Integrated Drainage-Wetland Systems for Reducing Nitrate Loads from Des Moines Lobe Watersheds

William G. Crumpton
Iowa State University, crumpton@iastate.edu

Matthew J. Helmers
Iowa State University, mhelmers@iastate.edu

Greg A. Stenback
Iowa State University, gastenba@iastate.edu

Dean W. Lemke
Iowa Department of Agriculture and Land Stewardship

Shawn Richmond
Iowa Department of Agriculture and Land Stewardship

Follow this and additional works at: http://lib.dr.iastate.edu/abe_eng_reports

Recommended Citation
http://lib.dr.iastate.edu/abe_eng_reports/13

This Report is brought to you for free and open access by the Agricultural and Biosystems Engineering at Iowa State University Digital Repository. It has been accepted for inclusion in Agricultural and Biosystems Engineering Technical Reports and White Papers by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
Integrated Drainage-Wetland Systems for Reducing Nitrate Loads from Des Moines Lobe Watersheds

Abstract
The main subject area for this project is the Des Moines Lobe in north-central Iowa drained mostly by the Des Moines, Raccoon, Iowa, and Skunk Rivers. The Des Moines Lobe represents the southernmost extent of the Prairie Pothole Region and is a relatively flat landscape with very poor natural drainage. However, as a result of extensive subsurface — tile drainage, the Des Moines Lobe has become some of the most valuable and productive land in the world. In 2011, the average land value in north-central Iowa was $7,356 an acre, and 80.5% of that area was in row-crops (42.9% in corn and 37.6% soybeans). Unfortunately, the Des Moines Lobe has also become a source of significant NO$_3^-$ loading to downstream waters.

The elevated nitrate loads from the Des Moines Lobe are largely a result of the changes in land-use and hydrology brought about by subsurface — tile drainage. Tile drainage has historically been treated and continues today as a conservation practice because it decreases surface runoff, thus reducing surface transported contaminants such as phosphorus, sediment, ammonium-nitrogen, pesticides and pathogens. However, tile drainage also increases subsurface flow and leaching losses of NO$_3^-$. This is due mostly to an increase in the subsurface fraction of the total annual discharge and the "short-circuiting" of subsurface flow, but also in part to the increased aeration of organic-rich soils with potentially increased mineralization and nitrification and decreased denitrification in the soil profile.

Iowa and the other Corn Belt states contribute a large share of the N and P loads transported by the Mississippi River and these have been identified as primary drivers of Gulf hypoxia. The Hypoxia Action Plan calls for 45% reductions in both N and P loads. Although some alternative cropping systems, such as small grains, alfalfa, or other sod-based crops/rotations, can substantially reduce both N and P losses, these alternatives have major economic implications as well as environmental concerns of increasing row crop production pressure on more highly erosive and environmentally sensitive lands. Improved N management has the potential to reduce NO$_3^-$ leaching, but that potential is much less than needed to address the problem of Gulf hypoxia. It is clear that a combination of approaches is needed, but it would be a mistake to approach practices on a piece meal basis, especially since some practices that reduce N loads can substantially increase P loads.

In this project, we evaluated a structural approach integrating nitrate-removal wetlands with the emerging technologies of drainage modification. In combination with in field management, the integration of drainage and wetland systems will provide a dual nutrient strategy with potential to reduce N loads, reduce P loads and increase crop production. We evaluated the effects of drainage systems designed to reduce subsurface flow (controlled drainage and shallow drainage), drainage systems designed to reduce surface runoff, and targeted wetland restoration. The integration of drainage and wetland systems has the potential to simultaneously increase the number of wetland sites, push those sites closer to the NO$_3^-$ source, and enhance wetland performance by increasing the average residence time in the wetlands.

The integration of these approaches also provides opportunities for developing market-based solutions. Private and public interests coincide if we are able to couple increased water-use efficiency and crop yield due to drainage modification with improved water quality due to integrating drainage and wetland systems. This

This report is available at Iowa State University Digital Repository: http://lib.dr.iastate.edu/abe_eng_reports/13
opens an array of possible strategies for leveraging funds, capabilities and activities of private and public sources.
Final Report

INTEGRATED DRAINAGE-WETLAND SYSTEMS FOR REDUCING NITRATE LOADS FROM DES MOINES LOBE WATERSHEDS

William G. Crumpton
Department of Ecology, Evolution and Organismal Biology, Iowa State University

Matthew Helmers
Department of Agricultural and Biosystems Engineering, Iowa State University

Greg Stenback
Department of Ecology, Evolution and Organismal Biology, Iowa State University

Dean W. Lemke
Iowa Department of Agriculture and Land Stewardship

Shawn Richmond
Iowa Department of Agriculture and Land Stewardship

Submitted by
Iowa Department of Agriculture and Land Stewardship
Department of Agricultural and Biosystems Engineering
Department of Ecology, Evolution and Organismal Biology
Iowa State University, Ames

FEBRUARY 2012
REVISED SUBMISSION APRIL 2012
REVISED SUBMISSION(2) MAY 2012
1. INTRODUCTION ........................................................................................................... 1
2. PROJECT DESCRIPTION ............................................................................................. 2
   2.1. Project objectives .................................................................................................. 3
3. RESULTS ....................................................................................................................... 4
   3.1. Watershed Characterization and Monitoring ......................................................... 4
      3.1.1. Synoptic monitoring results ............................................................................. 9
      3.1.1.2. Intensive monitoring results .......................................................................... 11
      3.1.2. Post Construction monitoring ......................................................................... 22
   3.2. Topographic analysis and performance forecast modeling .................................... 22
      3.2.1. Topographic analyses ...................................................................................... 22
      3.2.1.1. Applicability of Alternative Drainage Designs and Integration of Wetlands ... 22
      3.2.1.2. Prioritized List of Subwatersheds ................................................................. 27
      3.2.2. Modeling of alternative drainage-wetland systems ......................................... 30
      3.2.2.1. Crop and drainage modeling ......................................................................... 30
      3.2.2.2. Wetland Modeling ....................................................................................... 42
      3.2.2.3. Combined drainage & wetland systems modeling ........................................ 46
   3.3. Broader impacts ........................................................................................................ 48
      3.3.1. Sources and implications of variability of nitrate concentration ...................... 48
      3.3.2. Regional assessment: Potential N reduction using N management, cover crops, drainage water management, and wetlands ...................................................... 50
   3.4. Outreach, engineering and construction of drainage-wetland systems .................... 55
      3.4.1. System Design and Construction ....................................................................... 55
      3.4.2. Landowner responses ...................................................................................... 56
      3.4.3. Outreach and Publications .............................................................................. 56
4. MAJOR CONCLUSIONS AND PROJECT FINDINGS ..................................................... 60
5. FUTURE DIRECTIONS .................................................................................................... 62
6. REFERENCES ................................................................................................................. 63
7. APPENDIX A – PROJECT TIMELINE .......................................................................... 65
8. APPENDIX B – TABLE OF INPUT PARAMETERS ......................................................... 67
9. APPENDIX C .................................................................................................................. 68
1. INTRODUCTION

Iowa leads the nation in the production of corn, soybeans, pork and eggs. This region is characterized by intensive row crop and animal agriculture, and nitrogen and phosphorus loads to surface waters are among the highest in the Mississippi River Basin. Nitrate is a particular concern because of (1) the potential adverse impacts on both public health and ecosystem function, (2) the high mobility of nitrate in surface and groundwater, and (3) the widespread use of nitrogen fertilizer in modern agriculture. In addition to the potential local impacts on receiving waters in the Corn Belt, nutrient loads from the region are a significant source of nitrate and phosphorus contributing to hypoxia in the Gulf of Mexico.

In recognition of these concerns, Iowa Department of Agriculture and Land Stewardship in conjunction with Iowa State University College Agriculture and Life Sciences and Iowa Department of Natural Resources is developing a statewide nutrient reduction strategy based on the goals of reducing nutrient loads to the Gulf of Mexico. Iowa’s 3000 drainage districts consistently at their annual meetings review and discuss the concerns with transport of nutrients through drainage systems and the resulting effects upon Gulf hypoxia. Through the Iowa Drainage District Association (IDDA), an active initiative is underway to address these concerns through several efforts to support, advise, and assist the implementation of nitrate-removal wetland technologies being implemented in Iowa through the Iowa Conservation Reserve Enhancement Program (CREP). The IDDA partnered and collaborated with this project by providing the necessary linkage to and networking with drainage districts, boards of trustees, and affected landowners. Through this linkage, a watershed study site search was initiated for this project, and three watersheds received approval of the governing boards of trustees of the three drainage districts (Figure 1). These approvals provide ingress/egress for needed land surveys, engineering and design needed to conduct the project. The boards of trustees also committed to working with the landowners of their districts to call informational meetings and to facilitate landowner input and local decisions.

![Figure 1. Location of study drainage districts in relationship to the Des Moines Lobe and the state of Iowa.](image-url)
2. PROJECT DESCRIPTION

The main subject area for this project is the Des Moines Lobe in north-central Iowa drained mostly by the Des Moines, Raccoon, Iowa, and Skunk Rivers. The Des Moines Lobe represents the southernmost extent of the Prairie Pothole Region and is a relatively flat landscape with very poor natural drainage. However, as a result of extensive subsurface “tile drainage,” the Des Moines Lobe has become some of the most valuable and productive land in the world. In 2011, the average land value in north-central Iowa was $7,356 an acre, and 80.5% of that area was in row-crops (42.9% in corn and 37.6% soybeans). Unfortunately, the Des Moines Lobe has also become a source of significant NO$_3^-$ loading to downstream waters.

The elevated nitrate loads from the Des Moines Lobe are largely a result of the changes in land-use and hydrology brought about by subsurface “tile drainage”. Tile drainage has historically been treated and continues today as a conservation practice because it decreases surface runoff, thus reducing surface transported contaminants such as phosphorus, sediment, ammonium-nitrogen, pesticides and pathogens. However, tile drainage also increases subsurface flow and leaching losses of NO$_3^-$. This is due mostly to an increase in the subsurface fraction of the total annual discharge and the “short-circuiting” of subsurface flow, but also in part to the increased aeration of organic-rich soils with potentially increased mineralization and nitrification and decreased denitrification in the soil profile.

Iowa and the other Corn Belt states contribute a large share of the N and P loads transported by the Mississippi River and these have been identified as primary drivers of Gulf hypoxia. The Hypoxia Action Plan calls for 45% reductions in both N and P loads. Although some alternative cropping systems, such as small grains, alfalfa, or other sod-based crops/rotations, can substantially reduce both N and P losses, these alternatives have major economic implications as well as environmental concerns of increasing row crop production pressure on more highly erosive and environmentally sensitive lands. Improved N management has the potential to reduce NO$_3^-$ leaching, but that potential is much less than needed to address the problem of Gulf hypoxia. It is clear that a combination of approaches is needed, but it would be a mistake to approach practices on a piece meal basis, especially since some practices that reduce N loads can substantially increase P loads.

In this project, we evaluated a structural approach integrating nitrate -removal wetlands with the emerging technologies of drainage modification. In combination with in field management, the integration of drainage and wetland systems will provide a dual nutrient strategy with potential to reduce N loads, reduce P loads and increase crop production. We evaluated the effects of drainage systems designed to reduce subsurface flow (controlled drainage and shallow drainage), drainage systems designed to reduce surface runoff, and targeted wetland restoration. The integration of drainage and wetland systems has the potential to simultaneously increase the number of wetland sites, push those sites closer to the NO$_3^-$ source, and enhance wetland performance by increasing the average residence time in the wetlands.

The integration of these approaches also provides opportunities for developing market-based solutions. Private and public interests coincide if we are able to couple increased water-use efficiency and crop yield due to drainage modification with improved water quality due to integrating drainage and wetland systems. This opens an array of possible strategies for leveraging funds, capabilities and activities of private and public sources.
2.1. Project objectives

The primary objective of this project was to evaluate the potential environmental benefits of integrating NO$_3^-$-removal wetlands with alternative drainage systems and initially focused on systems designed to reduce subsurface drainage (controlled and shallow drainage). Previous research suggested the potential for greater nitrate reduction by integrating wetland restoration with drainage systems that reduced subsurface flow. However, preliminary results suggested that while these systems could reduce N export, they had the potential to increase P loads because of increased surface runoff. This concern was expressed in the 2007 hypoxia reassessment by the USEPA Science Advisory Board (Hypoxia in the Northern Gulf of Mexico: An Update by the EPA Science Advisory Board). The 2007 EPA SAB report specifically recommended that

“A strategy for implementation of alternative drainage design or management should be developed that includes consideration of potential trade-offs between reduced nitrate loss through tile drains and increased P loss through surface runoff.”

In addition, the EPA SAB’s 2007 hypoxia reassessment identified both N and P as major contributors to Gulf hypoxia and the 2008 Action Plan calls for a dual nutrient strategy of 45% reductions in both N and P loads. Both the EPA SAB report and preliminary results from this project argue against drainage designs targeting only N load reduction. As a result, the project was amended to evaluate the potential of integrating wetland restorations with alternative drainage systems to reduce both N and P loads and the scope of the project was amended to include:

- assessing the potential impacts of integrating wetlands with drainage systems designed to reduce subsurface flow and nitrate transport. This includes modeling the impacts of controlled and shallow drainage on crop yield and on discharge and nutrient export through surface and subsurface pathways and the potential for integrating wetlands with controlled and shallow drainage systems.

- assessing the potential impacts of integrating wetlands with drainage systems designed to reduce surface runoff and phosphorus transport. This includes modeling the impacts of drainage capacity on crop yield and on discharge and nutrient export through surface and subsurface pathways and the combined impact of integrating drainage and wetland systems.

- evaluating the sources and implications of variability in nitrate concentration and nitrate load. This includes evaluating the effect of nitrogen management on nitrate concentration and load variability and reviewing drainage designs for intensively monitored sites to assess the possible influence of drainage intensity on nitrate concentration and load variability.

- using LIDAR data to assess the applicability of alternative drainage designs and potential for integrating wetlands with drainage systems across the Des Moines Lobe project area.

- evaluating the potential of nutrient management, drainage management, cover crops and wetlands to reduce nitrate loads from the Des Moines Lobe of Iowa.
3. RESULTS

The three specific Des Moines Lobe subwatersheds that are the focus of this project are organized drainage districts (DD) in Palo Alto and Pocahontas counties. Palo Alto DD12 is an approximately 2000-acre watershed with detailed data on surface elevations and on locations of subsurface tile lines. Palo Alto DD80 and Pocahontas and Palo Alto Joint DD77 comprise approximately 123,000 acres in total. Aerial land survey using LiDAR was provided at drainage district expense. The survey provided digital elevation data accurate to at least 15 cm with 95% confidence, and provided an important resource to accomplish this project.

The project involves four major components (1) hydrologic and water quality monitoring of selected subwatersheds, (2) topographic analysis and performance forecast modeling of alternative drainage-wetland systems, (3) broad scale assessments of nitrate variability and the potential for nitrate reductions through combinations of practices (including nutrient management, cover crops, and wetlands), and (4) outreach, engineering design and construction of drainage-wetland systems. (An approximate timeline of activities is shown in Appendix A.)

3.1. Watershed Characterization and Monitoring

Twenty three locations were identified as potential sampling sites for baseline monitoring of drainage networks: two in Clay County, 12 in Palo Alto County, and nine in Pocahontas County. These sites were selected based on GIS analyses of topography, soils, land use and subsurface drainage networks so as to provide ecohydrological conditions representative of the Des Moines Lobe watershed. From these sites we identified 11 major tile networks as candidates for intensive monitoring of nutrient concentrations, discharge, and nutrient load. All of the candidate sites represented major tile networks draining areas of >500 acres in predominately row crop agriculture. Nine of these networks outlet at or just above public right of ways and were included in synoptic sampling initiated in late August 2006. These include CLA1, PAL 11, POC 2, and POC 8 in DD 77 and PAL 3, PAL 5, PAL 7, PAL 9, and PAL 10 in DD 80 (Figure 2).

In addition to synoptic sampling at the sites in DD77 and DD80 in Pocahontas and Palo Alto Counties we collected synoptic samples at an additional 22 sites in Cerro Gordo and Franklin Counties. These synoptic sampling sites represent 32 distinct drainage systems, each draining approximately 1000-3000 acres in predominately row crop agriculture. As part of a separate project, US Geologic Survey National Stream Quality Accounting Network (NASQAN) flow-weighted-average (FWA) nitrate concentrations were compared to monthly arithmetic average concentrations to determine a suitable measure and time frame for a surrogate of FWA concentration in the Upper Mississippi and Ohio River basins (Crumpton et al 2006). The results of those comparisons indicate that the average of the April-May-June (AMJ) nitrate concentrations should approximate the long term average annual FWA concentration reasonably well for drainage systems of 200 square miles or more. Comparison of the five-year average AMJ nitrate concentrations with the five-year FWA concentrations at the targeted watershed study sites shows a strong direct relationship ($R^2 = 0.85$). The 39 annual average AMJ concentrations compared with the annual FWA nitrate concentrations show an average 17% percent difference, although the AMJ average concentrations tend to be greater than the annual FWA concentration for the targeted watershed study sites. Because discharge was not measured at the synoptic sampling sites the FWA nitrate concentration could not be determined and so, in
view of the above considerations, the average AMJ nitrate concentration was used as a simple approximation of the annual FWA nitrate concentration.

Figure 2. Sampling sites and land use in DD 77 (top) and DD80 (bottom). Areas of corn and soybean based on 2004 satellite imagery are shown in yellow and green.
Seven of the synoptic sampling sites were instrumented with autosamplers and flow monitors in spring of 2007 (CLA 1, PAL 11, POC 2, POC 8 in DD77 and PAL 3, PAL 5, and PAL 7 in DD80) and an eighth site (PAL 16 in DD77) was instrumented in 2008. These eight sites were selected to have similar land use but to represent a wide range of nitrate concentrations. Grab water samples were collected year round during approximately weekly site visits. Autosamplers were deployed throughout the portion of each year having temperatures sufficiently above freezing to allow stream water to be pumped through tubing to an automated sample collection instrument – usually from about mid to late March through mid to late November. Autosamplers collected water samples as daily composites of four six-hour interval subsamples. Samples were preserved by acidification to pH <2 and subsequently analyzed for nitrogen and phosphorus concentrations using standard laboratory procedures (Appendix C).

Stream channel water depth measurements were taken at five minute intervals at each monitoring site using submerged stage recorders. (Duplicate stage recorders were installed at each location for redundancy.) Submerged area velocity (SAV) meters were deployed at each stream monitoring location during the non-freezing portion of the year and recorded water velocity at five minute intervals. A stream cross sectional profile was determined at each discharge measurement location from which a wetted cross section area versus stream depth relationship was determined. Discharge was calculated based on measured velocity and the cross sectional area at a given water depth. The SAV discharge was calibrated using manual velocity-area based discharge measurements collected during weekly site visits. Manual velocity-area discharge measurements were determined using the mid-section method whereby the stream depth is determined at 10 cm intervals across the stream and the water velocity is measured at the midpoint of each interval. Velocity was measured with a hand held Sontek Doppler water velocity probe using the 0.6 depth method where the velocity at 0.6 of the depth from the surface is taken as the mean velocity for the interval.

We used the engineering design records and GIS based estimates of drainage area to calculate the outlet drainage coefficient for each of the eight systems selected for intensive monitoring (Table 1). The systems range from 675-2801 ha in area with drainage coefficients ranging from 0.25-1.19 cm/day (0.1-0.47 inch/day).

<table>
<thead>
<tr>
<th>Watershed area (ha)</th>
<th>Drainage Coefficient (in./d)</th>
<th>Percent row crop (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLA1 2801</td>
<td>0.18</td>
<td>88</td>
</tr>
<tr>
<td>PAL3 1222</td>
<td>0.10</td>
<td>92</td>
</tr>
<tr>
<td>PAL5 1242</td>
<td>0.12</td>
<td>91</td>
</tr>
<tr>
<td>PAL7 711</td>
<td>0.38</td>
<td>94</td>
</tr>
<tr>
<td>PAL11 780</td>
<td>0.15</td>
<td>93</td>
</tr>
<tr>
<td>PAL16 1656</td>
<td>0.18</td>
<td>92</td>
</tr>
<tr>
<td>POC2 1219</td>
<td>0.17</td>
<td>89</td>
</tr>
<tr>
<td>POC8 675</td>
<td>0.47</td>
<td>91</td>
</tr>
</tbody>
</table>

We also estimated nitrogen input for three of systems (PAL 3, PAL 11, and POC 8) based on farmer surveys. The percent of crop land covered by survey respondents ranged from 49-58% of
the total area of each drainage system (Table 2). All responses reported taking soil samples at least every 4 years. In Pocahontas County (POC 8) only one respondent reported applying liquid swine manure, while two of the three respondents in PAL 11 applied liquid swine manure.

The reported nitrogen application rate for continuous corn was very close to the recommended application rate for Maximum Return to Nitrogen based on the Nitrogen Rate Calculator (http://extension.agron.iastate.edu/soilfertility/nrate.aspx). However, the reported application rate for corn-soybean rotations was higher than the recommended rate. With $0.50/lb-N and $5/bushel corn, the MRTN for corn following soybean is 149 kg-N/ha (133 lb-N/acre) and the MRTN for corn following corn is 213 kg-N/ha (190 lb-N/acre).

Average corn yield by watershed ranged from 181 to 190 bushels/acre for corn following soybean and 175-213 bushel/acre for corn following corn. Using estimates of nitrogen input, nitrogen harvested with grain, and nitrogen exported with drainage, a rough nitrogen budget can be computed. These estimates do not include outputs with water, gas fluxes, or atmospheric deposition but provide useful information on farmer inputs and outputs of nitrogen. In these estimates it is assumed that each bushel of corn contains 0.72 lbs of nitrogen, each bushel of soybean contains 3.36 lbs of nitrogen and the soybeans fix 2 lbs of nitrogen per bushel produced. These estimates are consistent with values used in developing the nitrogen and phosphorus budgets for the state of Iowa. Based on these estimates there is the potential that there is a negative net nitrogen balance. In other words more nitrogen is harvested with the grain than the combined input from fertilizer application and N fixation (Table 3). When combining grain inputs, grain harvest, and exports with water as measured in these drainage districts all three districts have a negative net nitrogen balance (Table 4). While these estimates have not accounted for atmospheric deposition or gas emissions they raise an important point about the potential for greater nitrogen export than input which over time could have negative soil quality impacts specifically that soil organic matter could be “mined” for the nitrogen need of the crops not supplied by external nitrogen inputs. This raises soil sustainability concerns as it indicates there is the possibility that the organic matter content of the soils would be reduced over time as it is mined for nitrogen needs of the crops. This is not only an agronomic concern, but an environmental concern as well since soils low in organic matter content become more erosive and environmentally sensitive. Also, at the scale monitored the variability in nitrogen export with water does not seem to be explained by nitrogen inputs and exports with grain. Specifically, there does not appear to be a direct correlation between the amount of nitrogen exported by drainage and the amount of nitrogen inputs or the amount of nitrogen harvested in the grain. From a mass balance perspective, one might expect there to be a correlation between the amount of nitrogen inputs and nitrogen harvested in the grain with the amount of nitrogen exported in drainage. This expected relationship is not clearly present in the sites monitored under this grant, indicating that there are other additional factors that affect the amount of nitrogen export in drainage aside from the amounts of inputs and exports in the grain.
Table 2. Summary of nitrogen input information

<table>
<thead>
<tr>
<th></th>
<th>Area Weighted Nitrogen Application Rate</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PAL 3</td>
<td>PAL 11</td>
<td>POC 8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ha</td>
<td>ha</td>
<td>ha</td>
</tr>
<tr>
<td>kg-N/ac</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CB</td>
<td>175</td>
<td>200</td>
<td>226</td>
<td>217</td>
</tr>
<tr>
<td>CC</td>
<td>207</td>
<td>139</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCB</td>
<td>224</td>
<td>75</td>
<td>197</td>
<td>140</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% of farm ground covered by respondents</th>
<th>Number of responses</th>
<th>% of farm ground covered by respondents</th>
<th>Number of responses</th>
<th>% of farm ground covered by respondents</th>
<th>Number of responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>7</td>
<td>49</td>
<td>3</td>
<td>58</td>
<td>5</td>
</tr>
</tbody>
</table>

CB- Corn in Corn-Soybean rotation, CC – Corn in a Continuous Corn rotation, and CCB – Corn in a Corn-Corn-Soybean rotation

Table 3. Summary of nitrogen balance based on nitrogen application, harvest with grain, and fixation of soybeans for PAL3, PAL11, and POC8.

<table>
<thead>
<tr>
<th>Summary</th>
<th>Nitrogen balance of corn (kg-N/ha)</th>
<th>Nitrogen balance of soybean (kg-N/ha)</th>
<th>Net nitrogen balance over two years accounting for area of corn and soybean (kg-N/ha)</th>
<th>Estimated annual net nitrogen balance considering corn and soybean inputs and exports (kg-N/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAL 3</td>
<td>50</td>
<td>-79</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>PAL 11</td>
<td>67</td>
<td>-78</td>
<td>-5</td>
<td>-3</td>
</tr>
<tr>
<td>POC 8</td>
<td>23</td>
<td>-79</td>
<td>-23</td>
<td>-11</td>
</tr>
</tbody>
</table>

Table 4. Summary of nitrogen balance based on grain inputs and harvest and water exports for PAL3, PAL11, and POC8.

<table>
<thead>
<tr>
<th>Summary</th>
<th>Estimated annual net nitrogen balance considering corn and soybean inputs and harvest (kg-N/ha)</th>
<th>Annual nitrogen export in water (kg-N/ha)</th>
<th>Estimated annual net nitrogen balance (kg-N/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAL 3</td>
<td>5</td>
<td>-35</td>
<td>-25</td>
</tr>
<tr>
<td>PAL 11</td>
<td>-3</td>
<td>-36</td>
<td>-39</td>
</tr>
<tr>
<td>POC 8</td>
<td>-11</td>
<td>-43</td>
<td>-54</td>
</tr>
</tbody>
</table>
3.1.1.1. Synoptic monitoring results

The synoptic sampling results reveal two major patterns 1) nitrate concentrations at drainage system scale are substantially higher than those typically reported by river monitoring programs and 2) nitrate concentrations can differ substantially between drainage systems despite similar geomorphology and land use characteristics.

1) *High nitrate concentrations at drainage system scale*: The higher nitrate concentrations measured at the drainage system scale reflect in part the proportionally greater influence of agriculture in these systems (over 80% of the contributing areas of these drainage systems are planted in corn and soybean) and in part the opportunity for in stream loss of nitrate and dilution between drainage system outlets and river monitoring stations. Crumpton et al. (2006) examined the relationship between nitrate concentration and land use for 52 NASQAN stations (Alexander et al., 1998) in the upper Mississippi and Ohio River basins selected to exclude sites with large upstream reservoirs or extensive upstream urban areas. Percent cropland (corn or soybean) accounted for 90% of the observed variation in the long term flow-weighted average nitrate concentrations for the 52 stations examined. This represents the relationship between percent cropland and the FWA nitrate concentration “delivered” at the major tributaries or the Upper Mississippi and Ohio Rivers. Based on the most recent SPARROW estimates (Alexander et al. 2008), in stream loss removes about 36% of the nitrate load from our region (their “Central Mississippi River”) by the time the loads are delivered to the Gulf of Mexico. After adjusting the relationship between percent cropland and the FWA nitrate concentration to account for in stream loss, the nitrate concentrations predicted for the synoptic sampling sites based on percent cropland are in the same range as the observed concentrations (Figure 3). For comparison, data are also included for 9 drainage systems where continuous flow monitoring and automated sampling allowed calculation of flow weighted average (FWA) nitrate concentrations. As in the case of the synoptic sampling sites, the observed FWA nitrate concentrations are about what would be predicted based on percent cropland (Figure 3).

2) *Variability in nitrate concentration at drainage system scale*: Nitrate concentrations are thought to be primarily determined by agricultural practices and drainage patterns, and are expected to be similar for tile drained watersheds in the same geographic area and with similar agricultural practices. However, our synoptic sampling results demonstrate a greater than two-fold range in average nitrate concentrations among drainage systems, despite similar patterns in the extent of cultivated cropland and subsurface drainage. Average nitrate concentrations for individual drainage systems range from about 10 to 25 mg N L$^{-1}$ (Figure 3). This is not due to temporal variability. Based on the now several years of data for the Cerro Gordo and Franklin County sites, average nitrate concentrations remain relatively consistent between years for individual drainage systems (Figure 4). Essentially, systems with low nitrate concentrations tend to have consistently low concentrations year after year and systems with high nitrate concentrations tend to have consistently high concentrations year after year. In section 3.3 of this report, we further examine the sources and implications of variability in nitrate concentrations.
Figure 3. Flow weighted average (FWA) and estimated FWA (April-June average) nitrate concentration versus percent cropland.

Figure 4. Average nitrate concentrations for Cerro Gordo / Franklin (Cer/Fra) and Palo Alto / Pocahontas (Pal/Poc) County synoptic sampling sites. April through June (AMJ) average nitrate concentrations for each year are plotted versus the median of the 2004 through 2007 AMJ averages for Cerro Gordo and Franklin County sites to illustrate the year to year repeatability of concentrations at individual sites. The 2007 Pal/Poc data are plotted to illustrate the similarity in the range of nitrate concentrations between the Cer/Fra and Pal/Poc sampling locations.
3.1.1.2. Intensive monitoring results

Nitrate and phosphorus show generally similar seasonal and flow related patterns at all eight project sites (Figures 5 and 6). Nitrate concentrations are generally elevated and relatively stable except for declines during periods of potential runoff (snowmelt or very high flow) or during periods of extended low flow. Nitrate concentrations often show a marked decline which may persist for several days or more during peak discharge events. This is thought to be associated with dilution of higher subsurface flow nitrate concentrations from the surface runoff component of discharge having lower concentration during and following storm events. On average, nitrate comprised 94% of TN and TN showed patterns similar to those of nitrate. On average, 80% of the nitrate load was delivered on about 31% of the days and 50% was delivered on about 12% of the days. Direct linear trends between discharge and nitrate load have coefficient of determination values ranging from $R^2 = 0.67$ to $R^2 = 0.91$ across sites and show that high nitrate load days tend to correspond with high flow days thus highlighting the importance of close interval sampling, and particularly sampling on high flow days, to accurately characterize nitrate loading for these systems.

Phosphorus concentrations are highly variable during periods of low discharge but are generally elevated during periods of high discharge (Figure 6) and phosphorus load is strongly related to discharge (Figure 7). On average TRP comprises 76% of the TP load and TRP and TP displayed similar patterns. TP and TRP are both transported primarily during a relatively few periods of high flow. On average, 80% of the total phosphorus load was delivered on less than 15% on the days and 50% was delivered on less than 5% of the days. On average 80% of the TRP load was delivered on 13% of the days and 50% was delivered on 4% of the days. These high load days tend to correspond with high flow days thus highlighting the importance of close interval sampling, and particularly sampling on high flow days, to accurately characterize phosphorus loads for these systems.

Nutrient concentrations and loads are thought to be primarily determined by agricultural practices and drainage patterns, and are expected to be similar for tile drained watersheds in the same geographic area and with similar agricultural practices. However, our monitoring results demonstrate a one to two fold range in long term average nitrate concentrations (Table 5; 8.8 to 12.4 mg N L$^{-1}$) and mass N yield (Table 6; 26 to 48 kg N/ha/year) among drainage systems, despite similar patterns in the extent of cultivated cropland and subsurface drainage (Table 1). For the period 2007-2011, the annual flow weighted average nitrate (FWA N) concentrations for the eight monitored systems varied by a factor of two, ranging from 6.05 to 14.86 mg N L$^{-1}$ (Table 5) with no significant relationship to land use or drainage coefficient. Annual nitrate-N yield varied by an order of magnitude, ranging from 8.9 to 94 kg N/ha/year (Table 6).

Our results also reveal a two to three fold range in long term average P concentrations (120 to 257 µg P L$^{-1}$) and mass P yield (0.33 to 1 kg P/ha/year) among drainage systems. For the period 2007-2011, the annual flow weighted average TP (FWA TP) concentrations for the eight monitored systems ranged from 71 to 473 µg P L$^{-1}$ (Table 5) with no significant relationship to land use or drainage coefficient. Annual TP yield varied by an order of magnitude, ranging from 0.12 to 2 kg P/ha/year (Table 6).
Figure 5. Nitrate concentration and discharge at currently monitored sites.
Figure 6. TP and TRP concentration and discharge at currently monitored sites.
Figure 7. TP load and discharge at currently monitored sites.
Table 5. FWA concentrations (no data collected during 2007 at PAL 16)

**FWA [NO3] mg/L**

<table>
<thead>
<tr>
<th></th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLA1</td>
<td>8.12</td>
<td>9.73</td>
<td>8.35</td>
<td>9.89</td>
<td>11.99</td>
<td>9.62</td>
</tr>
<tr>
<td>PAL3</td>
<td>8.96</td>
<td>10.96</td>
<td>11.73</td>
<td>9.26</td>
<td>9.02</td>
<td>9.99</td>
</tr>
<tr>
<td>PAL5</td>
<td>9.95</td>
<td>11.44</td>
<td>11.92</td>
<td>10.02</td>
<td>11.00</td>
<td>10.87</td>
</tr>
<tr>
<td>PAL7</td>
<td>10.90</td>
<td>11.80</td>
<td>11.04</td>
<td>13.47</td>
<td>14.86</td>
<td>12.42</td>
</tr>
<tr>
<td>PAL11</td>
<td>6.05</td>
<td>8.44</td>
<td>9.10</td>
<td>9.31</td>
<td>11.21</td>
<td>8.82</td>
</tr>
<tr>
<td>PAL16</td>
<td>10.45</td>
<td>11.06</td>
<td>10.70</td>
<td>13.10</td>
<td>11.33</td>
<td></td>
</tr>
<tr>
<td>POC2</td>
<td>12.22</td>
<td>11.98</td>
<td>11.60</td>
<td>10.52</td>
<td>13.56</td>
<td>11.98</td>
</tr>
<tr>
<td>POC8</td>
<td>14.04</td>
<td>12.88</td>
<td>11.70</td>
<td>10.34</td>
<td>12.65</td>
<td>12.32</td>
</tr>
</tbody>
</table>

**FWA [TN] mg/L**

<table>
<thead>
<tr>
<th></th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLA1</td>
<td></td>
<td>10.15</td>
<td>12.22</td>
<td>11.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAL3</td>
<td></td>
<td>10.03</td>
<td>9.76</td>
<td>9.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAL5</td>
<td></td>
<td>10.68</td>
<td>11.68</td>
<td>11.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAL7</td>
<td></td>
<td>13.98</td>
<td>15.29</td>
<td>14.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAL11</td>
<td></td>
<td>9.71</td>
<td>11.61</td>
<td>10.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAL16</td>
<td></td>
<td>11.05</td>
<td>13.52</td>
<td>12.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>POC2</td>
<td></td>
<td>10.69</td>
<td>13.77</td>
<td>12.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>POC8</td>
<td></td>
<td>10.80</td>
<td>12.91</td>
<td>11.85</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**FWA [TP] ppb**

<table>
<thead>
<tr>
<th></th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLA1</td>
<td>128</td>
<td>180</td>
<td>182</td>
<td>168</td>
<td>229</td>
<td>199</td>
</tr>
<tr>
<td>PAL3</td>
<td>163</td>
<td>147</td>
<td>142</td>
<td>266</td>
<td>200</td>
<td>233</td>
</tr>
<tr>
<td>PAL5</td>
<td>140</td>
<td>162</td>
<td>124</td>
<td>246</td>
<td>170</td>
<td>208</td>
</tr>
<tr>
<td>PAL7</td>
<td>174</td>
<td>338</td>
<td>473</td>
<td>296</td>
<td>217</td>
<td>257</td>
</tr>
<tr>
<td>PAL11</td>
<td>248</td>
<td>234</td>
<td>102</td>
<td>228</td>
<td>154</td>
<td>191</td>
</tr>
<tr>
<td>PAL16</td>
<td></td>
<td>198</td>
<td>161</td>
<td>172</td>
<td>148</td>
<td>160</td>
</tr>
<tr>
<td>POC2</td>
<td>71</td>
<td>121</td>
<td>129</td>
<td>114</td>
<td>126</td>
<td>120</td>
</tr>
<tr>
<td>POC8</td>
<td>103</td>
<td>169</td>
<td>220</td>
<td>200</td>
<td>75</td>
<td>138</td>
</tr>
</tbody>
</table>

**FWA [TRP] ppb**

<table>
<thead>
<tr>
<th></th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLA1</td>
<td>80</td>
<td>141</td>
<td>149</td>
<td>134</td>
<td>194</td>
<td>164</td>
</tr>
<tr>
<td>PAL3</td>
<td>113</td>
<td>83</td>
<td>110</td>
<td>188</td>
<td>164</td>
<td>176</td>
</tr>
<tr>
<td>PAL5</td>
<td>91</td>
<td>91</td>
<td>90</td>
<td>165</td>
<td>123</td>
<td>144</td>
</tr>
<tr>
<td>PAL7</td>
<td>123</td>
<td>229</td>
<td>405</td>
<td>250</td>
<td>186</td>
<td>218</td>
</tr>
<tr>
<td>PAL11</td>
<td>213</td>
<td>177</td>
<td>88</td>
<td>195</td>
<td>136</td>
<td>165</td>
</tr>
<tr>
<td>PAL16</td>
<td></td>
<td>134</td>
<td>124</td>
<td>140</td>
<td>130</td>
<td>135</td>
</tr>
<tr>
<td>POC2</td>
<td>38</td>
<td>82</td>
<td>121</td>
<td>87</td>
<td>110</td>
<td>99</td>
</tr>
<tr>
<td>POC8</td>
<td>57</td>
<td>96</td>
<td>191</td>
<td>171</td>
<td>67</td>
<td>119</td>
</tr>
</tbody>
</table>
Table 6. Nutrient yields (no data collected during 2007 at PAL 16)

<table>
<thead>
<tr>
<th>NO3 yield (kg/ha)</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLA1</td>
<td>24.15</td>
<td>13.59</td>
<td>8.87</td>
<td>50.73</td>
<td>32.37</td>
<td>25.94</td>
</tr>
<tr>
<td>PAL3</td>
<td>46.98</td>
<td>16.97</td>
<td>9.86</td>
<td>62.10</td>
<td>36.64</td>
<td>34.51</td>
</tr>
<tr>
<td>PAL5</td>
<td>73.09</td>
<td>23.51</td>
<td>14.93</td>
<td>72.23</td>
<td>44.05</td>
<td>45.56</td>
</tr>
<tr>
<td>PAL7</td>
<td>65.24</td>
<td>33.03</td>
<td>15.90</td>
<td>94.30</td>
<td>31.30</td>
<td>47.95</td>
</tr>
<tr>
<td>PAL11</td>
<td>39.45</td>
<td>19.41</td>
<td>16.28</td>
<td>64.58</td>
<td>38.43</td>
<td>35.63</td>
</tr>
<tr>
<td>PAL16</td>
<td>13.02</td>
<td>11.93</td>
<td>48.13</td>
<td>39.76</td>
<td>28.21</td>
<td></td>
</tr>
<tr>
<td>POC2</td>
<td>55.23</td>
<td>33.37</td>
<td>15.68</td>
<td>50.64</td>
<td>27.71</td>
<td>36.53</td>
</tr>
<tr>
<td>POC8</td>
<td>82.92</td>
<td>26.58</td>
<td>17.50</td>
<td>57.97</td>
<td>31.02</td>
<td>43.20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TN yield (kg/ha)</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLA1</td>
<td>52.08</td>
<td>33.00</td>
<td>42.54</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAL3</td>
<td>67.25</td>
<td>39.64</td>
<td>53.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAL5</td>
<td>76.94</td>
<td>46.77</td>
<td>61.85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAL7</td>
<td>97.81</td>
<td>32.19</td>
<td>65.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAL11</td>
<td>67.35</td>
<td>39.80</td>
<td>53.58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAL16</td>
<td>49.70</td>
<td>41.06</td>
<td>45.38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POC2</td>
<td>51.41</td>
<td>28.15</td>
<td>39.78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POC8</td>
<td>60.55</td>
<td>31.65</td>
<td>46.10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TP yield (kg/ha)</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLA1</td>
<td>0.381</td>
<td>0.251</td>
<td>0.194</td>
<td>0.861</td>
<td>0.619</td>
<td>0.46</td>
</tr>
<tr>
<td>PAL3</td>
<td>0.851</td>
<td>0.227</td>
<td>0.119</td>
<td>1.781</td>
<td>0.813</td>
<td>0.76</td>
</tr>
<tr>
<td>PAL5</td>
<td>0.940</td>
<td>0.305</td>
<td>0.143</td>
<td>1.626</td>
<td>0.624</td>
<td>0.73</td>
</tr>
<tr>
<td>PAL7</td>
<td>1.040</td>
<td>0.945</td>
<td>0.682</td>
<td>2.074</td>
<td>0.457</td>
<td>1.04</td>
</tr>
<tr>
<td>PAL11</td>
<td>1.597</td>
<td>0.539</td>
<td>0.183</td>
<td>1.579</td>
<td>0.527</td>
<td>0.88</td>
</tr>
<tr>
<td>PAL16</td>
<td>0.246</td>
<td>0.174</td>
<td>0.775</td>
<td>0.449</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>POC2</td>
<td>0.322</td>
<td>0.337</td>
<td>0.175</td>
<td>0.549</td>
<td>0.258</td>
<td>0.33</td>
</tr>
<tr>
<td>POC8</td>
<td>0.605</td>
<td>0.350</td>
<td>0.329</td>
<td>1.121</td>
<td>0.184</td>
<td>0.52</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TRP yield (kg/ha)</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLA1</td>
<td>0.237</td>
<td>0.196</td>
<td>0.158</td>
<td>0.687</td>
<td>0.524</td>
<td>0.36</td>
</tr>
<tr>
<td>PAL3</td>
<td>0.594</td>
<td>0.129</td>
<td>0.093</td>
<td>1.258</td>
<td>0.664</td>
<td>0.55</td>
</tr>
<tr>
<td>PAL5</td>
<td>0.614</td>
<td>0.171</td>
<td>0.103</td>
<td>1.092</td>
<td>0.450</td>
<td>0.49</td>
</tr>
<tr>
<td>PAL7</td>
<td>0.736</td>
<td>0.642</td>
<td>0.583</td>
<td>1.750</td>
<td>0.392</td>
<td>0.82</td>
</tr>
<tr>
<td>PAL11</td>
<td>1.374</td>
<td>0.407</td>
<td>0.157</td>
<td>1.354</td>
<td>0.465</td>
<td>0.75</td>
</tr>
<tr>
<td>PAL16</td>
<td>0.167</td>
<td>0.133</td>
<td>0.631</td>
<td>0.396</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>POC2</td>
<td>0.172</td>
<td>0.228</td>
<td>0.164</td>
<td>0.419</td>
<td>0.225</td>
<td>0.24</td>
</tr>
<tr>
<td>POC8</td>
<td>0.338</td>
<td>0.198</td>
<td>0.286</td>
<td>0.958</td>
<td>0.163</td>
<td>0.39</td>
</tr>
</tbody>
</table>
Nitrate and P have different transport pathways, with nitrate being transported almost entirely by subsurface flow while substantial P loads are transported with surface runoff. As a result, nitrate and P concentrations might be inversely related across otherwise similar systems having different amounts of surface and subsurface flow. Six of the study sites do show an inverse relationship between nitrate and P concentrations and an analysis of covariance (ANCOVA) model was used to assess the statistical significance of the trend (p-value = 0.015), while accounting for differences between the sites (Figure 8). The two sites, CLA 1 and POC 2 not showing this relationship showed no significant relationship at the 0.05 level of statistical significance. ANCOVA is a standard statistical procedure that allows comparison of two or more groups while taking into account (or to correct for) variability associated with other variables, called covariates. An ANCOVA can also perform a regression analysis to evaluate the relationship between one or more independent or predictor variables (the covariates) with the dependent variable(s) while allowing for differences between groups. In the following three instances we use an ANCOVA to assess the effect of the covariates as linear trends, while accounting for differences between the sites.

Figure 8. FWA TP concentration tends to decrease with increasing FWA nitrate-N concentration ($R^2 = 0.94$).
Annual flow-weighted average (FWA) nitrate concentration in discharge varied across locations but could not be directly related to land use or drainage coefficient. Since nitrate is transported almost entirely by subsurface flow and nitrate concentration in surface runoff is generally low, nitrate concentrations might be expected to decline with increased surface runoff during periods of high flow. When daily water sample nitrate concentrations were compared with discharge data at the sites in this study, concentrations were often observed to decline during and immediately following large flow events. During a storm event the stream discharge might increase by several orders of magnitude. However, the nitrate concentration in the stream is influenced by the sources of nitrate, either as subsurface flow or surface runoff, delivered to the stream. As a consequence, a storm event having substantial surface runoff might show reduced in-stream nitrate concentration due to the influx of surface runoff having low concentration -- all the mass of nitrate delivered to the stream is still there, but the concentration is diluted. Once in the stream, nitrate will be affected by in-stream processes but will primarily be carried downstream in the discharge water. Because of this, for land areas under similar land use, annual FWA nitrate concentration might be expected to show a declining trend with increasing water yield due to the increased surface runoff likely to occur in wetter years.

Four of the study sites show this expected pattern (Figure 9). This is demonstrated using an ANCOVA model where the four sites (PAL 11, PAL 3, PAL 5 and POC 2) are modeled as having a common slope with respect to water yield, but distinct intercepts to allow for differences between sites. In this case, interest is not in differences between sites, but in the relationship of annual FWA nitrate concentrations to water yield. The results show a significant negative slope supporting the expected relationship (p-value = 0.007, Figure 9). The remaining four sites show no significant relationship to water yield on the basis of linear regression slopes tested at the 0.05 level of statistical significance (individual p-values > 0.7). While it is possible that no relationship exists at the four sites not showing this trend, it is also likely that other factors masked the ability to detect the trend. This is because water yields sufficiently large enough to generate substantial surface runoff would be necessary to cause a detectable FWA nitrate concentration response. Sufficient water yield variation may not have occurred during this five year study. Two of the four sites not showing the trend, PAL 7 and POC 8, are high drainage coefficient sites which would tend to route more storm water through the subsurface tile system and minimize surface runoff, thus failing to show the anticipated nitrate concentration decline. Regarding the other two sites not showing the trend, PAL 16 had a maximum water yield that was lower than those at any of the sites where the trend was detected and CLA 1 had a maximum water yield that was lower than those at three of the four sites where the trend was detected.
Figure 9. FWA nitrate-N concentration tends to decline with increasing water yield ($R^2 = 0.68$).

Annual flow-weighted average (FWA) total phosphorus (TP) concentrations varied across locations but could not be directly related to land use or drainage coefficient. Since substantial phosphorus is transported by surface runoff, phosphorus concentrations might be expected to increase with increased surface runoff during periods of high flow. Daily water samples assayed for total phosphorus (TP) concentrations in tile-drained, row crop land on the Des Moines Lobe in Iowa are often observed to have variable TP concentrations during low discharge periods but tend to increase during moderate to high discharge periods. Some of this concentration increase with increasing discharge is thought to be associated with surface runoff carrying elevated phosphorus loads. Because of this, for a particular land area under similar land use, annual FWA TP concentration might be expected to increase with increasing water yield due to increased surface runoff which is more likely to occur in wetter years.

Three of the study sites show this expected pattern. This is demonstrated using an ANCOVA model where the three sites (PAL 11, PAL 3, and PAL 5) are modeled as having a common slope, but distinct intercepts to allow for differences between sites. In this case, interest is not in differences between sites, but in the relationship of FWA TP concentrations to water yield. The results show a significant positive slope supporting the expected relationship ($p$-value $= 0.014$, Figure 10). The remaining five sites show no significant relationship to water yield on the basis of linear regression slopes (individual $p$-values above 0.17).
Discharge and water yield (WY) varied by an order of magnitude across sites and years, ranging from 0.084 m/year at PAL3 in 2009 to 0.734 m/year at PAL 5 in 2007. There was no apparent relationship between water yield and drainage coefficient or other known site characteristics. Precipitation was the overwhelming determinant of water yield and it is possible that variability in precipitation would have obscured any more subtle effects due to differences in site characteristics. In some years, precipitation differed enough to cause large differences in flow between sites that were within a 10 mile radius. Rain gages were installed at CLA 1, PAL 5, and POC2 each year during the period of year having low likelihood of freezing conditions; generally about mid-April through October or November. For each year, at each of these three sites, the daily water yield corresponding to the rain gage data period was summed and plotted versus the measured total precipitation (Figure 11). These data show a linear pattern having an R$^2$ of 0.662 and suggest that annual precipitation total of less than about 0.2 m would result in little or no discharge (Figure 11).
To account for stored subsurface water that would contribute to discharge over longer time periods, we used the prior years precipitation as a surrogate for stored subsurface water, and developed a regression model to predict WY as a linear function of precipitation in the current and previous year. In effect, this model suggests that for the same amount of precipitation in the current year, WY would be lower following a dry year and higher following a wet year. The resulting model improves the prediction of WY from precipitation data resulting in an $R^2$ of 0.865 (Figure 12).

Figure 11. Relationship between water yield (WY) and precipitation

Figure 12. Relationship between predicted and observed water yield
3.1.2. Post Construction monitoring

Post construction monitoring did not occur during the project period. However, construction of pilot sites under the “Iowa Wetland Landscape Systems Initiative” will occur in the coming year and detailed water quality and hydrologic monitoring of these systems is planned. The project team was heavily involved in developing the work plan for the assessments and evaluations to be conducted as part of the pilot studies. These assessments include developing nutrient budgets, assessing crop yields, quantifying surface and subsurface flow and the impacts of drainage design, water quality impacts of drainage redesign, and water quality benefits of wetlands integrated with the redesigned drainage systems.

3.2. Topographic analysis and performance forecast modeling

We utilized multiple approaches to assess the potential for drainage management and drainage modification along with the potential for integrating wetlands with alternative drainage systems. These approaches included 1) topographic analyses to assess the applicability of alternative drainage designs, 2) modeling the projected performance of alternative drainage systems, 3) modeling the projected performance of wetlands, and 4) modeling the projected performance of combined drainage and wetland systems.

3.2.1. Topographic analyses

We used GIS technologies to assess the potential for implementation of drainage water management in our study region and across the Des Moines Lobe as well as to identify candidate sites for targeted wetland restoration within our study area. Topographic analysis of high resolution DEMs based on LIDAR data was used to (1) assess the applicability of controlled drainage and (2) identify subwatersheds and sites with the greatest potential for implementation of drainage-wetland systems (tile zone and floodplain).

3.2.1.1. Applicability of Alternative Drainage Designs and Integration of Wetlands

In considering the potential for controlled drainage or drainage water management it is important to assess the applicability or feasibility of implementing the practice. The applicability is limited by topography and a slope of 0.5% or less is recommended (Frankenberger et al., 2006). Drainage districts DD 12, 77, and 80 in Palo Alto and Pocahontas counties encompass 64,849 ha (160,177 acres) of extensively drained cropland. Although 50-75% of the cropland in these drainage districts is presumed to be tile drained (cropland soils considered somewhat poorly drained and wetter), only a portion of this is appropriate for retrofitting with controlled drainage. Controlled drainage requires relatively flat topography and slopes of less than 0.5% are recommended. Topographic analyses using LIDAR derived DEMs reveals that only about 10% of the area in DD’s 12, 77, and 80 presumed to be tile drained has a slope less than 0.5% and only about 7% of the entire Des Moines Lobe has slopes less than 0.5% (Table 8). In addition, these relatively flat areas are not in large contiguous areas (Figures 13 and 14).

Due to lack of detailed topographic information in most areas, there has been ongoing work by researchers working on controlled drainage to assess applicability of controlled drainage
using less detailed information. Specifically, soils maps and the slope classifications defined by the soils maps have been used to assess this applicability and the percent of land area in specific slope ranges. Based on this, we have used our study area, where detailed topography is available from the LiDAR data, to compare the results of detailed topographic analysis to assessments of applicability using the slope classes defined by soils maps. These soils maps break the slope classes into categories such as 0-1% slope, 0-2% slope, and 0-3% slope. Using equal portions of the land in each 0.5% slope increment the amount of land in the 0-0.5% and 0-1% slope range was summarized for the study area. Additionally, the percent of land in the 1-2% slope range was summarized using equal portions in each 1% slope increments for the 0-2% and 0-3% slope category. The purpose of this analysis was to assess the potential for using less detailed information such as soils maps to evaluate the applicability of controlled drainage. Using slope classes from the soils maps results in greater estimated acreage in the slope classes (Table 9). This analysis reveals that the use of the soils maps could grossly overestimate the applicability of controlled drainage and highlights the need for detailed topography to adequately assess the applicability of controlled drainage. Overall, this analysis indicates that the application of controlled drainage on the Des Moines Lobe is severely limited due to topography.
Table 8. Percent of land in slope ranges for (a) DD12, (b) DD77, (c) DD80, and the entire Des Moines Lobe. The total area of the drainage districts and soils in these areas is shown in (d).

(a) DD12

<table>
<thead>
<tr>
<th>Slope Range (%)</th>
<th>Slope Breakdown</th>
<th>Combination of Slope Breakdown &amp; Presumed Tile Drained Soils (Somewhat Poorly Drained and Wetter Soils)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope Range Area (ha)</td>
<td>% of Land in Slope Range</td>
</tr>
<tr>
<td>0-0.5</td>
<td>218.9</td>
<td>11.2</td>
</tr>
<tr>
<td>0.5-1</td>
<td>366.7</td>
<td>18.7</td>
</tr>
<tr>
<td>1-1.5</td>
<td>342.8</td>
<td>17.5</td>
</tr>
<tr>
<td>1.5-2</td>
<td>280.9</td>
<td>14.3</td>
</tr>
</tbody>
</table>

(b) DD77

<table>
<thead>
<tr>
<th>Slope Range (%)</th>
<th>Slope Breakdown</th>
<th>Slope &amp; Somewhat Poorly Drained and Wetter Soils</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope Range Area (ha)</td>
<td>% of Land in Slope Range</td>
</tr>
<tr>
<td>0-0.5</td>
<td>3562.8</td>
<td>10.4</td>
</tr>
<tr>
<td>0.5-1</td>
<td>6892.4</td>
<td>20.2</td>
</tr>
<tr>
<td>1-1.5</td>
<td>6533.2</td>
<td>19.2</td>
</tr>
<tr>
<td>1.5-2</td>
<td>4954.3</td>
<td>14.5</td>
</tr>
</tbody>
</table>

(c) DD80

<table>
<thead>
<tr>
<th>Slope Range (%)</th>
<th>Slope Breakdown</th>
<th>Slope &amp; Somewhat Poorly Drained and Wetter Soils</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope Range Area (ha)</td>
<td>% of Land in Slope Range</td>
</tr>
<tr>
<td>0-0.5</td>
<td>3269.1</td>
<td>11.4</td>
</tr>
<tr>
<td>0.5-1</td>
<td>5813.4</td>
<td>20.2</td>
</tr>
<tr>
<td>1-1.5</td>
<td>5536.8</td>
<td>19.2</td>
</tr>
<tr>
<td>1.5-2</td>
<td>4237.3</td>
<td>14.7</td>
</tr>
</tbody>
</table>

(d) Entire Des Moines Lobe

<table>
<thead>
<tr>
<th>Slope Range (%)</th>
<th>Slope Breakdown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope Range Area (ha)</td>
</tr>
<tr>
<td>0-0.5</td>
<td>218000.5</td>
</tr>
<tr>
<td>0.5-1</td>
<td>437967.6</td>
</tr>
<tr>
<td>1-1.5</td>
<td>464106.7</td>
</tr>
<tr>
<td>1.5-2</td>
<td>402364.0</td>
</tr>
</tbody>
</table>
Figure 13. Map of a portion of DD80 showing the 0-0.5% slope areas combined with the somewhat poor and wetter soils.

Figure 14. Map of the Des Moines Lobe showing 0-0.5% slope areas shown in blue. (Lakes and reservoirs show as relatively large expanses of contiguous flat area.)
Table 9. Comparison of percent of land in slope ranges for (a) DD12, (b) DD77, and (c) DD80 using LiDAR DEM data and soils data.

(a) DD12

<table>
<thead>
<tr>
<th>Slope Range (%)</th>
<th>% of Land in Slope Range using LIDAR Data</th>
<th>% of Land in Slope Range using Soils Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-0.5</td>
<td>9.3</td>
<td>16.7</td>
</tr>
<tr>
<td>0.5-1</td>
<td>15.8</td>
<td>16.7</td>
</tr>
<tr>
<td>1-2</td>
<td>26.4</td>
<td>26.0</td>
</tr>
</tbody>
</table>

(b) DD77

<table>
<thead>
<tr>
<th>Slope Range (%)</th>
<th>% of Land in Slope Range using LIDAR Data</th>
<th>% of Land in Slope Range using Soils Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-0.5</td>
<td>9.5</td>
<td>16.9</td>
</tr>
<tr>
<td>0.5-1</td>
<td>18.6</td>
<td>16.9</td>
</tr>
<tr>
<td>1-2</td>
<td>28.8</td>
<td>26.4</td>
</tr>
</tbody>
</table>

(c) DD80

<table>
<thead>
<tr>
<th>Slope Range (%)</th>
<th>% of Land in Slope Range using LIDAR Data</th>
<th>% of Land in Slope Range using Soils Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-0.5</td>
<td>9.5</td>
<td>14.8</td>
</tr>
<tr>
<td>0.5-1</td>
<td>17.9</td>
<td>14.8</td>
</tr>
<tr>
<td>1-2</td>
<td>29.0</td>
<td>22.9</td>
</tr>
</tbody>
</table>
3.2.1.2. Prioritized List of Subwatersheds

Based on assessments of this project and complementary research, three different wetland implementation scenarios have been identified. These include fractional flow wetland sites within the crop landscape where a portion of the drain flow is routed to a wetland for nitrate removal (tile zone wetlands); wetlands sited at the breakpoint in the landscape where the drainage mains can be outletted into wetlands (break point wetlands); and rerouting of flow to floodplain wetlands (floodplain wetlands).

- Tile zone wetlands: We completed preliminary topographic analyses of high resolution DEMs (LiDAR data) to identify potential sites for fractional flow wetlands within the tile zones of the target watershed. These are sites located within the tile zone where there is a potential to route a variable fraction of flow from tile mains without having to surface the entire flow. Based on analyses of DD80, DD77, and DD12, we have identified 40 potential sites for fractional flow wetlands (Figures 15, 16, and 17).

- Break point wetlands: The potential for break point wetlands was assessed for approximately half of the DD80 area. These locations are identified using DEMs to assess where wetlands meeting specific design criteria can be retrofit onto the landscape to intercept tile drainage water. The design criteria that these wetlands must meet include: wetland receives 500-3500 acres drainage, wetland size between 0.5% and 2.0% of the watershed area, no greater than 25% of wetland pool be deep water (>3ft), and design must protect the drainage rights of upstream and downstream landowners.

- Floodplain wetlands: Potential floodplain sites were identified by using topographic maps, aerial photography and LiDAR elevation data where available. The goal was to locate existing or historic water features that could have flow routed to them in order to provide water quality benefits. By use of the available data, potential areas to reroute flow to are identified by scanning the floodplain and looking for areas that are positioned topographically close to existing waterways and appear to be conducive to having flow routed to them. This analysis was specifically targeted to DD80 where it seems there is the greatest potential for implementation of this practice (Figure 15).

Four potential breakpoint wetland sites were identified in DD80 (Figure 15). Of these four potential sites, two were initially turned down by landowners and the other two landowners have been contacted and presented with conceptual designs. Of these two sites, one was actually requested for assessment by the landowner and the other is under landowner consideration.
Figure 15. Potential tile zone, break point, and flood plain wetland sites in DD80.
Figure 16. Potential fractional flow wetland sites for DD77.
3.2.2. Modeling of alternative drainage-wetland systems

Using crop, drainage, and wetland modeling we assessed the estimated impact of implementing these various practices using data from the study area. Practices considered include drainage water management through controlled and shallow drainage, optimized drainage through increased drainage outlet capacity and integration of wetlands with the most promising drainage scenarios.

3.2.2.1. Crop and drainage modeling

In addition to evaluating the applicability of drainage water management practices it was also important to quantify the performance or projected performance of these practices. While long-term field studies were not possible, crop and drainage modeling were utilized to assess the impacts of drainage modifications. Calibration and validation of the drainage model (DRAINMOD) was undertaken based on comparison of predicted and measured drainage outflow using data sets from the Gilmore City site in DD 77 (SW ¼, Section 27, T92N, and R31W Pocahontas County). Input parameters for DRAINMOD are defined in Table B.1 in Appendix B. Though DRAINMOD has been successfully used for different soil, water and crop conditions, its prediction capability depends on calibration and validation for local ecohydrological conditions. Model calibration is the process of determining indirectly the uncertain input parameters to minimize the differences between predicted and observed state variables (in this case subsurface drainage). Comparing predicted and observed subsurface drainage for an independent dataset validate the indirectly determined input parameters. To calibrate and validate DRAINMOD for Iowa’s tile landscapes, the dataset from Gilmore City
was used. A total of 10 plots were selected; five (0.25 ha) for Webster soil and five (0.25 ha) for Canisteo soil. Figure 18 shows the cumulative observed and predicted subsurface drainage during the calibration years (1990-1993), and Figure 19 shows the results from the validation years (1994-2003). The cumulative subsurface drainage over the years from 1990 to 2003 was predicted to be 2% higher than the observed subsurface drainage for WEBS_CC and 10% lower for CANI_CS. The overall values of Index of Agreement, IoA, and Model Efficiency, EF, were higher than 0.85 for both WEBS_CC and CANI_CS, and showed a close agreement between the predicted and observed subsurface drainage. The predicted outputs sufficiently represented the systems being simulated and provide useful information on the interseasonal relationships between the precipitation, surface runoff, evapotranspiration and subsurface drainage of Iowa’s drained landscapes. These results increase confidence in the model’s ability to predict subsurface drainage hydrology, and the calibrated and validated DRAINMOD provides a tool to estimate the impacts of hydrologic and drainage system modifications on the subsurface drainage.

Figure 18. Cumulative observed and predicted monthly subsurface drainage (cm) for (a) WEBS_CC and (b) CANI_CS during the calibration years from 1990 to 1993.

Figure 19. Cumulative observed and predicted annual subsurface drainage (cm) for (a) WEBS_CC and (b) CANI_CS during the validation years from 1994 to 2003.

Using the calibrated and validated DRAINMOD for a Webster soil (WEBS_CC) we investigated the long-term drainage patterns in north-central Iowa and the potential impacts of drainage water management. A conventional drainage design with a 30 m drain spacing and a
1.2 m drain depth over a 60 (1945-2004) years weather record from Humboldt County in the Des Moines Lobe of Iowa was simulated. The average annual subsurface drainage was 17.5 cm, which is about 22% of the average annual precipitation. Figure 20 shows the cumulative monthly distribution of average precipitation, evapotranspiration and subsurface drainage. Most of the precipitation occurred during the months of March through October with the highest average monthly precipitation of 12.0 cm in June. The time period from March through June contributed approximately 74% of the annual subsurface drainage with the time period of April through May contributing nearly 46% of the annual subsurface drainage. In cold months, where soil freeze/thaw occurs, little if any subsurface drainage occurs.

![Graph showing cumulative monthly distribution of average precipitation, evapotranspiration and subsurface drainage.](image)

Figure 20. Monthly cumulative distribution of average precipitation, evapotranspiration and subsurface drainage of WEBS_CC simulated with conventional drainage design (with a drain spacing of 30 m at a drain depth of 1.20 m) over a weather record of 60 (1945-2004) years.

DRAINMOD simulations were conducted with WEBS_CC for different drainage design and management modifications (shallow and controlled drainage) over the weather record of 60 (1945-2004) years. Placing the drains at different depths or spacing would change the drainage intensity, defined as amount of water (cm) removed per day. Using the Hooghoudt’s steady state equation, the calculated drainage intensity for WEBS_CC varied from 0.10 to 2.35 cm day⁻¹ with drain spacing ranging from 10 to 50 m. Controlled drainage was simulated maintaining an outlet control level at 0.60 m below soil surface during the winter (November through March) and summer (June through August) months. The control structure was opened to lower the water table during crop sowing (April to May) and harvesting (September-October) months. The simulations suggest that both shallow and controlled drainage have some potential to reduce subsurface drainage as compared to conventional (free) drainage (with a drain depth of 1.20 m and free drainage at the drain outlet) (Figure 21). The reduction in subsurface drainage due to shallow and controlled drainage was on the order of 8 to 18% with slightly higher reduction at
lower drainage intensities. However, the preliminary simulations indicate that much of this reduction in subsurface drainage was reflected in increased surface runoff (Figure 21). Also, the simulations indicate that shallow or controlled drainage might increase the excess water stress on crop production, thereby resulting in slightly lower relative yields.

Figure 21. Impact of (a) shallow drainage (drain depth of 0.75 m) and (b) controlled drainage on average annual subsurface drainage, surface runoff and relative yields of WEBS_CC simulated over a weather record of 60 (1945-2004) years.

As reported above there is some potential for reducing subsurface drainage through drainage water management but the applicability of the practice in Iowa may be limited due to topographic constraints. In addition, there is the potential for increased surface runoff with drainage water management practices. As a result, the project team evaluated the impacts of optimized drainage design for minimizing field-to-stream transport of agricultural pollutants. Many current drainage systems are underdesigned with drainage coefficients less than 0.635 cm/day (0.25 in/day) (modern design standards would be approximately 1.27 cm/day or 0.5 in/day). Modeling using the calibrated DRAINMOD model has been conducted to evaluate how changes in the drainage coefficient may impact surface runoff and subsurface drainage. Based on this modeling, drainage systems designed to a drainage coefficient of 1.27 cm/day would be expected to have greater subsurface drainage but less surface water runoff than a system with a drainage coefficient of less than 0.635 cm/day (Figure 22). While the change in drainage coefficient changes the pathways of water movement, the total outflow shows little change. If the drainage water was treated through a nitrate-removal wetland the overall result may be less field-to-stream transport of surface contaminants and treatment of the subsurface drainage water. The increased drainage coefficient is especially effective in reducing the peak discharge at the field scale (Figure 23). This work seems to indicate that the impacts of optimized drainage on subsurface drainage and surface runoff are important in considering a dual nutrient strategy of reducing nitrogen and phosphorus export.
The calibrated DRAINMOD model was also used to evaluate the potential crop yield impacts of changing the drainage coefficient. Changing the drainage coefficient will alter how quickly the water table drops after a rainfall event. Since crop production can be impacted by excess soil moisture it is expected that changing the drainage coefficient would have an impact on estimated crop production. Based on the simulations once the drainage coefficient is less than approximately 0.6 cm/day there is fairly rapid decline in relative yield of the crops (Figure 24). Thus, if drainage systems are redesigned with a greater drainage coefficient there is the potential to increase crop production.
Figure 23. Impact of drainage coefficient on daily discharge (surface + subsurface) for period in (a) 1982 and (b) 1991.
Figure 24. Impact of drainage coefficient on average relative crop yield

While the DRAINMOD results provide very useful information for field-scale assessment it is also useful to assess potential impacts of drainage outlet capacity on a larger-scale. Based on this we utilized a watershed-scale hydrologic model (MIKE SHE) to assess potential impacts of drainage capacity. Modeling was conducted in an area where monitoring was also conducted (PAL 3) (Figure 25). PAL3 contains the drainage district DD28 which contains over 2.6 miles of main line tile drainage infrastructure and many more miles in perforated field tile lines that drain the subsurface soil to a depth of 1.2 m. The area of PAL3 is approximately 1125 ha or about 2780 acres. Both watersheds drain into Cylinder Creek which is a tributary to the Upper Des Moines River.

The soil types of the site are similar to regional soil types and consist mainly of the Clarion-Nicollet-Webster series in the landscape positions of upland, slopes, and bottomland, respectively, as well as Canisteo soil series in the bottomland. Potholes within the study areas consist of Okoboji silty soils and Palms muck. Other minor soils and soil complexes like Harps, Storden, and Clarion-Storden are also in both watersheds. Figure 25 shows the distribution of soil type for the PAL3 watershed. Canisteo is the most prevalent soil in the watershed with 34.1%. Clarion-Nicollet-Webster soils accounted for 45% and potholes associated with Okoboji soils were 11.4%.

Clarion and Palms soils were considered to be not drained in this watershed. Clarion soils are typically well drained and in landscape positions were tile drainage is unnecessary. Palms soils can be effectively drained since they have poor natural drainage and occur at lower regions of landscape position; however, the Palms soil in PAL3 is in a non-farmed wetland that is not drained.
MIKE SHE is a deterministic, physically based, continuous and event based model capable of simulating both water quality and water management simulations. The program code is divided into several modules that can be simulated separately or simultaneously integrated. Model components used include climate, land use, overland, unsaturated and saturated zone interactions to predict the hydrology in the watershed.

Climate data for each watershed was collected from various weather station locations in the area of the watersheds. Precipitation data were collected from four different locations around the watershed locations. Each station was in one of four directions - northeast, southeast, southwest, and northwest. After inspection, the SW station location was chosen for the simulation because the site provided data that most closely matched the observed data, as well as the small relative distance to the sites compared to other local stations. Other climatic data, including wind speed, solar radiation, temperature, and humidity values were collected from Iowa Environmental Mesonet station locations administrated by the Iowa State University Department of Agronomy. These values were used to calculate evapotranspiration (ET) within MIKE SHE. The Kanawha weather station was used for this climatic data.

MIKE SHE is an input intensive model. The model has been tested using best estimate parameters. Some of the important parameters are shown in Table 10. A more extensive list of input parameters for MIKE SHE are defined in Table B.1 in Appendix B.

Values for these parameters were chosen based on site inspection and published findings of similar situations. The cell size in the model grid is considered to be 30m by 30m. This resolution helps keep error low and also helps to reduce computational time needed for each simulation. The Manning’s coefficient $n$ is the reciprocal of the Manning’s M and was based on cropland with a disk tillage system (Engman, 1986). Detention storage sets the limit on the amount of accumulated precipitation that will cause overland flow. A value too high will cause excessive ponding and evaporation and will decrease total flow at the outlet (Harder et al., 2007).
Unsaturated soil profiles were calculated using Web Soil Survey data (soil texture and bulk density) and the Rosetta soil model (empirical constants, hydraulic conductivity parameters) available through the USDA. Soil hydraulic conductivity values were based on values reported in the Web Soil Survey. Drainage level or depth was set to -1.2 m (relative to ground surface) and is typical for drainage practices in the region. Drainage flow is simulated in MIKE SHE using an empirical formula, which requires, for each cell, a drainage level and a time constant (leakage factor) (DTC). Mathematically, the time constant is exactly the same as a leakage coefficient – it is simply a factor that is used to regulate how quickly the water can drain. From the MIKE SHE manual a typical time constant is between 1e-6 and 1e-7 s. However, since the model has not been used in the U.S. we did have to slightly adjust this parameter during model assessment.

Table 10. Important input parameters in the MIKE SHE simulation.

<table>
<thead>
<tr>
<th>MIKE SHE Module</th>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topography</td>
<td>Cell Size</td>
<td>30 x 30</td>
<td>m²</td>
</tr>
<tr>
<td>Overland</td>
<td>Manning’s coefficient</td>
<td>1/6</td>
<td>s m⁻¹/³</td>
</tr>
<tr>
<td>Overland</td>
<td>Detention Storage</td>
<td>2</td>
<td>mm</td>
</tr>
<tr>
<td>Unsaturated</td>
<td>Profile Depth</td>
<td>Varies</td>
<td>m</td>
</tr>
<tr>
<td>Unsaturated</td>
<td>Soil Properties</td>
<td>Varies</td>
<td>varies</td>
</tr>
<tr>
<td>Saturated Zone</td>
<td>Hydraulic Conductivity</td>
<td>10⁻⁵ to 10⁻⁶</td>
<td>m s⁻¹</td>
</tr>
<tr>
<td>Saturated Zone</td>
<td>Drainage Depth</td>
<td>-1.2</td>
<td>m</td>
</tr>
<tr>
<td>Saturated Zone</td>
<td>Drainage Type</td>
<td>Grid Codes</td>
<td>---</td>
</tr>
<tr>
<td>Saturated Zone</td>
<td>Drainage Time Constant</td>
<td>10⁻⁷ to 10⁻⁸</td>
<td>s⁻¹</td>
</tr>
</tbody>
</table>

This is the first application of MIKE SHE that we know of in the U.S. examining the impacts of drainage on water flow at the drainage district scale. A critical parameter in this modeling is the drainage time constant (DTC). Cumulative annual flow (Table 11) and daily flow data including surface, sub surface, and total flow (Figures 26, 27, and 28) for the PAL3 watershed shows that as the drainage time constant decreases the predicted surface runoff increased. The modeled flow data is compared to observed flow data at the monitoring location near the outlet of the PAL3 watershed (Figure 28).

Daily surface runoff, subsurface drainage, and total flow data for the three drainage time constants from 2007 through 2011 are shown below in Figures 26, 27, and 28, respectively. Most simulations seem to have higher peaks than the observed data in most years. This could be due to a number of factors including precipitation, land use definition, overland, soil, and drainage parameters. However, using best estimates of input parameters and without fitting results to the observed data, the model provides reasonable predictions of the timing of flow and the monthly flows are in the satisfactory range defined by Moriasi et al. (2007) (Table 12). Since precipitation was not available on-site there is the potential that the on-site precipitation was different than that used for the model. If the actual precipitation at the site were higher than the precipitation measured at the off-site location, the model may under predict flow. Table 13
shows the annual precipitation for PAL3 from 2007 through 2011. Also, there are periods during low flow where the model over predicts the flow. This could be a result of slow groundwater seepage to the drains that in the field happens more quickly than modeled. This could be a result of the saturated zone hydraulic conductivity not accurately reflecting the actual soil conditions.

It is also useful to statistically analyze modeled flow data against observed flow data to determine the degree of fit of the model. As stated above, a negative percent bias (PBIAS) value means the model tends to over predict flow, especially peaks in 2010 and 2011, and low flow conditions late in most years. Daily Nash-Sutcliffe Efficiency (NSE) values for this simulation were as high as 0.42, with weekly values being as high as 0.58 (Table 12). These weekly and monthly NSE values are closer to the optimal NSE value of 1, and within the satisfactory limit set forth by Moraisi et al. (2007). From the surface runoff response we estimate that the DTC of $3.75 \times 10^{-8}$ s$^{-1}$ represent existing conditions and the DTC of $5 \times 10^{-8}$ s$^{-1}$ represents enhanced or optimized drainage. Consistent with the field-scale DRAINMOD simulations as the subsurface drainage is optimized there is potential to reduce surface water runoff. Future work should further investigate the impacts of subsurface drainage on watershed hydrology specifically examining the impacts at increasing scale.

Table 11. Total flow breakdown of surface and subsurface flow for various drainage time constants (DTC)

<table>
<thead>
<tr>
<th>Drainage Time Constant (s$^{-1}$)</th>
<th>Surface Flow (mm and %)</th>
<th>Subsurface Flow (mm and %)</th>
<th>Total Flow (mm and %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 x $10^{-8}$</td>
<td>178.9 (9.8)</td>
<td>1643 (90.2)</td>
<td>1822 (100)</td>
</tr>
<tr>
<td>3.75 x $10^{-8}$</td>
<td>270.2 (15)</td>
<td>1520 (85)</td>
<td>1790.2 (100)</td>
</tr>
<tr>
<td>2.5 x $10^{-8}$</td>
<td>473.5 (27)</td>
<td>1281 (73)</td>
<td>1754.5 (100)</td>
</tr>
</tbody>
</table>

Table 12. Drainage time constant statistical values considering daily and weekly flow of PAL3 using NSE and PBIAS tests

<table>
<thead>
<tr>
<th>Drainage Time Constant (s$^{-1}$)</th>
<th>NSE Daily</th>
<th>NSE Weekly</th>
<th>NSE Monthly</th>
<th>PBIAS Daily</th>
<th>PBIAS Weekly</th>
<th>PBIAS Monthly</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 x $10^{-8}$</td>
<td>0.42</td>
<td>0.58</td>
<td>0.64</td>
<td>-2.79</td>
<td>-2.78</td>
<td>2.71</td>
</tr>
<tr>
<td>3.75 x $10^{-8}$</td>
<td>0.30</td>
<td>0.53</td>
<td>0.66</td>
<td>-0.60</td>
<td>-0.45</td>
<td>0.38</td>
</tr>
<tr>
<td>2.5 x $10^{-8}$</td>
<td>0.01</td>
<td>0.38</td>
<td>0.52</td>
<td>-0.42</td>
<td>1.07</td>
<td>-1.13</td>
</tr>
</tbody>
</table>
Table 13. PAL3 precipitation total and averages from 2007 through 2011

<table>
<thead>
<tr>
<th>Year (2007 – 2011)</th>
<th>Precipitation (mm and in)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>1065.2 (41.9)</td>
</tr>
<tr>
<td>2008</td>
<td>767.0 (30.2)</td>
</tr>
<tr>
<td>2009</td>
<td>752.4 (29.6)</td>
</tr>
<tr>
<td>2010</td>
<td>1162.3 (45.7)</td>
</tr>
<tr>
<td>2011</td>
<td>796.9 (31.4)</td>
</tr>
<tr>
<td>Total</td>
<td>4543.8 (178.9)</td>
</tr>
<tr>
<td>Ave</td>
<td>908.8 (35.8)</td>
</tr>
</tbody>
</table>

Figure 26. Daily surface flow for years 2007 - 2011 predicted by MIKE SHE (charts a, b, c, d, and e, respectively)
Figure 27. Daily subsurface flow for 2007 - 2011 predicted by MIKE SHE (charts a, b, c, d, and e, respectively)
Figure 28. Total flow for 2007 – 2011 predicted by MIKE SHE and observed (charts a, b, c, d, and e, respectively)

3.2.2.2. Wetland Modeling

Over the past 10 years, Iowa State University and the Iowa Department of Agriculture and Land Stewardship have cooperated on a targeted research program addressing agricultural water and nutrient management. For its part, the ISU Wetlands Research Laboratory has focused on the development and application of performance forecast models for siting, design and assessment of wetland restorations in agricultural watersheds. This work provided the research foundation for the Iowa Conservation Reserve Enhancement Program (CREP), a targeted, performance-based strategy for reducing nitrate loads from tile-drained landscapes. As an integral part of the Iowa CREP, representative wetlands are monitored each year to document nitrate reduction. By design, the wetlands selected for monitoring span the 0.5% - 2% wetland/watershed area ratio range approved for Iowa CREP wetlands. The wetlands also span a range in average nitrate-N concentration from about 3 - 20 mg N/L. The wetlands thus provide a broad spectrum of those factors most affecting wetland performance: hydraulic loading rate, residence time, nitrate concentration, and nitrate loading rate. Wetlands are instrumented with automated samplers and flow meters at wetland inflows and outflows. Water levels are monitored continuously at outflow structures in order to calculate changes in wetland volume and wetland water temperatures are recorded to facilitate modeling of nutrient loss.
Wetlands receiving nonpoint source loads are subject to widely varying hydraulic and nutrient loading rates and successful nitrate loss models must adequately represent these variations in load and in residence time distributions. Tanks in series (TIS) models have been shown to realistically represent the residence time distributions of wetlands having a wide range of morphometries and aspect ratios. Nitrate removal in CREP wetlands has been successfully modeled as a temperature dependent first-order process in TIS models (Crumpton 2001, 2005, Crumpton et al. 2006, Crumpton and Stenback 2011). Input and output parameters for the TIS models are provided in Table B.1 in Appendix B. These models are able to replicate the short term and seasonal dynamics of nitrate loss in wetlands subject to highly variable loading patterns (Figure 29) and have been validated against observed nitrogen mass balances of wetlands monitored as part of the Iowa CREP (Figure 30). The models were used to estimate the variability in performance of Iowa CREP wetlands that would be expected due to spatial and temporal variability in temperature and precipitation patterns. The percent nitrate removal expected for Iowa CREP wetlands was estimated based on hindcast modeling over the 1980 through 2005 period (Figure 30) and results compare favorably with the percent nitrate removal measured for wetlands monitored from 2004 through 2011 (Figure 30).

The results in Figures 29 and 30 are provided here to demonstrate the fidelity of the model predictions over a range of conditions and scales since this modeling approach was used to assess the potential performance of wetlands based on the observed hydrologic and nitrate loading patterns at the TWG sites. Models were constructed for hypothetical wetlands sized at 0.5, 1, and 2% of the catchment area above each of the eight intensively monitored TWG sites in Clay, Palo Alto and Pocahontas Counties. The observed nitrate concentrations and discharge at each site were used as forcing functions for the models and simulations were run for the period from 2007 through 2011. The observed N export and the predicted N export for each model scenario are presented below (Table 14 and Figures 31 and 32). On average, wetlands representing 0.5 to 2% of the watershed areas at the monitored sites would have been expected to reduce nitrate export by 26 to 59% over the period from 2007 through 2011.

Figure 29. Measured and modeled performance of AL Wetlands in 2011 (Crumpton and Stenback 2011).
Figure 30. Measured (2004-2011) and modeled performance of wetlands monitored under Iowa CREP (Crumpton and Stenback 2011).

Table 14. Observed and predicted nitrate-N export (kg/ha/yr) and % loss (in parentheses) for the monitored watersheds with different size wetlands.

<table>
<thead>
<tr>
<th>Site</th>
<th>Observed export</th>
<th>Predicted export with 0.5% wetland</th>
<th>Predicted export with 1% wetland</th>
<th>Predicted export with 2% wetland</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLA 1</td>
<td>26</td>
<td>17 (33)</td>
<td>13 (50)</td>
<td>8 (68)</td>
</tr>
<tr>
<td>PAL 3</td>
<td>35</td>
<td>26 (24)</td>
<td>21 (39)</td>
<td>15 (57)</td>
</tr>
<tr>
<td>PAL 5</td>
<td>46</td>
<td>35 (23)</td>
<td>29 (37)</td>
<td>21 (55)</td>
</tr>
<tr>
<td>PAL 7</td>
<td>48</td>
<td>36 (24)</td>
<td>30 (38)</td>
<td>22 (54)</td>
</tr>
<tr>
<td>PAL 11</td>
<td>36</td>
<td>27 (23)</td>
<td>22 (37)</td>
<td>16 (55)</td>
</tr>
<tr>
<td>PAL 16</td>
<td>28</td>
<td>18 (34)</td>
<td>14 (51)</td>
<td>9 (69)</td>
</tr>
<tr>
<td>POC 2</td>
<td>37</td>
<td>26 (29)</td>
<td>21 (43)</td>
<td>14 (61)</td>
</tr>
<tr>
<td>POC 8</td>
<td>43</td>
<td>32 (27)</td>
<td>25 (42)</td>
<td>17 (60)</td>
</tr>
<tr>
<td>Average</td>
<td>37</td>
<td>27 (26)</td>
<td>22 (41)</td>
<td>15 (59)</td>
</tr>
</tbody>
</table>
Figure 31. Observed and predicted nitrate-N export (kg/ha/yr) with different size wetlands for the monitored watersheds (drainage coefficient shown in parentheses).

Figure 32. Average observed and predicted nitrate-N export (kg/ha/yr) for the monitored watersheds with different size wetlands.
3.2.2.3. Combined drainage & wetland systems modeling

As aging drainage systems are replaced with modern designs across the Des Moines Lobe of Iowa, wetlands can be integrated into most drainage networks to achieve nitrate reductions in conjunction with the environmental benefits of the drainage network itself. It is likely that most 20th century systems will be replaced with higher drainage coefficients based on modern design standards matched to the more row crop intensive landscape today, with potential crop yield increases on lands already in row crop production. We evaluated the combined effect of integrating wetlands of different sizes with drainage systems having different drainage coefficients using PAL 3 as a model system. The MIKE SHE output at various drainage time constants (DTC) were input into wetlands models to assess the impacts of drainage modifications on wetland performance. Overall, based on subsurface flow predicted by MIKE SHE, a DTC of $3.75 \times 10^{-8}$ is assumed to represent a low DC and a DTC of $5 \times 10^{-8}$ or greater is assumed to represent the higher DC of modern systems (modern DC).

Nitrate and phosphorus loads were estimated based on daily subsurface and surface flow predicted by MIKE SHE and estimates of the associated nitrate and phosphorus concentrations. Table B.1 in Appendix B shows where the MIKE SHE flow and measured nutrient concentrations were used as input parameters for the TIS models. Nitrate loads were estimated assuming nitrate nitrogen concentrations of 0.3 mg N/L in surface runoff and 13 mg N/L in subsurface flow (based on the long term FWA nitrate concentration observed for drainage districts monitored under the Iowa CREP). Phosphorus loads were estimated assuming phosphorus concentrations of 550 µg/L in surface runoff (based on the average TP concentrations measured in overland flow) and 150 µg/L in subsurface flow (based on the literature values and FWA TP concentrations measured during periods with no surface runoff). Nitrate loss in wetlands was modeled as a temperature dependent first-order process in TIS models (Crumpton 2001, 2005, Crumpton et al. 2006, Crumpton and Stenback 2011). Phosphorus retention by wetlands was modeled based on loss-load relationships developed from mass balances for 33 wetland years of monitoring data from Braskerud (2005) and Crumpton et al. (in prep.).

Results suggest that increasing drainage capacity as represented in the MIKE SHE simulations would be expected to increase subsurface flow and nitrate export by about 8%. However, when integrated with a wetland nitrate export is expected to decrease by 26-64% (Table 15 and Figure 33). The simulated increase in drainage capacity would be expected to decrease surface runoff by about one third and to decrease phosphorus loads by 10-20% depending on the TP concentration in subsurface flow. Reducing surface runoff would have less effect on phosphorus transport in systems with higher phosphorus concentrations in subsurface flow and much greater effect in systems with lower phosphorus concentrations in subsurface flow. When wetlands equivalent to 1% of the drainage area are integrated with systems having increased DC, phosphorus export is expected to decrease by more than 25% (Figure 34).
Table 15. Simulation modeling export (kg ha\(^{-1}\) yr\(^{-1}\)) results for drainage designs for two drainage coefficients (DC) with percent decline from the current condition (low DC) in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Low DC</th>
<th>Modern DC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate-N export</td>
<td>40 (0)</td>
<td>43 (-8*)</td>
</tr>
<tr>
<td>Export with 0.5 % wetland</td>
<td>27 (33)</td>
<td>30 (26)</td>
</tr>
<tr>
<td>Export with 1% wetland</td>
<td>20 (50)</td>
<td>22 (44)</td>
</tr>
<tr>
<td>Export with 2% wetland</td>
<td>12 (69)</td>
<td>14 (64)</td>
</tr>
</tbody>
</table>

* 8% increase from current condition

Figure 33. Simulation modeling results of nitrate export for drainage designs for two drainage coefficients (DC).

Figure 34. Simulation modeling results of phosphorus export for drainage designs for two drainage coefficients (DC) and wetlands sized to 1% of the watershed area. Values in parentheses are the percent decline from the low DC with no wetland condition.
3.3. Broader impacts

3.3.1. Sources and implications of variability of nitrate concentration

Nitrate concentrations are thought to be primarily determined by agricultural practices and drainage patterns, and are expected to be similar for tile drained watersheds in the same geographic area and with similar agricultural practices. However, our synoptic sampling results demonstrated a greater than two-fold range in average nitrate concentrations among drainage systems, despite similar patterns in the extent of cultivated cropland and subsurface drainage. In this section, we further examine the sources and implications of variability in nitrate concentrations, primarily focusing on the potential effects of land use and nutrient management on nitrate concentrations in agricultural watersheds.

Nitrate concentrations in stream flow are a function of contributions from subsurface flow and surface runoff. We estimated nitrate concentrations in subsurface flow as a function of land use and nitrogen application rates across the entire state. Land use data from the USDA National Agricultural Statistics Service for the years 2006 to 2010 were used to generate a 56 m land use grid for Iowa where each grid was classified into one of five categories: 1) CC: continuous corn (corn for at least four of the five years), 2) CB: corn/soybean rotation (corn and soybean each at least two of the five years), 3) PH: pasture or hay, 4) EXT: extended rotation (includes at least one year of perennial cover within a row crop system), and 5) other (non-agricultural land use) (grid developed by David James, USDA/ Agricultural Research Service). Nitrogen concentration grids were developed as functions of these five land use categories. Constant nitrate-N concentrations for pasture-hay (1.6 mg/L) and extended rotations (6.1 mg/L) were assumed based on literature in and nearby to Iowa. Other (non-agricultural) land uses were assumed to contribute negligible nitrate load. Nitrate loads in surface runoff from agricultural land are assumed to be negligible in comparison to loads associated with subsurface drainage (this is not assumed to be the case for other forms of N).

County scale fertilizer and manure nitrogen application rates were used to estimate nitrate concentrations for continuous corn and corn-soybean rotation based on relationships between nitrogen application rate and nitrate concentration developed for the study area (Lawlor et al. 2008). The county based nitrogen application rates were based on David et al., 2010 and included both manure and fertilizer. Since that study was designed to look at a total nitrogen balance for regions in the state, manure numbers included all cattle (both grain fed and pastured). Since manure from pastured cattle is not applied to production crops, these cattle were removed from the analysis (leaving grain fed cattle only). Replacement cattle numbers came from the 2002 Census of Agriculture (United States. National Agricultural Statistics Service, 2007). Adjustments were also made to manure nitrogen amounts by adjusting for nitrogen availability and storage losses to calculate plant available nitrogen applied to the crop. The methods for fertilizer application rates developed by David et al., 2010 used county level data from the 1997 and 2002 Census of Agriculture. The methods employed included distributing statewide fertilizer sales reported by the Association of American Plant Food Control Officials in 2008 to counties based on county level fertilizer, lime, and soil conditioner expenditure for 1997 and 2002 reported by the Census of Agriculture.
In addition to the eight sites that were intensively monitored for 5 years as part of the current project, we assembled data for thirty sample stations on Iowa rivers having at least 5 years of at least monthly nitrate concentration data and calculated flow-weighted-average (FWA) nitrogen concentrations for all sites. For each of these watersheds, the nitrate concentration grid described above was clipped and the average concentration was determined and compared with the observed FWA concentration. Based on these analyses, land use and nitrogen management explained most of the variability in nitrate concentration at larger watershed scales (Figure 35 – blue diamonds). Nitrate concentrations estimated based on land use and N application rates overestimate the observed nitrate concentrations by about 17% on the basis of a least squares statistical model. However, this 17% difference could be largely explained by in stream loss of nitrate and by dilution due to surface runoff. In contrast, land use and N application rates explained very little of the variability in nitrate concentration observed at the drainage district scale (Figure 35 – red squares). Nitrate concentrations of drainage districts varied much more than could be explained based on land use and N management.

Figure 35. GIS average nitrate concentration versus observed FWA concentration for 38 watersheds. The red squares are drainage district scale sites in Clay, Palo Alto and Pocahontas Counties.
3.3.2. Regional assessment: Potential N reduction using N management, cover crops, drainage water management, and wetlands

The Des Moines Lobe is characterized by intensive row crop agriculture and nitrogen loads to surface waters are among the highest in the region. The problem of excess nitrate loads can probably be ameliorated by a combination of in field and off site practices, but the limitations and appropriateness of alternative practices must be considered. We used a GIS based approach to conduct a regional assessment of the potential for nitrate load reductions on the Des Moines Lobe of Iowa due to improved N management, implementation of cover crops no-till acres, and targeted wetland restoration and drainage water management.

We estimated N yield based on nitrate concentration (as a function of land use and N management as described above) and water yield. Nitrate yield grids were generated as the products of the subsurface water yield and nitrate concentration grids and assumed nitrate loads associated with surface runoff were negligible. On the basis of considerations given above, the long term surface runoff component was assumed to be 17% of the total flow, leaving the remaining 83% as subsurface flow.

Water yield grids were generated based on precipitation grids and a stream flow versus precipitation regression developed for watersheds across Iowa. Daily precipitation data was downloaded from the National Climatic Data Center for the period 1980 through 2010. Data were obtained for 231 weather stations within Iowa and 127 stations in states surrounding Iowa within approximately 40 miles of Iowa. The data from these stations was approximately 30% incomplete. To complete the record for each station, missing daily values were estimated as the inverse distance weighted average of the 5 nearest stations having data on that day. These data were summed by year to obtain the total annual precipitation for each of the 358 weather stations.

Discharge data were downloaded from the USGS Water Watch web pages for 18 gage stations distributed across Iowa. The watershed for each of these stations was determined and annual water yields were calculated for the period 1980 through 2010. Annual precipitation data for all weather stations within (and sometimes near) each watershed were averaged and used to represent the annual precipitation for each watershed. Examination of the relationship between annual water yield and precipitation suggested that most of the annual variation in water yield could be explained by precipitation in the current and preceding year (equation 1):

\[ WY_t = \beta_1 P_t + \beta_2 P_t^2 + \beta_3 P_{t-1} + \epsilon_t \]  \hspace{1cm} \text{eq 1}

where the \( \beta_i \) are regression coefficients for each watershed, \( P_t \) and \( WY_t \) are the precipitation and water yield for year \( t \), and \( \epsilon_t \) is the prediction error for year \( t \). The \( R^2 \) for individual watersheds ranged from 0.51 to 0.91 (average 0.80). In most cases, all three regression coefficients were statistically significant at the 0.05 level of significance. In a few cases \( \beta_3 \) was not significant at that level but was retained to maintain the same functional form across all watersheds. For a few combinations of very low precipitation in two consecutive years, equation 1 returned a negative value in which case the water yield was set to zero.
Equation 1 was applied to the annual precipitation data to generate a water yield estimate at each weather station location. To account for the spatially variable relationship between precipitation and water yield, regression coefficients at each weather station were estimated as the inverse distance weighted values for the three nearest USGS watersheds using the distance from the approximate centroid of the watershed to the precipitation station. This generated an annual water yield at each of the 358 weather stations for 1981 to 2010. These water yields were used to generate a 300 m water yield grid for the state of Iowa for each year from 1981 to 2010 using the kriging procedure in ArcMap.

Comparisons of the 30 year average water yield grid developed on the basis of the precipitation data with water yields from 15 USGS gage stations scattered around Iowa indicate that the 30 year predicted water yield range from 1.5% low to 5.6% high and have annual water yield prediction efficiencies ranging from 0.48 to 0.94, with an average annual prediction efficiency of 0.804.

Results for nitrogen management, cover crops, drainage water management and wetlands

The average nitrogen application rates for Iowa and for the Des Moines Lobe are greater than the recommended application rate for Maximum Return to Nitrogen based on the Nitrogen Rate Calculator (http://extension.agron.iastate.edu/soilfertility/nrate.aspx). The statewide estimated nitrogen application rate to corn in a corn soybean rotation is approximately 169 kg-N/ha (151 lb-N/acre) compared to a MRTN rate of 149 kg-N/ha (133 lb-N/acre). Using the current application rate for each county as the starting point, we evaluated the nitrate reduction that might be achieved by moving the county based rate to the MRTN rate. For the Des Moines Lobe, results suggest that reducing to the MRTN could reduce nitrate export by about 18% (Table 16). The spatial pattern of changes in nitrate concentrations suggests that most of the Des Moines Lobe could benefit from improved N management (Figures 36 vs 37). The pattern also suggests substantial benefits for a few counties which are predicted to have especially high nitrate concentrations based on estimates of current application rates.

We evaluated the effect of establishing cover crops on all crop land where no-till was currently practiced. In many areas this is a very small percent of the acreage but since cover crops are used within a no-till system it is believed current no-till operators would be the early adopters. Since no-till acres are only a small portion of all row-crop acres on the Des Moines Lobe, there is only a 2% reduction due to the implementation of cover crops on no-till acres. When cover crops are implemented in combination with nutrient management the estimated nitrate reduction for the Des Moines Lobe increases from 18% to 20% (Table 16 and Figure 38).

We evaluated the potential effect of implementing drainage water management on all tile drained crop land on the Des Moines Lobe with slopes less than 0.5%. Based on LIDAR, about 7.1% of the Des Moines lobe has slopes less than 0.5%. Based on results for DD12, DD77, and DD80 (Table 8) we assumed 85% of this was tile drained cropland. Helmers et al. (in review) from a field location in Iowa report a subsurface flow reduction of about 40% using controlled drainage and similar magnitude reduction in nitrate export. Singh et al. (2007) from modeling data report approximately a 15% subsurface flow reduction using controlled drainage. Based on these studies, we estimated N loss assuming 30% removal efficiency and assuming drainage water management systems were implemented on all tile drained cropland with slopes less than 0.5%. Results suggest that drainage water management has the potential to reduce nitrate export from the Des Moines Lobe by about 2% (Table 16 and Figure 38). When drainage water
management is implemented in combination with nutrient management the estimated nitrate reduction for the Des Moines Lobe increases from 18% to 20% (Table 16 and Figure 38).

We evaluated the potential effect of establishing wetlands targeted to intercept nitrate loads from areas from the areas of Des Moines Lobe having the greatest nitrate yield. The wetlands were assumed to intercept flow from half of the total area of the Des Moines Lobe and sized to be 1% or 2% of their watershed's contributing area. Wetland performance was estimated based on HLR (calculated from the WY grid and wetland area) and N concentration (calculated based on N inputs and land use) (Crumpton et al. 2006, Crumpton and Stenback 2011, Crumpton and Stenback in prep). Wetlands alone reduced N export by 34-46% and in combination with N management reduced N export by 45-55% (Table 16 and Figure 38). The combination of N management and wetlands offered the greatest potential for meeting the 45% reduction goal set by the Hypoxia Action Plan.
Figure 36. Current nitrate concentration estimated from curves relating land use and county scale nitrogen application to FWA nitrate.

Figure 37. Nitrate concentration estimated from curves relating land use and maximum return to nitrogen (MRTN) application rate to FWA nitrate.
Table 16. Estimated long term average effects of using nutrient management, drainage water management (DMW), and wetland schemes on nitrate-N yield (kg/ha/yr) on the Des Moines Lobe in Iowa. The value in parentheses is the percent reduction in yield from the current condition.

<table>
<thead>
<tr>
<th></th>
<th>Current condition</th>
<th>Nutrient management</th>
<th>Nutrient management + cover crop</th>
<th>Nutrient management + DWM</th>
</tr>
</thead>
<tbody>
<tr>
<td>No wetlands</td>
<td>25.1</td>
<td>20.6 (18)</td>
<td>20.1 (20)</td>
<td>20.1 (20)</td>
</tr>
<tr>
<td>50% of highest yield land at 1% wetland/watershed area ratio</td>
<td>16.6 (34)</td>
<td>13.8 (45)</td>
<td>13.5 (46)</td>
<td>13.5 (46)</td>
</tr>
<tr>
<td>50% of highest yield land at 2% wetland/watershed area ratio</td>
<td>13.5 (46)</td>
<td>11.4 (55)</td>
<td>11.1 (56)</td>
<td>11.1 (56)</td>
</tr>
</tbody>
</table>

Figure 38. Estimated long term average effects of using nutrient management and wetland schemes on nitrate-N yield (kg/ha/yr) on the Des Moines Lobe in Iowa.
3.4. Outreach, engineering and construction of drainage-wetland systems

3.4.1. System Design and Construction

A major outcome of the work that has been funded under this grant is the development of a new initiative to look at watershed optimization for both environmental and agricultural benefits. The “Iowa Wetland Landscape Systems Initiative” has developed as a pilot project to assess the benefits of pairing modernized drainage infrastructure with restored wetlands to provide numerous environmental and agricultural benefits. Iowa has over 3000 organized drainage districts encompassing 6,000,000 acres of tile drained land. Most of these systems were installed in the early 20th century and are quickly reaching the end of their design life expectancy. As these systems age out economics are strong to maintain and update them in order to preserve and enhance the agricultural production in place today. This provides a unique window of opportunity to assess a new model that also incorporates environmental benefits by utilizing an integrated systems approach.

By pairing drainage and wetland systems together at a watershed scale it is expected that the modernized drainage system will increase water infiltration while reducing surface runoff, erosion, sediment, and phosphorous delivery to surface waters. The wetlands at the drainage system outlets round out the environmental benefits by removing nitrate delivered by the tile drainage and providing valuable habitat. Currently in Iowa, wetland restorations for nitrate removal are done under the Conservation Reserve Enhancement Program which retrofits wetlands to the existing drainage infrastructure. This retrofit approach has been very successful, but requires locations where the drainage infrastructure allows enough vertical separation between the wetland and tile outlet for gravity flow delivery of the tile drainage water into the wetland. The integrated systems approach provides the new ability to make more locations for wetlands feasible by designing the modernized drainage infrastructure with wetlands in mind and thereby enabling enough vertical separation to deliver the tile drainage water by gravity flow. Other expected benefits are increased yields on currently farmed land and numerous habitat and recreation benefits from the restored wetlands. The pilot project aims to demonstrate a means for farmers to achieve environmental goals while continuing to produce food, fuel, and fiber for an ever growing world population.

Seven pilot projects have been targeted to build and demonstrate modernized drainage infrastructure systems paired with wetlands at their outlets. Estimated cost for these projects is $15.0 million. Of this total, landowners will pay $7.11 million to fund the restorations and demonstrations with the remainder $7.89 million being provided by public cost-share support to provide the study sites needed for the monitoring and assessments. These pilot projects will be monitored for a minimum of 5 years to document the project outcomes for environmental and agricultural benefits.

The first step is to construct the modernized drainage infrastructure and restore wetlands at the 7 pilot demonstration and study sites. One site is completed in Pocahontas County and six more are planned for restoration and construction in 2012. The following pilot sites are proposed in drainage districts in 3 Iowa counties:
– Pocahontas DD 65 – completed in 2010
– Clay DD 8
– Pocahontas DD 48 & 81
– Palo Alto DD15 North
– Palo Alto DD15 South
– Pocahontas/Palo Alto Joint DD36
– Pocahontas DD178 – project to close 11 agricultural drainage wells

3.4.2. Landowner responses

Numerous presentations were conducted throughout Iowa to relay information from this project and specifically present information on drainage redesign and wetlands. Based on this the feedback on wetland potential was always excellent. Producers were interested in wetlands and excited about their environmental performance. Relative to controlled drainage feedback has been mixed. Producers recognize that the land may not be applicable to broad implementation and they recognize the potential for increased surface runoff. There is some limited interest in shallower drain placement but producers recognized the cost increase due to increased linear feet of tile per acre resulting from the narrower drain spacing. Overall, there is growing interest in redesign of the drainage system to increase the drainage coefficient to optimize drainage design. Most producers recognize that the most limiting part of their drainage system is the common outlet owned by the entire watershed group through the drainage district.

3.4.3. Outreach and Publications

As part of this project there was broad dissemination of project activities and results to a wide variety of audiences. In addition, conference abstracts, proceedings, and peer-reviewed publications were developed.

January 12, 2005 – Presentation on modified drainage for improved water quality at North Central Crop Clinic in Iowa Falls, IA by Dr. Matt Helmers (45 attendees).
January 20, 2005 – Presentation on integrated drainage-wetland systems for reducing nitrate loads from Des Moines lobe watersheds by Dr. William Crumpton at the 2004 Targeted Watershed Grants recipients conference.
January 26-27, 2005 – Presentation on drainage design and management at Heartland Water Quality Initiative Nitrogen Roundtable in Nebraska City, NE by Dr. Matt Helmers (30 attendees from Iowa, Nebraska, Kansas, Missouri, and USEPA).
March 15, 2005 – Project team met with Pocahontas Soil and Water Conservation District to discuss EPA Targeted Watershed Project and the concept of drainage water management.
May 2005 – Developed and distributed a newsletter to nearly 1200 landowners in the project area introducing the project and the potential for integration of drainage and wetland systems.
June 2005 – Presentation on subsurface drainage and treatment of drainage water to reduce nitrate-N at Heartland Water Quality Initiative Nitrogen Workshop in Nebraska City, NE by Dr. Matt Helmers (75 attendees from Iowa, Nebraska, Kansas, Missouri, and USEPA).

June 2005 – Presentation on design of drainage water treatment facilities at Heartland Water Quality Initiative Nitrogen Workshop in Nebraska City, NE by Dr. Matt Helmers (20 attendees from Iowa, Nebraska, Kansas, Missouri, and USEPA).

June 30, 2005 – A field day was organized at the Gilmore City, IA drainage research facility. The evening field day was attended by approximately 75 stakeholders. The topics discussed were current crop issues (Paul Kassel), nitrate-removal wetlands (Dr. William Crumpton), the Targeted Watershed Project (Dean Lemke and County Board of Supervisors), highlights from 15 years at Gilmore City (Dr. Stewart Melvin, Peter Lawlor, and Dr. James Baker), and controlled drainage (Dr. Matt Helmers).

July 28, 2005 – Presentation by Dr. Matt Helmers on subsurface drainage design and drainage water management in Iowa at Ag Insights: Water Management Solutions, meeting sponsored by Hancor in Oelwein, IA (50 attendees).

September 15, 2005 – Presentation by Dr. Matt Helmers on drainage water quality and drainage water management at a field day near Pekin, IA. The “8 to 80 Water Quality Field Day” was attended by approximately 100 students from surrounding schools.


November 2, 2005 – The 6th Annual IA-MN Drainage Research Forum was held in Dows, IA. The forum was attended by 80 stakeholders that included individuals from both Iowa and Minnesota. The program focused on drainage and water management issues including the implications of nitrogen management, water quality and drainage modeling at the watershed scale, preferential flow on drained lands, nitrate-removal wetlands, cropping strategies for nitrogen management and drainage water management. Presenters included researchers from Iowa State University, University of Minnesota, and the USDA ARS.

December 15, 2005 – Presentation on drainage management and cropping practices at Iowa Drainage District Association annual meeting in Fort Dodge, IA (75 attendees).

January 9, 2006 – Presentation on drainage management and cropping practices at Iowa Land Improvement Contractors annual meeting in Des Moines, IA by Dr. Matt Helmers (100 attendees). Invited by the Agricultural Drainage Management Coalition to present.


March 13-17, 2006 – Presentation on drainage water management at Iowa Drainage Design Workshops by Dr. Matt Helmers (~200 attendees).

March 13, 2007 – A drainage design workshop was held in West Bend, IA. This location is in Palo Alto County. Approximately 30 local stakeholders attended the meeting. The focus and purpose of the meeting was to discuss drainage design and specifically design of drainage systems for economic and environmental benefits. The topic of controlled drainage was discussed.

May 15-16, 2007 – Project team was involved with organizing a nitrate-removal wetland tour.
June 2007 – Presentation on “Economic and Environmental Considerations for Drainage Design” by Dr. Matt Helmers at the Iowa Farm Bureau Federation Conservation and Natural Resources Issues Conference.

September 11-13, 2007 – Presentation on design of drainage water management systems at the Iowa Drainage School.

October 15-16, 2007 – Project team was involved with presenting information on nitrate removal wetland performance at an IDALS organized meeting with representatives of USDA-FSA, USEPA, state agency, and other NGO personnel from across the cornbelt.

October 23, 2007 – organized a tour to review drainage projects with Iowa Secretary of Agriculture Bill Northey and Iowa Department of Natural Resources Director Rich Leopold.

February and March 2008 – four different Extension events were held in Iowa in which Drainage Water Management and Wetland Performance were major topics and the Targeted Watershed Grant was highlighted. These events were Drainage Workshops held February 29, 2008 in Fairfield, IA (30 attendees), March 7, 2008 in Jefferson, IA (32 attendees), and March 18, 2008 Rockwell City, IA (25 attendees) and a Drainage Meeting held in Humboldt, IA on March 20, 2008 (20 attendees).

April 2008 – Dr. Helmers gave an oral presentation on the project at the EPA 2008 Wetlands and Watersheds Conference in Kansas City, MO.

August 13-14, 2008 – Coordinated with Craig Schrader and Drs. Jeff Strock and Gary Sands from the University of Minnesota a drainage water management workshop in Lamberton, MN. There were 16 specially invited contractors at this meeting.

September 15, 2008 – Presentation on “Drainage water management and subirrigation” at producers field near Newell, IA (5 attendees).

August 20, 2008 – Presentation on “Controlled drainage and nitrate-removal wetland performance” at NRCS Area 2 Technician Tour near Stanhope, IA (25 attendees).

August 6, 2008 – Presentation on “Controlled drainage and impacts of conservation practices on runoff” at the Iowa Learning Farm Field Day near Otho, IA (135 attendees).


December 2, 2008 – Presentation “A pilot program for integrated drainage and wetland landscape systems” by Dean Lemke at the 9th IA-MN Drainage Research Forum in Owatonna, MN (95 attendees).

December 4, 2008 – Presentation “A pilot program for integrated drainage and wetland landscape systems” by Dean Lemke at Iowa Drainage Engineers meetings in Fort Dodge, IA (20 attendees).

January 22, 2009 – Presentation “Water Quality Research in Northern Iowa” by Dr. Matt Helmers at the ISU Extension Crop Advantage Series Meeting in Fort Dodge, IA (20 attendees).

February 4, 2009 – Presentation “Pilot Program for Integrated Drainage and Wetland Systems” by Dr. Matt Helmers at the Agriculture Clean Water Alliance Meeting in Ames, IA (15 attendees).

March 5, 2009 – Briefing to the Packard Foundation and Environmental Working Group on potential for integrated drainage wetland systems in Iowa by Dr. William Crumpton and Dr. Matt Helmers.
March 11, 2009 – Presentation “Wetlands and Drainage in Iowa” by Dr. Matt Helmers at the Minnesota Drainage Workshop in Willmar, MN (45 attendees).
March 17, 2009 – Presentation “Treatment of Drainage Water” by Dr. Matt Helmers at a Drainage Workshop in Storm Lake, IA (25 attendees).
March 20, 2009 – Presentation “Treatment of Drainage Water” by Dr. Matt Helmers at a Drainage Workshop in Storm Lake, IA (75 attendees).
April 1, 2009 – Presentation “Pilot Program for Integrated Drainage and Wetland Systems” by Dr. Matt Helmers at the Agricultural Drainage Management Systems Task Force and Agricultural Drainage Management Coalition meeting in Columbus, OH (50 attendees).
April 27, 2009 – Presentation on drainage design and management at the Mason City, IA Rotary Club meeting by Dr. Matt Helmers (100 attendees).
April 30, 2009 – Presentation on drainage design and management to Iowa DNR staff in Des Moines, IA by Dr. Matt Helmers and Dr. Bill Crumpton.
May 19, 2009 – Presentation “Drainage Main Rehabilitation in Iowa” by Dr. Matt Helmers at the EWRI World Environmental and Water Resources conference in Kansas City, MO (25 attendees).
July 21, 2009 – Presentation “Pilot Program for Integrated Drainage and Wetland Systems” by Dean Lemke, Dr. Matt Helmers, and Dr. William Crumpton to the Iowa Environmental Protection Commission.
August 17, 2009 – Presentation on “Pilot Program for Integrated Drainage and Wetland Systems” by Dean Lemke, Dr. Matt Helmers, and Dr. William Crumpton to local, regional, and national agency personnel including IDNR, USFWS, USEPA, NRCS, FSA, and COE.
September 21, 2009 – Presentation on “Pilot Program for Integrated Drainage and Wetland Systems” by Dean Lemke, Dr. Matt Helmers, and Dr. William Crumpton to Iowa Environmental Council.
September 23, 2009 – Presentation on “Pilot Program for Integrated Drainage and Wetland Systems” by Dean Lemke to Hypoxia Task Force.

Conference Abstract

Conference Proceedings

Peer-reviewed Publications
4. MAJOR CONCLUSIONS AND PROJECT FINDINGS

Below we summarize our major conclusions and project findings related to 1) patterns of nutrient concentrations and loads, 2) drainage systems designed to reduce subsurface flow, 3) drainage systems designed to reduce surface runoff, 4) regional assessments of potential effects of drainage water management, N management, cover crops, and wetlands.

**Patterns in nutrient concentrations and loads**

The eight intensively monitored sites displayed generally similar seasonal and flow related patterns in nitrogen and phosphorus. For all sites, nitrate concentrations tended to remain elevated and relatively stable except for declines during periods of potential runoff (snow melt or very high flow) or during periods of extended low flow. On average, nitrate comprised 94% of TN and TN showed patterns similar to those of nitrate. Phosphorus concentrations were highly variable but tended to increase during discharge events. Phosphorus loads were strongly related to flow and phosphorus was transported primarily during a relatively few periods of high flow. On average, 80% of the total phosphorus load was delivered on less than 15% on the days and 50% was delivered on less than 5% of the days. On average TRP comprised 76% of TP and displayed patterns similar to those of TP.

Nutrient concentrations and loads are thought to be primarily determined by agricultural practices and drainage patterns, and are expected to be similar for tile drained watersheds in the same geographic area and with similar agricultural practices. However, monitoring results demonstrate a large range in N and P concentrations and loads among drainage systems despite similar patterns in the extent of cultivated cropland and subsurface drainage. Neither concentrations nor loads of N or P displayed a significant relationship to land use or drainage coefficient for the intensively monitored drainage districts in this study.

It is assumed that nutrient management can significantly affect nutrient export in agricultural watersheds and our analyses revealed that land use and nitrogen management explained most of the variability in nitrate concentration at larger watershed scales. However, these factors explained very little of the variability in nitrate concentration observed at the drainage district scale. Nitrate concentrations of drainage districts monitored during this project varied much more than could be explained based on land use and available N management data.

**Drainage systems designed to reduce subsurface flow (controlled and shallow drainage)**

Modeling results indicate that controlled and shallow drainage have the potential to reduce subsurface drainage volume and nitrate loss. However, these results also indicate that the reduction in subsurface drainage would be achieved primarily as a result of increased surface runoff which would be expected to increase phosphorus loss. Modeling results suggest little if any consistent crop yield benefits from controlled and shallow drainage. The primary benefit is thus a reduction in subsurface drainage volume and concurrent reduction in nitrate export. However, as noted above this could come with greater surface water runoff and greater export of runoff-associated contaminants.

Analyses using LIDAR derived elevations revealed that topography severely limits applicability of controlled drainage in the study area. Results also reveal that using soil slope classifications can grossly overestimate the land area suitable for controlled drainage when compared to detailed topographic information such as LIDAR. Although not so limited by
topography, shallow drainage has limited feasibility since it would require reinstallation of entire field drainage systems.

Based on the assessments conducted as part of this project there appears to be 1) limited applicability of controlled drainage in the Des Moines Lobe and 2) a concern of increased surface runoff with controlled and shallow drainage. In addition, based on interactions with landowners during outreach activities there was limited interest in the use of controlled drainage due to topographic limitations and lack of consistent yield benefit.

**Drainage systems designed to reduce surface flow (referred to as optimized drainage designs)**

Although modern design standards call for drainage coefficients of 1.27 cm/day (0.5 in/day) or greater, many of the drainage systems in the study area have substantially lower drainage coefficients. The agricultural lands in the early 20th century when the drains were originally installed, were typically in a 50% crop use of hay and forage to support the animal power for agriculture of the day, but today are nearly in 100% row crop which requires higher drainage coefficient. Field scale modeling results using DRAINMOD indicate that systems with drainage coefficients lower than about 0.64 cm/day (0.25 in/day) produce substantially greater quantities of surface runoff. Field scale modeling results indicate that increasing drainage coefficients of these systems to 1.27 cm/day (0.5 in/day) would lead to reduced surface runoff as well as increased crop yield. These field-scale modeling results were consistent with the results of drainage district scale modeling using MIKE SHE. These results also suggested little change in overall flow with improved drainage but a reduction in surface water runoff. Modeling results suggest that optimized drainage could reduce phosphorus loss at the drainage district scale and slightly increase nitrate export. When wetlands are integrated with improved drainage systems, the result is a reduction in both N and P export. There is considerable potential to integrate wetlands where the wetland is considered in the drainage system design phase rather than a retrofit. As such there are great opportunities for implementation of wetland technologies explored under this grant, as the drainage districts update the aging drainage main infrastructure across the 3000 drainage district encompassing 6 million acres of these lands in Iowa.

**Potential N load reductions through N management, wetlands, cover crops and drainage management.**

Regional assessments suggested that nitrogen management and wetlands have the greatest potential to reduce N export. Total nitrate export from the Des Moines Lobe could potentially be reduced by about 18% if application rates were reduced to those recommended based on the Maximum Return to Nitrogen. Establishing wetlands targeted to intercept nitrate loads from 50% of the area of the Des Moines Lobe having the greatest nitrate yield has the potential to reduce nitrate export by 34 to 46%. The combination of nitrogen rate management and targeted wetland restoration has the potential to reduce nitrate export by 45-55%.

In contrast, implementing cover crops on all no-till acres would only reduce nitrate export by about 2% on the Des Moines Lobe. Similarly, implementing drainage water management on tile drained cropland with slopes less than 0.5% would only reduce nitrate export by about 2%.
5. FUTURE DIRECTIONS

We conducted substantial outreach relative to the practice of drainage water management as part of this project and overall the feedback from this was mixed. While producers were intrigued by the concept they were skeptical of the applicability due to slope limitations. In addition, they were concerned about increased surface runoff due to a wetter soil profile.

IDALS has been restoring nitrate removal wetlands for the past ten years through the Iowa Conservation Reserve Enhancement Program (CREP). The program is unique in that it relies on GIS analyses to identify targeted locations where wetlands will work best to provide large nutrient removal benefits and cold-calls to determine whether the landowners of those locations are interested. The wetlands technology of the Iowa CREP has proven to be well received by the landowners in the intensely row-cropped areas of the Des Moines Lobe. Early on the response rate was 25% of landowners were interested in restoring wetlands on their farms and most recently that response rate has grown to over 33% of landowners being interested. The Iowa CREP has landowner interest well beyond what can be supported with the limited amount of public funds available. The “Iowa Wetland Landscape Systems Initiative” is striving to demonstrate a new watershed model of integrated drainage and wetland systems implementation with market-driven forces to restore wetlands largely with private funds which will help to lessen the burden on limited public funding sources while increasing the implementation rate of wetland restorations and associated reductions in nutrient loadings to waters across the landscape. The expected outcome of this approach is that integrated systems can provide for mutual environmental and agricultural benefits.

IDALS has engaged the landowners of several drainage districts to ascertain their interest in combining the type of wetlands established under the Iowa CREP with modernized drainage infrastructure, which has been a key element of the work under this grant. The overwhelming preliminary response from all seven of the proposed pilot sites is that the landowners are interested in incorporating wetlands with their drainage infrastructure to demonstrate a new model of watershed optimization. This level of support is well evidenced by the over $7 million share of project costs that landowners have pledged towards the overall total of $15 million for the seven pilot projects.

The development of the Iowa Initiative is a direct output from the work performed under this grant and has provided a means to continue the important research of integrated drainage and wetland system designs which was made possible by the EPA funding to explore these concepts. The work performed under this grant was instrumental in laying the groundwork for the Iowa Wetland Landscape Systems Initiative concepts and will continue to be so as the pilot projects proceed to be implemented and intensively monitored for a minimum of five years to assess the project outcomes of this integrated systems approach.

From the project findings there are also future research directions that could be pursued to address some of the questions were left unanswered in this work. As discussed within the report, the variation in nitrogen inputs did not explain the differences in nitrate export. Additional work is needed to explain this variability in nitrate export at a smaller scale. A combination of more detailed accounting of inputs, outputs with grain, underlying geology, and soil testing may be needed to further assess this variability.
A major question from this study is the impact of drainage redesign on hydrology, there is a need for field, drainage district, scale studies that examine the impact of drainage redesign on pathways of water flow specifically assessing how drainage redesign may shift the pathways of water flow. This study was not set up to answer these questions but monitoring associated with the “Iowa Wetland Landscape Systems Initiative” is vitally needed specifically related to pathways of water flow. This information would be very essential to further validate results from the hydrologic modeling that should continue to be pursued on the drainage district and larger scale. There is a need to continue investigation on the hydrologic impacts of drainage and hydrologic modeling is one avenue to pursue this but the modeling should be based on solid monitoring data at an appropriate scale.

A critical factor in predicting potential nitrogen load reduction is knowledge about nitrogen inputs. Currently there is a lack of location specific information on nitrogen inputs. Future work should attempt to continue to pursue avenues to collect this type of information. To make gains in nitrate export and have some information about what gains can be made with nitrogen management it is critical that an accurate baseline condition is identified.

6. REFERENCES


7. APPENDIX A – PROJECT TIMELINE

The project timeline is shown in Table A.1. These project activities were completed as part of the project and the location they are discussed within the project report are noted in Table A.2.

<table>
<thead>
<tr>
<th>ACTIVITY</th>
<th>2010</th>
<th>2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>Work Element #1: Topographic analysis and performance forecast modeling of alternative drainage-wetland systems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crop and drainage modeling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wetland modeling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test models against existing data sets and perform validation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crop and drainage modeling of optimally designed drainage systems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Develop prioritized list of subwatersheds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Develop nutrient budgets for study areas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evaluate applicability of alternative drainage designs and integration of wetlands using LiDAR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Work Element #2: Outreach, engineering, and construction of drainage-wetland systems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Landowner contacts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>System design and construction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Work Element #3: Performance monitoring of drainage-wetland systems and regional assessments of potential system performance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conventional system monitoring (continuous sampling and synoptic sampling)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post construction monitoring (paired systems, continuous sampling)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regional assessment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evaluate regional effectiveness of nutrient management strategies</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table A.1: Project Timeline
Table A.2. Listing of project activities and section where they are discussed in the project report.

<table>
<thead>
<tr>
<th>Project Activity</th>
<th>Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crop and drainage modeling</td>
<td>3.2.2.1</td>
</tr>
<tr>
<td>Wetland modeling</td>
<td>3.2.2.2</td>
</tr>
<tr>
<td>Test models against existing data sets and perform validation</td>
<td>3.2.2.1, 3.2.2.2, 3.2.2.3</td>
</tr>
<tr>
<td>Crop and drainage modeling of optimally designed drainage systems</td>
<td>3.2.2.1, 3.2.2.3</td>
</tr>
<tr>
<td>Develop prioritized list of subwatersheds</td>
<td>3.2.1.2</td>
</tr>
<tr>
<td>Develop nutrient budgets for study areas</td>
<td>3.1.1.2</td>
</tr>
<tr>
<td>Evaluate applicability of alternative drainage designs and integration of wetlands using LIDAR</td>
<td>3.2.1.1</td>
</tr>
<tr>
<td>Landowner contacts</td>
<td>3.4.2</td>
</tr>
<tr>
<td>System design and construction</td>
<td>3.4.1</td>
</tr>
<tr>
<td>Conventional system monitoring (continuous sampling and synoptic sampling)</td>
<td>3.1</td>
</tr>
<tr>
<td>Post construction monitoring (paired systems, continuous sampling)</td>
<td>3.1.2</td>
</tr>
<tr>
<td>Regional assessment</td>
<td>3.3.2</td>
</tr>
<tr>
<td>Evaluate regional effectiveness of nutrient management strategies</td>
<td>3.3.2</td>
</tr>
</tbody>
</table>
## 8. APPENDIX B – TABLE OF INPUT PARAMETERS

Table B.1. Input and output parameters for DRAINMOD, MIKE SHE and the TIS Model

<table>
<thead>
<tr>
<th>Input Parameters</th>
<th>DRAINMOD</th>
<th>MIKE SHE</th>
<th>TIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precipitation Rate (cm/h)</td>
<td>Model Grid Cell Size (30 m x 30 m)</td>
<td>Bathymetry</td>
<td></td>
</tr>
<tr>
<td>Thornthwaite ET Parameters (monthly factors)</td>
<td>Topography (LIDAR Data Used)</td>
<td>Full pool area</td>
<td></td>
</tr>
<tr>
<td>Air Temperature</td>
<td>Precipitation Rate (mm/unit of time)(^1)</td>
<td>Full pool volume</td>
<td></td>
</tr>
<tr>
<td>Vegetation Parameters and Properties (Rooting depth and response to water stress)</td>
<td>Reference Evapotranspiration (mm/day)</td>
<td>(K_{20}) (rate constant)</td>
<td></td>
</tr>
<tr>
<td>Detention Storage (cm)</td>
<td>Air Temperature</td>
<td>(\Theta) (temperature coefficient)</td>
<td></td>
</tr>
<tr>
<td>Soil Profile Depth (cm)</td>
<td>Vegetation Parameters and Properties (Leaf area index and rooting depth)(^2)</td>
<td>Seepage rate (m/d)</td>
<td></td>
</tr>
<tr>
<td>Saturated Hydraulic Conductivity (cm/h)</td>
<td>Manning’s Number(^3)</td>
<td>Outflow structure dimensions</td>
<td></td>
</tr>
<tr>
<td>Soil Water Retention Properties</td>
<td>Detention Storage (mm)(^2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drain Depth (cm)</td>
<td>Soil Profile Depth (m)(^2)</td>
<td>Precipitation rate (m/d)</td>
<td></td>
</tr>
<tr>
<td>Drain Spacing (cm)</td>
<td>Bulk Density (kg/m(^3))(^3)</td>
<td>Evapotranspiration rate (m/d)</td>
<td></td>
</tr>
<tr>
<td>Effective Radius of Drains (cm)</td>
<td>Saturated Hydraulic Conductivity (m/s)(^2)</td>
<td>Water temperature</td>
<td></td>
</tr>
<tr>
<td>Depth to Impermeable Layer (cm)</td>
<td>Soil Water Retention Properties(^2)</td>
<td>Inflow discharge (m(^3)/day)</td>
<td></td>
</tr>
<tr>
<td>Drainage Coefficient (cm/d)</td>
<td>Drain Depth (m)(^2)</td>
<td>Subsurface inflow (From MIKE SHE)</td>
<td></td>
</tr>
<tr>
<td>Kirkham’s Depth for Flow to Drains (cm)</td>
<td>Drainage Time Constant (s(^{-1}))</td>
<td>Surface inflow (runoff) (From MIKE SHE)</td>
<td></td>
</tr>
<tr>
<td>Soil Temperature Parameters</td>
<td>Drainage Drain Codes</td>
<td>Nutrient concentrations (mg/L) (From measured data)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subsurface concentration</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surface water conc.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Output Parameters</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Overland Flow (cm/day)</td>
<td>Evapotranspiration (mm/unit of time)(^4)</td>
<td>Outflow discharge (m(^3)/day)</td>
<td></td>
</tr>
<tr>
<td>Drain Outflow (cm/day)</td>
<td>Overland Flow (mm/ unit of time)</td>
<td>Precipitation (m(^3)/day)</td>
<td></td>
</tr>
<tr>
<td>Deep Seepage (cm/day)</td>
<td>Drain Outflow (mm/ unit of time)</td>
<td>Evapotranspiration (m(^3)/day)</td>
<td></td>
</tr>
<tr>
<td>Lateral Seepage (cm/day)</td>
<td>Subsurface Outflow (mm/ unit of time)</td>
<td>Seepage loss (m(^3)/day)</td>
<td></td>
</tr>
<tr>
<td>Relative Yield for Crop Evapotranspiration (%)</td>
<td>Surface and Subsurface Storage (mm/ unit of time)</td>
<td>Outflow nitrate concentration (mg/L)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Error</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mass N in (g/d)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mass N out (g/d)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mass N removed (g/d)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) For MIKE SHE precipitation inputs this can be input on as fine a time scale as is available. Since only daily precipitation was available this was input and the model converted to hourly precipitation, the information is output in mm per unit of time input. For example you could output hourly flow. We accumulated the more frequent output into daily flow for comparison with observed values.

\(^2\)These parameters are input for each cell in the model domain and in some cases at multiple depths in each grid cell.

\(^3\)For MIKE SHE outputs the information is output in mm per unit of time input. For example you could output hourly flow. We accumulated the more frequent output into daily flow for comparison with observed values.
9. APPENDIX C

1. PREPARATION OF 0.1% SULFURIC ACID ACIDIFIED WATER

2. ACIDIFYING BOTTLES FOR SAMPLES

3. CALIBRATION OF VARIABLE VOLUME PIPETTE

4. PREPARATION OF COLOR DEVELOPING SOLUTION TO BE USED IN ORTHOPHOSPHATE ANALYSIS USING A MODIFIED MURPHY AND RILEY METHOD

5. WASHING DISHES FOR WETLAND RESEARCH LAB

6. EMPTYING AUTO SAMPLER BOTTLES

7. MARKET FORGE STERILMATIC STERILIZER AUTOCLAVE OPERATION

8. PREPARATION ON NITRATE STANDARDS

9. PREPARATION ON PHOSPHATE STANDARDS

10. PIPETTING TOTAL NITROGEN SAMPLES

11. PIPETTING TOTAL PHOSPHORUS SAMPLES

12. PIPETTING TOTAL REACTIVE PHOSPHORUS (TRP) SAMPLES

13. SPECTROPHOTOMETRIC ANALYSIS OF NITRATE-NITROGEN USING THE SPECTORSOCOPIC SECOND DERIVIATIVE METHOD

14. SPECTROPHOTOMETRIC ANALYSIS OF ORTHO PHOSPHORUS USING THE ASCORBIC ACID METHOD

15. SAMPLE DIGESTION FOR TOTAL NITROGEN ANALYSIS

16. SAMPLE DIGESTION FOR TOTAL PHOSPHORUS ANALYSIS
STANDARD OPERATING PROCEDURE FOR:
PREPARATION OF 0.1% SULFURIC ACID ACIDIFIED WATER

Summary
Standards used in nitrate, total nitrogen, total reactive phosphorus and total phosphorous analysis must be prepared using ultra pure water and acidified with sulfuric acid to the same normality as the field samples.

Materials
Repeater pipette (Eppendorf)
1000 ml volumetric flask
Concentrated sulfuric acid (36N H₂SO₄)
Deionized (DI) water

Precautions
Review MSDS for each of the chemicals used in this procedure. Wear protective clothing, goggles, and gloves when handling H₂SO₄. Use concentrated H₂SO₄ in the hood. If eye contact occurs, rinse with water or normal saline solution for 15-20 minutes and get medical attention immediately. A safety shower is located in Room 127A and outside room 129; eye washes in Room 150 and 127.

Preparation of Acidified Water
To make a 0.1% sulfuric acid acidified water add concentrated H₂SO₄ to fresh deionized water at a ratio of 1ml sulfuric acid: 1000ml DI water. Always add acid slowly to a large volume of water do not pour water into concentrated sulfuric acid as it will become very hot, boil and splatter. If the water is going to be used in preparation of a standard do not fill until the standard stock solution is added otherwise fill the volumetric flask to the line and cap and invert 10 times to distribute the acid.

Acidified water can be stored in brown glass bottles with Teflon lined lids. Label bottle with the date, Sulfuric Acid 0.1%, and the name of the preparer. Discard after two months.

Waste Disposal
All chemicals except DI water that remain following the preparation of acidified water must be placed in properly labeled waste bottles in the Waste Accumulation Area until collected by Environmental Health and Safety. NO CHEMICALS GO DOWN THE DRAIN.

Approved By: Jana Z. Stenback Date: 03 February 2011
STANDARD OPERATING PROCEDURE FOR:
ACIDIFYING BOTTLES FOR SAMPLES

Summary
Concentrated sulfuric acid is added to empty bottles prior to field sampling. The acid is added so that when water samples are collected in the bottle the pH in the sample is such that bacterial and fungal growth is inhibited.

Materials
Repeater pipette (Eppendorf)
Concentrated sulfuric acid (36N H₂SO₄)
Empty sample bottles that are both clean and dry

Precautions
Review MSDS for each of the chemicals used in this procedure. Wear protective clothing, goggles, and gloves when handling H₂SO₄. Use concentrated H₂SO₄ in the hood. If eye contact occurs, rinse with water or normal saline solution for 15-20 minutes and get medical attention immediately. A safety shower is located in Room 127A and outside 129; eye washes are located in Rooms 150, and 127A.

Acidifying Bottles
Using the Eppendorf, add concentrated H₂SO₄ to empty, clean sample bottles such that the final concentration of the sample will be 1 ml H₂SO₄ to 1000 ml sample or 0.1% H₂SO₄ by volume. For plastic sample bottles, collect sample within one week of adding acid to the bottle. If acidified sample bottles are not used after one week they must be washed, dried and re-acidified.

Example:

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Sulfuric acid added</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 ml</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>100 ml</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>25 ml</td>
<td>0.025ml</td>
</tr>
</tbody>
</table>

For 400ml auto sampler bottles
Use the 25ml Eppendorf tip with the repeater pipette set on 1 to deliver 0.4 ml

For the 150ml grab bottles
Use the 2.5ml Eppendorf tip with the repeater pipette set on 3 to deliver 0.15ml

Waste Disposal
All chemicals that remain following the acidification of bottles must be placed in properly labeled waste bottles in the Waste Accumulation Area until collected by Environmental Health and Safety. NO CHEMICALS GO DOWN THE DRAIN

Written and Approved By: Jana Z. Stenback  Date: 02 February 2011
STANDARD OPERATING PROCEDURE FOR:
CALIBRATION OF VARIABLE VOLUME PIPETTE

Summary
Variable volume pipettes are used in the preparation of standards. There are several volume ranges available. When standards are being prepared the pipette should be calibrated for each volume that it is set to deliver. Calibration is done gravimetrically using water at room temperature and a balance. The balance should be accurate to several decimal places in the masses of water to be weighed.

Materials
Clean beakers
Variable volume pipette
Deionized (DI) water at room temperature
Paper and pen for taking notes

Precautions
Gloves and eye protection need to be worn whenever working in the lab

Calibration Procedure
Fill a beaker with some deionized water at room temperature. The water must be at room temperature because water near room temperature has a volume in cubic centimeters that is equal to its mass in grams. Place a small beaker on the try of a balance. Have third beaker to use as a waste beaker.

1. Adjust the variable pipette to deliver a desired volume.
2. Fill the pipette tip twice with DI water and empty into the waste container.
3. Tare the balance so that it reads zero.
4. Fill the pipette with DI water and empty aliquot into the beaker on the balance.
5. If the volume is not what is desired adjust again and weigh repeat step 3 and 4 until the desired volume is reached
6. Fill and weigh the 5 aliquots of water recording the mass each time
7. Find the average and standard deviation of the 5 aliquots of water. This is the volume and error you use in calculating the stock solution to be added in preparation of your standards.

Pipetting and Delivery of Stock Solution
After the pipette is calibrated fill it with the stock solution and empty twice into the waste container then fill with stock solution and empty into the volumetric flask used to prepare standards. Be sure to rinse the pipette with DI water before returning to the calibration process.

Written By: Jana Z. Stenback  Date: 23 March 2011
STANDARD OPERATING PROCEDURE FOR:
PREPARATION OF COLOR DEVELOPING SOLUTION TO BE USED IN ORTHOPHOSPHATE
ANALYSIS USING A MODIFIED MURPHY AND RILEY METHOD

Summary
This procedure uses the ascorbic acid method originally described by Murphy and Riley (1962) and slightly
modified in AWWA (1998). The method has been further modified by this lab to allow for a smaller sample treated
by a more convenient volume of color developing solution. This was accomplished by altering the concentrations of
the stock solutions used to make the color developing solution. Briefly, in this reagent ammonium molybdate and
potassium antimonyl tartrate react in an acid medium with orthophosphate to form the heteropoly acid,
phosphomolybdic acid, that is reduced to an intense molybdenum blue by ascorbic acid (AWWA, 1997).

After addition of the color developing solution to water samples they are analyzed using the spectroscopic method
for the analysis of the orthophosphate ion in water. This method only detects orthophosphate. Analysis of total
phosphorus requires that a sample be digested to convert all phosphorous to orthophosphate.

Materials
Balance
Weighing boats
Appropriate graduated cylinders
Stir plate
Teflon coated stir bars
Brown bottles with Teflon seals
Deionized (DI) water
Concentrated sulfuric acid (H\(_2\)SO\(_4\))
Ammonium molybdate ([NH\(_4\)]\(_6\)Mo\(_7\)O\(_24\)·4H\(_2\)O)
Potassium antimonyl tartrate (K(SbO)C\(_4\)H\(_4\)O\(_6\)·1/2 H\(_2\)O)
Ascorbic acid
10 ml pipette
Water samples

Precautions
Review current MSDS for each of the chemicals used in this procedure. Protective goggles, clothing and gloves
must be worn at all times when following this procedure. Lab paper must cover all countertops and work surfaces,
as the chemicals used in this procedure can be corrosive.

Equipment Preparation
All glassware used in this analysis should be soaked at least 24 hours in phosphorus free surfactant and quadruple
rinsed in DI water (see SOP for washing dishes). All glassware should be dried, covered in foil or stored in a closed
container. Any glassware that is chipped or scratched should be discarded in the broken glass receptacle. The
preparation area should be covered in lab paper and be clean and dust free.

Reagent Preparation
The color developing solution (CDS) is prepared from stock solutions of sulfuric acid, ammonium molybdate,
potassium antimonyl tartrate and ascorbic acid. The sulfuric acid, ammonium molybdate and potassium antimonyl
tartrate can be prepared and stored in 1 liter brown bottles with Teflon lined lids and labeled with date, contents and
initials of preparer. The ascorbic acid stock solution must be made fresh daily. It is important to know ahead of
time how much of the CDS is needed for a set of samples because the same batch of CDS must be used for all
standards and samples to be analyzed.

Stock Solutions
1. 5 Normal sulfuric acid (H\(_2\)SO\(_4\)) solution- Preparation of sulfuric acid solution should be done in a fume
    hood. Use a graduated cylinder to measure 150 ml concentrated sulfuric acid to be diluted with DI water to
    1000 ml. The sulfuric acid solution should be prepared by slowly adding approximately 75 ml of
    concentrated sulfuric acid to about 400 ml DI water in a large glass graduated cylinder. Then add DI water
    until volume is at 700 ml and slowly add remaining sulfuric acid. Fill the graduated cylinder to 1000 ml,
empty into a beaker and stir slowly on a stir plate. This reaction will get very hot and care must be taken
to not boil the water and splash the acid. Continue to stir until the solution has cooled enough to transfer to
a storage bottle. Store in a brown bottle with a Teflon seal. Label 5N Sulfuric Acid, initial and date.

2. **Potassium antimonyl tartrate (K(SbO)C₄H₆O₆·1/2 H₂O) solution** - Measure out 1000 ml of DI water in a
   graduated cylinder, using a balance measure 2.90g potassium antimonyl tartrate on a weighing boat. Pour
   most of the water into a 2L beaker with a stir bar empty potassium antimonyl tartrate into the beaker with
   water and rinse the boat with remaining water into the beaker. Stir on a stir plate until the reagent is
dissolved. The solution can be stored in a brown bottle with a Teflon seal. Label Potassium Antimonyl
Tartrate, initial and date.

3. **Ammonium molybdate ((NH₄)₆Mo₇O₂₄·4H₂O) solution** - Measure out 1000 ml of DI water in a
   graduated cylinder, using a balance measure 42.2g ammonium molybdate on a weighing boat. Pour
   most of the water into a 2L beaker with a stir bar empty ammonium molybdate into the beaker with water
   and rinse the boat with remaining water into the beaker. Stir on a stir plate until the reagent is dissolved. The solution can be
   stored in a brown bottle with a Teflon seal. Label Ammonium Molybdate, initial and date.

4. **Ascorbic acid (MUST BE PREPARED FRESH DAILY)** - Determine how much ascorbic acid to prepare
   from Table 1. Weigh out the ascorbic acid in a weighing boat, put it into a beaker with the corresponding
   volume of water with rinsing, place on a stir plate and stir until dissolved. This is the last reagent to be
   added in preparation of the CDS.

### Preparation of Color Development Solution

*ALL SOLUTIONS MUST BE AT ROOM TEMPERATURE BEFORE MIXING*

&

**MIXED IN THE ORDER SHOWN**

Note: Before combining any of the reagents together prepare the desired amount of ascorbic acid solution
as described above and have it stirring while other reagents are being combined.

Table 1 gives the mixing ratios to prepare different volumes of CDS.

<table>
<thead>
<tr>
<th>Volume CDS (ml)</th>
<th>Sulfuric acid (ml)</th>
<th>Potassium antimonyl tartrate (ml)</th>
<th>Ammonium molybdate (ml)</th>
<th>Mass Ascorbic Acid (g)</th>
<th>Volume DI water used to dissolve Ascorbic Acid (ml)</th>
<th>~number of racks of test tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>50</td>
<td>5</td>
<td>15</td>
<td>0.558</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>180</td>
<td>90</td>
<td>9</td>
<td>27</td>
<td>1.004</td>
<td>54</td>
<td>2</td>
</tr>
<tr>
<td>300</td>
<td>150</td>
<td>15</td>
<td>45</td>
<td>1.674</td>
<td>90</td>
<td>3</td>
</tr>
<tr>
<td>400</td>
<td>200</td>
<td>20</td>
<td>60</td>
<td>2.232</td>
<td>120</td>
<td>4.5</td>
</tr>
<tr>
<td>500</td>
<td>250</td>
<td>25</td>
<td>75</td>
<td>2.79</td>
<td>150</td>
<td>6</td>
</tr>
<tr>
<td>600</td>
<td>300</td>
<td>30</td>
<td>90</td>
<td>3.348</td>
<td>180</td>
<td>7</td>
</tr>
</tbody>
</table>

As an example the following is the procedure to prepare 100ml of CDS:

Measure out the above stock solutions using graduated cylinders and prepare the ascorbic acid solution mix
into a beaker with stirring between each addition in the following order:

1. 50 ml sulfuric acid solution
2. 5 ml potassium antimonyl tartrate solution
3. 15 ml ammonium molybdate solution
4. 30 ml ascorbic acid solution

The final color developing solution will be light yellow in color and can be used for 8 hours. This solution is added
to water samples digested and undigested to measure orthophosphorus. After 6 hours of use the solution should be
checked by doing a standard check with the phosphorus standards.
References

STANDARD OPERATION PROCEDURE FOR:
WASHING DISHES FOR WETLAND RESEARCH LAB

Summary
All dishes must be washed in a manner that prevents contamination of samples.

Materials
P-Free RBS
Isopropyl Alcohol
Deionized water
Ultra-pure water
Clean bench tops covered with clean bench paper—the absorbent side up

Precautions
Review MSDS for each of the chemicals used in this procedure. Gloves, lab coat and goggles should be worn at all times when washing dishes to protect hands and keep dishes free of contamination.

Preparation of Stock Solution
Mix cleaning agent P-Free RBS in water; manufacture recommendation is to mix 20 ml per liter of distilled water. The dish tubs have been marked at the 8L line, so if you fill the tub to 8L you need to add 160 ml of RBS.

Preparation of Dishes for Washing
1. Grab bottles that have never been used just need to have their caps removed.
2. Any bottle and cap that has been acidified or contained a water sample needs to be rinsed. Be very careful when rinsing bottles if they contain concentrated sulfuric acid as it can splash out.
3. Remove dirt and debris from inside and outside of bottle and loosen interior debris with a brush if needed.
4. Glassware that contains sediment plant and or animal material should be rinsed before soaking.
5. Isopropyl alcohol can be used to remove any labels or writing on non-glass dishes. (Blue marker will come off glass easily with soaking.)

Procedure
Soak caps and bottles in separate tubs. Soak ISCO and QCEC caps separately from American Sigma caps and grab bottle caps; soak small test tube caps separate from large test tube caps. Bottles can be filled and stacked in an empty tub; the bottles do not need to be submerged in RBS solution. Caps need to be fully submerged, use plastic strainers placed on top of the soaking caps to keep them submerged.

All dishes should be soaked, completely filled or covered in RBS solution for at least 12 hr. Following soaking, if more rigorous cleaning is needed, use green scrubber or bottlebrush to clean the dishes.

When dishes are finished soaking they must be completely filled and emptied with clean distilled or DI water A MINIMUM OF 4 TIMES and after that continued to be rinsed until no sign of cleaning agent remains, no bubbles should form. Dishes may be air dried on drying racks or placed on a clean bench covered with clean bench paper.

Be sure to place all dishes with their open ends down so they do not collect dust. Cover stir bars with a clean beaker while drying.

Before leaving the dishes area make sure thing are tidied up. Rinse all empty buckets and invert on the counter or shelves if there is room, wipe down counters, and organize any dishes that need to be rinsed or soaked.

Update the white board so that the next person that does dishes knows the full status of everything in the dish room.

When putting dishes away, wear gloves to protect dishes from contamination. Cover all glassware—such as graduated cylinders—with foil if it is stored open end up. Glassware such as beakers can be stored open side down on shelves or in drawers lined with clean bench paper.
**Waste Disposal**
All hazardous chemicals that are removed from laboratory dishes must be placed in properly labeled waste bottles in the Waste Accumulation Area until collected by Environmental Health and Safety. Rinse water from rinsing bottles before soaking can be washed down the drain. Isopropyl alcohol used to remove labels on dishes can be flushed down the drain with water. The wash and rinse water can be flushed down the drain.

Written and Approved By:  Jana Z. Stenback

Date: 02 February 2011

**Dish Washing Notes**

1. Washing laboratory dishes is nothing like washing dishes at home. The idea is for them to get cleaned of any contaminants that would interfere with our research. We analyze phosphorus at the parts per billion levels so a very small amount of contamination can skew results. The RBS solution we use is a very strong surfactant but will not work unless it is in direct contact with the item being washed. Therefore things must be fully submerged or filled.

2. To help keep caps submerged use the rectangular sieves on top to force the lids under the solution.

3. Top off bottles that have been filled with RBS solution after the bubbles have popped.

4. When rinsing you must completely fill and empty the item a minimum of four times. If there is any sign of bubbles that persist then continue rinsing until there are no bubbles.

5. Scrub dirt and grime off of the threads of the auto sampler bottles with a bottle brush before soaking them.

6. Always rinse caps and bottles at the same time in separate tubs. Dry bottles without caps cannot be used or put away but only take up space on the drying racks.

7. Always rinse auto sampler bottles or grab bottles and their caps that have acid in them before putting them into the dirty dish containers.

8. When you are finished washing dishes please tidy up the space.
   1. Rinse any empty dish buckets and place them on the counter upside down.
   2. Try to organize bottles that need to be soaked. Make sure there is room to walk around and work.
   3. Carts of bottles needing to be soaked or rinsed can be stored in the main lab just put a note on them saying what their status is, “Need to be soaked” or “Need to be rinsed”.
   4. Throw away the diaper paper if it has acid on it, wipe down the counters and rinse out the sink of any debris.

9. All dry dishes that are stored should be dry and must have caps on them. Bottles in boxes without lids will be considered dirty because we cannot insure that they are contaminant free.
STANDARD OPERATING PROCEDURE FOR:
EMPTYING AUTO SAMPLER BOTTLES

Summary
Samples collected in auto sampler bottles are divided into two smaller 125ml bottles for analysis and storage.

Materials
Clean 125ml bottles
Labels
Auto sampler collected water samples

Precautions
The water samples in the auto sampler bottles are acidified with sulfuric acid. Protective clothing and gloves as well as eye protection should be used when emptying auto sampler bottles.

Emptying Auto Sampler Bottles
Before emptying auto sampler bottles either hand write labels or use the label generating program to print them off. They should include the name of the site, the date and what type of sample “A” for Auto Sampler. Since each auto sampler sample is split into two samples for storage those are distinguished by and X and a Y so for each auto sampler sample there should be an “AX” and an “AY”. In some cases there is not enough water to fill two bottles so there will only be a and “AX” this is a special case and should be noted (see below Special Cases).

Once the 125 ml bottles are labeled then they can be filled with the water from the auto sampler bottle. It is important that we get a well-mixed sample into the 125 ml bottles. To do this follow the following procedure:
1. Open the two labeled 125 ml bottles that will be filled
2. Tighten the cap on the auto sampler bottle and shake vigorously
3. Fill each 125 ml bottle half way
4. Cap and shake auto sampler bottle
5. Fill each 125 bottle all the way or split what is left in the auto sampler bottle between the 125 ml bottles.

Special Cases
Listed are some special cases and what to do in each of them.
1. Very small sample- if there isn’t enough sample in the auto sampler bottle to fill each 125 ml bottle half way put the entire auto sampler sample in one bottle, the AX bottle. Make the following note on this bottle: "NO Y"
2. Insects- sometimes there are insects in the auto sampler bottle. This needs to be noted on the label whether or not the insects get into the 125 ml bottle. This is because just having the insects in the bottle can greatly affect the phosphorus content of the water sample. Make a note by writing “BUGS” on the 125 ml bottle label.
3. Leaches- Very often towards the end of summer we get one or more leach in an auto sampler bottle. Leaches cause huge spikes in the phosphorus content of the water. Write “Leach” on the label of the 125 ml bottle.
4. Twigs, Leafs, Algae and other Botanicals- If there are bits of plants in an auto sampler. It should be noted on the bottle that these things were in the auto sampler bottle even if they do not get into the 125 ml bottle. Write “Leaf”, “Twig” or “Algae” on the 125 ml bottle label. If you can identify the plants for example lemna write the “Lemna” on the bottle.

Waste Disposal
Unused sample from the auto sampler bottles can be put down the sanitary sewer drain. Make sure to use a drain that is equipped with a soil trap to empty any leftover samples that contain solid material such as leaches or plants.

Written and Approved By: Jana Z. Stenback Date: 18 April 2011
STANDARD OPERATION PROCEDURE FOR:
MARKET FORGE STERILMATIC STERILIZER AUTOCLAVE OPERATION

Summary
High heat and pressure are used to sterilize and digest organics in samples.

Materials
Autoclave (Room 120 Bessey Hall)
Samples to be digested/sterilized
Autoclave gloves

Precautions
1. Review standard operating procedures and MSDSs for the materials to be autoclaved.
2. Wear loose-fitting, temperature-resistant autoclave gloves when removing items from the autoclave.
3. The autoclave may be hot, even when not on.
4. Avoid contact with steam to prevent burns.
5. Avoid contact with copper pipe on floor as it gets hot.
6. Steam is exhausted through the copper pipe causing steam to flow into the room.

Procedure for Digestion of Nutrients in Water Samples
1. First make certain that the drain valve is closed and the chamber is empty. Empty chamber if necessary by opening the valve and allowing it to drain completely. With the drain closed fill bottom of chamber with a mixture of ½ distilled water and ½ tap water to a total volume of approximately six quarts or to just below the ledge at the bottom of the door opening.
2. Load the sterilizer with a single layer of samples only; do not stack samples. Latch and lock the Sterilmatic door.
3. Set the exhaust selector switch to SLOW. The switch is located at the center of the control panel which is mounted on the top of the unit.
4. Turn the Timer located at the upper right front of the Sterilizer to the desired length of the sterilizing period. This turns the power supply on to the heaters. The timed cycle starts only after the pressure-temperature combination has been reached. The pilot light will not light until the unit is up to temperature and pressure and the timer is running. Because of this it is a good idea to check the autoclave after about 10 min. to make sure it is on and warming up. You can do this by checking the temperature and pressure dials to see whether or not they are rising.
5. When the sterilizing cycle is complete and the timer has returned to 0, all electrical components are shut off except the exhaust solenoid and the indicator light. After two more minutes have elapsed, the light will shut off and the exhaust solenoid will de-energize.
6. Do NOT open until pressure is 0 and temp is less than 60 degrees C.
7. When opening the door, allow a few seconds for the steam to escape from the chamber before you open it completely. (Using protective gloves, release the handle and LET GO to avoid possible contact with the remaining steam.)
8. Open drain valve to empty fluid chamber.

Cleaning
After each use:
Remove pan supports by lifting front of supports up and off studs. Pull back of support forward and off stud. Wash interior, pan supports, and drain plug area with mild detergent and water. Rinse and dry thoroughly. Wipe gasket clean. Leave door open.

Monthly:
1. Open door fully.
2. Disengage door spring from each of its studs by holding the spring down with one hand and pulling it off the studs with the other.
3. Slip door lift springs off studs.
4. Rotate door out opening, handle first, followed by spring then rest of assembly.
5. Remove gasket and wash, if necessary.
6. Clean exhaust silencer by rinsing in mild detergent and water. Replace if clogged.
7. Follow daily cleaning procedures.

Times for Digesting
Total P: 15 psi @ 121° C for 60 minutes
Total N: 15 psi @ 121° C for 30 minutes
Waste Disposal
All chemicals that remain following the use of the autoclave must be placed in properly labeled waste bottles in the Waste Accumulation Area until collected by Environmental Health and Safety.  NO CHEMICALS GO DOWN THE DRAIN.

Reviewed Approved By: Jana Z. Stenback  Date: 02 February 2011
STANDARD OPERATING PROCEDURE FOR:
PREPARATION ON NITRATE STANDARDS

Summary
Working nitrate standards are prepared by adding known volumes of a stock solution of sodium nitrate (NaNO₃) to acidified, deionized water and diluting. These standards are used to construct a standard curve for spectrometric determination of nitrate in water samples.

Materials
ACS certified reagent grade sodium nitrate NaNO₃
Concentrated sulfuric acid H₂SO₄ (36N)
0.2 and 1.0 ml adjustable pipette and tips
Repeater (Eppendorf) pipette 50 ml tips
2- 100 ml volumetric flasks
1- 200 ml volumetric flasks
Multiple- 1000 ml volumetric flasks
Drying oven

Precautions
Review MSDS for each of the chemicals used in this procedure. Protective lab coat, goggles and gloves must be worn at all times when following this procedure. Lab paper must be on all surfaces because chemicals in this procedure can be corrosive to countertops and instruments.

Preparation Stock Solutions
Dry sodium nitrate in a drying oven at 200°C for a minimum of two hours. Remove from the drying oven and cool to room temperature in a desicator with desicant before using. Stock solutions are prepared by weighing and recording a mass of sodium nitrate and adding to a volumetric flask. Water is added to the flask at a level below the line and capped and inverted until sodium nitrate is dissolved. Because the process of dissolving sodium nitrate in water is endothermic the solution should be allowed to stand in the volumetric overnight so that it can reach room temperature. Once it is room temperature, fill to the line with DI water and invert 30 times to mix.

3 stock solutions are prepared to be used in preparing the standards. They are as follows:

<table>
<thead>
<tr>
<th>Target Concentration mgN/l</th>
<th>Mass Sodium Nitrate g</th>
<th>Volumetric flask volume l (DI water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>1.2136</td>
<td>0.200</td>
</tr>
<tr>
<td>10000</td>
<td>12.136</td>
<td>0.200</td>
</tr>
<tr>
<td>40000</td>
<td>24.272</td>
<td>0.100</td>
</tr>
</tbody>
</table>

Preparation of Working Standards
Use 1 liter volumetric flasks for preperation of standards. In addition to the sodium nitrate concentrated sulfuric acid is added to the volumetric flask so that the final stock solution has 0.1% (volume to volume) sulfuric acid. To each flask fill at least half full with DI water, add 1ml of concentrated sulfuric acid, invert several times until the acid is mixed in. Once the acid is added and mixed the stock solution can be added.

Use the following table to determine which stock solution and how much to add for each standard. Calibrate the pipette for each addition by following the Standard Operating Procedure for Pipette Calibration. Once the stock solution is added cap and invert at least three times and then fill to the line with DI water cap and invert 30 times.
<table>
<thead>
<tr>
<th>Target Concentration mg/l</th>
<th>Target Volume ml</th>
<th>Stock Solution mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.1</td>
<td>1000</td>
</tr>
<tr>
<td>0.3</td>
<td>0.3</td>
<td>1000</td>
</tr>
<tr>
<td>0.6</td>
<td>0.6</td>
<td>1000</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1000</td>
</tr>
<tr>
<td>2</td>
<td>.2</td>
<td>10000</td>
</tr>
<tr>
<td>3</td>
<td>.3</td>
<td>10000</td>
</tr>
<tr>
<td>4</td>
<td>.4</td>
<td>10000</td>
</tr>
<tr>
<td>6</td>
<td>.6</td>
<td>10000</td>
</tr>
<tr>
<td>8</td>
<td>.8</td>
<td>10000</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>10000</td>
</tr>
<tr>
<td>12</td>
<td>.3</td>
<td>40000</td>
</tr>
<tr>
<td>14</td>
<td>.35</td>
<td>40000</td>
</tr>
<tr>
<td>16</td>
<td>.4</td>
<td>40000</td>
</tr>
<tr>
<td>18</td>
<td>.45</td>
<td>40000</td>
</tr>
<tr>
<td>20</td>
<td>.5</td>
<td>40000</td>
</tr>
<tr>
<td>22</td>
<td>.55</td>
<td>40000</td>
</tr>
<tr>
<td>24</td>
<td>.6</td>
<td>40000</td>
</tr>
<tr>
<td>26</td>
<td>.65</td>
<td>40000</td>
</tr>
<tr>
<td>28</td>
<td>.7</td>
<td>40000</td>
</tr>
<tr>
<td>30</td>
<td>.75</td>
<td>40000</td>
</tr>
</tbody>
</table>

**Waste Disposal**

All chemicals that remain following the preparation of nitrate standards must be placed in properly labeled waste bottles in the Waste Accumulation Area until collected by Environmental Health and Safety. NO CHEMICALS GO DOWN THE DRAIN.

**Written By:** Jana Z. Stenback  
**Date:** 25 February 2011
STANDARD OPERATING PROCEDURE FOR:
PREPARATION ON PHOSPHATE STANDARDS

Summary
Working nitrate standards are prepared by adding known volumes of a stock solution of potassium phosphate monobasic (KH₂PO₄) to acidified, deionized water and diluting. These standards are used to construct a standard curve for spectrometric determination of ortho phosphate in water samples.

Materials
ACS certified reagent grade potassium phosphate monobasic KH₂PO₄
Concentrated sulfuric acid H₂SO₄ (36N)
0.2 and 1.0 ml adjustable pipette and tips
Repeater (Eppeendorf) pipette 50 ml tips
Multiple- 1 liter volumetric flasks
Drying oven

Precautions
Review MSDS for each of the chemicals used in this procedure. Protective lab coat, goggles and gloves must be worn at all times when following this procedure. Lab paper must be on all surfaces because chemicals in this procedure can be corrosive to countertops and instruments.

Preparation Stock Solutions
Dry potassium phosphate monobasic in a drying oven at 200°C for a minimum of two hours. Remove from the drying oven and cool to room temperature in a desicator with desicant before using. Stock solutions are prepared by weighing and recording a mass of potassium phosphate monobasic and adding to a volumetric flask. Water is added to the flask at a level below the line and capped and inverted until potassium phosphate monobasic is dissolved then fill to the line with DI water and invert 30 times to mix.

3 stock solutions are prepared to be used in preparing the standards. They are as follows:

<table>
<thead>
<tr>
<th>Target Concentration mgP/l</th>
<th>Mass Potassium Phosphate Monobasic g</th>
<th>Volumetric flask volume l (DI water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>.4394</td>
<td>1</td>
</tr>
<tr>
<td>500</td>
<td>2.197</td>
<td>1</td>
</tr>
<tr>
<td>2000</td>
<td>8.788</td>
<td>1</td>
</tr>
</tbody>
</table>

Preparation of Working Standards
Use 1 liter volumetric flasks for preparation of standards. In addition to the potassium phosphate monobasic concentrated sulfuric acid is added to the volumetric flask so that the final stock solution has 0.1% (volume to volume) sulfuric acid. To each flask fill at least half full with DI water, add 1ml of concentrated sulfuric acid, invert several times until the acid is mixed in. Once the acid is added and mixed the stock solution can be added.

Use the following table to determine which stock solution and how much to add for each standard. Calibrate the pipette for each addition by following the Standard Operating Procedure for Pipette Calibration. Once the stock solution is added cap and invert at least three times and then fill to the line with DI water cap and invert 30 times.
<table>
<thead>
<tr>
<th>Target Concentration ppb</th>
<th>Target Volume ml</th>
<th>Stock Solution mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.1</td>
<td>100</td>
</tr>
<tr>
<td>25</td>
<td>0.25</td>
<td>100</td>
</tr>
<tr>
<td>50</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>150</td>
<td>0.3</td>
<td>500</td>
</tr>
<tr>
<td>200</td>
<td>0.4</td>
<td>500</td>
</tr>
<tr>
<td>250</td>
<td>0.5</td>
<td>500</td>
</tr>
<tr>
<td>300</td>
<td>0.6</td>
<td>500</td>
</tr>
<tr>
<td>350</td>
<td>0.7</td>
<td>500</td>
</tr>
<tr>
<td>400</td>
<td>0.8</td>
<td>500</td>
</tr>
<tr>
<td>600</td>
<td>0.3</td>
<td>2000</td>
</tr>
<tr>
<td>800</td>
<td>0.4</td>
<td>2000</td>
</tr>
<tr>
<td>1000</td>
<td>0.5</td>
<td>2000</td>
</tr>
<tr>
<td>1200</td>
<td>0.6</td>
<td>2000</td>
</tr>
<tr>
<td>1400</td>
<td>0.7</td>
<td>2000</td>
</tr>
</tbody>
</table>

**Waste Disposal**

All chemicals that remain following the preparation of nitrate standards must be placed in properly labeled waste bottles in the Waste Accumulation Area until collected by Environmental Health and Safety. **NO CHEMICALS GO DOWN THE DRAIN.**

**Written By:** Jana Z. Stenback  
**Date:** 25 February 2011
STANDARD OPERATING PROCEDURE FOR:
PIPETTING TOTAL NITROGEN SAMPLES

Summary
Water samples are sub-sampled for the analysis of total nitrogen. It is important to follow all of the directions so that the sample is representative of the field water sample.

Materials
Water samples preserved in 0.1 sulfuric acid
Clean test tubes and lids
Test tube racks
Blue Sharpie
Post-It Notes
Purple Sharpie

Precautions
Review MSDS for each of the chemicals used in this procedure. Field water samples are preserved in dilute sulfuric acid so handle them carefully. Wear protective clothing, goggles, and gloves when handling samples. A safety shower is located in Room 127A and outside room 129, eye washes in Room 150 and 127A.

Methods
The following are the steps to be followed while pipetting total nitrogen samples:

1. Open the box of bottles and check the box sheet by surveying for X’s in the TN columns to see if there are samples that require total nitrogen analysis. If there are no samples in the box that need total nitrogen analysis then write N/A in the TN check box on the front of the box. Select the next box of samples and begin again.
2. Remove necessary bottles and place in order.
3. Using a blue sharpie, number test tubes with the same numbers as the bottles to be sampled. The last or 10th row in each rack will be repeated and used to do spikes. When you get to the 10th row of test tubes in the rack duplicate the numbers in the 10th row, these test tubes will be stored in a different rack. Mark each of the spike tubes with an “S” for Spike below the sample number and put tubes in the spike rack.
4. Shake the bottle, even if you have already shaken it to dispense TP or TRP samples, and dispense 10 ml using the 10ml fixed volume pipette into the test tube with same number as the bottle. Be sure to hold the test tube by the base rather than at the top to avoid contamination of the sample. Bottles should be shaken between the 10th row samples and the spikes.
5. Replace the pipette tip with a clean tip between each sample. If concurrently pipetting TP and TRP samples, the same tip may be used for all of the samples drawn from one bottle but they must be shaken between drawing samples.
6. Cap and store test tube in test tube rack.
7. When beginning a new rack, label a post-it “TN” and write the beginning sample number of the rack. Attach to the end of the rack where the low numbers are. Write the final sample number when rack is finished.
8. Check off the box list with the purple sharpie as you use each sample bottle to fill the test tube.
9. Mark the finished bottles’ tops with a purple sharpie to show it has been processed for total nitrogen.
10. When a rack is filled with samples and spikes take it to room 121 to be processed for total nitrogen.
11. When all the TN samples have been taken from a box use the purple sharpie to make a check in the check box on the front of the box. Put the box away in its correct place (in numerical order) so that we can find them again if needed.

Approved By: Jana Z. Stenback
Date: 02 February 2011
STANDARD OPERATING PROCEDURE FOR:
PIPETTING TOTAL PHOSPHORUS SAMPLES

Summary
Water samples are sub-sampled for the analysis of total phosphorus. It is important to follow all of the directions so that the sample is representative of the field water sample.

Materials
Water samples preserved in 0.1 sulfuric acid
Clean test tubes and lids
Test tube racks
Post-It Notes
Blue Sharpie
Orange Sharpie

Precautions
Review MSDS for each of the chemicals used in this procedure. Field water samples are preserved in dilute sulfuric acid so handle them carefully. Wear protective clothing, goggles, and gloves when handling samples. A safety shower is located in Room 127A and outside room 129, eye washes in Room 150 and 127A.

Methods
The following are the steps to be followed while pipetting total phosphorus samples:

1. Open the box of bottles and check the box sheet by surveying for X’s in the TP columns to see if there are samples that require total phosphorus analysis. If there are no samples in the box that need total phosphorus analysis then write N/A in the TP check box on the front of the box. Select the next box of samples and begin again.
2. Remove necessary bottles and place in order.
3. Using a blue sharpie, number test tubes with the same numbers as the bottles to be sampled. The last or 10th row in each rack will be repeated and used to do spikes. When you get to the 10th row of test tubes in the rack duplicate the numbers in the 10th row, these test tubes will be stored in a different rack. Mark each of the spike tubes with an “S” for Spike (below the sample number) and put tubes in the spike rack.
4. Shake the bottle even if you have already shaken it to dispense TN or TRP samples and dispense 10 ml using the 10ml fixed volume pipette into the test tube with same number as the bottle. Be sure to hold the test tube by the base rather than at the top to avoid contamination of the sample. Bottles should be capped and shaken between the 10th row samples and the spikes.
5. Replace the pipette tip with a clean tip between each sample. If concurrently pipetting TN and TRP samples, the same tip may be used for all of the samples drawn from one bottle.
6. Cap and store test tube in test tube rack.
7. When beginning a new rack, label a post-it “TP” and write the beginning sample number of the rack. Attach to the end of the rack where the low numbers are. Write the final sample number when finished with the rack.
8. Check off the box list with the orange sharpie as you use each sample bottle to fill the test tube
9. Mark the finished bottles’ tops’ with an orange sharpie to show it has been processed for total phosphorus.
10. When a rack is filled with samples and spikes take it to room 121 to be processed for total phosphorus.
11. When all the TP samples have been taken from a box use the orange sharpie to make a check in the check box on the front of the box. Put the box away in its correct place (in numerical order) so that we can find them again if needed.

Approved By: Jana Z. Stenback
Date: 14 February 2011
STANDARD OPERATING PROCEDURE FOR:
PIPETTING TOTAL REACTIVE PHOSPHORUS (TRP) SAMPLES

Summary
Water samples are sub-sampled for the analysis of total reactive phosphorus. It is important to follow all of the directions so that the sample is representative of the field water sample.

Materials
Water samples preserved in 0.1 sulfuric acid
Clean test tubes and lids
Test tube racks
Post-It Notes
Blue Sharpie
Red Sharpie

Precautions
Review MSDS for each of the chemicals used in this procedure. Field water samples are preserved in dilute sulfuric acid handle them carefully. Wear protective clothing, goggles, and gloves when handling samples. A safety shower is located in Room 127A and outside room 129, eye washes in Room 150 and 127A.

Methods
The following are the steps to be followed while pipetting total reactive phosphorus samples:

1. Open the box of bottles and check the box sheet by surveying for X’s in the TRP columns to see if there are samples that require total reactive phosphorus analysis. If there are no samples in the box that need total reactive phosphorus analysis then write N/A in the TRP check box on the front of the box. Select the next box of samples and begin again.
2. Remove necessary bottles and place in order.
3. Using a blue Sharpie, number test tubes with the same numbers as on the bottles to be sampled.
4. Shake the bottle even if you have already shaken it to dispense TN or TP samples and dispense 10 ml using the 10ml fixed volume pipette into the test tube with same number as bottle. Be sure to hold the test tube by the base rather than at the top to avoid contamination of the sample.
5. Replace the pipette tip with a clean tip between each sample. If concurrently pipetting TP and TN samples, the same tip may be used for all of the samples drawn from one bottle.
6. Cap and store test tube in test tube rack.
7. When beginning a new rack, label a post-it “TRP” and write the beginning sample number of the rack. Attach to the end of the rack where the low numbers are. Write the final sample number when rack is finished.
8. Check off the box list with the red sharpie as you use each sample bottle to fill the test tube
9. Mark the finished bottles’ tops with a red sharpie to show it has been processed for total reactive phosphorus.
10. When a rack is filled with samples to be processed for total reactive phosphorus transfer it to the TRP holding area.
11. When all the TRP samples have been taken from a box use the red sharpie to make a check in the check box on the front of the box. Put the box away in its correct place (in numerical order) so that we can find them again if needed.

Approved By: Jana Z. Stenback Date: 14 February 2011
STANDARD OPERATING PROCEDURE FOR:
SPECTROPHOTOMETRIC ANALYSIS OF NITRATE-NITROGEN USING THE SPECTROSCOPIC SECOND DERIVATIVE METHOD

Summary
This procedure uses the UV/Vis spectrophotometric second derivative method to analyze for nitrate-nitrogen in natural water samples Crumpton (1992) and AWWA (2005). In this procedure and set of standards is used to generate a calibration curve which is then used to determine the amount of nitrate-nitrogen in a water sample.

Note: This method only detects nitrate-nitrogen. Analysis of total nitrogen requires that a sample be digested so as to convert all nitrogen to nitrate see SOP: Sample digestion for total nitrogen analysis.

Materials
1. For nitrate analysis: nitrate-nitrogen standards and water samples
2. For total nitrogen analysis: pipetted standards, spikes, organic digestion checks and samples that have been digested
3. 1cm quartz cuvette
4. 4 L Erlenmeyer flask with a plastic funnel on top to be used as a waste container

Precautions
Review current MSDS for each of the chemicals used in this procedure. Protective goggles, clothing and gloves must be worn at all times following this procedure. Lab paper must be on all surfaces.

Operation of Spectrophotometers

Note: Cuvettes are expensive and very fragile. Handle the cuvettes only on the etched surfaces do not touch the clear surfaces that the light shines through. It is also important that the cuvette be very clean. After the cuvette has been filled, it should be carefully wiped off using a Kimwipe. Never use anything other than a Kimwipe to clean the cuvettes. This is because Kimwipes are 100% cotton and will not scratch the cuvette. After the cuvette is wiped off check to make sure there are no streaks or spots on the surfaces and no bubbles on the inner surface that the light shines through.

Turning on the spec and setting parameters
1. Turn on PC that is connected to the spectrophotometer (spec).
2. Open the HP-Bootp (CAG Bootp) program if not already running.
3. Turn on the spectrophotometer the HP-Bootp window should show when the connection is made between the computer and the spectrophotometer.
4. Open the Chemstations software (UV/Vis instrument online) when the logon menu appears there is no need to enter anything, just select the “OK” button.
5. In the instrument menu, turn on the UV lamp, click on the icon of the UV lamp (oblong and yellow if on) and choose “lamp on”. If the visible lamp is on click on the icon of the visible lamp (round and red if on) and choose “lamp off”. When the lamp is on the icon will change to yellow for the UV lamp.  Allow the light bulb to warm up for 15 minutes.
6. Set up the instrument program to do quantification by opening the menu in the Task box and selecting “quantification”. At this point a setup menu will open. Set up the following parameters and leave the others blank:

Use wavelength: 235 nm
Background correction: none
Analyte name: Nitrate for total nitrate-nitrogen analysis and TN for total nitrogen analysis
Calibration Curve type: QuadraticOffset
Select concentration and use mg/l for the unit
Data type: 2nd Derivative
From: 220 nm
To: 250 nm

The box to the right is how the window should appear for total phosphorus analysis. For total nitrogen it should read TN in the “Analyte name” box.
Standard and Sample Analysis
Because this analysis is done in the ultraviolet region of the spectrum a quartz cuvette must be used. Glass is opaque in the ultraviolet. Once the spectrophotometer is ready a blank will be run using DI water to subtract any absorbance due to water and the cuvette from the standards and samples. Once an acceptable blank has been run standards and samples can be analyzed.

Setting up Excel Spreadsheet (Waiting for the spectrophotometer to warm up the Excel Spreadsheet can be set up.)
The following example is written for Spectrophotometer #3. If using another spectrophotometer the 3 in the file names would be changed to the number of the spec being used.
1. In the file C:\Spec 3 data open the “Excel Template” Excel spreadsheet.
2. Save the Excel spreadsheet in the appropriate folder (TN or Nitrate) by date, followed by spec number, followed by the type of analysis. For example an Excel file would be saved as “02-24-10 Spec 3 NO3” for nitrate-nitrogen analyses run on 24 February 2010 on spec number 3 or for spec 1 “03-25-10 Spec 1 TN” for low total nitrogen analysis run on 25 March 2010.

Running the blank
1. To run the blank, rinse the quartz cuvette three or four times with DI water and then fill with DI water. Wipe the cuvette off with a Kimwipe until spotless, place in cuvette holder and press the “Blank” icon on the screen with cursor while pressing the left mouse button OR press the ”F4” button on the keyboard OR press the blank button on the front of the spec. When the blank scan is completed check to see that the value at 235 nm is within ± 5.0 X 10⁻³ units of zero. You can get a tabular display of the data by double-clicking on the graph that appears on the screen. Find the absorbance for 235 nm by scrolling down through the table. If the value is not small enough rescan until within acceptable range.
2. Empty cuvette contents into the waste container.

Running the standards
1. Standards are run first, working from low to high concentration, emptying and rinsing the cuvette three times with the next standard before filling and scanning. All waste is put into the waste container.
2. To scan a standard press “Standard” on the screen with the cursor while pressing the left mouse button OR press the “F6” button on the keyboard OR press the standard button on the front of the spec.
3. Scan each standard three times. The two closest values as read in the column headed “d2(Abs)<235nm>” should be saved. They should be within 0.10 of each other. Sometimes there are particles or bubbles that drift in the light path causing the values to drift so that you may have to continue scanning a sample to get the absorbance to converge. Keep scanning until there is convergence and delete all but the 2 closest scans.
4. Fill in the table on in the spec software with the concentration and name of the standard (same thing) by entering them into the Standard Name column and the concentration column. For nitrate-nitrogen the concentration column heading will be “NITRATE(MG/L)”
5. Empty cuvette contents into a waste container and proceed to the next standard.
6. Save the standards at regular intervals, such as every 20 scans. Do this by going to File/Save/Standards. Save the standards in the correct folder (TN/Standard or Nitrate/Standard) by date, followed by spec number, followed by the type of analysis. For example: “022410” for standards at 24 February 2010. The spec program only allows 8 characters for a file name.
7. When calibration standards have all been run first save the data as explained in #6 then generate a calibration curve by selecting the “Calibrate” icon on the screen, and then press the “Show Coefficients” button to view the calibration curve and summary statistics. The correlation coefficient should be between 0.995 and 1.0.
8. If you do not get a correlation coefficient within this range or the standards do not seem to form a smooth curve please check with Jana ASAP to make sure there are no problems.
9. DO NOT COPY THE DATA FROM THE SPEC PROGRAM THEN OPEN EXCEL; THIS WILL RESULT IN A “STACK OVERFLOW” WHICH REQUIRES COMPLETE REBOOTING OF THE COMPUTER AND LOSS OF ALL UNSAVED DATA. To copy the standards into Excel. First make sure Excel is open to the page where you want to save the data. While in the Chemstations program highlight the table by starting at the top or bottom of the table of standards and pressing left mouse button while icon displays a black arrow on left side of the table and dragging until all of table is highlighted. Once they are all highlighted press the “Edit” button on the top of the tool bar and select “Copy” OR press Control “c”. Open the excel spreadsheet and paste the data into the work page labeled “standards”. Do this by highlighting cell A1 and pasting OR press Control “v”. Do not leave any empty rows above the data or empty columns to the left of the data.
10. Using the same process as above copy, the Coefficients table onto the standards worksheet in Excel. Paste in on the right side of the standards.
11. Enter appropriate information into the appropriate Spec Logbook. Be sure to put the start time as the time when running of standards is complete, date, names of all operators and analysis type.

Running the samples
1. Samples are run by first rinsing three times, then filling the cuvette with sample, then scanning by pressing “Sample” on the screen with the cursor while pressing the left mouse button OR press the”F5” button on the keyboard OR press the sample button on the front of the spec.
2. Scan each sample three times. Check to make sure the absorbance at least 2 of the scans is within 10% of each other, if they are not repeat scanning until there are 2 scans within an acceptable range. Sometimes there are particles or bubbles that drift in the light path causing the values to drift so that you may have to continue scanning a sample to get the absorbance to converge. Keep scanning until there is convergence and delete all but the 2 closest scans.

3. Fill in the sample number in the “Name” column on the data table.

4. About every 20 scans save the data to the appropriate file by going to File/Save/Samples As. Save the spec data by date followed by a letter designation. For example, the first set of scans run on 02-24-2010 would be labeled “022410A” and the second set would be “022410B”. Continue to save the data to the same file until a total of 80 scans.

5. Once there are a total of 80 scans (one rack) save the data, and copy it into the same excel workbook where the standards are stored but to a spec data worksheet. Highlight the table by starting at the top or bottom of the table of samples and pressing left mouse button while icon displays a black arrow on left side of the table and dragging until all of table is highlighted. Once all the sample data are highlighted press the “Edit” button on the top of the tool bar and select “Copy” OR press Control “c”. Open the excel spreadsheet and paste the data into the work page labeled “spec data a”. Do this by highlighting cell A1 and pasting. Do not leave any empty rows above the data or empty columns to the left of the data. Use the worksheet spec data A for the first set of scans followed by spec data B, spec data C and so on for subsequent sets of data.

6. Save the Excel spreadsheet.

7. If the scans are not saved and cleared after 80 scans the spectrophotometer begins to run very slowly.

Running spikes and organic checks for total nitrogen samples

1. Spikes and organic checks are run in exactly the same way as samples. In the name column for spikes write “Spike 8” for a 8 mg/l spike followed by the sample number, for example a spiked sample 21985 with a 8 mg/l spike of organic nitrogen you would write “Spike 8 21985”. For organics put ORGX where X is the concentration of organic P. There are usually six sets of organics, 0, 4, 8, 12, 16, and 20 mg/l. For example for an 8 ppb organic you would write “N ORG 8”.

2. The spikes are saved by the date and “sp” while the organics are saved by the date and “og” for example for 24 February 2010 they would be “022410sp” and “022410og”.

3. They are saved on the spikes and organics worksheet in the Excel workbook.

6 Hour drift check

1. If running a set of samples extends beyond 6 hours standard drift checks need to be run.

2. Select three standards with low, middle and high concentrations for each of the spectrophotometers.

3. Run the check standards as samples and note the how close they are to the actual standards.

4. Calculate the percent drift by dividing the ((measure concentration-actual concentration)/actual concentration)*100. Enter the percent drift check into the spec logbook.

5. The drifts are saved by the date and “dr” for example for 24 February 2010 they would be “022410dr”.

Shutting Down and Cleaning up

1. Make sure all data and files are saved. Exit Excel and the Spec program. Turn off the spec. Turn off CAG Bootp Server (must do before logging off). Turn off computer. Turn off monitor.

2. Empty waste into appropriate waste container in the waste accumulation area and rinse waste container.

3. All test tubes and caps should be soaked in P-free RBS.

4. Remove the stage from spec and swish in P-free RBS, rinse with DI and set to dry. Wipe down the spec and area around the spec.

5. Clean cuvette by first filling and emptying of RBS solution several times. Then rinse thoroughly with DI water and put open side down on diaper paper in the cuvette storage area.

6. If the laboratory bench paper needs to be changed, do so.

References

Written and Approved By: Jana Z. Stenback Date: 15 February 2011
STANDARD OPERATING PROCEDURE FOR:
SPECTROPHOTOMETRIC ANALYSIS OF ORTHO PHOSPHORUS USING THE ASCORBIC ACID METHOD

**Summary**
This procedure uses the ascorbic acid method originally described by Murphy and Riley (1962) and slightly modified in AWWA (1998). The preparation of the Color Developing Solution is described in the SOP: Preparation of color developing solution to be used in orthophosphate analysis using a modified Murphy and Riley method.

This is a spectroscopic method for the analysis of the orthophosphate ion in water. Ammonium molybdate and potassium antimonyl tartrate react in an acid medium with orthophosphate to form the heteropoly acid phosphomolybdic acid, that is reduced to an intense molybdenum blue by ascorbic acid (AWWA, 1997).

The standards for this analysis are divided into two groups and are analyzed using two different cuvette path lengths. This is because the ranges of concentrations analyzed are not all detectable using one cuvette length. The lower concentrations from 10 ppb to 350 ppb use the 5 cm path length cuvette and the high concentrations from 100 ppb to 1400 ppb use a 1 cm path length cuvette.

<table>
<thead>
<tr>
<th>Light Path cm</th>
<th>Approximate P Range ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>100 to 1300</td>
</tr>
<tr>
<td>5.0</td>
<td>10 to 350</td>
</tr>
</tbody>
</table>

Note: This method only detects orthophosphate. Analysis of total phosphorus requires that a sample be digested so as to convert all phosphorous to orthophosphate see SOP: Sample digestion for total phosphorus analysis.

**Materials**
1. Color Developing Solution (CDS)
2. Repeater (Eppendorf) pipette
3. 25 ml repeater tip and collar
4. Pipetted standards and water samples in test tubes for TRP analysis
5. Pipetted standards and water samples in test tubes that have been digested for TP analysis
6. Spectrophotometer
7. 1cm cuvette
8. 5cm cuvette
9. 4 L erlenmeyer flask with a plastic funnel on top to be used as a waste container

**Precautions**
Review current MSDS for each of the chemicals used in this procedure. Protective goggles, clothing and gloves must be worn at all times when following this procedure. Lab paper must be on all surfaces because chemicals in this procedure can be corrosive to countertops and instruments.

**Addition of the Color Developing Solution and Color Developing Process**
For preparation of CDS see SOP: Preparation of color developing solution to be used in orthophosphate analysis using a modified Murphy and Riley method.

Note: The next procedure involves the addition of the CDS to the samples and standards; this is a time sensitive step. The reaction between the reagents yields a blue color that over time has a shift in intensity. It is best to do the analysis within 4 hours of adding the CDS. Because of this it is important to add the CDS to only as many test tubes as can be analyzed in a three hour period. It is also important to make sure your standards are good before adding the CDS to the samples.

Using an Eppendof repeat pipette with a 25 ml tip set on 4, add 2.0 ml of CDS as follows:
1. First add the CDS to all the standards, both high and low.
2. Then add CDS to one or two racks of samples if doing TRPs or the spikes and organics rack and a rack of samples if doing TPs.
3. Once the samples have developed they should turn blue, with the higher the concentration of orthophosphate being the darrest blue. If this does not occur there is a problem with the CDS or the standards.
4. Allow the color to develop in the standards or samples for at least 15 min after which the standards or samples will be ready to analyze using the UV/Vis spectrophotometer.

**Operation of Spectrophotometers**
Because there is no way to tell the concentration of phosphorus in a set of samples, two spectrophotometers are set up and run simultaneously— one for low phosphorus concentrations and one for high phosphorus concentrations. The low concentration spectrophotometer uses the 5 cm quartz or glass cuvette and the high concentration spectrophotometer uses a 1 cm quartz or glass cuvette. The following procedures are the same for both spectrophotometers.

Note: Cuvettes are expensive and very fragile. Handle the cuvettes only on the etched surfaces do not touch the clear surfaces that the light shines through. It is also important that the cuvette be very clean. After the cuvette has been filled, it should be carefully wiped off using a Kimwipe. Never use anything other than a Kimwipe to clean the cuvettes. This is because Kimwipes are 100% cotton and will not scratch the cuvette. After the cuvette is wiped off check to make sure there are no streaks or spots on the surfaces and no bubbles on the inner surface that the light shines through.

Turning on the spec and setting parameters
5. Turn on PC that is connected to the spectrophotometer (spec).
6. Open the HP-Bootp (CAG Bootp) program if not already running.
7. Turn on the spectrophotometer, the HP-Bootp window should show when the connection is made between the computer and the spectrophotometer.
8. Open the Chemstations software (UV/Vis instrument online) when the logon menu appears there is no need to enter anything, just select the “OK” button.
9. In the instrument menu, turn on the visible lamp, click on the icon of the visible lamp (round & red) and choose “lamp on”. If the UV lamp is on click on the icon of the UV lamp (long & yellow) and choose “lamp off”. When the lamp is on the icon will change to red for the visible lamp. Allow the light bulb to warm up for 15 minutes.
10. Set up the instrument program to do quantification by opening the menu in the Task box and selecting “quantification”. At this point a setup menu will open. Set up the following parameters and leave the others blank:

Use wavelength: 885 nm
Background correction: none
Analyte name: TP for total phosphorus analysis and TRP for total reactive phosphorus analysis
Calibration Curve type: QuadraticOffSet
Select concentration and use ppb for the unit
Data type: Absorbance
From: 600 nm
To: 950 nm

Below is an image of how the window should appear for total phosphorus analysis. For total reactive phosphorus it should read TRP in the “Analyte name” box.
Standard and Sample Analysis
Once the spectrophotometer(s) are ready a blank will be run using DI water to subtract any absorbance due to water and the cuvette from the standards and samples. The high concentration standards are run on the spectrophotometer with the 1 cm cuvette and the low concentration standards are run on the spectrophotometer with the 5 cm cuvette. To determine whether a sample is to be run on the high or the low spectrophotometer the first and second from the lowest of the high standards (around 100 and 200 ppb) are saved as a visual check standard. If a sample is as dark as or darker than the check standards it is run on the high end spec. After running each of the ~100 and ~200 standards combine what is remaining of the like concentration test tubes to yield enough volume to use as a visual check standard.

Setting up Excel Spreadsheet
The following example is written for Spectrophotometer #3. If using another spectrophotometer the 3 in the file names would been changed to the number of the spec being used.
11. In the file C:\Spec 3 data open the “Excel Template” Excel spreadsheet.
12. Save the Excel spreadsheet in the appropriate folder by date, followed by spec number, followed by concentration range and type of analysis. For example an Excel file would be saved as “02-24-10 Spec 3 high TP” for high concentration total phosphorus analysis run on 24 February 2010 on spec number 3 or “03-25-10 Spec 1 low TRP” for low concentration total reactive phosphorus analysis run on 25 March 2010 on spec number 1.

Running the blank
13. To run the blank, rinse the quartz cuvette three or four times with DI water and then fill with DI water. Wipe the cuvette off with a Kimwipe until spotless, place in cuvette holder and press “Blank” icon on the screen with cursor while pressing the left mouse button OR press the “F4” button on the keyboard OR press the blank button on the front of the spec. When the blank scan is completed check to see that the value at 885 nm is within ± 5.0 x 10⁻² units of zero. You can get a tabular display of the data by double-clicking on the graph that appears on the screen. Find the absorbance for 885 nm by scrolling down through the table. If the value is not small enough rescan until within acceptable range.
14. Empty cuvette contents into the waste container.

Running the standards
15. Standards are run first, working from low to high concentration, emptying and rinsing the cuvette three times with the next standard before filling and scanning. All waste is put into the waste container.
16. To scan a standard press “Standard” on the screen with the cursor while pressing the left mouse button OR press the “F6” button on the keyboard OR press the standard button on the front of the spec.
17. Scan each standard once then let the sample rest for about 20 seconds, delete the first scan, then do 4 consecutive scans. Check to make sure the absorbance of the 4 scans are within 10% of each other if they are not repeat scanning until there are 4 scans within an acceptable range. There is a tendency for the scans to drift if you do not wait the 20 seconds after the first scan. Sometimes there are particles or bubbles that drift in the light path causing the values to drift so that you may have to continue scanning a sample to get the absorbance to converge. Keep scanning until there is convergence and delete all but the 4 closest scans.
18. Remember to retain the second from the lowest of the high standards as a check standard.
19. Fill in the table on in the spec software with the concentration and name of the standard (same thing) by entering them into the Standard Name column and the concentration column. For TP the concentration column heading will be “TP(ppb)”.
20. Empty cuvette contents into a waste container and proceed to the next standard.
21. Save the standards at regular intervals, such as every 20 scans. Do this by going to File/Save/Standards As. Save the standards in the correct folder by the current date using format of two digits for each month, day, year followed by ‘hi’ for the high end standard or ‘lo’ for the low end standards. For example: “022410hi” for the high standards and “022410lo” for the low standards run on 24 February 2010. The spec program only allows 8 characters for a name.
22. When calibration standards have all been run first save the data as explained in #7 then generate a calibration curve by selecting the “Calibrate” icon on the screen, and then press the “Show Coefficients” button to view the calibration curve and summary statistics. The correlation coefficient should be between 0.995 and 1.0.
23. If you do not get a correlation coefficient within this range or the standards do not seem to form a smooth curve please check with Jana ASAP to make sure there are no problems.
24. DO NOT COPY THE DATA FROM THE SPEC PROGRAM THEN OPEN EXCEL; THIS WILL RESULT IN A STACK OVERFLOW WHICH REQUIRES COMPLETE REBOOTING OF THE COMPUTER AND LOSS OF ALL UNSAVED DATA. To copy the standards into Excel. First make sure Excel is open to the page where you want to save the data. Highlight the table by selecting at the top or bottom of the table of standards and pasting OR press Control “c”.
25. Using the same process as above copy, the Coefficients table onto the standards worksheet in Excel. Paste in on the right side of the standards.
26. Enter appropriate information into the appropriate Spec Logbook. Be sure to put the start time as the time when running of standards is complete, date, names of all operators and analysis type.

Running spikes and organic checks for total phosphorus samples
27. Spikes and organic checks are run in exactly the same way as standards. In the name column for spikes write “Spike 200” for a 200 ppb spike followed by the sample number, for example a spiked sample 21985 with a 200 ppb spike of organic phosphorus you would write “Spike 200 21985”. For organics put ORGx where X is the concentration of organic P. There are usually six sets of organics, 0, 100, 200, 300, 400, and 500ppb. For example for a 300ppb organic you would write “Org 300”.
28. The spikes are saved by the date and “sp” while the organics are saved by the date and “og” for example for 24 February 2010 they would be “022410sp” and “022410og”. The spec program only allows 8 characters for a name.
29. Spikes and organics are saved on the spikes and organics worksheet in the Excel workbook.

Running the samples
30. Samples are run by first rinsing three times, then filling the cuvette with sample, then scanning by pressing “Sample” on the screen with the cursor while pressing the left mouse button OR press the “F5” button on the keyboard OR press the sample button on the front of the spec.
31. Scan each sample once then let the sample rest for 20 sec., delete the first scan, then do 4 consecutive scans. Check to make sure the absorbance of the 4 scans are within 10% of each other, if they are not repeat scanning until there are 4 scans within an acceptable range. There is a tendency for the scans to drift if you do not wait the 20 seconds after the first scan. Sometimes there are particles or bubbles that drift in the light path causing the values to drift so that you may have to continue scanning a sample to get the absorbance to converge. Keep scanning until there is convergence and delete all but the 4 closest scans.
32. Fill in the sample number in the “Name” column on the data table.
33. About every 20 scans save the data to the appropriate file by going to File/Save/Samples As. Save the spec data by date followed by a letter designation. For example, the first set of scans run on 02-24-2010 would be labeled “022410A” and the second set would be “022410B”. Continue to save the data to the same file until a total of 80 scans.
34. Once there are a total of 80 scans (half a rack) save the data, and copy it into the same excel workbook where the standards are stored but to a spec data worksheet. Highlight the table by starting at the top or bottom of the table of samples and pressing left mouse button while icon displays a black arrow on left side of the table and dragging until all of table is highlighted. Once all the sample data are highlighted press the “Edit” button on the top of the tool bar and select “Copy” OR press Control “c”. Open the excel spreadsheet and paste the data into the work page labeled “spec data a”. Do this by highlighting cell A1 and pasting OR press Control “v”. Do not leave any empty rows above the data or empty columns to the left of the data. Use the worksheet spec data A for the first set of scans followed by spec data B, spec data C and so on for subsequent sets of data.
35. Save the Excel spreadsheet.
36. Once the Excel spreadsheet is saved go back to the spec program and clear the samples by using the cursor to select the “Clear” button followed by using the cursor to select the “Sample” button. This should clear all of the data from the samples spreadsheet.
37. If the scans are not saved and cleared after 80 scans the spectrophotometer begins to run very slowly.

6 Hour drift check
38. If running a set of samples extends beyond 6 hours standard drift checks need to be run.
39. Select three standards with low, middle and high concentrations for each of the spectrophotometers. Add the CDS to them and allow them to sit for at least 15 min.
40. Run the check standards as samples and note the how close they are to the actual standards.
41. Calculate the percent drift by doing the following ((measure concentration-actual concentration)/actual concentration)*100. Enter the percent drift check into the spec logbook.
42. The drifts are saved by the date and “dr” for example for 24 February 2010 they would be “022410dr” Save the drift check data in the Excel workbook on the standards spreadsheet.

Shutting Down and Cleaning up
43. Make sure all data and files are saved. Exit Excel and the Spec program. Turn off the spec. Turn off CAG Bootp Server (must do before shutting down the computer). Turn off computer. Turn off monitor.
44. Empty waste into appropriate waste container in the waste accumulation area and rinse waste container.
45. All test tubes and caps should be soaked in P-free RBS.
46. Remove the stage from spec and swish in P-free RBS, rinse with DI and set to dry. Wipe down the spec and area around the spec.
47. Clean cuvette by first filling with RBS solution and emptying the solution several times. Then rinse thoroughly with DI water and put open side down on diaper paper in the cuvette storage area.
48. If the laboratory bench paper needs to be changed, do so.

References
STANDARD OPERATING PROCEDURE FOR:
SAMPLE DIGESTION FOR TOTAL NITROGEN ANALYSIS

Summary
Organic nitrogen in a water sample is converted to nitrate by persulfate digestion. Some forms of nitrogen may not be converted to nitrate by this method so in some cases a more rigorous method may be needed. The potassium persulfate solution that is used has sodium hydroxide added to it to adjust the pH of the solution being digested. Following digestion the samples are analyzed using second derivative method to analyze for nitrate-nitrogen in natural water samples Crumpton (1992) and AWWA (2005). See SOP: Spectrophotometric analysis of Nitrate-Nitrogen using the spectroscopic second derivative method.

Materials
500 ml volumetric flask
1000 ml graduated cylinder
1000 ml beaker
5 ml beaker
10 ml fixed volume pipette
Repeater (Eppendorf) pipette and 0.5 ml & 50 ml tips
Potassium Persulfate (K₂S₂O₈)
Sodium Hydroxide (NaOH)
Urea (CH₄N₂O)
Deionized Water

Precautions
Review MSDS for each of the chemicals used in this procedure. Protective lab coat, goggles and gloves must be worn at all times when following this procedure. Lab paper must be on all surfaces because chemicals in this procedure can be corrosive to countertops and instruments.

Equipment Preparation
All glassware used in this analysis should be soaked at least 24 hours in phosphorus free surfactant and quadruple-rinsed in DI water (see SOP for washing dishes). All glassware should be dried, covered in foil or stored in a closed container. Any glassware that is chipped or scratched should be discarded in the broken glass receptacle. The preparation area should be covered in lab paper, clean and dust free.

Preparation Organic Nitrogen Solution
A solution that is approximately 4 g organic N/l is prepared to use as a digestion check and as an organic spike to be added to samples to check for digestion and any matrix interference. The compound used is urea (CH₄N₂O)

The molecular weight of urea is 60.06g. Weigh out about ~4.29 g of urea and dissolve in 500ml of deionized water. The final concentration should be around 4g N/l.

The molecular weight of urea is 60.06g and the weight of nitrogen per mole is 28.014g. To prepare the urea solution that is about 4gN/l dissolve ~04.29 g urea in a 500 ml of deionized water using a volumetric flask. Record the exact mass used and calculate the exact concentration. The prepared solution can be stored in a brown bottle with a Teflon lined cap. Label the bottle with chemical name, concentration, date and initials of the preparer.
An example of the calculations where 4.29 g is dissolved in 500 ml of water, given the molecular weight of urea is 60.06 g, has two molecules of nitrogen per molecule with a molecular weight of 28.014 g. The concentration of nitrogen in the stock solution is:

\[
((4.29 \text{ g urea} \cdot (28.014 \text{ g N} / 60.06 \text{ g urea}))/0.5 \text{ l})= 4 \text{ gN/l}
\]

the concentration of organic N when 0.01 ml of urea stock solution is added to 10 ml of sample is:

\[
4 \text{ gN/l } \cdot ((0.01 \text{ ml})/(10 \text{ ml})) \cdot (1000 \text{ mg/g}) = 4 \text{ mg/l}
\]

---

### Preparation Potassium Persulfate Solution

The potassium persulfate solution should be prepared at a ratio of 4.6 g potassium persulfate / 100 ml DI water. Added to this is sodium hydroxide at a ratio of 1.15 g / 100 ml DI water. The sodium hydroxide is added after potassium persulfate has been mixed with water. **DO NOT** mix dry sodium hydroxide and dry potassium persulfate together. Determine how much potassium persulfate you need if each test tube receives 3 ml. Be sure to prepare about 25 ml more than you need to account for rinsing and mistakes.

Unused potassium persulfate solution must be discarded in waste container; it cannot be stored or put down the drain.

### Mass potassium persulfate (g) | Mass sodium hydroxide (g) | Volume of DI water (ml) | ~Number of test tubes treated (with 25 ml buffer) | ~Number of racks of test tubes
---|---|---|---|---
4.6 | 1.15 | 100 | 25 | 0.5
6.9 | 1.725 | 150 | 41 | 1
13.8 | 3.45 | 300 | 91 | 2
20.7 | 5.175 | 450 | 141 | 3.5
27.6 | 6.9 | 600 | 191 | 4.5
34.5 | 8.625 | 750 | 241 | 6

### Standard, Spike, Organic and Zero Sample Preparation

1. **Standards** - Dispense 10.0 ml duplicates of inorganic standards to labeled test tubes.
2. **Organic Digestion Checks** - Using the 10 ml fixed volume pipette, dispense 10.0 ml acidified DI water (see SOP for preparation of acidified water) into each of 12 test tubes. Label two each of the test tubes ORG N 0, ORG N 4, ORG N 8, ORG N 12, ORG N 16, ORG N 20; these are the zero and organic checks. The ORG N 0 is a zero check and does not get any urea added to it. For the rest of the test tubes fill a 5 ml beaker with 2 to 3 ml of the urea solution and using the repeater pipette with a 0.5 ml tip set on 1 deliver one aliquot of urea solution to each of the test tubes labeled ORG N 4, cap and shake. Then change the setting to 2 and add one aliquot to each of the ORG N 8 test tubes cap and shake. Repeat, changing the setting and pipetting into the remainder of the test tubes respectively.
3. **Samples** – See SOP for pipetting Total Nitrogen Samples.
4. **Spikes** – Spiked samples are used to determine if there is matrix interference in the samples. About 10% of the samples are spiked for quality assurance procedures. Using an Eppendorf repeater pipette with a 0.5 ml tip set on 2, deliver one aliquot of urea solution to each of the spike test tubes. Cap and shake. Write “8” on the label following the “S”.

### Adding Potassium Persulfate/Sodium Hydroxide and Digestion

1. Using the Eppendorf repeater pipette with a 50 ml tip, set it on 3 to add 3 ml (one aliquot) of the prepared potassium persulfate/Sodium Hydroxide solution to each of the test tubes. Cap and shake.
2. Autoclave (see SOP for AUTOCLAVE OPERATION) samples and standards at 15 psi/121°C for 30 minutes.
3. When the autoclave has shut off and cooled remove the test tube racks from autoclave and allow the samples and standards to cool to room temperature.
4. During the autoclaving process, the caps can come loose so tighten all the caps. Place the racks on the counter, tape together with lab tape, and label with initials of preparer, type of sample (TN) and date autoclaved.

Note: Samples that are processed together and autoclaved together need to all be analyzed at the same time, so it is important to not get different batches of processed samples mixed with each other. This is why it is a good idea to tape them all together when they are removed from the autoclave. Tape them together and label with initials and date.

Waste Disposal
All chemicals that remain following the preparation of sample for total nitrogen analysis must be placed in properly labeled waste bottles in the Waste Accumulation Area until collected by Environmental Health and Safety. NO CHEMICALS GO DOWN THE DRAIN.

References


Written By: Jana Z. Stenback  
Date: 15 February 2011
STANDARD OPERATING PROCEDURE FOR:
SAMPLE DIGESTION FOR TOTAL PHOSPHORUS ANALYSIS

Summary
Organic phosphorus in a water sample is converted to orthophosphate by persulfate digestion. Some forms of phosphorus, especially those bound to sediment particles, may not be converted to orthophosphate by this method so in some cases a more rigorous method may be needed. Following digestion the samples are analyzed using the ascorbic acid method originally described by Murphy and Riley (1962) and slightly modified in AWWA (1998). See SOP: Spectrophotometric analysis of orthophosphorus using the ascorbic acid method.

Materials
100 ml volumetric flask
1000 ml graduated cylinder
1000 ml beaker
5 ml beaker
Repeater (Eppendorf) pipette and 0.5 ml & 50 ml tips
Potassium Persulfate (K₂S₂O₈)
Glycerophosphate (C₃H₇O₆PNa₂)
Deionized Water
10 ml fixed volume pipette

Precautions
Review MSDS for each of the chemicals used in this procedure. Protective lab coat, goggles and gloves must be worn at all times when following this procedure. Lab paper must be on all surfaces because chemicals in this procedure can be corrosive to countertops and instruments.

Equipment Preparation
All glassware used in this analysis should be soaked at least 24 hours in phosphorus free surfactant and quadruple-rinsed in DI water (see SOP for washing dishes). All glassware should be dried, covered in foil or stored in a closed container. Any glassware that is chipped or scratched should be discarded in the broken glass receptacle. The preparation area should be covered in lab paper, clean and dust free.

Preparation Organic Phosphorus Solution
A solution that is approximately 100 mg organic P/l is prepared to use as a digestion check and as an organic spike to be added to samples to check for digestion and any matrix interference. The compound used is glycerophosphate. This chemical has water associated with the molecule at different molecular ratios. Because of this the molecular weight of glycerophosphate can vary.

Check the bottle of the glycerophosphate (C₃H₇O₆PNa₂) and determine the molecular weight. Weigh out about ~0.10 g C₃H₇O₆PNa₂ and dissolve in 100ml of deionized water. The final concentration should be around 100mg P/l.

To prepare the glycerophosphate solution dissolve ~0.1g Glycerophosphate in a 100 ml of deionized water using a volumetric flask. Record the exact mass used and calculate the exact concentration. The prepared solution can be stored in a brown bottle with a Teflon lined cap. Label the bottle with chemical name, concentration, date and initials of the preparer.
Preparation Potassium Persulfate Solution

The potassium persulfate solution should be prepared at a ratio of 4.6 g potassium persulfate / 100 ml DI water. Determine how much potassium persulfate you need if each test tube receives 3 ml. Be sure to prepare about 25 ml more than you need to account for rinsing and mistakes. Weigh the amount of potassium persulfate out and add to measured water using a graduated cylinder, stir (700 RPM) and warm (60°C) slightly to dissolve. Below is a table of mixing ratios and the number of tubes that can be treated.

Unused potassium persulfate solution must be discarded in waste container; it cannot be stored or put down the drain.

<table>
<thead>
<tr>
<th>Mass potassium persulfate (g)</th>
<th>Volume of DI water (ml)</th>
<th>~Number of test tubes treated (with 25 ml buffer)</th>
<th>~Number of racks of test tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.6</td>
<td>100</td>
<td>25</td>
<td>0.5</td>
</tr>
<tr>
<td>6.9</td>
<td>150</td>
<td>41</td>
<td>1</td>
</tr>
<tr>
<td>13.8</td>
<td>300</td>
<td>91</td>
<td>2</td>
</tr>
<tr>
<td>20.7</td>
<td>450</td>
<td>141</td>
<td>3.5</td>
</tr>
<tr>
<td>27.6</td>
<td>600</td>
<td>191</td>
<td>4.5</td>
</tr>
<tr>
<td>34.5</td>
<td>750</td>
<td>241</td>
<td>6</td>
</tr>
</tbody>
</table>

An example of the calculations where 0.1 g is dissolved in 100 ml of water, given glycerophosphate has 5.5 waters per molecule where \( C_3H_7O_6PNa_2O_{0.5} \) has a molecular weight is 315.177 g glycerophosphate/mole and the weight of P is 30.974 g P/mole glycerophosphate.

\[
\text{mg P/liter of stock solution} = \frac{(0.10 \text{ g glycerophosphate} \times 30.974 \text{ g P/315.177 g } C_3H_7O_6PNa_2O_{0.5})}{(0.1 \text{ liter})} \times (1000 \text{ mg/g}) = 98.27 \text{ mg P/l}
\]

Standard, Spike, Organic and Zero Sample Preparation

5. Standards - Dispense 10.0 ml duplicates of inorganic standards to labeled test tubes.

6. Organic Digestion Checks - Using the 10 ml fixed volume pipette, dispense 10.0 ml acidified DI water (see SOP for preparation of acidified water) into each of 12 test tubes. Label two each of the test tubes ORG P 0, ORG P 100, ORG P 200, ORG P 300, ORG P 400, ORG P 500; these are the zero and organic checks. The ORG P 0 is a zero check and does not get any glycerophosphate added to it. For the rest of the test tubes fill a 5 ml beaker with 2 to 3 ml of the glycerophosphate solution and using the repeater pipette with a 0.5 ml tip set on 1 deliver one aliquot of glycerophosphate solution to each of the test tubes labeled ORG P 100, cap and shake. Then change the setting to 2 and add one aliquot to each of the ORG P 200 test tubes cap and shake. Repeat, changing the setting and pipetting into the remainder of the test tubes respectively.

7. Samples – See SOP for pipetting Total Phosphorus Samples.

8. Spikes – Spiked samples are used to determine if there is matrix interference in the samples. About 10% of the samples are spiked for quality assurance procedures. Using an Eppendorf repeater pipette with a 0.5 ml tip set on 2, deliver one aliquot of glycerophosphate solution to each of the spike test tubes. Cap and shake. Write “200” on the label following the “S”.

Adding Potassium Persulfate and Digestion

9. Using the Eppendorf repeater pipette with a 50 ml tip, set it on 3 to add 3 ml (one aliquot) of the prepared potassium persulfate solution to each of the test tubes. Cap and shake.

10. Autoclave (see SOP for AUTOCLAVE OPERATION) samples and standards at 15psi/121C for 60 minutes.

11. Remove from autoclave and allow the samples and standards to cool to room temperature.

12. During the autoclaving process, the caps can come loose so tighten all the caps. Place the racks on the counter, tape together with lab tape, and label with initials of preparer, type of sample (TP) and date autoclaved.
Note: Samples that are processed together and autoclaved together need to all be analyzed at the same time, so it is important to not get different batches of processed samples mixed with each other. This is why it is a good idea to tape them all together when they are removed from the autoclave. Tape them together and label with initials and date.

**Waste Disposal**
All chemicals that remain following the preparation of sample for total phosphorus analysis must be placed in properly labeled waste bottles in the Waste Accumulation Area until collected by Environmental Health and Safety. NO CHEMICALS GO DOWN THE DRAIN.

Written By: Jana Z. Stenback                                      Date: 15 February 2011