

Fate and Effects of Nitrogen and Phosphorus in Shallow Vegetated Aquatic Ecosystems



Fate and Effects of Nitrogen and Phosphorus in Shallow Vegetated Aquatic Ecosystems

James F. Fairchild
U.S. Geological Survey
Columbia Environmental Research Center
4200 New Haven Road
Columbia, Missouri 65201

Leigh Ann Vradenburg
Currently with: Willow Creek Reclamation Project
28 Tyndal Street
Monte Vista, Colorado 81144

Interagency Agreement No. DW14938599-01

Project Officer
Timothy J. Canfield
Ground Water and Ecosystems Restoration Division
National Risk Management Research Laboratory
Ada, Oklahoma 74820

National Risk Management Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

Notice

The U.S. Environmental Protection Agency through its Office of Research and Development funded, managed, and collaborated in the research described here under contract IAG DW14938599-01 to the USGS, Columbia, MO. It has been subjected to the Agency's peer and administrative review and has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

All research projects making conclusions or recommendations based on environmental data and funded by the U.S. Environmental Protection Agency are required to participate in the Agency Quality Assurance Program. This project did not involve the collection or use of environmental data and, as such, did not require a Quality Assurance Project Plan.


Foreword

The U.S. Environmental Protection Agency is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The National Risk Management Research Laboratory is the Agency's center for investigation of technological and management approaches for preventing and reducing risks from pollution that threatens human health and the environment. The focus of the Laboratory's research program is on methods and their cost-effectiveness for prevention and control of pollution to air, land, water, and subsurface resources; protection of water quality in public water systems; remediation of contaminated sites, sediments and ground water; prevention and control of indoor air pollution; and restoration of ecosystems. NRMRL collaborates with both public and private sector partners to foster technologies that reduce the cost of compliance and to anticipate emerging problems. NRMRL's research provides solutions to environmental problems by: developing and promoting technologies that protect and improve the environment; advancing scientific and engineering information to support regulatory and policy decisions; and providing the technical support and information transfer to ensure implementation of environmental regulations and strategies at the national, state, and community levels.

This publication has been produced as part of the Laboratory's strategic long-term research plan. It is published and made available by EPA's Office of Research and Development to assist the user community and to link researchers with their clients.

The U.S. Geological Survey's Columbia Environmental Research Center is located in Columbia, MO. The Center conducts interdisciplinary research on the existing and potential effects of various chemical, physical, and biological stressors on fish and aquatic communities to provide scientific understanding and technologies needed to support sound management and conservation of natural resources. The Center includes five research branches: Ecology, Toxicology, Environmental Chemistry, Biochemistry and Physiology, and Field Research. Activities are integrated among the Research Branches of the Center via multi-disciplinary research teams. These research teams conduct research and provide technical assistance for use by federal and state agencies to predict and evaluate the effects of contaminants and other stressors on fish, invertebrates, aquatic plants, and other components of aquatic ecosystems. Research results are widely recognized and applied by national and international research and management organizations.



Stephen G. Schmelling, Director
Ground Water and Ecosystems Restoration Division
National Risk Management Research Laboratory

Contents

| | |
|---|------|
| Foreword | iii |
| Figures | ix |
| Tables | xii |
| Acknowledgments | xiii |
| Executive Summary | xv |
| Introduction..... | 1 |
| Methods..... | 2 |
| Study Site..... | 2 |
| Corral Construction and Design..... | 2 |
| Dosing | 3 |
| Water Chemistry | 3 |
| Phytoplankton | 4 |
| Periphyton | 4 |
| Zooplankton | 4 |
| Macrophytes..... | 4 |
| Sediment..... | 5 |
| System Metabolism..... | 5 |
| Statistical Analysis | 5 |
| Quality Assurance Summary for Nutrient Analyses..... | 5 |
| STUDY 1: Effects of N:P Ratio and Biweekly Nutrient Loading | 7 |
| Experimental Design..... | 7 |
| Results | 7 |
| Macrophytes | 7 |
| Macrophyte Biomass..... | 7 |
| Macrophyte Taxa | 10 |
| Macrophyte Nutrients..... | 10 |
| Macrophyte Nutrient Stock..... | 12 |
| Water Chemistry..... | 13 |
| Phosphorus | 13 |
| Nitrogen..... | 16 |
| Nitrogen:Phosphorus Ratio | 22 |
| pH..... | 23 |
| Alkalinity and Hardness | 24 |
| Conductivity..... | 26 |
| Turbidity | 27 |
| Phytoplankton..... | 28 |
| Periphyton..... | 33 |
| Zooplankton | 34 |
| Sediment | 37 |
| System Metabolism | 39 |
| Net Nutrient Balance | 41 |

| | |
|---|-----|
| STUDY 2: Effects of Dosing Prior to Macrophyte Development..... | 43 |
| Experimental Design..... | 43 |
| Results | 43 |
| Macrophytes | 43 |
| Macrophyte Taxa | 43 |
| Macrophyte Biomass..... | 45 |
| Macrophyte Nutrients..... | 46 |
| Macrophyte Nutrient Stock..... | 48 |
| Water Chemistry..... | 50 |
| Phosphorus..... | 50 |
| Nitrogen..... | 52 |
| Nitrogen:Phosphorus Ratio..... | 55 |
| pH..... | 56 |
| Alkalinity and Hardness | 57 |
| Conductivity..... | 59 |
| Turbidity..... | 60 |
| Phytoplankton..... | 61 |
| Periphyton..... | 68 |
| Zooplankton..... | 69 |
| Sediment | 72 |
| System Metabolism | 73 |
| Net Nutrient Balance | 75 |
| STUDY 3: Effects of Dosing in Relation to Macrophyte Stage..... | 77 |
| Experimental Design..... | 77 |
| Results | 77 |
| Macrophytes | 77 |
| Macrophyte Taxa | 77 |
| Macrophyte Biomass..... | 79 |
| Macrophyte Nutrients..... | 80 |
| Macrophyte Nutrient Stock..... | 82 |
| Water Chemistry..... | 84 |
| Phosphorus..... | 84 |
| Nitrogen..... | 87 |
| Nitrogen:Phosphorus Ratio..... | 91 |
| pH..... | 92 |
| Alkalinity and Hardness | 93 |
| Conductivity..... | 95 |
| Turbidity..... | 96 |
| Phytoplankton..... | 97 |
| Periphyton..... | 101 |
| Zooplankton..... | 102 |
| Sediment | 103 |
| System Metabolism | 105 |
| Net Nutrient Balance | 106 |

| | |
|---|-----|
| Discussion | 107 |
| Nutrient Dissipation Rates | 107 |
| Nutrient Stocks..... | 107 |
| Phytoplankton Dynamics..... | 107 |
| Phytoplankton Biomass and Growth Rates..... | 107 |
| Phytoplankton Species Composition..... | 108 |
| Periphyton Dynamics | 109 |
| Periphyton Biomass and Growth Rates..... | 109 |
| Macrophyte Dynamics..... | 110 |
| Macrophyte Biomass and Growth Rates..... | 110 |
| Macrophyte Species Composition..... | 111 |
| Macrophytes as Nutrient Sinks..... | 112 |
| Macrophytes as Nutrient Sources | 112 |
| Zooplankton Dynamics | 113 |
| Sediments as Nutrient Sources and Sinks | 113 |
| Conclusions, Management Implications, and Research Needs..... | 115 |
| Literature Cited..... | 117 |
| Appendix 1 | 121 |
| Figures | 126 |
| Tables | 141 |

Figures

STUDY 1

| | |
|--|----|
| 1. Pond and corral diagram for Study 1 experiments indicating corral orientation and diameter..... | 8 |
| 2. Changes in macrophyte biomass over time | 9 |
| 3. Changes in macrophyte nitrogen content over time..... | 10 |
| 4. Changes in macrophyte phosphorus content over time | 11 |
| 5. Changes in macrophyte nitrogen stock over time | 12 |
| 6. Changes in macrophyte phosphorus stock over time | 13 |
| 7. Changes in total phosphorus by phosphorus and nitrogen dose levels over time..... | 14 |
| 8. Changes in soluble reactive phosphorus by phosphorus dose levels over time..... | 15 |
| 9. Changes in soluble reactive phosphorus dissipation rates over dose periods | 16 |
| 10. Changes in total nitrogen by nitrogen dose levels over time..... | 17 |
| 11. Total and nitrate nitrogen over the season pooled by N-dose level | 18 |
| 12. Changes in nitrate by nitrogen dose levels over time..... | 19 |
| 13. Changes in nitrate dissipation rates over dose periods | 19 |
| 14. Nitrate concentrations by N-dose over the 4-week extended monitoring periods at the midseason and the end..... | 20 |
| 15. Changes in ammonia by nitrogen dose levels over time..... | 21 |
| 16. Changes in the ratio of total nitrogen to total phosphorus by treatment over time | 22 |
| 17. Changes in pH by nitrogen dose levels over time | 23 |
| 18. Changes in alkalinity by nitrogen dose levels over time..... | 24 |
| 19. Changes in hardness by nitrogen dose levels over time..... | 25 |
| 20. Changes in conductivity by nitrogen dose levels over time..... | 26 |
| 21. Changes in turbidity by nitrogen dose levels over time | 27 |
| 22. Changes in phytoplankton chlorophyll by treatment over time..... | 28 |
| 23. Changes in phytoplankton abundance of the four dominant divisions over time | 32 |
| 24. Changes in periphyton chlorophyll accrual rates over time..... | 33 |
| 25. Changes in total numbers of copepods over time..... | 34 |
| 26. Changes in zooplankton species richness over time | 35 |
| 27. Changes in sediment nitrogen pool over time..... | 37 |
| 28. Changes in sediment phosphorus pool over time | 38 |
| 29. Changes in community gross oxygen production by phosphorus dose levels over time..... | 39 |
| 30. Changes in community gross respiration of oxygen by phosphorus dose levels over time..... | 40 |

STUDY 2

| | |
|---|----|
| 31. Corral and pond diagram for Study 2 experiments indicating orientation of corrals | 44 |
| 32. Changes in macrophyte biomass over time | 45 |
| 33. Changes in macrophyte nitrogen content over time..... | 46 |
| 34. Changes in macrophyte phosphorus content over time | 47 |
| 35. Changes in macrophyte nitrogen stock over time | 48 |
| 36. Changes in macrophyte phosphorus stock over time | 49 |
| 37. Changes in total phosphorus concentrations over time | 50 |
| 38. Changes in soluble reactive phosphorus concentrations over time | 51 |
| 39. Changes in total nitrogen concentrations over time | 52 |
| 40. Changes in nitrate concentrations over time | 53 |
| 41. Changes in nitrate dissipation rates over dose periods | 53 |
| 42. Changes in ammonia over time..... | 54 |
| 43. Changes in the ratio of total nitrogen to total phosphorus over time | 55 |
| 44. Changes in pH over time..... | 56 |
| 45. Changes in alkalinity over time..... | 57 |
| 46. Changes in hardness over time..... | 58 |
| 47. Changes in conductivity over time..... | 59 |
| 48. Changes in turbidity over time..... | 60 |
| 49. Changes in phytoplankton chlorophyll concentrations over time | 61 |
| 50. Changes in particulate organic carbon concentrations over time | 62 |
| 51. Changes in the ratio of particulate organic carbon to phytoplankton chlorophyll over time..... | 63 |
| 52. Changes in phytoplankton abundance of the dominant divisions over time | 63 |
| 53. Changes in accrual rates of periphyton chlorophyll in 1- and 2-week exposures over time..... | 68 |
| 54. Changes in zooplankton abundance over time | 69 |
| 55. Changes in the sediment nitrogen pool over time..... | 72 |
| 56. Changes in the sediment phosphorus pool over time..... | 73 |
| 57. Changes in community oxygen and respiration production over time..... | 74 |

STUDY 3

| | |
|---|----|
| 58. Corral and pond diagram for Study 3 experiments indicating orientation of corrals | 78 |
| 59. Changes in macrophyte biomass over time | 79 |
| 60. Changes in macrophyte nitrogen content over time..... | 80 |
| 61. Changes in macrophyte phosphorus content over time..... | 81 |
| 62. Changes in macrophyte nitrogen stock over time | 82 |
| 63. Changes in macrophyte phosphorus stock over time | 83 |
| 64. Changes in total phosphorus concentrations over time | 84 |

| | |
|---|-----|
| 65. Changes in soluble reactive phosphorus concentrations over time | 85 |
| 66. Changes in soluble reactive phosphorus dissipation over dose periods | 86 |
| 67. Changes in total nitrogen concentrations over time | 87 |
| 68. Changes in nitrate concentrations over time | 88 |
| 69. Changes in nitrate dissipation over dose periods | 89 |
| 70. Changes in ammonia concentrations over time | 90 |
| 71. Changes in the ratio of total nitrogen to total phosphorus over time | 91 |
| 72. Changes in pH over time..... | 92 |
| 73. Changes in alkalinity over time..... | 93 |
| 74. Changes in hardness over time..... | 94 |
| 75. Changes in conductivity over time..... | 95 |
| 76. Changes in turbidity over time | 96 |
| 77. Changes in phytoplankton chlorophyll concentrations over time | 97 |
| 78. Changes in particulate organic carbon concentrations over time | 98 |
| 79. Changes in the ratio of particulate organic carbon to phytoplankton chlorophyll over time..... | 99 |
| 80. Changes in phytoplankton abundance of the dominant divisions | 100 |
| 81. Changes in accrual rates of periphyton chlorophyll in 1- and 2-week exposures over time..... | 101 |
| 82. Changes in zooplankton abundance over time | 102 |
| 83. Changes in sediment nitrogen pool over time..... | 103 |
| 84. Changes in sediment phosphorus pool over time..... | 104 |
| 85. Changes in community oxygen and respiration production over time..... | 105 |

Tables

| | |
|--|-----|
| 1. Summary of Recovery Data for Nutrient Analyses Recoveries | 6 |
| 2. Target Dose Ratios (N:P) for Treatments Receiving Both Nutrients in the Early Dosing Period of Study 1 | 8 |
| 3. Target Dose Ratios (N:P) for Treatments Receiving Both Nutrients in the Late Dosing Period of Study 1 | 8 |
| 4. List of Phytoplankton Species Collected during Study 1 | 29 |
| 5. List of Zooplankton Species Collected during Study 1 | 36 |
| 6. Summary Table of the Final Store of P and N in Water and Macrophytes in Study 1 | 41 |
| 7. List of Phytoplankton Species Collected during Studies 2 and 3 | 64 |
| 8. List of Zooplankton Species Collected during Studies 2 and 3 | 70 |
| 9. Summary Table of the Final Store of P and N in Water and Macrophytes in Study 2 | 75 |
| 10. Summary Table of the Final Store of P and N in Water and Macrophytes in Study 3 ... | 106 |

Acknowledgments

Rachael Bredfelt, Dan Carnegy, Brian Fuhr, Curt Gately, Mandi Hapner, Travis Hill, David Hughes, Lynne Johnson, Julie Kisely, Patti Kohn, Ben Lakish, Steve Olson, Jennifer Riddle, and Chris Witte provided technical assistance in data collection, entry, and analysis during the course of the study. Bill Mabee (Missouri Dept. of Conservation, Columbia, MO) and John Beaver (BSA Environmental Services, Beechwood, OH) identified the zooplankton. John Beaver (BSA Environmental, Beechwood, OH) and Cheryl Gilpin (New Braunfels, TX) identified the phytoplankton. Dr. William Richardson (USGS, LaCrosse, WI), Nile Kemble (USGS, Columbia, MO), and three anonymous reviewers from the U.S. EPA provided insightful reviews of the report. This research was funded by the U.S. Environmental Protection Agency under IAG DW14938599-01.

Executive Summary

Nitrate concentrations have greatly increased in streams and rivers draining agricultural regions of the Midwestern United States. Increasing nitrate transport to the Gulf of Mexico has been implicated in the hypoxic conditions that threaten the productivity of marine fisheries. Increases in nitrate concentrations have been attributed to a combination of factors including agricultural expansion, increased nitrogen application rates, increased tile drainage, and loss of riparian wetlands. These landscape-level changes have resulted in a decreased natural capacity for nitrogen uptake, removal, and cycling back to the atmosphere. Land managers are increasingly interested in using wetland construction and rehabilitation as a management practice to reduce loss of nitrate from the terrestrial systems. Yet, relatively little is known about the limnological factors involved in nitrate removal by wetland systems.

We conducted a series of studies from 1999-2000 to investigate the functional capacity of shallow, macrophyte-dominated pond wetland systems for uptake, assimilation, and retention of nitrogen (N) and phosphorus (P). We evaluated four factors that were hypothesized to influence nutrient uptake and assimilation: 1) nitrate loading rates; 2) nitrogen to phosphorus (N:P) ratios; 3) frequency of dosing/application; and 4) timing of dose initiation.

Nutrient assimilation was rapid; more than 90% of added nutrients were removed from the water column in all treatments. Neither variation in N:P ratios (evaluated range: <13:1 to >114:1), frequency of application (weekly or bi-weekly), nor timing of dose initiation relative to macrophyte development (0%, 15–25%, or 75–90% maximum biomass) had significant effects on nutrient assimilation or wetland community dynamics. Maximum loading of nitrate (60 g N/m²; 2.4 g P/m²) applied as six weekly doses stimulated algal communities, but inhibited macrophyte communities.

Predicted shifts from a stable state of macrophyte- to phytoplankton-dominance did not occur due to nutrient additions. Macrophytes, phytoplankton, and the sediment surface were all significant factors in the removal of nitrate from the water column. Overall, these shallow, macrophyte-dominated systems provided an efficient means of removing nutrients from the water column. Construction or rehabilitation of shallow, vegetated wetlands may offer promise as land management practices for nutrient removal in agricultural watersheds.

Introduction

Anthropogenic eutrophication of water bodies has been a major aquatic research and management focus since the 1950's (Jansson et al. 1994; Smith, 2003). Point sources of nutrients, such as effluents from municipal and industrial facilities, have largely been identified and controlled via mechanical and engineering approaches to water pollution prevention. However, non-point sources of nutrients to water bodies have continued to rise due to expanded agricultural activities, increased application of fertilizers, fossil-fuel combustion, over-application of manure to crops, and runoff from urban areas (Vitousek et al. 1997; Carpenter et al. 1998). These non-point sources of nutrients are continuing to rise due to difficulties in source identification, lack of effective nutrient management strategies, and the lack of regulatory focus (Carpenter et al. 1998).

Shallow aquatic systems such as wetlands and ponds can act as sinks for nutrients, thereby significantly decreasing watershed export (Vitousek et al. 1997). Jansson et al. (1994) suggest that shallow ponds can provide the best means of nitrogen retention through sedimentation, uptake by vegetation, and denitrification. Assimilative processes may be facilitated in shallow environments because of the high surface areas of sediments and aquatic plants compared to pelagic systems (Gasith and Hoyer 1998). Phosphorus may likewise be assimilated into vegetation or retained in sediments during periods of high nutrient loading. However, under senescent or anoxic conditions, sediments may act as a nutrient source and result in release of nutrients to the water column (Scheffer 1998).

The establishment of macrophyte stands in shallow systems can increase nutrient retention and recycling (de Haan et al. 1993). During the growing season, macrophytes act as a sink by accumulating nutrients in developing tissues (Engel 1990). Weisner et al. (1994) demonstrated that removal of nitrate from the water column was significantly higher in vegetated than non-vegetated mesocosms due to uptake and denitrification. Macrophytes stimulate denitrification by lowering the redox potential in microzones at the sediment surface and releasing dissolved organic carbon. Therefore, shallow ponds utilized to reduce nutrients in surface waters may be most effective if macrophyte communities develop and persist (Jansson et al. 1994).

One factor that may diminish the establishment and persistence of macrophyte stands is a dense community of phytoplankton that may develop with nutrient enrichment. Lake and reservoir investigations have generally found an inverse relationship between macrophyte and phytoplankton communities where two alternative conditions may exist: 1) a macrophyte-dominated system containing clear water and low phytoplankton biomass, or 2) high phytoplankton biomass, with turbid water and poor macrophyte development. These "alternative stable states" of macrophyte or phytoplankton dominance are relatively persistent and do not readily alternate unless conditions are disrupted by external or internal forces (Scheffer 1990, 1998).

Nutrient ratio is a primary determinant of primary production in aquatic systems (Sakamoto 1966, Wetzel 1983). Optimum ratios of nitrogen:phosphorus (N:P ratio) are approximately 13 (mass:mass basis) in aquatic systems; ratios that are under 10 are generally considered nitrogen-limited, and ratios above 17 are generally considered phosphorus-limited (Redfield et al. 1963; Sakamoto 1966). Thus, aquatic systems that receive nutrient inputs near the optimum level will achieve a maximum level of primary productivity with efficient utilization of nutrients and minimal dissolved nutrient accumulation in the water column. Nutrient ratios that are limiting in one nutrient frequently exhibit elevated dissolved forms of the nutrient in excess. Thus, nutrient uptake and retention is maximal when the N:P ratio is near the 13:1 optimum. Nutrients other than nitrogen or phosphorus can be limiting (e.g., silica, carbon dioxide) in some systems, but most are typically limited by phosphorus or nitrogen. Other factors can cause departures from expectations based on ratios of dissolved nutrients. For example, internal sources of nutrients from sediments are an especially critical component in shallow aquatic systems such as wetlands (Sand-Jensen and Borum 1991; Scheffer 1998). In addition, intense grazing of phytoplankton by zooplankton (such as in the absence of fish predators) can increase turnover rates of phosphorus and sustain productivity under conditions of low P supply and low algal biomass (Wetzel 1983).

Another primary factor influencing whether a system is macrophyte or phytoplankton-dominated is nutrient loading (Scheffer 1998). When total phosphorus is below 20 $\mu\text{g P/L}$, algal turbidity and shading are minimal, thereby allowing for the proliferation of the macrophyte community (Mjelde and Faafeng 1997). Conversely, at high N and/or P loadings, algal biomass can rapidly increase beyond zooplankton grazing demands and thus dominate aquatic systems due to shading of macrophytes. Dominance, however, is not absolute because other factors such as non-algal turbidity, water depth, and season can alter predictions and outcomes (Scheffer 1998).

Timing of nutrient loading, rather than the absolute amount of nutrient input, may also be influential in the determination of macrophyte or phytoplankton dominance. Algal communities stimulated by nutrient enrichment early in the growing season can result in significant shading effects and thus hinder development of macrophytes (Phillips et al. 1978). However, some macrophytes may out-compete phytoplankton by early season accumulation and storage of available nutrients (Ozimek et al. 1990). It has been demonstrated that established macrophyte stands can maintain dominance despite increases in loading (Balls et al. 1989). Such communities may respond with a change in composition to tall-growing species that are better able to compete with epiphytes and phytoplankton shading (Moss 1990). Established macrophyte stands can also reduce the amount of nitrogen in the water column, thereby inhibiting algal taxa that are not able to fix atmospheric nitrogen. These nitrogen decreases may be the result of macrophyte uptake or the facilitation of denitrification. Much less is known regarding the uptake and assimilation of phosphorus in shallow vegetated systems (Scheffer 1998).

There are other biological factors that may influence the relative contribution of algal and macrophyte communities to aquatic productivity and nutrient cycling. For example, shallow ponds and wetlands may not support fish communities because of extreme temperature fluctuations and low dissolved oxygen (Bronmark and Hansson 1998). In the absence of fish predation, large-bodied zooplankton frequently dominate and exert extreme grazing pressure on the phytoplankton (Brooks and Dodson 1965) which can promote water clarity and increase growth and stability of macrophyte communities (Moss 1995, Scheffer 1998). Zooplankton can exert variable grazing pressure on phytoplankton, though, and therefore may not always be inversely related to phytoplankton biomass (Mitchell et al. 1988). Grazing may be ineffectual in controlling a filamentous algal community, which is less palatable to grazers than smaller-celled micro-algal species (Mayer et al. 1997). It has also been observed that nitrogen-limited systems may have decreased grazing pressure by zooplankton due to proliferation of large and generally unpalatable cyanobacteria (Jensen et al. 1994).

Much of the research regarding eutrophication of aquatic systems has been conducted using fertilization experiments. Studies have demonstrated that macrophytes show a variable response to nutrient loading, and that the relative capacity of a system for nutrient retention may depend on the resulting dominant community. Mulligan et al. (1976) used experiments at two fertilization levels in shallow ponds without fish to evaluate the fate of added nutrients and effects on the macrophyte community. With the highest load, they found that dense communities of phytoplankton inhibited or eliminated macrophyte development. Balls et al. (1989) conducted enrichment experiments in constructed ponds to explore the mechanisms of macrophyte loss in local water bodies that had lost submerged plant communities. In their experiments, macrophytes strongly buffered against all levels of nutrient enrichment and maintained dominance whether or not fish were present; however, experimental treatments included several phosphorus levels but only one nitrogen level. Stachowicz et al. (1994) fertilized a field pond over several years across various states of macrophyte and phytoplankton dominance to evaluate nutrient retention. They found that phosphorus retention was high under both phytoplankton and macrophyte dominance; however, macrophytes were far more effective at reducing the export of nitrogen. Therefore, research has demonstrated the complexities between nutrient enrichment and community interactions; yet few studies have comprehensively evaluated the full range of factors that may influence the uptake, assimilation, and retention of nitrogen and phosphorus in experimental wetland systems. Such extensive studies are logistically complex, but are necessary to isolate the relative effects of nutrient loading, ratios, frequency, and timing on retention and community dynamics (Moss 1995; Havens et al. 1999).

The objective of this study was to systematically evaluate the assimilative capacity of shallow, vegetated experimental wetlands for the uptake, removal, and retention of nitrogen and phosphorus. Three factors were evaluated: 1) the effect of N:P ratios; 2) the effect of loading or dosing rates of N and P; and 3) the effect of timing of dosing of N and P. The studies were conducted to explore the response of these experimental systems to nutrient manipulation under controlled, experimental conditions. The results are provided to explore the functional utility of using constructed wetlands as mitigation tools for removal of nutrients in runoff from agricultural watersheds.

Methods

Study Site

Studies were conducted at the experimental mesocosm facility located at the U.S. Geological Survey's Columbia Environmental Research Center (CERC), Columbia, MO. This facility was constructed in 1968 to provide a controlled experimental complex to evaluate the effects of environmental stressors on shallow aquatic systems. Individual impoundments are approximately 0.1 ha in area and range in depth from 0.1 to 1.5 m. Macrophyte communities are dominated by *Najas guadalupensis* and *Chara* sp. Physical, chemical, and biological characteristics of the mesocosms have been previously described (Fairchild et al. 1992, 1994; Fairchild and Sappington, 2002).

Corral Construction and Design

Circular corrals were used as the experimental treatment unit because they are highly replicable and reduce statistical variation typical of whole mesocosms. Multiple corrals were placed within each of 4 mesocosms; the mesocosm served as the experimental block.

Corrals were constructed of impermeable Scrimweave™ (StoCote Products, Chicago, IL) to create a circular enclosure of approximately 4-m diameter. Corral walls were secured to a circular ring of 2.5 cm diameter black polyethylene water pipe supported on steel fence posts (driven outside the corral) to maintain the upper edge of the corral approximately 20 cm above the water surface. The bottom edges of the corral wall were wrapped outward beneath a piece of circular metal garden edging, which was then driven approximately 8 cm into the sediments before the ponds were filled with water. The sides of the corrals were then weighted down with bricks while the ponds were filled with well water over a 2-d period. Once flooded, the mesocosms were allowed to mix and allow mobile biota to freely move within the system. Prior to dosing, the corral edges were raised and secured to effectively isolate the contents (water, sediment, and biota) within each individual corral.

Water exchange between the corral and the outside water was minimal as indicated by visual inspection (i.e., turbidity during wading on the outside of the corral) and analytical chemical data. Ponds were occasionally refilled with water during the season to maintain an average depth near 1 meter; water additions were conducted during non-critical periods of the dose/monitoring schedule to minimize artifacts of corral management. Depths ranged among the corrals from 0.74–1.16 m and averaged 0.91 m. Levels of water in each individual corral remained similar to that outside of the corral due to slow water diffusion through the sediments.

Dosing

The ranges of dose concentrations were selected based on the range of published spring and summer concentrations of N and P from Midwestern streams subject to agricultural runoff (Hauck et al. 1997). Magnitude of dosing was chosen based on literature reviews of studies (Johnston 1991; Mitsch et al. 1999) that indicate that natural wetlands can assimilate a range of 0.03 - 28 g N /m²/yr and 0.07-3.48 g P/m²/yr depending on a range of factors including wetland type, depth, vegetative structure, and hydrologic residence time.

Granular agricultural fertilizers (soda of nitrate and triple super phosphate) were used to dose the corrals. Amendments were calculated according to corral volumes and the percent of available N and P in the fertilizer. Fertilizer was pre-weighed, placed into a cotton bag, and agitated under the surface of the water inside the perimeter of the corral for approximately 5 minutes. The water was then gently mixed with a paddle to ensure nutrient distribution. Once granules were mostly dissolved, the bag was suspended in the water column to allow for release of residual nutrients in the material. Laboratory experiments prior to the start of the study indicated that nutrients were rapidly released into the water column. Rapid nutrient dissolution was also verified by measured nutrient concentrations in the corrals (see results). Nutrient bags were specific for each corral and were used throughout the experiment. Control corrals were similarly mixed to prevent experimental bias due to the physical disturbance of mixing.

Water Chemistry

Water samples were collected with a tube sampler (cylindrical sampler 7.62 cm diameter by 1 m length; vol.= 4560 cm³) deployed in a rapid, vertical motion to collect a depth-integrated water sample. Three vertical samples were composited in a clean 20-L polyethylene bucket. The composite was thoroughly mixed and then sub-sampled with a 1-L polyethylene bottle. The 1-L samples were immediately chilled on ice and transported to the laboratory for analysis. Unfiltered samples were kept on ice or refrigerated until processed or analyzed. All samples were analyzed within recommended time limits according to EPA standards (U.S. EPA 1979).

Approximately 250 mL of each water sample was filtered for dissolved nutrients using a 0.45 µm membrane (47 mm nitrocellulose filter; Whatman Inc., Clifton, NJ). All filtering equipment was thoroughly rinsed with deionized water between samples to minimize contamination. The filtrate (for dissolved constituents) was stored refrigerated ($\leq 4^{\circ}\text{C}$) in 60-mL Nalgene HDPE bottles or 10-mL capped, borosilicate disposable tubes until analysis.

Soluble reactive phosphorus (SRP) was determined using a color reagent and an MR 1201 Spectrophotometer as described by U.S. EPA Method 365.3 (U.S. EPA 1979). A Lachat 8000 Flow Injection Analyzer (Lachat Instruments, Milwaukee, WI) was used for analysis of nitrogen. The combined total of nitrate (NO₃-N) and nitrite (NO₂-N) was measured by a colorimeter after cadmium reduction to nitrite (Lachat Instruments 1997). Ammonia (NH₃-N) was measured colorimetrically after reactions with alkaline phenol, sodium hypochlorite, and sodium nitroprusside (Lachat Instruments 1997).

Unfiltered water was analyzed for total phosphorus and total nitrogen. Samples to be analyzed for total nitrogen and total phosphorus were stored frozen (<0°C) in 60-mL HDPE bottles. Before analysis, the samples were thawed, mixed, and pipetted into 10-mL glass tubes. Samples for total phosphorus were oxidized using potassium persulfate (Fisher Scientific, Fairlawn, NJ) and then analyzed for orthophosphorus on an MR 1201 Spectrophotometer (U.S. EPA 1979). The spectrophotometer was also used to determine total nitrogen as N after persulfate oxidation (Crumpton et al 1992).

The pH of each sample was determined using an Orion Model SA 290A pH meter in accordance with the manufacturer's recommendations. Alkalinity was determined by burette titration with 0.02 N sulfuric acid and expressed as mg

CaCO₃/L. Hardness (mg CaCO₃/L) was measured using a color indicator and burette titration with EDTA (APHA 1992). Conductivity was determined using an YSI Model 33 S-C-T meter (YSI Corp., Yellow Springs, OH) and expressed as μS/cm at room temperature (20–30°C). The HACH Model 2100A Turbidimeter (Hach Co., Loveland, CO) was used to estimate the turbidity (NTU's).

Phytoplankton

Phytoplankton biomass was based on the chlorophyll *a* content of algae. A measured amount of sample (25–250 ml) was filtered through a 47 mm glass fiber filter (Gelman type A/E; Fisher Scientific, Fairlawn, NJ). The filter was then placed in a 15-ml vial of 90% buffered acetone and refrigerated overnight for extraction. This extract was subsequently analyzed using a fluorometer (Turner Designs 10-AU-Fluorometer; La Jolla, CA) using EPA Method 445.0 (APHA 1992). Particulate organic carbon samples were filtered onto a 47-mm Gelman type A/E filter and then combusted and analyzed using a Coulometrics Model 2010 Total Carbon Analyzer (UIC Corporation, Wheaton, IL). Phytoplankton were sampled and preserved for taxonomic analysis on a monthly basis by preservation of 40 mL of the unfiltered water sample using 1 mL Lugol's solution. Phytoplankton were counted and identified by John Beaver of BSA Environmental Services, Beachwood, OH.

Periphyton

Periphyton biomass and accrual rates in the corrals were evaluated as chlorophyll concentrations extracted from growth on artificial substrates (1 cm by 10 cm strips of Scrimweave™ suspended vertically just below the surface of the water). In June of 1999, four strips were exposed for four weeks and then analyzed individually for chlorophyll. Future exposures were shorter to reflect the rapid growth of periphyton that was observed. In July of 1999, four strips were deployed. Two replicate strips were collected after 1-week and 2-week exposure intervals. In August and September, six strips were deployed, and three strips were collected and analyzed as a composite after each of 1-week and 2-week exposures. Strips were carefully collected with forceps in the field and immediately put on ice in vials of 15 mL of 90% buffered acetone. Periphyton chlorophyll was estimated using the same methods as for the phytoplankton; however, values were expressed as accrual rates (μg Chl/cm²/wk).

Zooplankton

Monthly zooplankton samples were collected. On May 12, 1999, (Study 1) zooplankton samples were collected using a 63 μm Wisconsin net and vertical tows to effectively sample a 10-L volume. Thereafter, zooplankton were sampled using vertical migration samplers modified from the design of Whiteside et al. (1978). Samplers consisted of a funnel and 2-L bottle assembly inverted and positioned in the water column just above the macrophyte layer. The funnel and bottle used were clear so as to minimize avoidance due to darkened conditions. Samplers were deployed at dusk and retrieved at dawn. These samplers passively trapped zooplankton during diurnal feeding movements. On retrieval, the samplers were poured through a 63 μm Wisconsin net to isolate the zooplankton. Samples were stored in 90% ethanol. Samples were analyzed by Bill Mabee, Missouri Dept. of Conservation, Columbia, MO. Sample numbers were then calculated on an area basis by dividing zooplankton number by the surface area of the funnel surface.

Macrophytes

Macrophytes were qualitatively assessed each month based on visual assessment and ranking of the benthic plant and filamentous algae communities; separate estimates were made within each of four quadrants of each corral. Assessments included estimates of percent cover, height, species composition, and color. There were only two species of macrophytes in the corrals (*Chara* sp. and *Najas guadalupensis*) which were easily distinguishable based on color and morphometrics. *Chara* sp. is a macroalgae and has an upright and branched thallus, and is attached to the substrate by rhizoids (Smith 1950; Kufel and Kufel 2002). *Najas* sp. is a submerged, branched macrophyte.

Macrophytes were quantitatively sampled each month from pre-set, buried standardized rings to minimize disturbance and sampling bias. Each ring (5 cm height; 10 cm diameter) was cut from a cross-section of white PVC water pipe. Replicate sampling rings were deployed in each of four corral quadrants to account for spatial variation within each corral. Prior to the study initiation, the rings were pushed into the sediments until flush with the top of the sediment layer. This technique made the rings easy to locate, but minimized shading or enclosure effects.

Monthly composite macrophyte samples were collected (one ring from each of the four quadrants) from each corral by divers wearing Neoprene wet suits. Wet suits allowed the divers to maintain neutral buoyancy and caused minimal disturbance to surrounding sediments and macrophytes (Madsen 1993). Collection involved diving to locate a ring and digging underneath it with a Plexiglas board. The board created a bottom for the encircled sediment and macrophyte sample, and enabled it to be brought to the surface for careful processing. Macrophyte material originating from the area enclosed by the ring was collected, including all above and belowground biomass. Composites of four rings per corral were stored in plastic bags on ice during transport to the lab. In the lab, the macrophytes were washed on a small mesh screen (<1 mm mesh) and any debris or attached sediment was carefully removed. Samples were then

placed in pre-weighed aluminum foil packets, dried at 105°C, and weighed to get an estimate of dry weight biomass (Madsen 1993). Biomass was expressed as dry weight (g/m²). A Wiley Mill was used to grind the dried samples, which were then stored in airtight vials.

Dried and ground macrophyte samples were subsequently analyzed for total nitrogen and total phosphorus content. Samples analyzed for N content were weighed (0.2 g) and then combusted in a LECO FP-528 Analyzer. This apparatus transformed sample nitrogen to N₂, which was then measured by thermal conductivity detection and expressed as percent of dry weight.

Total phosphorus in macrophyte tissues was determined in pre-weighed samples (0.2 g) using perchloric acid digestion (6% perchloric acid) based on the procedure of Sommers and Nelson (1972). This digestion process converts all phosphorus to orthophosphate in a clear supernatant. Orthophosphate was then determined using the Lachat 8000 FIA (Lachat Instruments, Inc. 2000). The results, expressed as µg P/L, were converted to a percent basis (mass:mass) normalized to the amount of plant material used in the digestion.

Sediment

Sediment samples were collected concurrently with macrophytes. One sediment plug of the top 2–5 cm of sediment was taken from each macrophyte ring and deposited in a plastic bag. Care was taken not to include any plant material with the sediment plug. The composite of sediment plugs for the four rings sampled in each corral was homogenized by hand and dried in foil pans at 105°C. Dried samples were ground using a Wiley mill and stored in airtight vials. Methods for analysis of nitrogen and phosphorus content of the sediments were the same as for macrophytes. At the beginning of the season, four separate rings were placed in the ponds. The sediment enclosed by them was collected, dried, and weighed to determine an average mass of sediment within a ring. Estimates of sediment nutrient pools (g N or P/(m²*5 cm deep)) were calculated by multiplying the percent content of nutrient by the average mass of sediment enclosed by the sampling ring, and then converting to a square meter of surface area.

System Metabolism

System metabolism was measured each week as a variation of the diurnal oxygen method outlined by Lind (1985). This method was chosen over traditional light-dark bottle techniques because evaluations were desired for the total system including macrophytes, phytoplankton, and sediments. Dissolved oxygen and temperature readings were taken with a YSI Model 54 Oxygen Meter in every corral on a consecutive morning, evening, and morning sequence. The first readings of each sequence coincided with water collection. Before field use, probes were calibrated in saturated air according to manufacturers' specifications. Dissolved oxygen and temperature were measured by submerging the probe to mid-depth of the water column to ensure homogeneity. All corrals were sampled in less than an hour to decrease temporal variability. Oxygen readings (mg O₂/L) were designated M1 (morning 1), E1 (evening 1), and M2 (morning 2). Gross production (GP) was calculated by: GP = (E1-M1)+(E1-M2). Gross respiration (GR) was calculated by: GR=2*(E1-M2) as a modification of Lind (1985).

Statistical Analysis

Data were tested for normality of distribution by treatment using Proc Univariate in the Statistical Analysis System, Release 6.12 (1996). The strong seasonal nature of the data (see Appendix 1) resulted in data that were not normally distributed with homogeneous variance. Therefore, all datasets were subsequently transformed using the rank procedure prior to analysis (Conover and Iman 1981). Although some statistical power was lost by rank transformation, this method provided the best means of analyzing all of the datasets uniformly (Snedecor and Cochran 1967, Green 1979). Transformed data were analyzed as a randomized complete block design using Analysis of Variance (ANOVA) to determine influences due to N-dose, P-dose, time, and their interactions (using pond as the block and corrals as experimental units). When ANOVA indicated significant main effects, we statistically compared individual treatments using the Student-T test. Significant differences between rank-transformed values were determined at the p≤0.05 level.

Quality Assurance Summary for Nutrient Analyses

A summary of quality assurance results for nutrient analyses is presented in Table 1. Results indicated that recovery of spiked standards ranged from 79–108% across the two years of study. Recoveries were within the range of acceptable results for these analyses.

Table 1. Summary of Recovery Data for Nutrient Analyses Recoveries. Numbers Represent Mean + 1 Standard Deviation. Number in Parenthesis is Number of Independent Standards Analyzed Each Year

| Nutrient | Year | |
|---------------------------------|------------------|------------------|
| | 1999 | 2000 |
| NH ₃ | 90.6 + 15.2 (63) | 85.9 + 14.7 (38) |
| NO ₂ NO ₃ | 87.6 + 19.0 (64) | 95.4 + 15.7 (38) |
| SRP | 94.2 + 4.6 (64) | 108 + 18.6 (38) |
| TN | 85.9 + 21.1 (30) | 79.1 + 19.4 (18) |
| TP | 94.3 + 2.0 (30) | 101.6 + 3.7 (18) |