald W. Stanley	Professor	East Carolina University	07

Problem and Research Objectives

For several decades eutrophication has been one of the most important water quality issues for our nation's estuaries. Many citizens, elected officials, and scientists believe that many estuaries are more eutrophic now than they were several years or decades ago. However, previous studies of historical trends in nutrient concentrations, dissolved oxygen and chlorophyll a in some estuaries, including the

Tar-Pamlico and Neuse Rivers in North Carolina, do not provide strong support for this hypothesis. Because there are significant contradictions between results of the statistical trend analyses and our perceptions of the history of trophic status of the estuaries, it would seem prudent to continue to examine the accumulating data. Failure to understand whether or not changes are taking place will lead to two serious problems: (1) scientists will formulate and test hypotheses about eutrophication based on misinformation, and (2) legislators and regulatory agencies will have inadequate information upon which to base decisions about implementation of nutrient reduction strategies and to assess the success of those strategies. There are many recent concerns about water quality in North Carolina estuaries (including the Tar-Pamlico River estuary and the Neuse River estuary). The combined drainage area of the Pamlico and Neuse River systems is over 25,000 square kilometers. Algal blooms, toxic algae, eutrophication, low oxygen, shellfish bed closure, decline of submerged aquatic vegetation, and fish kills are just some of the issues. Many of these problems are related, and have not been historically monitored in these systems. The history of algal communities, especially toxic algae, are of particular interest in relation to fish kills and human health concerns in these estuaries, as well as other coastal systems. The proposed research will build on previous work quantifying the degree of anthropogenic disturbance in the estuaries of the Pamlico and Neuse Rivers by providing evidence of changes in abundance and productivity of different algal groups, including toxic algae and other biological and chemical parameters back through time and in relation to specific events. The proposed research will help address specific questions raised about water quality trends through time and provide insights into the effects of nitrogen loading reductions on the algal community in these estuaries. The first component will investigate the effect of a proposed 30% nitrogen reduction on the present phytoplankton community, an evaluation of the N2 fixing potential and rates under reduced N-loading, baseline data for monitoring the effectiveness of N-loading reductions, and identification of other factors that may have to be managed to achieve the desired level of algal productivity and water quality in these estuaries. The second component will assess trophic status changes in the Tar-Pamlico and Neuse River estuaries during the period 1950-1998. The assessment will be based on three measures including: watershed nutrient production, nutrient loading to the estuaries, and riverine and estuarine water quality. The third component will characterize the environmental conditions (including anoxia), water quality, and algal communities of the Pamlico and Neuse River estuaries as they existed prior to anthropogenic influences, and through time as land use in the watersheds of these systems has evolved with growing populations and industries.

Methodology

Paerl et al. (1) Biweekly sampling at eight fixed locations, in situ continuous monitoring of water quality at four locations, routine genetic screening of planktonic diazotrophs, and seasonal mesocosm nutrient addition bioassays provide the necessary infrastructure, complementary data, and historical perspective needed to address the specific project objectives for the proposed research. This component will use nutrient dilution bioassays (Paerl & Bowles 1987, Carrick et al. 1993, Dodds et al. 1993) to determine the effect of DIN reduction on Neuse River phytoplankton communities. Nutrient manipulation experiments will be conducted at the UNC Institute of Marine Sciences (IMS) using bulk water samples from the Neuse River. Bioassays will be run seven times during the project to account for seasonal variations in phytoplankton community composition and response to reductions in the ambient DIN concentrations. The nutrient dilution bioassay technique is a simple manipulative method for reducing the concentrations of ambient dissolved ions in natural water samples. In this assay, concentrations of potential growth-limiting nutrients, chiefly N and P compounds, are reduced while maintaining non-growth limiting ions at naturally occurring levels. For details on the dilution bioassay procedure see Paerl and Bowles 1987. Neuse River water will be obtained from an estuarine site (25.9 km) and riverine site (7.6 km). Treatments will consist of: (1) control (unamended river water); (2) 30% dilution;

(3) 30% dilution with P, dissolved inorganic carbon (DIC), and trace metals replenished to ambient levels; and (4) 30% dilution with N, P, DIC, and trace metals replenished to ambient levels. Community composition will be characterized using standard microscopic (direct enumeration) and chemosystematic (photopigment) techniques. For microscopic enumeration, phytoplankton samples (100 ml) will be preserved with Lugol's iodine solution and later concentrated to a 5 ml slurry using settling chambers. For each sample, ca. 1.0 ml of slurry will be dispensed into a counting cell and the phytoplankton enumerated using a Zeiss (research grade) microscope (480x or 750x). Photopigments will be analyzed using high performance liquid chromatography (HPLC). N2 fixation rates (nitrogenase activity) will be estimated using the acetylene reduction assay (Stewart et al. 1967). Water samples (50 ml) will be taken from Cubitainers following bioassays and transferred to serum vials for measurements of N2 fixation rates (nitrogenase activity). Sealed vials will be injected with acetylene and incubated under ambient light and temperature conditions. Nitrogenase activities will be measured for all experimental treatments (controls and nutrient manipulations) as well as under light and dark incubation conditions. Gas samples will be taken at the end of the assay (ca. 3 h) and analyzed using gas chromatography with flame ionization detection (GC FID) to quantify ethylene concentrations. Acetylene is converted to ethylene by the nitrogenase enzyme and is a relative measure of N2 fixation rates. Limitations of the acetylene inhibition technique for measuring denitrification in sediments have recently been documented (Thompson et al. 1995, Seitzinger et al. 1993). Acetylene inhibition of the reduction of N2O to N2 has been shown to be ineffective at low (<10 m M) NO3- concentrations (Kaspar 1982, Oremland et al. 1984, Slater and Capone 1989). Acetylene also inhibits nitrification (Hynes and Knowles 1981), yielding underestimates of denitrification in systems where nitrification is a significant source of NO3- for (i.e. coupled to) denitrification. However, the simplicity of the acetylene inhibition technique allows for greater spatial replication relative to other techniques and the short incubation time (minutes to hours) permits the measure of short-term changes in denitrification. The acetylene inhibition technique has been adapted for use in estuarine sediments by measuring denitrification rates at incremental NO3- additions to determine saturation kinetics, providing both potential (Vmax) and an estimate of in situ denitrification activity. This technique has been successfully utilized in Dr. Paerl's laboratory during the past several years for measuring denitrification in estuarine headwater creeks and salt marsh sediments. Water samples (surface and bottom) and porewater from surface sediments collected bi-weekly from Neuse transect stations will be analyzed for NOx and NH4+ and for CHN content. Water samples will be filtered through muffled glass fiber filters and analyzed with a Lachat QuickChem automated ion analyzer. Porewater NOx and NH4+ will be extracted from the sediments with 2N KCl, filtered and analyzed with the Lachat autoanalyzer. Filters and surface sediments will be analyzed for CHN with a Perkin Elmer 2400 Series II elemental CHN analyzer. Water samples will be analyzed for dissolved organic N (catalytic oxidation) and dissolved organic C (infrared analysis). Water column parameters including profiles of dissolved oxygen, salinity, temperature, pH and depth will be obtained with a Hydrolab Surveyor 3 data logging system. Biological parameters, including primary productivity (14C Method), phytoplankton species (M3, gravity filtration/centrifugation), photopigments (HPLC-PDAS) and growth rates (Packard 525a Flow Scint Counter) are measured bi-weekly at the same stations as part of USDA project: Nitrogen-Driven Eutrophication of the Neuse River, NC. Surface sediment O2 dynamics in parallel will be determined by using a stirred core technique. Biological and chemical oxygen demand are determined by micro Winkler titration of water overlying sealed sediment cores incubated for 6-48 hrs. This analysis, which is supported by other research funds (USDA) will be of value to this project by helping to clarify temporal and spatial relationships between bottom water hypoxia/anoxia and denitrification dynamics (i.e., PO2 as an environmental regulator of denitrification potential in the Neuse Estuary). The results of the nutrient dilution bioassays will be analyzed using a 2-way ANOVA (factors = nutrient dilution treatment, experiment date; variables = Chl a, productivity, photopigment, nitrogenase activity, etc.). Stepwise multiple regressions will be performed to explain the variation in both potential and calculated in situ denitrification rates in terms of chemical, biological and physical variables. Stanley (2)

Calculations of annual total N and P production in the watersheds of the two estuaries will be made using procedures similar to those of Stanley (1993), with several modifications (some of which are based on methods used by McMahon and Woodside 1997). Production will be computed at 2-to-3 year intervals for the period 1950 to present. Details concerning data sources, methods for converting "mixed fertilizer" data to elemental N and P values, methods for estimating production by municipal wastewater treatment plants, etc. are given in Stanley (1992, 1993). Annual nutrient loadings from a large portion of the Tar River will be computed using concentration data collected at a site just above the normal tidal reach (near Grimesland, NC) every other week since 1989. About 85% of the Tar-Pamlico basin is upstream of this site. The first step will be to determine whether the nutrient concentration data are flow dependent. If not, then interpolation will be used to provide daily values. If there is flow dependence, loads will be calculated using the MVUE log-linear regression model described above. In either case, daily flows will be extrapolated from the Tarboro, NC, gauging site by means of land-area ratios. It may be possible to compute loadings for each year, depending on whether or not the MVUE method has to be used and if so, how many concentration values are needed to estimate the regression parameters. The same method will be used for calculating Neuse River loadings from about 80% of the basin above a site on the lower river near Cowpen Landing. The Tar-Pamlico River estuary water quality data to be analyzed for long term trends come from a monitoring program that began in the late 1960s and has continued uninterrupted except for an 18-month period in 1973-74 (Stanley 1993). Data on water temperature, salinity, surface and bottom water dissolved oxygen, phosphorus (three fractions), nitrogen (four fractions), and chlorophyll a are collected approximately every other week at twenty stations spread across the estuary from near Washington, NC, to the mouth at Pamlico Sound. There is considerably less water quality data for the Neuse River estuary, but enough to permit trend analyses. The earliest is from a two-year monitoring project in the early 1970s. Temperature, salinity, dissolved oxygen, nitrogen, phosphorus, and chlorophyll a data from appendices in the project completion report (Hobbie and Smith 1972) will be transcribed to computer spreadsheets. Since the late 1970s a considerable amount of Neuse River estuary nutrient data have been collected by the North Carolina Department of Environment, Health, and Natural Resources (DEHNR), by the U.S. Geological Survey (e.g., Garrett and Bales (1991) and Garrett (1992, 1994)) and by university investigators (Bob Christian and Don Stanley at East Carolina University and Hans Paerl at the UNC Institute of Marine Science). Most, if not all, of the DEHNR data is available in electronic format through STORET. Most of the data that Christian and Stanley collected is already in spreadsheet format. Some of it is from the 1983-to-1985 period, but the bulk of it was collected between 1985 and 1988. Paerl's data are from the 1980s and 1990s. The seasonal Kendall-tau test will be used to analyze the water quality data for monotonic (one direction) trends over time. This nonparametric procedure, developed by U.S. Geological Survey investigators (Hirsch et al. 1982, 1991; Hirsch and Slack 1984; Helsel 1993), is suitable for application to water quality data, which are often skewed, serially correlated, and affected by seasonality. Also, missing values or values less than the laboratory detection limit present no problems (Hirsch et al. 1991). In addition to establishing the significance of a trend, the test provides a slope estimator, which is the average rate of change over the whole test period. The test cannot detect reversals of direction in trends within a test period. Cooper (3) Sediment cores from seven different sites were collected by standard piston coring methods from the Neuse and Pamlico estuaries during the summer of 1997. Selection of sites was based on personal communications, as well as reports on sedimentation, circulation, depth, and monitoring of bottom water oxygen in the estuaries (Riggs, pers.comm., Benninger, pers. comm., Wells, pers. comm., Bales & Robbins 1995; Robbins & Bales 1995; Treece 1993; Riggs et al. 1992; Garrett 1992, 1994). The cores were 82-148 cm in length and 5-10 cm in diameter. Sediment cores were x-rayed, carefully characterized and subsampled into 2 cm sections. Dating of sediments and determination of sedimentation rates for recent sediments is being accomplished in part using standard Pb-210 and Cs-137 techniques and measured with an Ortec EG&G gamma spectrometer in the Wetland Center at Duke University. Sedimentation rates will also be

determined using pollen dated horizons (such as the agricultural horizon characterized by an increase in ragweed percent abundance) and pollen concentration techniques (Brush 1984, 1989). This method is based on the fact that the majority of pollen grains found in estuarine sediments originate from terrestrial vegetation, particularly from plants whose pollen is wind-dispersed (such as oak, pine, ragweed, etc.). The concentration of the tracer particle (pollen in this case) in any interval of sediment will reflect the rate of accumulation of the other particles that make up the sediment. The validity of dating sediments using this method has been demonstrated by identifying historically dated events (Brush 1989). Bulk density, LOI, TOC, P, N, S, acid-soluble Fe, heavy metals, and other chemical parameters are being measured from subsamples in the cores. These data will be correlated to sediment dates, pollen, diatom species, and dinoflagellate cyst abundance. Analyses of sediments at the Duke Wetland Center will follow standard methods of EPA, USGS or other certified approved methods for nutrients, metals or geochemical analysis and follow standard QA/QC protocols on file at the Wetland Center. Accumulation or preservation of parameters will be calculated using appropriate sedimentation rates and bulk density for each subsample of the sediment cores. Heavy metals and other elements of interest are being measured using an inductively coupled plasma-mass spectrometer (ICP-MS) located in the Earth Sciences Division of the Nicholas School of the Environment. This data will provide an alternate dating method for the recent sediments in addition to information on toxic metal concentrations in the sediments. Other elements being analyzed include: Cr, Ni, Cu, Cd, Ti, V, Mn, Co, Zn, Th, U, and Hg. Diatoms and pollen are being extracted and identified at subsampled intervals in the cores (for example, diatoms are being counted at every 10 cm depth interval for the current project). All identifications will be done using light microscopy, according to available taxonomic references (e. g., Faegri & Iverson 1989, Krammer & Lange-Bertalot 1986-1991, Hustedt 1955, Cooper 1995b). Pollen is extracted from sediments following the methods of Faegri & Iverson (1989) and Brush (1989). Slides of pollen are prepared using silicon oil. Dinoflagellate cysts and certain forams are extracted and preserved with the pollen. The dinoflagellate cysts will be enumerated, as will the forams. All the cyst forms that resemble the cyst stages of Pfiesteria will be enumerated separately. Dr. Peter Leavitt is at the forefront of pigment analysis and paleopigment research using high pressure liquid chromatography (HPLC) methods for separation and characterization of algal pigments and pigment degradation products (Leavitt & Findlay 1994, Leavitt 1993, Leavitt & Carpenter 1990, Leavitt et al. 1989). Further analyses for pigments and pigment degradation products from the Neuse and Pamlico estuarine sediment samples will be done by Dr. Leavitt at his lab. Abundance or concentration of all indicators will be plotted along a time axis in a series of equal time intervals for each of the sediment cores analyzed. Time intervals in relation to depth in each core will depend on sedimentation rates determined for each core. The vertical (time) profiles of changes in paleoecological indicators of water quality will be matched with the history of land use at each site in order to determine the most obvious correlations with each indicator. Diatom species counts will be made at a level to ensure good statistical reproducibility (see section above). Diversity and similarity indices will be employed to evaluate changes in diatom communities over time and between samples. Distance between communities identified at each depth in each core, and between cores, will be computed and used for cluster analysis of the species data. These data will then be compared with other algal indicators, including dinoflagellate cyst abundances, and algal pigments and paleopigments. Mulitvariate techniques such as principal components analysis, canonical correspondence analysis and detrended correspondence analysis will also be used in analyses of the paleoecological data to uncover linkages and patterns among indicators through time and space. This research plan is intended to build on current work that is determining sedimentation rates and chronologies and researching indicators of community structure, water quality and sediment chemistry for sediment cores collected from two sites in each of the Neuse and Pamlico River estuaries. Data will be collected on additional indicators, as well as at three additional sites. The data will also be used for comparisons between the Pamlico and the Neuse estuaries and between the North Carolina estuaries with the Chesapeake Bay.

Principal Findings and Significance

Paerl et al. (Progress Report) Preliminary results from this study indicate that approximately 30% of the NO3- load to the Neuse River is denitrified. Seasonal and spatial variations in denitrification are currently being assessed. Additionally, correlations of denitrification and other environmental factors (e.g. dissolved oxygen and inorganic nutrient concentrations) are being examined. The 30% reduction in N-loading to the Neuse River has been targeted as a preliminary goal for water quality improvement. However, changes in N-loading may result in shifts in the ratio of dissolved nitrogen to phosphorus (N:P) loadings and concentrations in the Neuse River if they are not accompanied by parallel reductions in P loading. Alterations in N:P in the water may have significant impacts on aquatic communities beyond a simple reduction in phytoplankton productivity and biomass (standing stock), including shifts in species composition and possible selection for species adapted to growth in waters with reduced N:P (e.g. fixing cyanobacteria). Significant rates of cyanobacterial (Anabaena sp.) N2 fixation rates were higher when N alone was reduced as compared to reduction of both N and P. However, diazotroph responses to dilution bioassays were seasonally variable. The results of these experiments are being incorporated into the N compartment of a hydrodynamic model (MODMON) currently under development. Efforts will begin to analyze the genetic potential for N2 fixation in the Neuse River after the enactment of the 30% reduction in N loading. The phylogenetic tree of the cyanobacteria gene nifH (a gene which codes for the enzyme nitrogenase) will be developed. Preliminary results indicate that Anabaena sp., which is among the most commonly observed cyanobacterium in the Neuse River, appears to contain this cyanobacteria gene. Stanley (Progress Report) Nitrogen, phosphorus, and chlorophyll a trends in the estuaries do not give clear evidence of increased eutrophication during the past two-to-three decades. Data from three stations in the upper, middle, and lower Pamlico River estuary (1969-1997) were analyzed for trends using the seasonal Kendall tau test. Ammonium nitrogen levels have decreased throughout the estuary, and nitrate nitrogen decreased in the middle region. Phosphorus levels increased in the 1970s and 1980s but have declined sharply since 1992 when PCS Phosphate reduced their P loading to the estuary by about 90%. Chlorophyll a increased in the upper two-thirds of the estuary, perhaps because of decreased light limitation resulting from decreased TSS loading to the estuary. Bottom water dissolved oxygen decreased in the upper estuary also, but the change has been very small. In the Neuse estuary, ammonium nitrogen concentrations appear to have decreased by 50-70% between 1970 and 1980, but they have changed little since then. Phosphorus (both orthophosphate and total phosphorus) has decreased by 25-70% throughout the estuary since the late 1980s following implementation of the phosphate detergent ban. Since the late 1980s chlorophyll a appears to have decreased in the tidal freshwater portion of the estuary, but has shown no trend since the early 1970s in the middle and lower estuary. Total N and P production from human-related activities in the Neuse and Pamlico watersheds increased substantially during the past 30 years. Four anthropogenic nutrient sources were considered: 1) atmospheric, 2) fertilizer, 3) farm animals, and 4) point sources. Atmospheric nitrogen may be increasing, but there is little historical data. Fertilizer application in these watersheds increased rapidly following World War II, but has stabilized or decreased slightly since the mid-1970s. Point source loadings have risen since 1970 (except that P in the Pamlico has decreased), but this production source is minor compared to the others. Farm animal N and P production has risen sharply in the 1990s. Strong differences in the magnitudes and trends in nutrient production, in-stream loading, and estuarine concentrations for these two estuaries suggest that eutrophication needs to be assessed from as many angles as possible. Only 5-15% of the anthropogenic nitrogen produced in the basins reaches the estuaries (at least via surface runoff). Even less of the anthropogenic phosphorus (2-7%) gets to the estuaries. Apparently there is not a tight coupling between increasing nonpoint source nutrient production and patterns of nutrient concentrations in the estuaries. For example, the great increase in animal nitrogen production in the Neuse basin seems not to

have had an impact on nutrients or algal biomass (as measured by chlorophyll a) in the estuary. This suggests there is a great "buffering" capacity in the watershed for this source. Cooper (Progress Report) Paleoecological methods can be used in these estuarine systems. Sedimentation rates and sedimentation patterns were adequate to study the geochemical and bio-indicators at a resolution of 10 years or less per 2 cm subsample for recent sediment (last 50 years) and at slightly lower resolution for older sediments, back over 1,000 years. Sedimentation rates and nutrient and trace metal flux to both the Pamlico and Neuse estuaries have increased dramatically in the past 40-50 years. Trace metal levels in surface sediments exceed "Threshold Effect Levels" as reported by EPA at several sites, and should be reduced. Industrial sources are apparently responsible for some of the increase in nutrient and trace metal accumulations in estuarine sediments. This influence appears to be substantial. Diatoms assemblages have changed significantly in the past 40-50 years These changes may be related to eutrophication, increased turbidity, loss of submerged aquatic vegetation, and increased freshwater flow to the estuaries. Preliminary results indicate that hypoxic and anoxic bottom water can most likely be reduced in the Pamlico with proper management of nutrients and sedimentation. However, hypoxic and anoxic waters in the Neuse may not be related to anthropogenic influences. Human impacts on estuarine water quality is evident, especially over the past 40-50 years. Population trends and land clearance also appear to have an influence on the estuaries, especially for the Neuse. The time frame of water quality changes seen in the Pamlico and Neuse estuaries occurs more recently than similar changes in the Chesapeake Bay. These differences may be due to the history of population and land use near the estuaries, and geomorphology of the estuaries.

Descriptors

Water quality, Nutrient loading, Eutrophication, Estuarine ecosystems, Algae, Land use, Nitrogen, Historical trends

Articles in Refereed Scientific Journals

Book Chapters

Dissertations

Kim, Sunghea, 1998, Heavy Metal Assessment in the Pamilico and Neuse River Estuaries of North Carolina, Masters Project to partially fulfill the requirements of the Masters of Environmental Management Degree from Nicholas School of the Environment, Duke University, Durham, NC, 47. (Under the direction of S. Cooper)

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Conference Proceedings

Other Publications

Cooper, S.R., 1999, A Journey Through Time: Paleoecology of Estuaries, Geotimes, 44(5), 14-18.

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Enviromental and Human Health Impact of Swine Waste Management Practices (70170, 70173)
Project Number	C-08
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Focus Category #2	Non Point Pollution
Focus Category #3	Waste Water
Lead Institution	Water Resources Research Institute of The University of North Carolina

Principal Investigators

Principal Investigators			
Name	**Title During Project Period**	**Affiliated Organization**	**Order**
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Mark D. Sobsey	Professor	University of North Carolina at Chapel Hill	08

Problem and Research Objectives

Nationwide, agriculture is the most important nonpoint source polluter (Sharpley and Meyer 1994). The swine industry is an increasingly important agricultural sector in the Southeastern United States. Rapid regional industry expansion has been accompanied by a shift from family farms to industrial scale animal production in confined quarters. This unprecedented growth presents enormous challenges with regard to disposal of swine manure. For example, swine waste production in North Carolina alone is 8 Tg yr-1, which includes 48 Gg N (Crouse 1995). Regionally, waste is commonly stored in anaerobic lagoons and the liquid phase is land-applied as fertilizer at no more than the agronomic nutrient (in this case N) requirement for the host crop. Improper management may encourage offsite transport, leading to enhanced N loading in adjoining ecosystems, particularly N-sensitive rivers, estuaries and coastal waterways. Clearly, agricultural water quality priorities relative to disposal of swine waste must include development and implementation of Best Management Practices (BMPs) and land use policies that both minimize loss of plant-available N from the site of application and meet the nutritional N requirements of the host crop. This is best accomplished through comprehensive studies that encompass all aspects of N cycling dynamics in spray fields, including simultaneous analysis of changes in pool sizes and rates of microbial and plant N transformations. Regional information of this nature is lacking. Traditional BMPs for agricultural animal wastes have focused primarily on nutrients. However, it is now recognized that

these waste materials can harbor numerous enteric human pathogens that will contaminate the environment and pose a human health risk if not properly managed. In particular, the protozoans Cryptosporidium parvum and various species of Salmonella bacteria are present in hog waste. These pathogens have been responsible for numerous outbreaks of gastrointestional illness caused by contaminated drinking water (MMWR 1996). Although Cryptosporidium, Salmonella and other human pathogens of animal origin are widespread in animal wastes, little is known about the relative contribution of agricultural animal wastes to environmental contamination of water resources. On a more basic level, even less is known about the effectiveness of waste treatment systems such as anaerobic lagoons in reducing pathogens or microbial indicators of fecal contamination. In order to better characterize the potential risks to human health and to identify appropriate regional (BMPs), information is needed on: (a) the occurrence, survival and treatment or Cryptosporidium, Salmonella and other human pathogens in stored hog waste; and (b) the effectiveness of current and candidate waste treatment processes at removing and destroying these pathogens before they are discharged into the environment. This two part project is aimed at: (a) obtaining a comprehensive N mass balance for liquid swine effluent applied seasonally to mesoscale (4m X 4m) experimental plots at different loading rates to determine the relative importance and relevant time scales for physical (NH3 volatilization, leaching) and biological (plant and microbial activities) processes involved in N transformations and transfers among reservoirs; and (b) characterizing and quantifying the reductions of pathogens and microbial indicators by conventional and alternative treatment systems for swine waste. These objectives are directly responsive to the several target areas of the 1998 Regional Water Resources Competitive Grants Program (Southeast and Island Region); namely, "problems from non-point sources of both municipal and agricultural sources," "use and user impacts on water quality," and "water quality problems associated with eutrophication and weed control".

Methodology

Whalen (1) General Experimental Design and Field Sample Collection Triplicate 4 m x 4 m (16 m2) and 2 m x 2 m (4 m2) experimental plots within the spray field will be fertilized with liquid swine effluent at volumes corresponding to 0.63, 1.25 or 2.5 cm additions (one plot each size and volume). These dosages agree with standard facility and industry practices. Collector cups (125 ml) will be positioned along a grid at 0.5 m intervals within plots to ensure homogeneous application with a backpack sprayer. Random samples will be analyzed for NH4+ and total-N. All plots will be spaced at 20 m intervals along a transect perpendicular to the direction of the prevailing wind with the larger plots arranged adjacent to one another along the transect. This spacing and orientation will facilitate utilization of a shared power source at the larger plots (see below, Section 12.1d) and will prevent cross-fertilization with volatilized NH3 via wind drift. Plots will be fertilized no sooner than 7 d following the previous whole field application. The 16 m2 plots will be intensely sampled for 14 d post-fertilization. A tipping bucket rain gauge will be deployed at the site to record precipitation. Installation of permanent sampling devices (gas flux chambers; Section 12.1g) and core collection in each 16 m2 plot will be limited to the area at least 0.5 m interior to the plot boundary to eliminate edge effects (c.f. Weed and Kanwar 1996). Cores removed from the 16 m2 plots will be immediately replaced with cores from similarly fertilized 4 m2 plots to minimize the effect of destructive sampling. The spray field shows no slope and sandy soils to a depth of at least 2 m, hence we expect no lateral post-spray transport of effluent. During each sampling session, a soil temperature profile will be taken at each site with a portable multi-thermister probe (2 cm intervals to 20 cm) and the mean soil temperature will be calculated. Unless noted, cores will be collected with a 5 cm diameter punch auger capable of being fitted with removable stainless steel inserts. Where appropriate (noted below in descriptions of specific methodologies) the following physicochemical determinations will be made on sieved (2 mm mesh), homogenized cores according to standard procedures (Carter 1993): soil pH, organic content, gravimetric moisture content, % water

holding capacity (WHC), KCl-extractable NO3--N and NH4+-N (hereafter referred to simply as NO3--N and NH4+-N) and total-C and total-N (dry combustion). Liquid waste samples will be analyzed for NH4+-N and total-N according to Parsons et al. (1984). Soil and liquid waste samples for nutrient analyses will rapidly be frozen in liquid N2 upon collection and transported on ice to the lab. Following are specific methods that will be used to assess N pool sizes and rates of transfer among compartments in Fig. 1. Efforts at assessing internal cycling will focus on the aboveground biomass and soil zone to 20 cm. Our ongoing research at this site shows that microbial activity is confined to this soil zone and soil roots are concentrated in the upper 10 cm. Hereafter, the surface 20 cm soil zone will be referred to as the "active soil zone." Zero Time and Final Time N Pool Sizes Pretreatment (zero time; T0) N pools will be assessed in soil and plant samples collected adjacent to the 16 m2 plots. Quadruplicate soil cores to 100 cm will each be homogenized (10 cm sections in the active zone and 20 cm sections thereafter) and analyzed for NH4+, NO3- and total-N (T0-OUT). Quadruplicate 20 cm diameter cores will be collected in the active zone adjacent to the chambers and cleaned of surface detritus. Roots will be separated from the soil by hydropneumatic elutriation (Smucker et al. 1982). Above- and belowground biomass and detritus will be dried, weighed and assayed for total-N. Soil microbial biomass N in the active zone will be analyzed by the fumigation-extraction procedure (Brookes et al. 1985; Vance et al. 1987). An additional set of samples collected after 14 d (final time; TF) from within (TF-IN) and outside (TF-OUT) each 16 m2 plot after and will be analyzed as described above. Bulk dry mass and concentration data will be used to calculate N pool sizes in soil, plant, detritus and microbial N in the active zone. Data for TF-OUT - T0-OUT will be used to assess changes in pools in the absence of fertilization, while data for TF-IN - T0-OUT will be used to calculate changes that occurred in the fertilized plot. Data will be corrected for changes that occurred outside the plot during the experimental period to evaluate the direct effect of fertilization. These data only evaluate the net change in pool sizes within the active soil zone. Following are methods used to assess rates of physical and biological processes that mediate N transfers among compartments within the active soil zone or N export from the active soil zone. Soil Emission of NH3 to the Atmosphere A dynamic chamber method (Ruess and McNaughton 1988) will be used to measure NH3 emission from the soil. Air will be drawn with a vacuum pump through a foil-covered chamber (25 cm diameter x 25 cm long) driven 3 cm into the soil. The flow rate will mimic the mean monthly wind velocity. The foil cover and air flow will minimize the heat load and inhibit formation of a soil surface boundary layer. Each chamber will be fitted with a thermistor to monitor air temperature. The intake will be oriented into the prevailing winds and will consist of a 1 cm diameter tube that extends at ground level to 1 m outside the 16 m2 plot. In-line gas (1 N H2SO4) and distilled water traps will strip NH3 and humidify the incoming air. Five percent of the chamber exhaust will be directed to a gas trap (1 N H2SO4) to collect NH3 emitted from the soil surface. A single chamber will be deployed in each plot and flux measurements will be made for 1 h at 4 h intervals from the time of fertilizer application to 12 h and at 18 and 24 h. Additional measurements of 4 h duration will be made at 48 h and at 2 d intervals thereafter to 14 d. Fresh gas traps will be used for each flux measurement and the NH4+ concentration of each trap will be determined as described above. This original system is 97% efficient at trapping NH3 volatilized in the chamber. We have modified some aspects of the original published method to accommodate the needs of this study after extensive discussion with the senior author, R.W. Ruess. Inorganic-N Export Below the Active Soil Zone Loss of inorganic-N from the active zone will be assessed by comparing soil NO3- and NH4+ concentrations in the 20 cm to 1 m zone in quadruplicate cores collected outside the plots prior to fertilization with similar data for cores collected inside and outside the plots 14 d post-fertilization (Section 12.1c). Bulk density and concentration data will be used as outlined above (Section 12.1c) to calculate mass N loss from the active zone as a result of fertilization. Suction lysimetry in conjunction with the addition of a stable conservative tracer (Cl- or Br-) is frequently employed to assess water and solute transport in the vadose zone. Spatial changes in the NO3-/tracer ratio are used to infer N loss to denitrification or volatilization in mass balance studies (e.g. Kessavalou et al. 1996). This methodology is unacceptable for the proposed research for three reasons. First, Groffman et al. (1995) demonstrated that both Cl-

and Br- significantly inhibit N mineralization and nitrification and depress denitrification at typical loading rates. These microbial processes are a primary focus of the proposed research. Second, N loss through volatilization (Section 12.1d) and denitrification (Section 12.1g) will be directly measured here, hence there is no need for a tracer to infer losses from these processes. Third, initial lysimeter deployment disturbs the soil and may influence the region of lysimeter influence (Wu et al. 1995), which are both factors of concern in short duration (<1 yr) experiments. Net Transfers and Transformations Within the Soil Inorganic-N Pool Net transfers and transformations within the soil inorganic-N pool will be assessed by measuring NO3- and NH4+ concentrations in 20 cm cores partitioned into 5 cm sections. Changes in nutrient pools will be computed from concentration and dry soil mass data. These changes will reflect the net influence of processes depicted in Fig. 1 on inorganic-N pools. Duplicate cores will be collected immediately following fertilization and at 3, 6, 12, 24 and 48 h post-fertilization and at 2 d intervals thereafter to 14 d. Many field incubation schemes have been proposed to simultaneously measure net N-mineralization, net nitrification and possibly plant assimilation and leaching loss (Raison et al. 1987; Debosz and Vinther 1989; Hart et al. 1994). To some degree, all involve long-term incubations and soil confinement that can both lead to experimental artifacts and disrupt natural feedbacks and interactions. Further, these methods require high replication to account for field-scale heterogeneity. The technique proposed here involves few samples collected from a relatively small area (heterogeneity minimized) and requires no soil confinement or lengthy incubation. Gaseous N2 and N2O Loss from Denitrification Denitrification rates will be determined by a static core technique (Groffman et al. 1993). Duplicate 20 cm cores will be collected in 30 cm core liners immediately following fertilization and at 3, 6, 12, 24 and 48 h post-fertilization and at 2 d intervals thereafter to 14 d. Liners will be sealed, the headspace will be amended with C2H2 to 10kPa, and the core (plus liner) will be incubated in situ (replaced in the collection hole) for a 1 h equilibration period. Core headspaces will be syringe-sampled following the equilibration period and at 0.5 and 1 h thereafter. Syringe gas samples will be transported to the laboratory and analyzed for N2O by electron capture gas chromatography (GC-ECD) within 24 h of collection. The internal headspace volume of each core will be determined with a pressure transducer according to Parkin et al. (1984). Acetylene inhibits the reduction of N2O to N2. The rate of N2O accumulation will therefore be used in conjunction with the internal headspace volume to obtain an area-based estimate N2O + N2 production by denitrification. A static chamber technique (Whalen and Reeburgh 1988) will be used to determine rates of N2O emission from denitrification within each plot. Essentially, an inverted cylinder (20 cm diameter X 12 cm height) will be placed over a permanently deployed soil collar (base) to isolate a parcel of air (headspace). The time-linear rate of concentration change of N2O in the headspace over 40 min (samples collected at 10 min intervals) will be used to calculate an area-based flux and the chamber top will be removed between sampling sessions to allow natural soil-atmosphere gas exchange. Flux determinations will be coordinated with core-based denitrification estimates (above) to determine the percent contribution of N2O to total gas (N2 + N2O) emission from denitrification. Duplicate soil collars will be permanently deployed at each plot for N2O flux determinations. Sampling frequency for determinations of N2O flux and denitrification will be increased following any post-fertilization rainfall to match the level proposed for the initial 24 h after fertilizer application. Denitrification is stimulated by rainfall (Tiedje 1988). Data Analysis Experiments are designed to provide a firmly based analysis of N cycling dynamics in response to fertilization, including exports and transformations that influence plant and soil N pools. The scope and labor-intensive nature of the proposed research precludes replication of treatments (loading rates) or soil types. Our ongoing work on various N transformations at this and other spray fields indicates that the proposed methodology is proven and sampling frequencies are adequate to capture anticipated fluxes during each experimental fertilization. Sufficient information will be collected to assign values (rates and mass) to transformations and reservoirs (surface water loss assumed nil). Methods of flux and mass calculation are given above when each technique is discussed. Rate data will be time-integrated to compare observed and predicted TF pool sizes and assess an error of closure. Rates of physical and

biological processes affecting N cycling will be correlated with environmental measures such as rainfall, soil temperature and N pool sizes to gain insight into the near-term (single event) importance of these drivers as a function of season and loading rate. Finally, these data can be used to determine whether loading rates or seasonality or combinations of the two promote N accumulation in the soil, which could ultimately lead to offsite transport via surface or groundwater in a time scale beyond the scope of these experiments. Finally, on a regional scale, these experiments will give some indication whether spray fields should be more closely evaluated as a point source of atmospheric N2O. Field-scale, long-term experiments involve considerable labor and expense and are beyond the anticipated funding level of this initiative. This mesoscale (several m2), event-based (individual applications) investigation on a regionally common soil type is less expensive and provides a detailed, conceptual understanding of physicochemical and biological interactions involved in soil N cycling dynamics and soil-atmosphere exchange of gaseous N that are mediated seasonally by a spray event. Data collected here will be the first of its kind and will therefore be invaluable to assess the performance of current BMPs for swine waste disposal and to intelligently modify these BMPs. Sobsey (2) Pathogen Research This research will characterize the pathogen reduction effectiveness of anaerobic lagoons, constructed wetlands, solids separation, and hog house waste collection using state-of-the-art methodology to characterize and quantify the fate of indicator organisms and selected pathogens. Changes in concentrations of total nitrogen, ammonium nitrogen (NH4+-N) and total phosphorous also will be tracked to evaluate how the different treatment techniques and management options affect both pathogen and nutrient removal. Analytical Procedures for Indicator Organisms Fecal coliforms, Eschericia coli, enterococci, Clostridium perfringens, somatic coliphages and male-specific (F+) coliphages are the indicators to be analyzed. Bacterial analyses will be by membrane filtration methods using standard media (Standard Methods for the Examination of Water and Wastewater 1995). Media are: mFC agar for fecal coliforms, nutrient agar-MUG for identification of which fecal coliforms are E. coli, modified mE agar for enterococci, and mCP agar and anaerobic incubation for C. perfringens followed by ammonium hydroxide fume exposure of presumptive colonies. Somatic and male-specific coliphages are analyzed by double agar layer (DAL) and single agar layer (SAL) plaque assay methods on hosts E. coli CN-13 and Salmonella typhimurium WG-49, respectively (Adams 1959; Grabow and Coubrough 1986; Sobsey et al 1990; 1995). Representative male-specific coliphage plaques in wastewater samples will be tested for RNAse sensitivity and, if RNA phages, they will be serotyped to one of four groups: I (animal origin), II (human origin), III (human origin), or IV (animal origin) (Hsu et al. 1995). Analytical Procedures for Pathogens. Field and laboratory samples will be analyzed for Salmonella spp. as important bacterial pathogens and Cryptosporidium parvum as a key protozoan pathogen. Salmonella spp. will be enriched in modified Rappaport-Vassilidis (RV) enrichment media (Vassilidis 1983; Vassilidis et al. 1978) followed by plating onto XLD, SS, and other Salmonella selective agars. Presumptive Salmonella colonies will be biochemically analyzed using API-20 or Enterotube kits (Oragui et al. 1993), and serologically confirmed (Perales and Audicana 1989; Watson 1985; Emperanza-Knorr and Torrella 1995). Another approach will be enrichment followed by RNA extraction and detection by Salmonella gene probe (non-radioactive 16S ribosomal RNA oligonucleotide probe) and hybrid detection by chemiluminescent methods. Cryptosporidium oocysts are recovered from 1-L wastewater samples by centrifugation, purified by immunomagnetic separation (IMS), and examined microscopically after fluorescent-antibody labeling. Oocyst will be assayed for viability by vital dye staining with DAPI and propidium iodide (Campbell et al. 1992; Grimason et al. 1994; Robertson et al. 1992) and for cell culture infectivity of MDCK cells followed by fluorescent antibody detection with C3C3-monoclonal antibody labeled with rhodamine (Arrowood et al. 1994; 1996). Analytical Methods for Nutrients Total nitrogen, NH4+-N, and phosphorous will be analyzed according to Standard Methods for the Examination of Water and Wastewater (1995). Pathogen and Indicator Reduction Characterization Samples of influent and effluent will be collected monthly for a period of one year from anaerobic lagoons at four hog farms representing differing types of swine operations (small-scale, large-scale, nursery, finishing, etc.) to characterize the effectiveness of currently operating lagoons in reducing

levels of indicators, Salmonella spp. and Cryptosporidium. The monthly samples will also be analyzed for TKN, nitrate, NH4+-N, and total phosphorous. These data will be combined with previous data to provide results for an entire year. Hydraulic Studies In order to investigate potential modifications of anaerobic lagoon design to improve pathogen reductions, one or more bench-scale models will be built to model observed field conditions. Tracer studies (fluoride, lithium or fluorescent dyes) will be performed (using these bench-scale units at three hydraulic residence times (HRT) to determine differences between actual and theoretical HRT for the bench-scale units. Wastewater from the field lagoon sites will be collected, transported to the laboratory, and stored at 4EC or -20EC for subsequent use in bench-scale tracer and treatment studies. After the bench-scale tracer studies are completed, the system(s) will continue to be operated. Influent and effluent samples will be collected and analyzed for indicator organisms and nutrients to generate to baseline performance estimate for the bench-scale unit (s). Once a baseline has been developed for the HRT, indicator/pathogen removal, and nutrient reduction achieved by the bench-scale model(s), the effect of installing baffles on the performance of the bench-scale unit(s) will be investigated. Our hypothesis is that the installation of baffles into existing lagoons modifies the hydraulics of the lagoon system from a theoretical complete-mix batch reactor to more of a plug flow system. Plug flow hydraulics may allow for increased, but directed, variation in the composition of the microbial communities in the lagoons. The increased HRT of the baffled lagoons and the differential metabolic activities of the spectrum of microbial communities that from plug flow dynamics may improve pathogen and indicator reductions. After analyzing the indicator and nutrient removal effectiveness of the baffled system(s), the influent to the bench-scale unit(s) will be spiked (in separate studies) with known quantities of Salmonella spp. organisms and Cryptosporidium oocysts to investigate whether installation of baffles in the bench-scale lagoon units improves the removal of these pathogens. If the results of the bench-scale tests demonstrate potential benefits of baffled lagoons, baffles will be installed in 2 currently operating, full-scale units previously selected for tracer studies. Two tracer studies will be done on each of the baffled full-scale lagoons during the same season(s) that the baseline tracer studies were performed, with monthly samples collected for analysis of microbial indicators and nutrient parameters. Microbial Sedimentation and Survival Studies Sedimentation columns and batch containers will be used in lab studies on sedimentation and survival (die-off). These studies are conducted to associate HRT with pathogen removal due to particle settling/natural die-off in anaerobic lagoons. Test wastewater will be from one or more of the four field sites. Sedimentation studies will be performed to characterize the rates of reduction of microbial indicators as well as added Salmonella bacteria and Cryptosporidium oocysts due to sedimentation/natural die-off by withdrawing wastewater samples from different points in a column of wastewater over time and analyzing for the indicators and the selected pathogens. Die-off Studies Die-off kinetics of microbial indicators, Salmonella spp. and Cryptosporidium will be studies in one or more of the lagoons at the selected study sites using microbial dialysis chambers. These chambers will be filled with lagoon wastewater and spiked with known quantities of Salmonella typhimurium and Cryptosporidium oocysts. The removal and die-off rates of these pathogens will then be tracked over time. Cryptosporidium oocysts viability and infectivity will be enumerated and assayed using previously described methods. Salmonella typhimurium will be assayed by the enrichment MPN methods described previously. Microbial Indicator Reduction Characterization Samples will be collected monthly for a period of one year from the pilot-scale constructed wetlands system at a Duplin County, NC, study site. This system is composed of three sets of parallel wetlands units, each comprised of two cells in series. The constructed wetlands were designed as surface flow (SF). Wastewater samples from two of the parallel SF wetlands units will be analyzed for microbial indicators. These two wetlands units will be operated at different HRTs to investigate the effect of HRT on indicator and nutrient reduction efficiency. Two replicate tracer studies will be performed for both the units, one during the summer and the other during the winter. The measured HRT from these tracer studies will be compared to the theoretical HRT for the units. Pathogen and Indicator Reduction Characterization in Mini-wetlands Small (approximately 1 m by 0.5

m) wetland cells will be used to evaluate the effectiveness of SF and subsurface flow (SSF) constructed wetlands for reducing the concentrations of indicators, Salmonella spp. and Cryptosporidium in effluent from primary treatment (e.g., anaerobic lagoons). These small-scale cells will be planted with vegetation similar to the larger pilot-scale units at the Duplin County site. Wastewater (anaerobic lagoon effluent or solids separated wastewater) from one or more of the study sites will be transported to the lab, stored refrigerated, continuously mixed and pumped into six mini-wetland units (four SF units and 2 SSF units). Pumping rates to the mini-wetland units will be varied to evaluate performance at different HRTs. Units will be operated at selected HRTs for 2 month periods. Samples will be collected weekly (and sometimes daily) to determine how the mini-wetlands perform as they stabilize (over a period of weeks), as well as within a diurnal cycle. In addition to determining mini-wetland performance for reducing microbial indicators and nutrients, influent wastewater to the small-scale cells will be dosed with Salmonella typhimurium and Cryptosporidium parvuum oocysts to determine the removal performance for these pathogens. Solids Separators A field study will be done to evaluate microbial indicator reductions using solids separators for primary treatment of wastewater from hog houses. At swine operations currently using solids separators, influent and effluent wastewater samples will be collected and analyzed for microbial indicators and nutrient parameters. The removal efficiencies for the solids separators will be compared to the removal efficiencies achieved by the currently operating anaerobic lagoons. Pit-Plug vs. Water-Wash House Flushing Systems A laboratory study will be performed to evaluate whether there is a pathogen reduction benefit of pit-plug vs. water-wash systems. Pit-plug systems are those in which the waste collection channels beneath the hog house pig pens are filled with water and collected hog waste is periodically removed from beneath the hog house by 'pulling the plug' and letting the wastewater flow by gravity into the primary treatment unit. Water-wash systems utilize recirculated water from a wastewater lagoon to periodically flush hog waste from beneath the hog house into the receiving lagoon. Pit plug systems may achieve some pathogen reduction because wastewater sits beneath the hog house for approximately one-half to 1 week before being released to the receiving lagoon. The laboratory study will investigate the die-off rate of microbial indicators under laboratory conditions simulated to be similar to those present in the waste collection channels in pit-plug systems. Data Analysis and Interpretation The data on concentrations of pathogens, indicators and nutrients in treated and untreated wastewater will be analyzed to determine the extent of reduction of these parameters. The frequency and concentrations of pathogens and indicators in raw, untreated wastes will be compared to that of the wastes subjected to specific treatment processes (i.e., lagooning, solids separation, constructed wetlands). By construction of and comparisons between or among the frequency distributions of pathogen concentrations in raw and treated wastes, quantitative reductions of the microbes by the specific treatment processes will be determined. Nonparametric t-tests will be performed on influent and effluent data to determine if the various treatment units achieve significant reductions in mean concentrations of each indicator, pathogen, and nutrient. T-tests also will be performed to determine whether alternative treatment units achieve significantly different reductions of indicators, pathogens,and nutrients. For studies where HRT is varied, regression analysis will be performed to relate indicator, pathogen and nutrient reduction performance to HRT. For constructed wetlands, regression analysis also will be performed to evaluate the significance of hydraulic loading rate, influent concentration, and ambient temperature in predicting wetland performance. Statistical analyses also will be performed to determine which indicators are most reliable in predicting the presence and concentrations of pathogens. Indicators will be evaluated on the basis of sensitivity, specificity, accuracy, reliability, technical difficulty, speed and cost. One goal of the analyses is to determine if levels of indicators (fecal coliforms, enterococci, C. perfringens, coliphages, etc.) in wastewater samples can be used to predict and quantify the presence and levels of the study pathogens (Salmonella spp., Cryptosporidium parvum) in wastewater. A 2x2 table is created to cross tabulate the level of a specific pathogen (high versus zero or low) against levels of an indicator (high versus zero or low). Sensitivity, specificity and measures of agreement (e.g., kappa statistic) will be calculated. This analysis is repeated to compare each pathogen to each indicator, in turn. Data on pathogen and indicator

survival (die-off) in swine wastewater will be analyzed by regression methods to determine the rate and extent of pathogen and indicator reduction as a function of time. Data will be expressed as the log10 of initial organisms surviving or remaining as a function of time: log10NtNo where No is the initial concentration of organisms at zero time, and Nt is the concentration of organisms at time = t. If die-off data are first-order, then the slopes of their regression lines can be used to compare die-off rates among the different pathogens and indicators. If the data are not first-order, then alternative regression or other quantitative measures of persistence will be used. These die-off data will be compared among and between indicators and pathogens. These comparisons will make it possible to determine not only how persistent pathogens are under certain waste and incubation conditions, but also which indicators, if any, are adequately predictive of pathogen persistence (or die-off). Similar statistical approaches will be used to determine if there are relationships between microbe reductions and nutrient reductions.

Principal Findings and Significance

1. Whalen (Progress Report) Environmental influences and controls on rates of nitrification and denitrification in agricultural soils were studied with intact soil cores and homogenized soil composites under controlled laboratory conditions. The time sources for accumulation of NO3-N, N2O-N+N2-Nor CO2-C were used as measures of net nitrification, denitrification and overall microbial activity, respectively, in response to the manipulated variable. Depth profiles of nitrifying activity and denitrifying enzyme activity induced that >85% of the potential activity of these two microbial groups was located in the upper 20 cm of soil. Hence, efforts focused on this soil zone. Net nitrification rates in intact soil cores showed a positive response to increasing temperature for cores incubated at 10, 20, 30 0C. Similarly, homogenized soil composites showed increased rates of nitrification to a temperature optimum of 33 0C, with rates declining at higher temperatures. The Q10 for nitrification was 3.2. Net nitrification rates in both intact soil cores and homogenized composites were relatively insensitive to changes in soil moisture to the point of saturation. However, rates at 30% water-holding capacity (WHC) were significantly higher than rates at 100% WHC. Net rates of nitrification clearly exhibited a dose response, with successively more NH4-N oxidized as the loading rate for swine waste increased from 0.7 to 2.6 cm. An older spray field (10 years of fertilization) showed two-fold higher rates of nitrification at all doses than a fallow field, suggesting the "aged" spray fields develop an endogenous microbial population that is highly efficient at processing applied waste. A nitrogen mass balance on cores consistently indicated a disappearance of inorganic-N 24 hours after application and a reappearance by the end of the experiment at 10 days, suggesting immobilization and subsequent remineralization. This is supported by analysis of CO2-C efflux from soil cores. Denitrification rates in homogenized soil composites showed a positive response to increasing temperature. The calculated temperature optimum and Q10 were 49 0C and 1.8, respectively, pointing to a higher optimum and decreasing sensitivity to temperature change with respect to nitrifiers. Denitrification rates were low and relatively insensitive to changes in soil moisture until soils became water-saturated. Rates then increased dramatically, indicating a threshold for denitrification at or near the point of saturation. Denitrification also increased with increasing dose of waste. Roughly 3 to 5% of the added waste was lost to coupled nitrification-denitrification immediately upon application. Studies of substrate limitation of denitrification show that denitrification is stimulated by both labile-C and NO3-N addition, but the most stimulatory response is a combination of the two. Overall, these soils show an endogenous population of nitrifiers and denitrifiers that is capable of responding instantly to a spray event. Rapid nitrification of these soils allows for the accumulation of NO3, but these sandy soils drain rapidly, permitting only a brief post-spray period of coupled nitrification-denitrification. Subsequent rain will stimulate denitrification and promote more fertilizer loss as N2 (2) Sobsey (Progress Report) This study continues to evaluate microbial reductions in conventional, alternative and additional swine waste treatment processes, including anaerobic lagoons, constructed wetlands, biofilters, an electro-reactor and UV

irradiation systems. Two-stage anaerobic lagoons as well as constructed wetlands continue to demonstrate considerable reductions of indicator and pathogenic microbes, as does a biofilter (Ekocan process) and UV irradiation of biologically treated swine effluent. Each of these processes reduce enteric microbes by about 99-99.99%, depending on swine waste quality, treatment operation conditions and environmental conditions. Untreated swine waste contains high concentrations of Salmonella bacteria, with concentrations in the thousands per 100 ml. Salmonella levels in the swine waste are reduced by treatment processes similar to the reductions of fecal coliform and E. coli bacteria. When the most effective treatment processes are used, Salmonella are reduced to low and even undetectable levels. However, swine waste treatment by single stage anaerobic lagoons achieves only modest Salmonella reductions and high concentrations sometimes remain in the treated effluent. Using two or more of these processes in series dramatically reduced enteric microbes in swine wastes to levels comparable to those in disinfected, secondary treated, municipal sewage effluent. These results suggest that adequately treated swine waste may be treatable to sufficient microbial quality for land application, surface water discharges, or beneficial reuse (greenhouse production of produce or horticulture plants). The laboratory wetlands study was conducted with an ambient temperature of 30 0EC and loading rate of 40 KG TKN. The efficacy of nitrogen metabolism by the different wetlands configurations and the reductions of fecal indicators microbes and Salmonella bacteria differ with the type of wetland (surface flow -SF and subsurface flow -SSF) and the presence of vegetation (SSF with plants and SSF without plants). It appears that the subsurface wetland system is more efficient in reducing microbes than the surface wetland system. Also, the presence of plants in the subsurface wetland system improved microbial reduction even more.

Descriptors

Soil microbiology, Soil chemistry, Nitrogen, Animal waste, Fertilizer, Pathogens, Viruses, Lagoons

Articles in Refereed Scientific Journals

Whalen, S.C., 1999, Simplified Procedure for Rapid Manual Analysis of Nitrate in Soil Extracts by Copper-Cadmium Reduction. Communications in Soil Science and Plant Analysis, 30, 1633-1641.
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Book Chapters

None

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Brown, Dan, 1999, The Effect of Environmental Variables on Nitrification in Agricultural Soils Amended with Liquid Lagoonal Swine Waste, M.S. Dissertation, Dept of Environmenal Science and Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC, . (Under the direction of Steve Whalen)

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Other Publications

Information Transfer Program

Publications The Institute follows an established review process for all of its publications. It has a stated policy that research completion reports shall be reviewed by at least three peer reviewers prior to publication. An editorial review committee has been created to assist with decisions regarding the inclusion of a report in the WRRI series. Newsletters - The Institute NEWS, a 16-page newsletter, was distributed bimonthly to more than 4,100 federal and state agencies, university personnel, multi-county planning regions, city and local officials, and engineers. The NEWS regularly covers a wide range of water-related topics from current federal and state activities and new research findings to special announcements and listings of new publications. This newsletter can also be electronically obtained through our homepage on the world wide web: (URL=http://www2.ncsu.edu/ncsu/CIL/WRRI). The Sediments News, a 6-page newsletter, is published quarterly and 5,600 copies are distributed by the Institute for the N.C. Sedimentation Control Commission. The newsletter provides information and assistance to the regulated community and facilitates communication among personnel of state and local erosion and sediment control programs. New Research Reports - A strong demand for Institute reports continues. During the year, the Institute published the following reports for distribution to users throughout the state and nation: WRRI-312 Management of Forested Filter Zones for Dispersion and Treatment of Agricultural Runoff WRRI-313 Nutrient Limitation and Eutrophication Potential in the Cape Fear and New River Estuaries WRRI-314 Water Quality Effects of Above-Stream Fish Feeders in Low-Nutrient North Carolina Mountain Streams WRRI-315 Effect of Organic and Inorganic Nutrient Loading on Photosynthetic and Heterotrophic Plankton Communities in Blackwater Rivers WRRI-316 Impact of Wastewater Quality on the Long-Term Acceptance Rate of Soils for On-Site Wastewater Disposal Systems WRRI-317 Denitrification Dynamics of an Estuarine Headwater Creek Receiving Agriculture Runoff WRRI-318 An In Vitro Test for Estrogenicity Combining Cultured Hepatocytes and an Enzyme-Linked Immunosorbant Assay (ELISA) WRRI-319 A New Method for Characterizing Aquatic Organic Matter WRRI-320 Slurry-Phase Bioremediation of Contaminated Soil From a Former Manufactured-Gas Plant Site Publications List - A 24-page listing of all the publications that the Institute has published to date was revised and made available. This includes 364 individual publications. Conferences, Workshops, Forums and Seminars During the past year the Institute co-sponsored and helped plan two conferences, five workshops, four forums, and eight seminars on key water issues. The following information is a brief description of them. Stream Restoration. In April, WRRI and the North Carolina Chapter of the American Water Resources Association conducted a forum on stream restoration. Individuals representing four different perspectives of stream restoration gave presentations on the Neuse Buffer Rule, restoration options, data collection, and practical examples in urban settings. Water Quality Issues in North Carolina: Gauging the Environment. In September, WRRI and the North Carolina Chapter of the American Water Resources Association presented a forum on stream gauging stations. Robert Hirsch, U.S. Geological Survey-National Headquarters, discussed the role of USGS data in addressing water quantity and quality issues. He focused his presentation on the USGS stream gauging network and how the data are being utilized in North Carolina. Erosion and Sediment Control Fall Design Workshops. In September and October, WRRI, the N.C. Sedimentation Control Commission and the North Carolina Department of Environment and Natural Resources (NCDENR), Division of Land Resources, Land Quality Section, held two, 2-day workshops in Raleigh and Hickory, North Carolina, on erosion and sediment control design. The workshops were held to familiarize design professionals with the erosion and sediment control requirements and design applications. Topics such as overview of regulations and governor's action plan, plan requirements, channel design, basic hydrology, efficiency based sediment basin design, turbidity, storm water, and buffers, pipe and dissipater design, and engineering planning were discussed. Storm Water Management Conference. Also in October, WRRI and the North Carolina Chapter of the American Public Works Association conducted a conference on storm water management. Participants learned more about how closely storm water BMPs and watershed management are linked. Other topics such as regulatory update, NPDES Phase II, cooperative efforts, meeting NPDES requirements, stream corridor management, innovative erosion control practices, and urban storm water

management BMPs were discussed. Neuse River Basin Nutrient Management Strategies. In December, WRRI and the North Carolina Chapter of the American Water Resources Association presented a forum on nutrient management strategies for the Neuse River Basin. A staff member from the N.C. Division of Water Quality provided an overview of the Neuse River Basin. An individual representing the Upper Neuse River Basin Association and the Lower Neuse River Basin Association provided their perspective on the issues facing this basin and what each association is doing to address nutrient management. Erosion and Sediment Control Workshop for Local Programs. In January, WRRI, the N.C. Sedimentation Control Commission and NCDENR, Division of Land Resources, Land Quality Section, held a two-day workshop to allow local programs to get together and exchange ideas and practices utilized at the local level. Topics such as sedimentation control commission action plan, gray areas in enforcement, turf reinforcement matting, plan design and review, three local program presentations, and an open forum were discussed. Erosion and Sediment Control Spring Design Workshops. In February and March, WRRI, the N.C. Sedimentation Control Commission and the NCDENR, Division of Land Resources, Land Quality Section, presented two, 2-day workshops in Greenville and Hickory, North Carolina, on erosion and sediment control design. The workshops were held to familiarize design professionals with the erosion and sediment control requirements and design applications. Topics such as current regulations, basic hydrology, storm water management, design for construction, new technology, vegetation cover, and design/install were discussed. There was also an open discussion with panel members representing regulators, developers and design professionals. Sediment: Approaches to Reducing Impacts. Also in February, WRRI, and the North Carolina Chapter of the American Water Resources Association conducted a forum on sedimentation. The two speakers addressed agriculture and construction as major sources of sediment. They reviewed practices that would greatly reduce sediment movement to streams. A three-hour workshop earlier in the day was held to tour local sediment control measures and discuss them in more detail. WRRI Annual Water Resources Research Conference. Also in March, WRRI held their annual conference with this year's theme being water quality and enhancement. There were 27 speakers providing their research findings to over 400 conference participants. Presentations were divided into six themes of: Neuse River monitoring and modeling; river, lake and stream processes; tools for assessing impacts on water quality; best management practices/storm water; and water and wastewater treatment. The morning plenary session had two speakers discussing the water supply needs in North Carolina. During lunch, the speaker addressed the importance of citizen involvement in watershed management. Water Resources Research Seminar Series. WRRI continued a seminar series on current water resource issues and research projects funded by the Institute. The seminars occurred once a month during the year except for the months of June, July, August, and December. The following seminars were given during FY 1998-99: A Review of Research Concerning On-Site Wastewater Systems - Professor Aziz Amoozegar - Department of Soil Science, North Carolina State University Neuse River Water Quality Monitoring - Associate Professor Richard Luettich - Institute of Marine Science, University of North Carolina at Chapel Hill and Professor Larry Crowder - Nicholas School of the Environment, Duke University Marine Laboratory Ponds and Wetlands for Water Supply Protection - Assistant Professor Sarah Liehr - Department of Biological & Agricultural Engineering, North Carolina State University Two Approaches to Modeling the Neuse River Estuary: Mechanistic and Network Analysis Models - Assistant Professor James Bowen - Department of Engineering, University of North Carolina at Charlotte and Professor Robert Christian - Department of Biology, East Carolina University Microbial Impacts from Animal Waste - Professor Mark Sobsey - Department of Environmental Sciences and Engineering, University of North Carolina at Chapel Hill Water Quality Trends in the Neuse and Pamlico Basins - Professor Donald Stanley - Institute of Marine and Coastal Resources, East Carolina University Effect of Management Practices on Land Application of Swine Waste - Assistant Professor Steven Whalen - Department of Environmental Sciences and Engineering, University of North Carolina at Chapel Hill Optimizing Buffers to Reduce Pollutants in Runoff - Associate Professor Richard McLaughlin - Department of Soil Science, North Carolina State University

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Erosion and Sediment Control Design Workshops
Description	These 4-workshops (2 in the fall and 2 in the spring) are jointly conducted by the N.C. Sedimentation Control Commission, N.C. Division of Land Resources - Land Quality Section, and N.C. Water Resources Research Institute. The workshops are held to familiarize design professionals with the erosion and sediment control design.
Start Date	02/01/1998
End Date	03/28/1999
Type	Conferences
Lead Institution	Water Resources Research Institute of The University of North Carolina

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Basic Project Information	
Category	**Data**
Title	The Institute NEWS
Description	A 16-page newsletter that covers a wide range of water-related topics from current federal and state activities and new research findings to special announcements and listings of new publications. This newsletter is published bi-monthly.
Start Date	03/01/1998

End Date	02/28/1999
Type	Newsletter
Lead Institution	Water Resources Research Institute of The University of North Carolina

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Basic Project Information	
Category	**Data**
Title	The Sediments News
Description	A 6-page newsletter that provides information and assitance to the regulated community and facilitates communication among personnel of state and local erosion and sediment control programs. This newsletter is published quarterly.
Start Date	03/01/1998
End Date	02/28/1999
Type	Newsletter
Lead Institution	Water Resources Research Institute of The University of North Carolina

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Basic Project Information	
Category	**Data**
Title	NCWRA/WRRI Forums
Description	Five forums were held jointly by the N.C. American Water Resources Association and N.C. Water Resources Research Institute. Over 250 people attended these forums covering topics such as stream restoration, stream gauging stations, storm water management, Neuse River nutrient management strategies,and approaches to reducing the impact of sediment.
Start Date	03/01/1998
End Date	02/28/1999
Type	Conferences
Lead Institution	Water Resources Research Institute of The University of North Carolina

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Basic Project Information	
Category	**Data**
Title	Water Resources Research Seminar Series
Description	This is an annual series of seminars on current water resources issues and research projects funded by the Institute. The seminars occur one afternoon each month during the year except for the months of June, July, August and December.
Start Date	03/01/1998
End Date	02/28/1999
Type	Conferences
Lead Institution	Water Resources Research Institute of The University of North Carolina

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Basic Project Information	
Category	**Data**
Title	Erosion and Sediment Control Workshop for Local Programs
Description	This 2-day workshop is jointly conducted by the Sedimentation Control Commission, N.C. Division of Land Resources - Land Quality Section, and N.C. Water Resources Research Institute. The annual workshop allows local erosion and sediment control programs to get together and exchange ideas and practices utilized at the local level.
Start Date	01/26/1999
End Date	01/27/1999
Type	Conferences
Lead Institution	Water Resources Research Institute of The University of North Carolina

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Basic Project Information

Basic Project Information	
Category	**Data**
Title	WRRI Annual Water Resources Research Conference
Description	The annual conference draws over 400 people that listen to 27 speakers provide information on their research findings. This year's presentations were divided into six themes of:colon; Neuse River monitoring and modeling, river, lake and stream processes, tools for assessing the impacts on water quality, best management practices/storm water, and water and wastewater treatment.

	[illegible]
Start Date	03/25/1999
End Date	03/25/1999
Type	Conferences
Lead Institution	Water Resources Research Institute of The University of North Carolina

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USGS Internship Program

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 RCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	0	4	1	20	25
Masters	0	4	1	41	46
Ph.D.	0	3	0	15	18
Post-Doc.	0	1	0	0	1
Total	0	12	2	76	90

Awards & Achievements

Three key staff members of the Institute with over 78 years of combined dedication to WRRI retired during this last year. The retiring members include: Linda Lambert, Administrative Officer with 30 years of service; Eva Walters, Program Assistant with 29 years of service; and Frances Yeargan, Accounting Technician with 20 years of service. The Institute will miss the experience these ladies provided in "keeping the WRRI running smoothly" over the years. Dr. Kenneth H. Reckhow, Director of the Water Resources Research Institute and Professor, Nicholas School of the Environment, Duke University, was awarded an additional $720,000 by the Department of Environment and Natural Resources for the Neuse River Modeling and Monitoring Project (ModMon). This award was to continue the development of Total Maximum Daily Loads (TMDL) for the Neuse River Basin. The project pulls together researchers from six universities and staff from the U.S. Geological Survey. Dr. Viney P. Aneja, Professor, Department of Earth and Atmospheric Sciences, North Carolina State University, was presented in 1998 with the Frank A. Chambers Award for outstanding achievement in the science and art of air pollution control. The Air & Waste Management Association presents this annual award for accomplishments of a technical nature on the part of the recipient, which are considered to be major contributions to the science and art of air pollution control, the merit of which has been widely recognized by persons in the field. The coverage is intentionally broad since it is expected to recognize achievement in any line of technical endeavor in air pollution control, from pure research to applied science. Dr. James E. Shelton, Associate Professor Emeritus, Soil Science Department, North Carolina State University, received two awards in 1996 that have not been reported. The U.S. Environmental Protection Agency, Region 4 awarded Dr. Shelton with the "Beneficial Use of Biosolid Award." He also received the "Recycler of the Year Award" from the North Carolina Recycling Asociation. The Second Annual Water Resources Research Conference titled "North Carolina Resources: Water Quality Trends and Enhancement" was successful in drawing over 400 participants to hear from 28 speakers. Topics included: Aspects of the Neuse River; River, Lake and Stream Processes; Tools for Assessing Impacts of Water Quality; Best Management Practices/Storm Water; and Water/Wastewater Treatment. There also was a poster session with over 15 projects

represented. The Water Resources Research Institute has a website(http//www2. ncsu.edu/ncsu/CIL/WRRI/) that was developed for the World Wide Web (WWW) in 1995. Within the last year, Jeri Gray our Technology Transfer Specialist and Editor has developed three new websites. These sites have been developed for the North Carolina Division of Land Resources - Land Quality Section(http//www.dlr.enr.state. nc.us/), Urban Water Consortium - Water and Wastewater Group (http//www2.ncsu.edu/ncsu/CIL/WRRI/uwc) and Urban Water Consortium - Storm Water Group(http//www2.ncsu.edu/ncsu/CIL/WRRI/stormwater /index.html). All of the sites have been created for WWW users to obtain current information on these subjects.

Publications from Prior Projects

Articles in Refereed Scientific Journals

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Book Chapters

None

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