

## ABSTRACT

Title of Dissertation: THE IMPACT OF AGRICULTURAL WETLAND RESTORATION ON ADJACENT TEMPORARY AND PERENNIAL STREAMS

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Wetlands are known for the ecosystem services they provide, including hydrologic storage, sediment retention, nutrient processing, habitat provision, and carbon sequestration. Since European settlement, however, it is estimated that > 50% of wetlands within the conterminous United States have been lost, with a majority of loss attributed to drainage of freshwater wetlands for agriculture. In efforts to offset loss and restore ecosystem services, agricultural wetland restoration has become common. How wetland restoration impacts adjacent stream ecosystem structure and function, however, is poorly understood. Additionally, many freshwater wetlands have historically been considered geographically isolated and disconnected from adjacent surface waters. Recent U.S. Supreme Court rulings have called into question the jurisdictional status of so-called

isolated wetlands and non-perennial streams, making investigation of wetland–stream connectivity particularly critical.

Comparing native forested, historical (i.e., prior-converted cropland), and hydrologically restored freshwater wetlands within the headwaters of the Choptank River watershed (Delmarva Peninsula, Maryland, USA), I examined the impact of agricultural wetland restoration on within-wetland structure and function and influences on adjacent temporary and perennial streams. In Chapter 1, I present evidence that recently restored wetland soils, although similar to historical wetland soils in physicochemical properties and denitrification potential, may be sediment and nutrient sinks. Chapter 2 shows that so-called isolated Delmarva bay wetlands may in fact be intimately linked to perennial stream networks via temporary stream flow and that land use influences connectivity. In Chapter 3, I investigate the role of temporary stream sediment drying and wetting on denitrification potential in restored and forested wetland–stream pairs and find that alterations in flow regime, a likely outcome of both land use change and climate change, may alter the capacity of temporary streams to denitrify. Chapter 4 considers the impact of cultivation on perennial stream dissolved organic matter (DOM) quantity and quality, and suggests agricultural wetland restoration may be a tool to recover more natural fluvial DOM.

Results from this research suggest geographically isolated wetlands may be both hydrologically and ecologically linked to adjacent temporary and perennial streams and that cultivation and subsequent restoration of historical wetlands exerts strong influence on these connections.

THE IMPACT OF AGRICULTURAL WETLAND RESTORATION ON ADJACENT  
TEMPORARY AND PERENNIAL STREAMS

by

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## Preface

This dissertation consists of an introduction, four research chapters, and a summary section. All research chapters are presented in manuscript form with introduction, methods, results, discussion, and conclusion. Tables, figures, and captions occur in line with the text. A single literature cited section occurs at the end for references made throughout the dissertation.

## Dedication

For Greg Capelli, who inspired in me and in many a passion for aquatic ecology and the environmental sciences.

## Acknowledgements

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Finally, I thank my family and friends, in particular Sara Nunley, for their love and support. I certainly couldn't have done this without them.

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## Introduction

It is estimated that more than half of wetlands within the conterminous United States have been lost since European settlement (Dahl 1990). A majority of this loss has been ascribed to drainage of freshwater depressional wetlands for agriculture (Dahl 1990, Zedler and Kercher 2005). In efforts to mitigate losses and enhance valuable ecosystem services provided by wetlands (e.g., nutrient and sediment retention, carbon sequestration, habitat provision, water storage), agricultural wetland restoration has become common (Mitsch and Gosselink 2000).

In an undisturbed state, depressional wetlands are characterized by hydric soils and a bowl-like structure often surrounded by an upland rim that reduces surface outflow (Tiner 2003, McCauley and Jenkins 2005). North American examples include prairie potholes, playas, vernal pools, sinkhole wetlands, interdunal and intradunal wetlands, desert springs, bogs, fens, and Carolina and Delmarva bays. These wetlands may be continuously connected to nearby streams by surface flow or *geographically isolated*, that is, lacking perennial surface hydrologic connectivity to adjacent waters (Tiner 2003).

Referring to depressional wetlands as isolated, however, may be inappropriate as they can be connected to nearby waters via groundwater flowpaths, episodic basin spillage and overland flow, or temporary stream flow (Tiner 2003, Winter and LaBaugh 2003, Wilcox et al. 2011). Furthermore, there may be ecologically significant exchanges of material and energy through these flowpaths (Gibbons 2003, Leibowitz et al. 2008). While understanding of geographically isolated wetland–stream connectivity is generally limited, interest in the hydrological and ecological links between isolated wetlands and

adjacent stream networks is growing (Leibowitz and Nadeau 2003, Leibowitz et al. 2008, Wilcox et al. 2011), in part sparked by recent U.S. Supreme Court cases (e.g., *Solid Waste Agency of Northern Cook County v US Army Corps of Engineers* 2001, *Rapanos v United States* 2006) creating new legal standards for determining the regulatory status of wetlands and non-perennial streams under Section 404 of the Clean Water Act (CWA). Briefly, the decisions suggest that surface hydrological permanence between wetlands and non-perennial streams and adjacent navigable waters and the influence of wetlands and non-perennial streams on the chemical, physical, and biological integrity of navigable waters (i.e., “significant nexus”) may be used to determine CWA jurisdiction.

My research is part of the wetland component of the U.S. Department of Agriculture Natural Resources Conservation Service (USDA-NRCS) Conservation Effects Assessment Project (CEAP) that seeks to quantify the effects of conservation practices and resource management on the ecosystem services provided by wetlands in agricultural landscapes. I specifically sought to address the degree to which ecosystem benefits generally attributed to wetlands extend beyond the wetland and to adjacent streams.

My goals for this dissertation were to assess the impact of agricultural wetland restoration on 1) within-wetland structure and function and 2) adjacent temporary and perennial stream structure and function. To accomplish these objectives, I used a combination of field observational studies, laboratory assays and experiments, and modeling exercises to study freshwater depressional wetlands and adjacent streams along an alteration gradient (forested, hydrologically restored, and historical [i.e., wetlands now



in cropland, aka *prior-converted cropland*<sup>1</sup>) at the headwaters of the Choptank River Watershed (Delmarva Peninsula, Maryland, USA). The Choptank catchment provided an ideal location for this research, as both cropland and freshwater depressional wetlands known as Delmarva bays are plentiful. A brief description of each chapter follows.

## **Chapter 1: The impact of agricultural wetland restoration on Delmarva bay soils**

To assess the impact of agricultural wetland restoration on a suite of soil physicochemical properties, I compared soils in forested, historical, and hydrologically restored Delmarva bay wetlands. Additionally, I compared net vertical accretion, sediment, and nutrient accumulation rates in forested and restored wetlands using feldspar pads. I found forested wetland soils had significantly greater moisture content, soil organic matter (SOM), and C:N, and significantly lower bulk density relative to historical and restored wetland soils. Historical and restored wetland soils did not differ with respect to any of the aforementioned metrics. Using a denitrification enzyme activity (DEA) assay, I found that denitrification potential was positively correlated with moisture content and SOM and negatively correlated with bulk density. Denitrification potential rates were significantly greater in forested relative to both historical and restored wetland soils which did not differ from one another. Feldspar pads revealed greater annual net

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<sup>1</sup> Prior-converted cropland is a converted wetland where the conversion occurred prior to December 23, 1985, an agricultural commodity had been produced at least once before December 23, 1985, and as of December 23, 1985, the converted wetland did not support woody vegetation and met the following hydrologic criteria: (i) Inundation was less than 15 consecutive days during the growing season or 10 percent of the growing season, whichever is less, in most years; and (ii) If a pothole, playa or pocosin, ponding was less than 7 consecutive days during the growing season in most years and saturation was less than 14 consecutive days during the growing season most years. Code of Federal Regulations - Title 7: Agriculture; 12.2.

carbon, nitrogen, and phosphorus accumulation rates in restored relative to forested wetlands, the result of substantial sediment accumulation within restored basins. Results from this study suggest little differences in soil physicochemical characteristics or denitrifying enzyme pools exist between historical and recently restored Delmarva bays, and that recovery of wetland soils from an agriculturally-impacted state to a more natural condition has not occurred since restoration. Results also suggest that restored Delmarva bays may serve as sediment and nutrient sinks.

## **Chapter 2: The impact of agricultural wetland restoration on surface hydrologic connectivity between depression wetlands and adjacent streams**

Although recent U.S. Supreme Court rulings indicate surface hydrologic connectivity (SHC) between wetlands and adjacent streams may be used, in part, to determine wetland jurisdictional status, wetland–stream SHC has rarely been quantified. Furthermore, the impact of cultivation and restoration on wetland–stream SHC is particularly unknown. To help fill these knowledge gaps, I recorded surface hydrologic connectivity (SHC) patterns between Delmarva bay wetlands and adjacent perennial streams and asked how hydrologic wetland restoration impacts SHC relative to historical and native forested wetlands. For the 2010 water year I quantified cumulative connection duration, total number of connections, mean connection duration, and maximum individual connection duration ( $D_{max-c}$ ). I found that forested wetlands were connected to perennial streams for a greater cumulative duration but fewer times relative to both historical and restored wetlands. Surface hydrologic connectivity between historical and

restored wetlands and adjacent perennial streams did not differ with respect to any of the calculated metrics. Forested SHC was seasonally continuous from mid-autumn to late-spring, while historical and restored SHC was largely ephemeral. Differences in wetland–stream SHC have potential ecological implications, including provision of dispersal corridors for biota, biogeochemical processing of nutrients, and downstream delivery of energy, matter, and organisms. Understanding how cultivation and restoration impact wetland–stream SHC is critical toward recovering freshwater ecosystems in agricultural landscapes.

### **Chapter 3: The impact of repeated drying and wetting on denitrification in a restored temporary stream**

After quantifying surface hydrologic connectivity patterns between Delmarva bay wetlands and adjacent streams (Chapter 2), I investigated the influence of repeated drying and wetting on denitrification potential in temporary streams linking restored and forested bays to nearby perennial streams. The hydrologic process of repeated drying and wetting has been shown to enhance denitrification rates in a variety of ecosystems, including wetlands, lakes, and more recently temporary streams. Typically positioned at the headwaters, temporary streams may be effective nutrient processors, yet particularly sensitive to anthropogenic disturbance. Increasing denitrification may be a desired goal when restoring temporary streams in nutrient-rich landscapes (e.g., agricultural, urbanized).

In response to drying and wetting, I asked if the pattern of denitrification potential in recently restored temporary stream sediments is similar to the pattern in undisturbed forested temporary stream sediments. In a laboratory experiment, temporary stream sediments were subjected to three different flow treatments for 28 days: continuously dry (*dry*), continuously wet (*wet*), and repeatedly dry / wet at 7-day recurrence intervals (*cycled*). Cores were processed for a suite of physicochemical metrics and denitrification potential throughout the experiment. Denitrification potential, which correlated strongly with sediment reduction potential and moisture content, increased in wet forested and wet restored sediments, and decreased in dry forested and dry restored sediments. Hysteresis in denitrification potential occurred in response to cycled drying and wetting in restored temporary stream sediments whereby rates increased more rapidly upon rewetting vs. drying. Alternatively, forested temporary stream sediments exhibited greater resistance to changes in hydrology. Over the 28-day duration of the experiment, estimated cumulative denitrification potential in both forested and restored temporary streams was lowest among dry sediments, intermediate among cycled sediments, and greatest among wet sediments. These results demonstrate that alterations in temporary stream hydrology, a likely outcome of land use change, restoration, and climate change, has potential to significantly impact sediment nitrogen processing. To my knowledge, this is the first study addressing outcomes of temporary stream restoration.

## **Chapter 4: Looking toward wetlands to restore stream dissolved organic matter**

Dissolved organic matter (DOM) plays a key role in fluvial ecosystems, influencing metabolism, nutrient processing, and light and temperature regulation. Wetlands may be critical sources of DOM to fluvial networks. In response to land use change (e.g., agriculture, urbanization) natural stream DOM may be fundamentally altered. Despite its significance with regard to stream function, however, restoration efforts are rarely taken to manage DOM. The objectives of this study were to quantify the impact of cultivation in a once wetland-dominated region on stream DOM quantity and quality and to test the hypothesis that agricultural wetland restoration contributes to restoration of stream DOM. Across twenty perennial headwater catchments on the Delmarva Peninsula with a range of cropland coverage (1 - 89% of total watershed area), I found that cropland coverage was negatively correlated with stream DOM concentration, molecular weight, aromaticity, humic-like fluorescence, and allochthonous origin. Conversely, cropland coverage was positively correlated with stream DOM protein-like fluorescence and DOC bioavailability. Along a wetland alteration gradient within the study region, DOM concentration was significantly greater in forested relative to historical and restored wetland outflows. However, the composition and lability of DOM exported from restored wetlands show signs of recovery. A comparison of wetland outflow and perennial stream DOM suggests there may be potential to restore stream DOM in cultivated landscapes via wetland restoration.

# **Chapter 1: The impact of agricultural wetland restoration on Delmarva bay soils**

## **INTRODUCTION**

It is estimated that more than 50% of wetlands within the continental United States have been lost since 1780 (Dahl 1990) with the majority of loss attributed to agriculture (Dahl 1990, Zedler and Kercher 2005). Although historically viewed as wastelands or areas prime for drainage and cropping (Mitsch and Gosselink 2000), wetlands are now recognized for their support of ecosystem services including flood mitigation, nutrient and sediment storage, nutrient processing, habitat provision, and carbon sequestration (Zedler and Kercher 2005). Restoration of wetlands in cultivated landscapes has become common in attempts to mitigate loss and promote valuable wetland functions (Mitsch and Gosselink 2000).

Many agriculturally-impacted wetlands are pool-like depressions, and restoration efforts generally focus on reestablishing a more natural hydroperiod — a factor considered a master variable with respect to a wetland's ecological condition (Mitsch and Gosselink 2000) that exerts strong control over redox, pH, nutrient cycling, biodiversity, and succession (Bridgham and Richardson 1993). While hydrologic restoration is necessary, it may not be sufficient for full ecosystem recovery, and it is important to determine whether other wetland structures and functions — such as those associated with soils — have been restored.

Soils are considered the physical foundation of wetland ecosystems (Stolt et al. 2003) and play a significant role in many wetland functions. Soils provide a medium for plant rooting and seed germination, supply water and nutrients for flora and fauna, provide suitable habitat for a diverse microbial community, support biogeochemical transformation of nutrients, trap contaminants and sediments, and maintain or enhance water quality (Zedler 2004). Unlike hydrology, however, soil properties may be more challenging to restore, are considered less frequently in restoration plans, and are rarely monitored post-restoration (Shaffer and Ernst 1999, Bruland et al. 2003). Whether wetland restoration promotes accumulation and storage of sediment and nutrients — an important function of wetlands with respect to the health of downstream ecosystems — is also not yet well known.

I investigated the impact of agricultural wetland restoration on wetland soils and sediment and nutrient accumulation on the Delmarva Peninsula. Comparing restored wetlands to native forested and historical end-members (i.e., wetlands now in cropland, aka *prior-converted cropland*), I sought to quantify the impact of restoration on: 1) wetland soil physicochemical properties and denitrification potential, and 2) annual rates of vertical accretion and sediment and nutrient accumulation. Additionally, I investigated possible controls on denitrification by analyzing correlations between denitrification potential and soil physicochemical properties. I predicted decreased soil organic matter (SOM), decreased denitrification potential, and increased bulk density in historical compared to forested wetlands as previous work has shown that cultivation within wetlands decreases SOM (Richter et al. 1999, Compton and Boone 2000), alters native microbial communities (Young and Ritz 2000), and increases soil compaction and

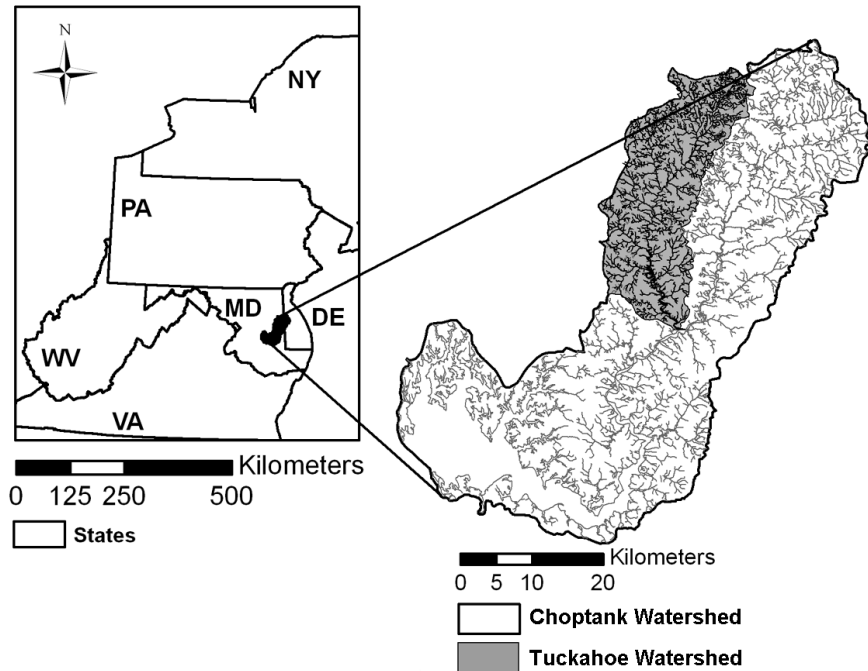
erosion (Martin and Hartman 1987, Braekke 1999). Due to a history of cultivation, I predicted restored wetlands would have greater sediment and nutrient accumulation rates compared to forested wetlands, and that soils in restored wetlands would be in a transition between forested and cultivated end-members.

## **METHODS**

### **Site Description**

This study was conducted in Delmarva bay freshwater depressional wetlands in the headwaters of the Tuckahoe Creek watershed (Maryland, USA), a sub-basin of the Choptank River watershed (Fig. 1.1). Located on the Delmarva Peninsula, the Choptank watershed is entirely situated in the Coastal Plain physiographic province. Topographic relief is low, and elliptical depressional wetlands known as Delmarva bays are plentiful (Tiner and Burke 1995). Fenstermacher (2012) estimated there to be some 17,000 Delmarva bays on the Peninsula, of which 65% have been impacted by agriculture. Nearly 65% of the Choptank watershed is in agricultural use, and nutrient and sediment loading to the river network and Chesapeake Bay are high (Fisher et al. 2006).





**Figure 1.1:** Choptank River and Tuckahoe Creek watersheds and stream networks. Modified from Lang et al. (2012).

Four forested, 3 historical, and 3 hydrologically restored Delmarva bay wetlands were selected for this study. Each wetland was considered a representative ecosystem within its type. Forested wetlands are characterized by an alternating hydrology, acting as discharge wetlands in winter and spring and recharge basins in summer and fall (Phillips and Shedlock 1993). Surface water is typically present within forested Delmarva bays from late fall through late spring. Forested sites are characterized by closed canopy palustrine forest (Cowardin et al. 1979). Dominant tree species include *Acer rubrum*, *Liquidambar styraciflua*, *Ilex opaca*, *Quercus rubra*, and *Prunus serotina*, and the understory is dominated by *Smilax rotundifolia*, *Lindera benzoin*, and *Leucothoe racemosa*.

Historical wetlands have been drained via ditches to enhance crop production and are characterized by brief periods of standing water following rainfall events. Vegetation within historical wetlands rotates annually between corn (*Zea mays*) and soybean (*Glycine max*). Prior to drainage and cultivation, historical wetlands were forested bays (Whigham et al. 2002).

Restored wetlands were removed from agricultural use 7-8 years prior to this study, and restoration efforts involved plugging drainage ditches with earthen mounds to reestablish a more natural hydroperiod. Restored Delmarva bays typically exhibit standing water from late fall to late spring. Dominant restored wetland vegetation consists primarily of emergent and floating plants including *Typha latifolia*, *Phragmites australis*, *Schoenoplectus americanus*, *Carex* sp., *Scirpus cyperinus*, and *Lemna minor*. Mature forested upland canopy has not yet developed at any of the restorations. Restored wetlands were once forested Delmarva bays located in topographic lows (Whigham et al. 2002).

### **Soil Sampling**

Soils were collected in June 2010 using a 2.85 cm ID soil probe in triplicate cores at 0-10 cm depth within each wetland. Triplicate cores were collected randomly within each wetland. Soils were placed in re-sealable plastic bags, kept on ice in the dark, and returned to the laboratory where they were refrigerated at 4°C until analyzed.

## Soil Analysis

Soils were analyzed for moisture content, bulk density, soil organic matter (SOM) content, total carbon, total nitrogen, and soil nitrate. Prior to analyses, soil samples were homogenized and any rocks and roots were removed. A subsample from each core was weighed and oven-dried at 70°C to determine moisture content and establish a constant dry weight basis. Bulk density was calculated as dry mass per unit volume of soil collected ( $\text{g cm}^{-3}$ ). A subsample of the oven-dried soil was ground using mortar and pestle and analyzed for total carbon, total nitrogen, and SOM. Soil total carbon and total nitrogen (presented as C:N) were analyzed by dry combustion using a CHNSO elemental analyzer (ECS 4010 CHNSO Analyzer, Costech Analytical Technologies, Valencia, CA). SOM was determined as mass loss-on-ignition at 450°C for 16 h. Soil nitrate was determined on subsamples of field moist soil using 2M KCl extraction followed by filtration through 0.7  $\mu\text{m}$  glass fiber filter paper (Whatman International Ltd, Maidstone, England). Extracts were analyzed via automated cadmium reduction on a QuikChem 8500 Series 2 flow injection analyzer (method # 12-107-04-1-B, Lachat Instruments / Hach Company, Loveland, CO).

Soils were analyzed for denitrification potential using the denitrification enzyme activity (DEA) assay method described by Groffman et al. (1999). DEA is a short-term assay to determine denitrification potential and extant denitrifying enzymes given unlimited organic carbon and nitrate substrate. While DEA does not directly measure actual denitrification, it is useful for site comparisons as it offers a method by which

nitrate reduction potential can be compared across different soil types (Hunter and Faulkner 2001).

For the DEA assay, 10 g of well-mixed, field-moist soil was measured into 125 mL Erlenmeyer flasks with tapered ground-glass necks. Flasks were fitted with rubber septa stoppers to allow for gas-tight seals and headspace gas sample collection via syringe. Soils used for the DEA assay were amended with a solution of glucose and  $\text{KNO}_3$  to ensure non-limiting substrate conditions and chloramphenicol to inhibit microbial reproduction (final slurry concentrations:  $100 \text{ mg kg}^{-1} \text{ N}$ ,  $200 \text{ mg kg}^{-1}$  glucose,  $125 \text{ mg kg}^{-1}$  chloramphenicol). Soil slurries were made anaerobic via repeated evacuation and  $\text{N}_2$  gas flushing. Flasks were injected with 5 mL acetylene gas ( $\text{C}_2\text{H}_2$ ) to inhibit reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  and shaken at 100 rpm for 90 min on an orbital shaker. At 90 min, 9 mL headspace gas samples were taken from each flask via syringe and transferred to pre-evacuated 9 mL septa sealed glass vials.  $\text{N}_2\text{O}$  concentrations were determined using a Shimadzu GC-14B gas chromatograph outfitted with an electron capture detector and Porapak Q column (Shimadzu Scientific Instruments, Columbia, MD). Soil  $\text{N}_2\text{O}$  flux was calculated as the rate of  $\text{N}_2\text{O}$  increase in the headspace of the incubation flask during the incubation period. Headspace measurement of total  $\text{N}_2\text{O}$  production was corrected for  $\text{N}_2\text{O}$  dissolved in slurry water using the Bunsen equation (Groffman et al. 1999) at the incubation temperature. Denitrification potential was calculated as the hourly rate of  $\text{N}_2\text{O-N}$  production per dry mass of soil and is indicative of the biomass of the denitrifying enzyme pool present in each soil sample.

## **Sediment and Nutrient Accumulation**

Feldspar clay pads were deployed in the forested and restored Delmarva bays to measure rates of net vertical accretion and net sediment, carbon, nitrogen, and phosphorus accumulation. Pads were not deployed in the historical wetlands as they would have been destroyed by tilling. Feldspar creates a bright white marker horizon easily distinguished from material deposited on top of it and may be used to inexpensively measure accumulation rates of matter in a variety of settings (Cahoon and Turner 1989). In June 2010 (time-zero), 10 circular feldspar pads measuring 25 cm in diameter and 0.5 cm thick were randomly positioned in each wetland. Net vertical accretion atop each pad was measured in June 2011. The accumulated material on each pad was collected using thin-walled aluminum cylinders as coring devices (65 mm ID) and returned to the laboratory to be processed for total dry mass, SOM, mineral fraction, total C, and total N as described above. Total phosphorus content of dried soils was measured via hot nitric acid digestion (EPA Method 3050B) followed by colorimetric determination using a molybdate reagent in the presence of ascorbic acid (Kuo 1996). Net vertical accretion rates are presented as  $\text{mm y}^{-1}$ . Net accumulation rates for total sediment, mineral sediment, C, N, and P are reported on a  $\text{g m}^{-2} \text{y}^{-1}$  basis.

## **Statistical Analysis**

Differences in wetland soil metrics among the three wetland types were assessed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) multiple mean comparisons tests. Feldspar pad accumulation results were compared between forested and restored wetlands using two-tailed Student's t-tests.

Pearson's product-moment correlation analyses were performed to determine relationships between soil properties and denitrification potential. When necessary, data were log-transformed to meet the assumption of normality. All univariate statistical analyses were conducted using R version 2.12.2 (R Development Core Team, Vienna, Austria) with differences deemed significant at  $\alpha = 0.05$ .

To gain an understanding of the variability in soil properties across wetland types, I ran principal component analysis (PCA). A PCA ordination diagram was plotted to visualize how wetland types cluster with axes generated using the suite of soil metrics. PCA analyses were conducted using the Vegan package in R version 2.12.2 (R Development Core Team, Vienna, Austria).

## **RESULTS**

### **Wetland Soil Physicochemical Properties and Denitrification Potential**

Significant differences existed among wetland types with respect to all soil physicochemical properties (Fig. 1.2). Forested Delmarva bay soils were characterized by high moisture content, high SOM, high C:N, low bulk density, and low soil nitrate relative to historical and restored wetland soils, which had low moisture content, SOM, and C:N, and high bulk density and soil nitrate (Fig. 1.2). Multiple mean comparisons revealed significantly greater moisture content, SOM, and C:N, and significantly lower bulk density in forested compared to historical and restored wetland soils (Fig. 1.2). SOM content was 10-fold greater in forested compared to historical wetland soils and 8-fold

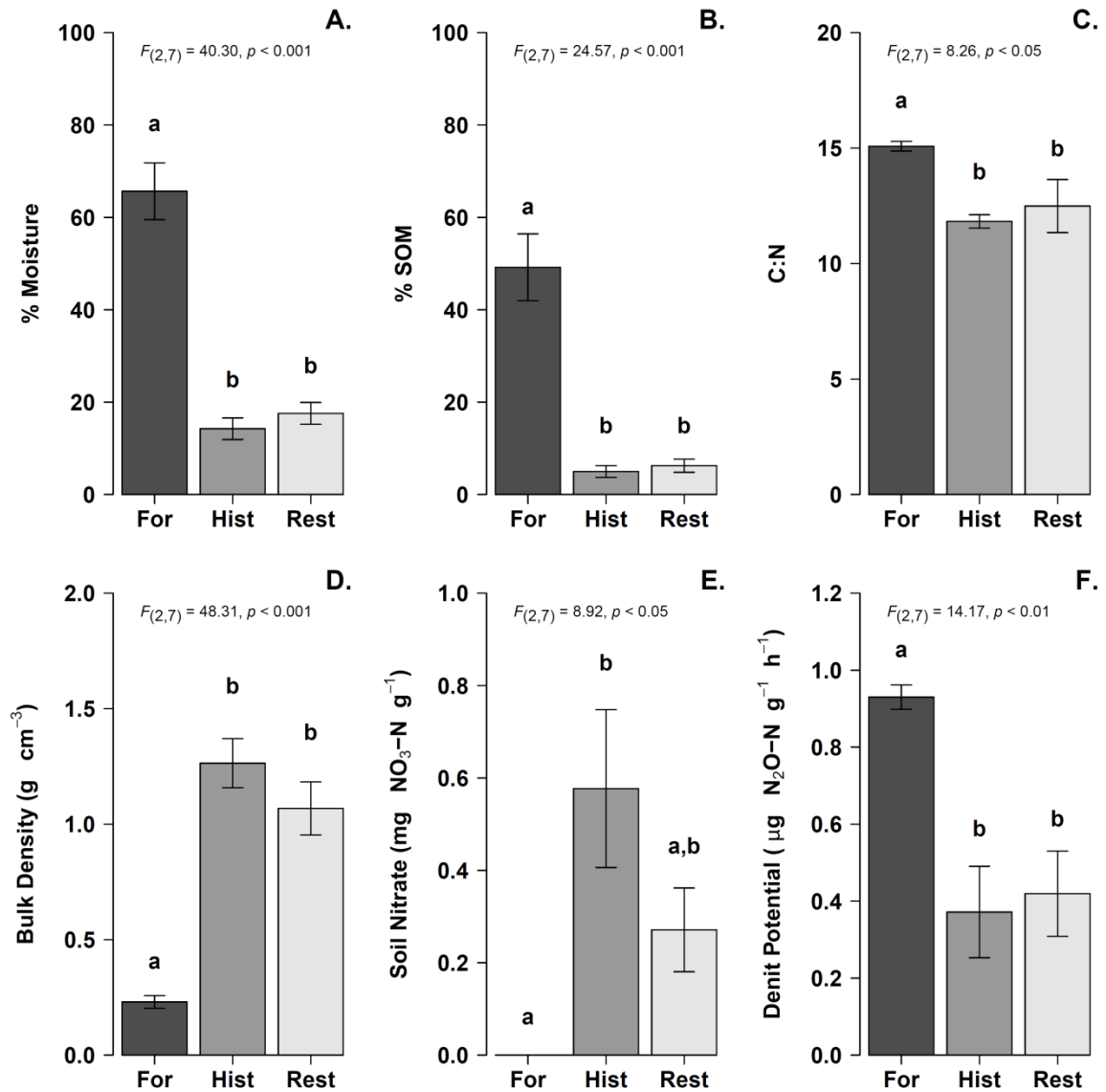
greater compared to restored soils (Fig. 1.2B). Conversely, bulk density was approximately 5 times lower in forested relative to both historical and restored wetland soils (Fig. 1.2D). Soil nitrate was significantly greater in historical relative to forested wetlands (Fig. 1.2E). Soil properties did not differ significantly between restored and historical wetlands with respect to any analyzed metrics (Fig. 1.2A-E).

Highest denitrification potential rates were measured in forested wetland soils with a mean ( $\pm$  SE) of  $0.93 \pm 0.32 \mu\text{g N}_2\text{O-N g}^{-1} \text{ h}^{-1}$ . This rate was 150% greater than rates measured in historical wetlands and nearly 125% greater than those in restored wetlands (Fig. 1.2F). Denitrification potential did not differ significantly between historical and restored wetlands (Fig. 1.2F).

Together, the first two components of the PCA incorporating soil physicochemical properties and denitrification potential explained 95% of the variance between wetlands (Fig. 1.3). Forested wetlands had positive scores on component 1 and grouped together closely, while historical and restored wetlands had negative scores and exhibited greater variability (Fig. 1.3).

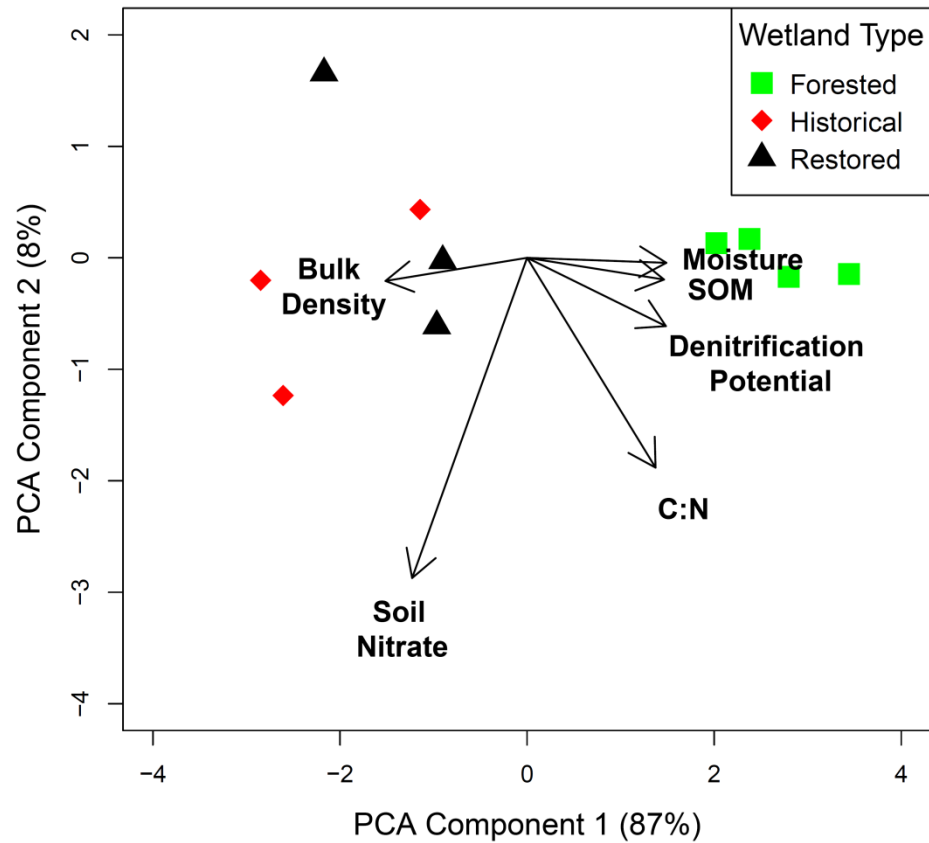
### **Correlations between Soil Physicochemical Properties and Denitrification Potential**

Across all wetland types, moisture content and SOM were positively correlated with denitrification potential ( $r = 0.67, p < 0.001$ ;  $r = 0.70, p < 0.001$ , respectively). Bulk density and denitrification potential were negatively correlated ( $r = -0.65, p < 0.001$ ). No relationships existed between C:N or soil nitrate and denitrification potential.



**Figure 1.2:** Wetland soil properties by wetland type (forested,  $n = 4$ ; historical,  $n = 3$ ; restored,  $n = 3$ ). **A)** Moisture content, **B)** soil organic matter (SOM) content, **C)** C:N, **D)** bulk density, **E)** soil nitrate, and **F)** denitrification potential. Error bars represent standard errors of the means. Means with different letters are significantly different from one another (Tukey's HSD,  $p < 0.05$ ).

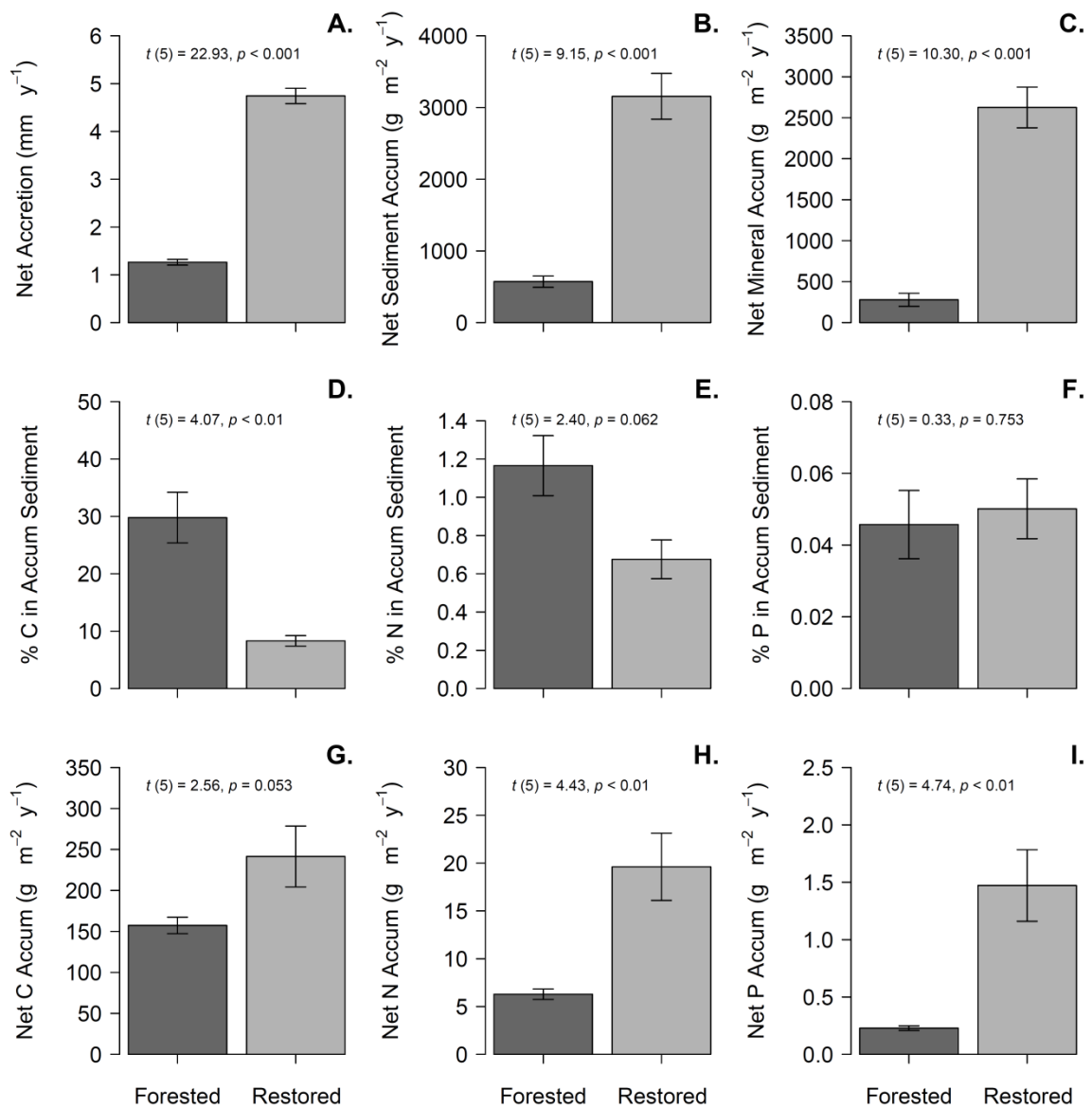




**Figure 1.3:** Principal component analysis (PCA) ordination diagram grouping forested, historical, and restored wetland soils with respect to physicochemical properties and denitrification potential. Percent values on each axis represent percent of explained variance.

## **Sediment and Nutrient Accumulation**

I recorded significant net vertical accretion and net sediment, mineral, C, N, and total P accumulation in both forested and restored Delmarva bays over the course of one year (Fig. 1.4). Net vertical accretion, sediment accumulation, mineral accumulation, N accumulation, and total P accumulation rates were all significantly greater in restored Delmarva bays compared to forested reference wetlands (Fig. 1.4). Nitrogen content within accumulated sediment tended to be lower (Fig. 1.4E) while C accumulation tended to be higher (Fig. 1.4G) in restored compared to forested Delmarva bays. Annual net vertical accretion measured  $4.7 \pm 0.2$  mm (mean,  $\pm$  SE) in restored wetlands — nearly 4 times the value in forested bays ( $1.3 \pm 0.1$  mm [mean,  $\pm$  SE]; Fig. 1.4A). Net sediment accumulation was more than 5 times greater in restored relative to forested Delmarva bays, averaging  $> 3,100$  g m<sup>-2</sup> y<sup>-1</sup> (Fig. 1.4B). Although C and N content in the accumulated sediment were lower in restored compared to forested wetlands (Fig. 1.4D,E), the substantial amount of net sediment accumulated in restored sites resulted in 53% greater C accumulation and 212% greater N accumulation relative to forested basins (Fig. 1.4G,H). While total P content in accumulated sediment did not differ between forested and restored sites (Fig. 1.4F), total P accumulation was  $> 500\%$  more in restored compared to forested wetlands due to greater sediment accumulation in restored sites (Fig. 1.4I).



**Figure 1.4:** Feldspar pad results for a 1-year deployment (June 2010 – June 2011) in forested ( $n = 4$ ) and restored ( $n = 3$ ) Delmarva bay wetlands. **A)** Net vertical accretion, **B)** net sediment accumulation, **C)** net mineral accumulation, **D)** C content in accumulated sediment, **E)** N content in accumulated sediment, **F)** total P content in accumulated sediment, **G)** net C accumulation, **H)** net N accumulation, and **I)** net total P accumulation. Error bars represent standard errors of the means.

## **DISCUSSION**

This research demonstrates that soil physicochemical properties and denitrification potential in hydrologically restored Delmarva bay wetlands are significantly different from forested wetland soils shortly following restoration (i.e., 7 to 8 years post restoration). Additionally, recently restored wetland soils remain structurally and functionally similar to historical wetland soils. While reestablishing wetland hydrology is critical to ecosystem restoration (Mitsch and Gosselink 2000), it may not be sufficient for rapid recovery of key wetland soil properties and processes (Bruland et al. 2003). This may be particularly true in once-farmed wetlands due to physical and chemical soil alteration caused by cultivation (Zedler 2003a).

### **Soil Restoration Effectiveness in Once-Cultivated Wetlands**

Results from this study corroborate previous work examining the impacts of agricultural wetland restoration on soil properties in morphologically similar settings (e.g., coastal plain bays), which have generally found comparable soil properties in historical and restored wetlands as unique from reference wetland soils (Bruland et al. 2003, Fenstermacher 2012). In particular, restored agricultural wetland soils often have lower SOM and higher bulk density relative to reference soils (Galatowitsch and van der Valk 1996, Bruland et al. 2003, Fenstermacher 2012). This has been shown even when restoration of wetland hydrology was deemed successful (Bruland et al. 2003), and has been attributed to intense alteration of the restored wetland soil while it had been actively drained and cultivated (Galatowitsch and van der Valk 1996, Bruland et al. 2003). Drainage and cultivation result in decreased SOM due to increased oxidation and

decomposition rates (Richter et al. 1999, Compton and Boone 2000) and increased bulk density due to tillage and soil compaction (Braekke 1999, Fenstermacher 2012). Moreover, it has been suggested that SOM remaining after wetland drainage and cultivation may largely be bound to clay and have limited bioavailability (Richardson and Bigler 1982). Zedler (2003a) stresses that restoring agricultural wetland soils to some historical or reference condition is particularly difficult where decades of cultivation may have fundamentally altered soil physicochemical properties. A history of cultivation combined with a relatively short period of time since restoration are likely the reason I found little difference between historical and restored wetland soils in this study.

It is important to note that SOM accumulation in restored and created wetlands is often slow (Hossler and Bouchard 2010) and may not increase as rapidly as expected (Bishel-Machung et al. 1996, Shaffer and Ernst 1999, Bruland et al. 2009). I found SOM content in historical and restored wetland to be only 10% and 12%, respectively, of that in forested wetlands. Although I did not measure SOM change over time, the fact that I observed no significant differences in SOM between historical wetlands and sites restored 7 to 8 years previously suggests restoration of natural SOM levels will take substantial time. Corroborating this, Fenstermacher (2012) estimated it would take restored Delmarva bay wetlands approximately 70 years to achieve the level of carbon in natural wetland soils. Others have estimated that restoring soil carbon to natural levels takes 30-500 years or longer, depending on the ecosystem (Jastrow 1996, Zedler and Callaway 1999, Wigginton et al. 2000, Craft et al. 2002, Edwards and Proffitt 2003, Hossler and Bouchard 2010). However, wetland soils may not be on a trajectory toward a

reference condition post-restoration (Zedler and Callaway 1999), but may instead be approaching alternative stable states (Moreno-Mateos et al. 2012).

Denitrification in nitrate-rich agricultural landscapes is of particular interest as a means to mitigate excess nutrient loading (Howarth et al. 1996). While restored and created wetlands may be effective nitrogen sinks (Lowrance et al. 1995), results from this study suggest wetland cultivation decreases soil denitrification potential relative to reference conditions and that the capacity to denitrify has not fully recovered in recently restored Delmarva bays. This finding corroborates those of others among a variety of restored wetland types (Hunter and Faulkner 2001, Bruland et al. 2006, Peralta et al. 2010). Across many ecosystems, research has shown that denitrification is limited primarily by organic carbon availability (Ingersoll and Baker 1998, Dolda et al. 2008, Sutton-Grier et al. 2010). Organic matter fuels heterotrophic respiration and oxygen consumption, both of which promote denitrification (Reddy and Patrick 1984). This suggests the low SOM content I measured in recently restored Delmarva bays ( $6.3 \pm 1.4\%$ ; mean,  $\pm$  SE) may in part be responsible for limited denitrifying enzyme activity therein.

Denitrification potential may also be lower in restored relative to reference wetland soils due in part to differences in the microbial communities themselves (Ronn et al. 1996, Cavigelli and Robertson 2000). Denitrification rates have been shown to correlate positively with soil microbial diversity (Griffiths et al. 2000). Agricultural disturbance can decrease microbial heterogeneity and, in turn, the ability of microbial communities to process nitrogen (Young and Ritz 2000). Peralta et al. (2010) found

significantly different bacterial community structure between restored and reference wetlands and higher denitrification potential in reference relative to restored sites, suggesting differences in denitrification potential may be due to differences in microbial assemblage. Poor microbial community development in newly restored or created soils is thought to be due in part to low SOM pools (Duncan and Groffman 1994).

I note that denitrification potential rates reported here were only measured once due to both the intensive nature of soil sampling and laboratory processing. The DEA assay provides a moment-in-time snapshot of soils at the time of sampling, and the patterns observed in Summer 2010 may not be consistent seasonally or inter-annually. Yet, DEA assay results have been shown to be highly correlated with annual soil denitrification rates (Groffman and Tiedje 1989). Moreover, the soil properties that correlated with denitrification potential, particularly SOM and bulk density, are unlikely to undergo rapid changes (Hossler and Bouchard 2010), and these patterns are expected to hold for some time.

Additionally, it is important to note that the DEA assay used in this study provided unlimited fuel (i.e., glucose) and substrate (i.e.,  $\text{KNO}_3$ ) to denitrifiers, and thus reflects *potential* denitrification. Actual denitrification may be greater in nitrate-rich historical and restored wetland soils relative to nitrate-poor forested wetland soils (see Fig. 1.2E). Moreover, forested Delmarva bays are often positioned along the watershed divide and thus likely to experience lower fluxes of water and nutrients relative to wetlands more distant from the watershed boundary. To better prioritize

restoration efforts to mitigate downstream eutrophication, future research should address *in situ* denitrification and the role of wetland landscape position on nitrate reduction.

### **Sediment and Nutrient Accumulation**

The high net vertical accretion and sediment accumulation rates observed in restored Delmarva bays compared to reference forested wetlands and various depressional wetland types (Table 1.1) are likely the result of continued upland erosion post-cultivation. Cropland tillage has been shown to accelerate sedimentation in depressional wetlands (Martin and Hartman 1987). Additionally, depressional wetlands are the terminus of closed basins and thereby subject to potentially large accumulation of runoff-transported sediments (Skagen et al. 2008).

Sediment accumulation from upland erosion may decrease wetland volume and hydrologic storage capacity and negatively impact plant community structure by burying seed banks and hindering germination (Luo et al. 1997). I observed a mean net vertical accretion rate of  $4.7 \text{ mm y}^{-1}$  in restored Delmarva bays, and note that sediment burial depths of 5 mm have been shown to cause marked reductions in seedling and invertebrate emergence within wetlands (Gleason et al. 2003). Alternatively, high accumulation rates in restored wetlands may be mitigating downstream sediment and nutrient delivery. Whether restored Delmarva bays are acting as sediment and nutrient sources or sinks, however, would require an investigation incorporating mass balances and represents a potential focus for future study.



**Table 1.1:** Comparison of net vertical accretion ( $\text{mm y}^{-1}$ ) and net sediment and nutrient (C, N, P) accumulation ( $\text{g m}^{-2} \text{y}^{-1}$ ) in freshwater depressional wetland soils.

Location	Wetland Type	Vertical Accretion	Sediment	Organic C	N	P
Florida <sup>1</sup>	Cypress Dome	—	—	—	0.8 - 2.8	0.01
Georgia <sup>2</sup>	Cypress Depressions	0 - 0.9	120 - 950	21 - 70	1.5 - 5.3	0.08 - 0.25
Maryland	Delmarva Bays (forested)	1.1 - 1.4	436 - 788	143 - 186	4.8 - 7.5	0.19 - 0.29
(this study)	Delmarva Bays (restored)	4.4 - 5.0	2525 - 3545	177 - 306	14.2 - 26.2	0.94 - 2.02
North Dakota <sup>3</sup>	Prairie Potholes	—	500 - 3600	—	—	0.01
Texas <sup>4</sup>	Playas (prior-converted cropland)	4.8 - 9.7	—	—	—	—

<sup>1</sup> Dierberg and Brezonik (1983) (\*accumulation in soil plus roots)

<sup>2</sup> Craft and Casey (2000)

<sup>3</sup> Freeland et al. (1999)

<sup>4</sup> Luo et al. (1997)

Although the mineral fraction of accumulated material within restored wetlands was nearly double that in forested wetlands (82.1% and 45.2%, respectively), net mean annual C accumulation was greater in restored compared to forested sites due to the substantial total mass of accumulated material in the restored basins (Fig. 1.4). These results suggest rapid C accumulation in restored sites is due to the erosion and redistribution of low organic upland soils to the wetland basins. The redistribution of low organic C soils into wetlands may dilute soil C content and stimulate C sequestration as soil C levels re-equilibrate (McCarty and Ritchie 2002). Additionally, upland erosion followed by wetland sedimentation may promote C sequestration by providing physical substrate (e.g., mineral surface area) for organic C stabilization (McCarty et al. 2009). These findings suggest the possibility of increased C sequestration within restored Delmarva bays over time as low organic C upland soils are delivered to wetland basins and transition to more C-rich hydric soils.

## **CONCLUSION**

Wetland restoration has become a common practice in agricultural landscapes throughout the U.S. both in response to a Federal “no net loss” policy aiming to prevent and offset the destruction or degradation of wetlands and to enhance desirable ecosystem services (Mitsch and Gosselink 2000). A more natural hydrology may be quickly reestablished in wetlands removed from cultivation via ditch-plugging or subsurface drain removal (Bruland et al. 2003), yet this research adds to the growing body of evidence that restoration of wetland soils is often more difficult (Bruland et al. 2003,

Zedler 2003a, Hossler and Bouchard 2010). While I acknowledge that the restorations investigated here are still relatively young (7 to 8 years post-restoration at the time of study), soil properties conducive to denitrification (e.g., high SOM content, low bulk density) may have been so significantly altered via previous cultivation practices that it may take decades to centuries until they compare favorably to reference wetland soils (Hossler and Bouchard 2010). To promote natural wetland soil development, I suggest efforts be made to enhance SOM accumulation, decrease bulk density, and increase water filled pore space. Supplemental addition of organic C and avoiding additional compaction during the restoration process may help to jump-start soil development and nitrogen processing (Bruland et al. 2009, Sutton-Grier et al. 2009).

Rapid sediment and nutrient accumulation is occurring in restored Delmarva bays, likely the result of continued upland erosion into the basins post-cultivation. Sedimentation may be detrimental to both wetland hydrology and biota (Luo et al. 1997, Gleason et al. 2003). Increased efforts could be employed to minimize upland runoff and subsequent basin sedimentation in restored wetlands (e.g., increased vegetative ground-cover, topsoil replacement). Alternatively, accumulation of sediments and nutrients within restored Delmarva bays may indicate reduced loading to downstream waters and ultimately Chesapeake Bay.

## **Chapter 2: The impact of agricultural wetland restoration on surface hydrologic connectivity between depressional wetlands and adjacent streams**

### **INTRODUCTION**

It is estimated that more than 50% of wetlands in the United States have been lost since European settlement (Dahl 1990), with substantial loss attributed to depressional wetland drainage for agriculture (Mitsch and Gosselink 2000). To promote wetland ecosystem services and mitigate loss, wetland restoration has become common in cultivated landscapes and typically aims to reestablish reference wetland hydrology, a master variable with respect to wetland ecological structure and function (Mitsch and Gosselink 2000).

In an undisturbed state, depressional wetlands are characterized by hydric soils and a bowl-like structure often surrounded by an upland rim that reduces surface outflow (Mitsch and Gosselink 2000, Tiner 2003, McCauley and Jenkins 2005). North American examples include prairie potholes, playas, vernal pools, sinkhole wetlands, interdunal and intradunal wetlands, desert springs, bogs, fens, and Carolina and Delmarva bays. These wetlands may be continuously connected to nearby streams by surface flow or geographically isolated, that is, lacking perennial surface hydrologic connectivity (hereafter, SHC) to other water bodies (Tiner 2003).

Referring to depressional wetlands as isolated, however, may be inappropriate as they can be connected to other surface waters via groundwater flowpaths, episodic basin

spillage and overland flow, or temporary stream flow (Tiner 2003, Winter and LaBaugh 2003, Wilcox et al. 2011). Furthermore, there may be ecologically significant exchanges of material and energy through these flowpaths (Gibbons 2003, Leibowitz et al. 2008). Geographically isolated wetlands may export carbon and nutrients to adjacent waters (Nessel and Bayley 1984, Wise et al. 2000) and may enable dispersal of aquatic flora (Galatowitsch and van der Valk 1996) and fauna (Babbitt and Tanner 2000). So-called isolated wetlands may at times sustain baseflow in nearby perennial headwater streams (Richardson 2003, Sharitz 2003, Zedler 2003b) and provide valuable water quality benefits to receiving waters (Whigham and Jordan 2003).

While subsurface connections between geographically isolated wetlands and other waters have been reported elsewhere (Winter and LaBaugh 2003, Rains et al. 2006, Min et al. 2010), surface connections have rarely been quantified (but see Martin 2011, Wilcox et al. 2011). Although understanding of geographically isolated wetland–stream connectivity is generally limited, interest in the hydrological and ecological links between isolated wetlands and adjacent stream networks is growing (Leibowitz and Nadeau 2003, Leibowitz et al. 2008, Wilcox et al. 2011), in part sparked by recent U.S. Supreme Court cases creating new legal standards for determining the regulatory status of wetlands and non-perennial streams under Section 404 of the Clean Water Act (Leibowitz et al. 2008). Currently, SHC between wetlands and non-perennial streams and adjacent navigable waters represents a key criterion for determining jurisdictional waters (Leibowitz et al. 2008). Moreover, without knowledge of the natural hydrologic connections between geographically isolated wetlands and nearby waters, we cannot assess changes likely to

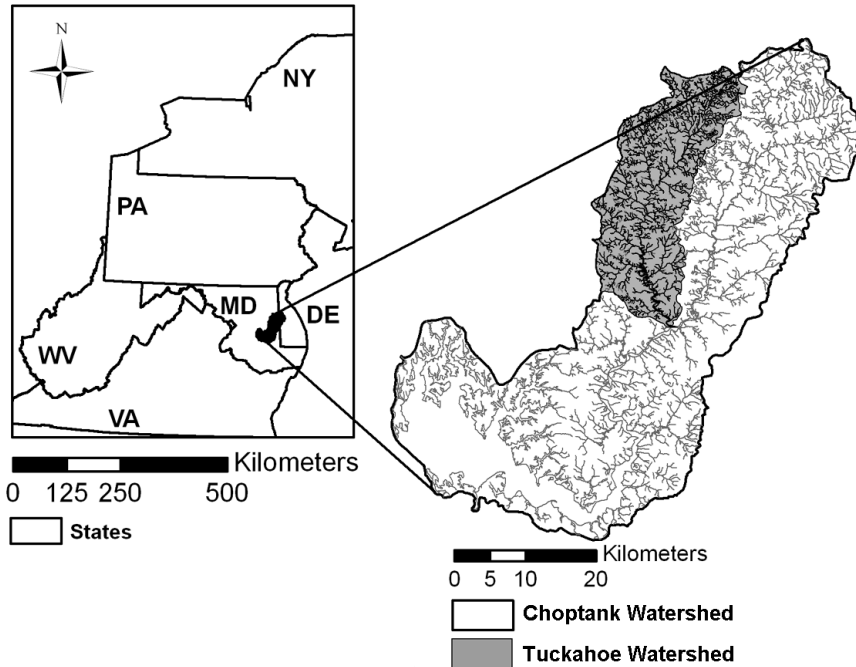
result from anthropogenic perturbations (e.g., land use change, climate change) or subsequent attempts at restoration.

I quantified SHC patterns between wetlands historically considered to be geographically isolated and adjacent perennial streams on the Delmarva Peninsula (Maryland, USA). From a restoration perspective, I asked how hydrologic wetland restoration impacts SHC relative to historical wetlands (i.e., wetlands now in cropland, aka *prior-converted cropland*) and native forested wetlands. This is the first study I am aware of quantifying SHC between multiple so-called isolated wetlands and nearby streams as well as the role of restoration with respect to connectivity.

## **METHODS**

### **Site Descriptions**

This study was conducted in Coastal Plain temporary streams connecting depressional wetlands (i.e., Delmarva bays) and wetland flats to adjacent perennial streams in the headwaters of the Tuckahoe Creek watershed, a sub-basin within the Choptank River watershed (Fig. 2.1). Nearly 65% of the Choptank catchment is in agricultural use, with smaller amounts of forest (26%) and urban (6%) land cover (Fisher et al. 2006). The region is characterized by a humid, temperate climate with average annual precipitation of 120 cm (Ator et al. 2005).

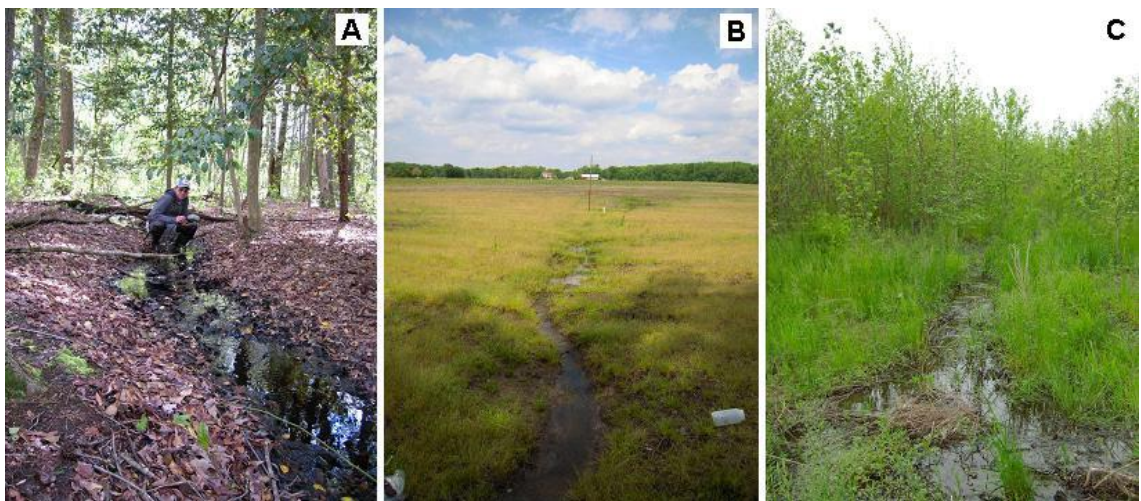


**Figure 2.1:** Choptank River and Tuckahoe Creek watersheds and stream networks. Adapted from Lang et al. (2012)

Delmarva bay wetlands are generally elliptical and characterized by a sandy upland rim (Tiner and Burke 1995). They have historically been considered geographically isolated (Tiner 2003). In their native forested state, Delmarva bays typically serve as discharge areas from late autumn through late spring when evapotranspiration is low and recharge basins during summer months when evapotranspiration is high (Phillips and Shedlock 1993). Although often small in size (< 1 ha; Phillips and Shedlock 1993), they are ubiquitous on the Delmarva Peninsula, numbering some 17,000 basins (Fenstermacher 2012). It is estimated that the bays originated between 16,000 and 21,000 years ago as saturated spots in interdunal areas or wind blowouts in sand barrens (Stolt and Rabenhorst 1987).

Due in part to agricultural ditching, Delmarva bays are among the most threatened ecosystems on the Peninsula (McAvoy and Bowman 2002) where it is estimated that 65% of all bays have been impacted by agriculture (Fenstermacher 2012). Historical wetlands are drained via ditches, cultivated, and exhibit brief periods of standing water following rainfall events. In response to agricultural losses, hydrological restoration of Delmarva bays has become common in recent decades. Restored wetlands are removed from active agricultural use and drainage ditches are plugged with earthen mounds in efforts to reestablish a natural Delmarva bay hydroperiod.

Four forested wetland complexes consisting of Delmarva bays and wetland flats (F1-F4), 4 historical wetlands (H1-H4), and 3 hydrologically restored wetlands (R1-R3) were selected for this study. Wetlands R1, R2, and R3 were restored in 2002, 2002, and 2003, respectively. Each site is considered a representative ecosystem within its wetland type. A temporary stream channel is adjacent to each study wetland through which the wetland episodically outflows to a nearby perennial stream (Fig. 2.2).

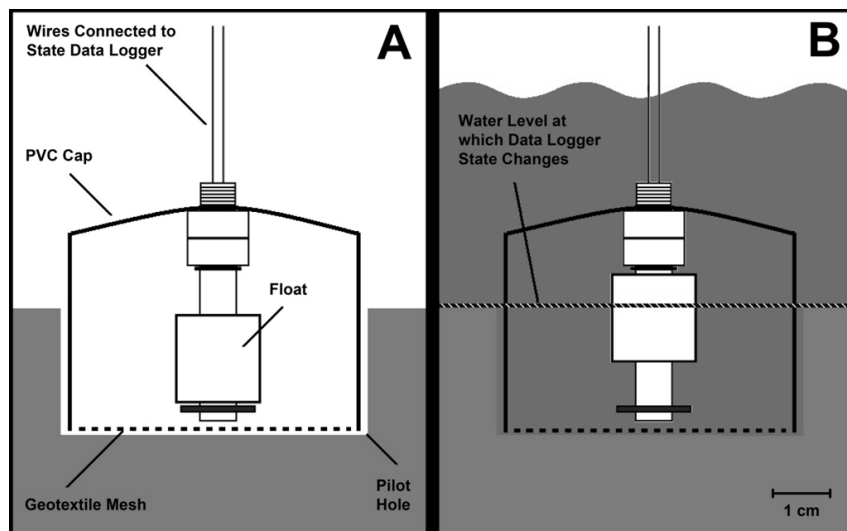


**Figure 2.2:** Examples of temporary streams connecting forested (A), historical [i.e., prior-converted cropland] (B), and restored (C) Delmarva bay wetlands to adjacent perennial streams via surface flow.



## Monitoring Surface Hydrologic Connectivity and Climate

During the 2010 water year (1 Oct 2009 – 30 Sept 2010) a state data logging method was used to monitor patterns of SHC at the 11 study sites. A binary polypropylene float switch (SMD Fluid Switch, Wallingford, CT) was positioned on the bed of the temporary stream connecting the wetland and adjacent perennial stream. The float switch closed a circuit when surface water was present and opened the circuit when surface water was absent (Fig. 2.3). A state data logger (HOBO model U9-001; Onset Computer Corp., Bourne, MA) connected to the float switch recorded the timing and duration of SHC as state changes in the circuit (i.e., surface water presence or absence as binary events).



**Figure 2.3:** Schematic of buoyant polypropylene float switch. The float switch state is open when water is absent (A) and closed when water is present (B). An associated state data logger records the timing of state changes.

Float switches were placed in the thalweg (i.e., center of flow) of the temporary streams and within 5 m of the temporary-perennial stream confluence. I avoided placing float switches in local pools where standing water could falsely indicate the presence of surface flow. State data loggers and float switches were visited monthly to offload data and ensure proper functionality. SHC results were compared to and plotted with local rainfall data collected using a HOBO weather station (Onset Computer Corp., Bourne, MA) located within 10 km of all study sites.

### **Watershed and Wetland Physical Attributes**

For each of the study wetland–stream pairs, I calculated watershed drainage area and wetland area using 1 m resolution light detection and ranging (LiDAR) derived digital elevation models (Lang et al. 2012) and ArcGIS software (Environmental Systems Research Institute, Redlands, CA) (Table 2.1). Wetland area was calculated using the threshold of a relief-enhanced topographic wetness index with areas in the wettest 4 classes considered wetlands (Lang et al., *in press*).

### **Statistical Analyses**

Using data logger records (Fig. 2.4), I quantified the cumulative duration, total number, mean duration, and maximum individual event duration of surface flow events in each temporary stream. Maximum individual event duration was defined as the longest duration in days of continuous surface connectivity ( $D_{max-c}$ ; Leibowitz et al. 2008). Correlations between wetland area and SHC metrics were tested using Pearson's product moment correlation coefficient,  $r$ , with wetland area log-transformed to meet assumptions

of normality. I tested the null hypothesis that the four metrics did not differ between the three wetland types using one-way analysis of covariance (ANCOVA) with wetland area as a covariate followed by Tukey's honestly significant difference (HSD) multiple mean comparisons test. For each wetland, I used Student's t-tests to assess differences in mean daily rainfall between days when a temporary stream connection did and did not occur between the wetland and adjacent perennial stream. All statistical analyses were conducted using R version 2.12.2 (R Development Core Team, Vienna, Austria) with a significance level set at  $\alpha = 0.05$ .

**Table 2.1:** Watershed and wetland physical attributes.

<b>Site</b>	<b>Wetland Type (year of restoration)</b>	<b>Location</b>	<b>Wetland Area (m<sup>2</sup>)</b>	<b>Watershed Drainage Area (m<sup>2</sup>)</b>
F1	Forested	39° 3'24.52"N, 75°49'30.93"W	335052	567304
F2	Forested	39° 3'28.34"N, 75°49'53.05"W	25166	36291
F3	Forested	39° 3'16.50"N, 75°50'4.77"W	17208	43400
F4	Forested	39° 3'25.09"N, 75°49'57.97"W	127853	324890
R1	Restored (2002)	39° 4'8.31"N, 75°45'31.99"W	9334	42080
R2	Restored (2002)	38°57'55.68"N, 75°57'55.74"W	67145	245387
R3	Restored (2003)	39° 2'54.14"N, 75°45'19.24"W	1433	13317
H1	Historical	39° 3'31.96"N, 75°49'44.61"W	8060	18652
H2	Historical	39° 3'35.88"N, 75°49'47.63"W	2571	14620
H3	Historical	39° 3'34.89"N, 75°51'4.21"W	3955	11348
H4	Historical	39° 3'39.73"N, 75°50'56.85"W	10276	18101

## RESULTS

Precipitation during the 2010 water year totaled 102.2 cm, slightly below the regional average of 120 cm  $y^{-1}$  (Ator et al. 2005). Data loggers revealed significantly different surface hydrologic connectivity (SHC) patterns among the three wetland types (Fig. 2.4, Table 2.2). Forested wetlands tended to have relatively continuous seasonal connectivity to perennial streams between mid-autumn and late-spring (Fig. 2.4a-d) when temperatures and evapotranspiration were low. Historical and restored wetland–stream SHC was typically marked by multiple ephemeral connections that occurred during and immediately following significant rain events during periods of low evapotranspiration (Fig. 2.4h-k). However, connections lasting ~ 50 days were observed at larger restored wetlands R1 (Fig. 2.4e) and R2 (Fig. 2.4f) that occurred when temperature and evapotranspiration were low.

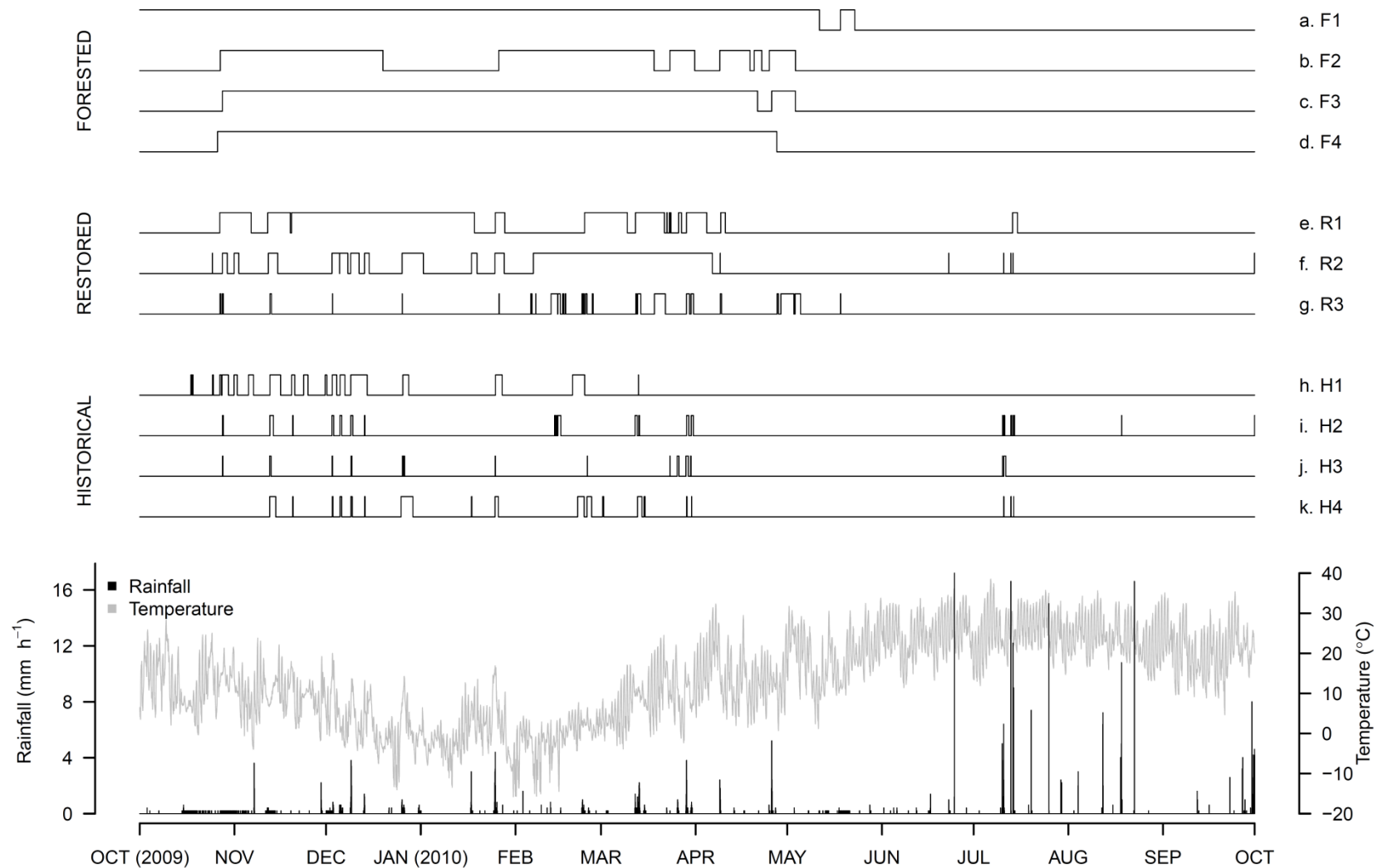
All SHC metrics were significantly correlated with log-wetland area (total time connected:  $r = 0.83$ ,  $p < 0.01$ ; # of connections:  $r = -0.75$ ,  $p < 0.01$ ; mean connection duration:  $r = 0.72$ ,  $p < 0.05$ ;  $D_{max,c}$ :  $r = 0.82$ ,  $p < 0.01$ ). ANCOVA (covariate: log-wetland area) revealed a significant effect of wetland area with respect to all SHC metrics (total connection duration:  $F_{1,5} = 46.80$ ,  $p < 0.01$ ; # of connections:  $F_{1,5} = 20.92$ ,  $p < 0.01$ ; mean connection duration:  $F_{1,5} = 11.69$ ,  $p < 0.05$ ;  $D_{max,c}$ :  $F_{1,5} = 25.25$ ,  $p < 0.01$ ), and the slopes of the relationships did not differ among the three wetland types. The effect of wetland type was only significant with respect to total connection duration and number of connections (Fig 2.5A,B). Total duration of connectivity between forested wetlands and adjacent perennial streams was approximately 12 and 2.5 times greater compared to

historical and restored wetlands, respectively (Fig. 2.5A). Forested wetlands were connected to perennial streams a significantly fewer number of times relative to both historical and restored wetlands. No significant differences were found with respect to SHC metrics between historical and restored wetlands (Fig. 2.5A-D).

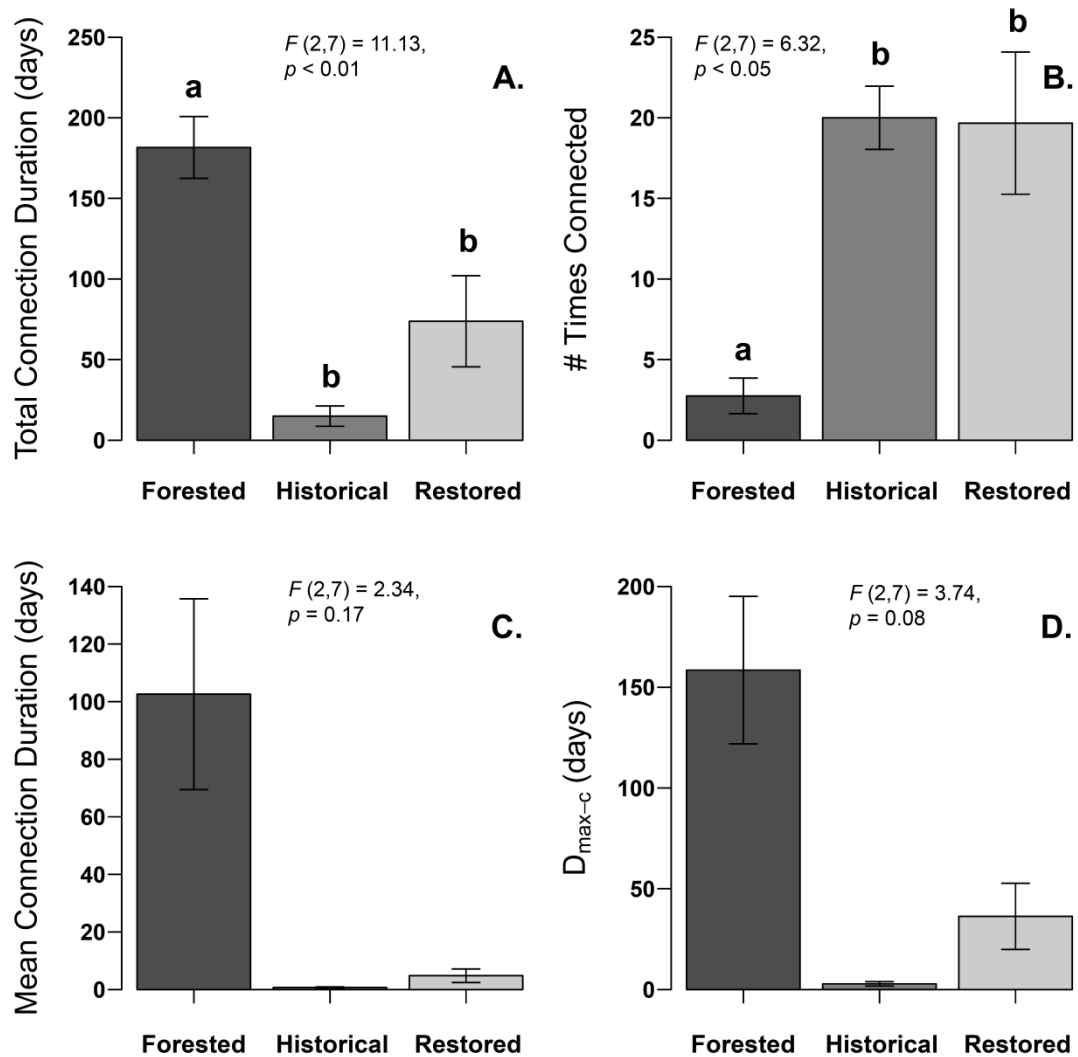
At both historical and restored wetlands, mean daily rainfall totals were significantly greater on days when a temporary stream connection occurred between the wetland and perennial stream compared to rainfall totals on those days lacking a connection (Table 2.3). No significant differences in mean daily rainfall were observed on days with and without temporary stream connections at any of the forested wetlands (Table 2.3).

**Table 2.2:** Surface hydrologic connectivity (SHC) metrics for each study site. Values in parentheses are standard errors of the means.

Site	Wetland Type	Total Time Connected (d)	# Individual Connections	Mean Connection Duration (d)	$D_{max-c}$ (d)
F1	Forested	227.2	2	113.6 (113.6)	222.5
F2	Forested	133.2	6	22.2 (9.3)	53.3
F3	Forested	182.9	2	91.5 (70.0)	175.2
F4	Forested	183.1	1	183.1	183.1
R1	Restored	115.3	13	8.9 (4.2)	46.0
R2	Restored	86.4	18	4.8 (3.0)	58.6
R3	Restored	19.7	28	0.7 (0.2)	4.3
H1	Historical	32.5	24	1.4 (0.3)	5.4
H2	Historical	8.7	22	0.4 (0.1)	1.2
H3	Historical	3.8	15	0.3 (0.1)	0.8
H4	Historical	14.8	19	0.8 (0.2)	3.9



**Figure 2.4: Top Panel)** State data logger surface hydrologic connectivity (SHC) records during the 2010 water year between forested (a-d), restored (e-g), and historical (h-k) Delmarva bays and adjacent perennial streams. The ‘up’ and ‘down’ positions indicate presence and absence of SHC, respectively. **Bottom Panel)** Rainfall and temperature records for the 2010 water year. Top and bottom panels share the same x-axis.



**Figure 2.5:** Summary of surface hydrologic connectivity (SHC) metrics between forested ( $n = 4$ ), historical ( $n = 4$ ), and restored ( $n = 3$ ) Delmarva bay wetlands and adjacent perennial streams during the 2010 water year. **A)** Wetland–stream total surface hydrologic connection duration, **B)** number of individual connections, **C)** individual connection duration, and **D)** maximum individual connection duration. Error bars represent standard errors of the means. Means with different letters are significantly different after accounting for wetland area via ANCOVA (Tukey’s HSD,  $p < 0.05$ ).

**Table 2.3:** Student’s t-tests between mean daily rainfall totals on days during which a temporary stream connection did and did not occur between wetlands and adjacent perennial streams. Values in parentheses are standard errors of the means.

Site	Wetland Type	Mean Daily Rainfall When Connected (mm d <sup>-1</sup> )	Mean Daily Rainfall When Not Connected (mm d <sup>-1</sup> )	t-Test Statistics
F1	Forested	2.5 (0.5)	3.3 (1.1)	$t(363) = 0.78, p = 0.43$
F2	Forested	3.1 (0.7)	2.6 (0.7)	$t(363) = 0.47, p = 0.64$
F3	Forested	2.9 (0.6)	2.7 (0.8)	$t(363) = 0.15, p = 0.88$
F4	Forested	2.9 (0.6)	2.7 (0.8)	$t(363) = 0.17, p = 0.86$
R1	Restored	4.3 (1.0)	2.1 (0.6)	$t(363) = 2.00, p < 0.05$
R2	Restored	6.2 (1.5)	1.5 (0.3)	$t(363) = 4.47, p < 0.01$
R3	Restored	5.8 (1.7)	2.5 (0.5)	$t(363) = 1.98, p < 0.05$
H1	Historical	7.4 (1.9)	2.0 (0.5)	$t(363) = 4.08, p < 0.01$
H2	Historical	21.2 (5.3)	1.5 (0.3)	$t(363) = 11.57, p < 0.01$
H3	Historical	16.7 (4.3)	2.1 (0.4)	$t(363) = 6.59, p < 0.01$
H4	Historical	14.4 (3.1)	1.7 (0.4)	$t(363) = 7.92, p < 0.01$

## DISCUSSION

State data loggers provided evidence of surface hydrologic connectivity (SHC) between Delmarva bay wetlands, which are commonly considered geographically isolated (Tiner 2003), and adjacent streams. Moreover, fundamentally different patterns of SHC occurred between forested wetlands and adjacent perennial streams relative to patterns at historical and hydrologically restored wetlands. This study suggests that restoring historical Delmarva bays has not resulted in recovery of reference wetland–stream SHC patterns in the 7 to 8 years since restoration.



## **Ecological and Regulatory Implications of Wetland–Stream Surface Hydrologic Connectivity**

Differences in SHC observed at forested, historical, and restored wetlands may have potential ecological implications. Surface hydrology, including patterns in duration and timing of flows, is considered a master variable controlling stream geomorphology, habitat suitability, thermal regulation, metabolism, biogeochemical cycling, and downstream fluxes of energy, matter, and biota (Poff et al. 1997). The differences in flow regime I observed in temporary streams linking wetlands and perennial reaches could, therefore, significantly influence ecosystem properties both within and among the waters they connect. For example, when surface water is present, temporary streams may serve as connectivity corridors influencing the dispersal of animals and plants (Galatowitsch and van der Valk 1996, Babbitt and Tanner 2000). Wetlands temporarily connected to perennial stream networks may provide refugia for stream amphibians or habitat for fish spawning and rearing (*sensu* Dodds et al. 2004, Cunningham et al. 2007). Dispersal throughout temporarily connected networks may also promote genetic exchange and opportunities for recolonization of periodically disconnected waters. Although some aquatic taxa or individuals may disperse via flight or passive attachment to migrating animals, most animal and plant dispersal throughout freshwater networks occurs via flowing water-mediated transport (Bohonak and Jenkins 2003). Dispersal of most aquatic taxa, therefore, requires surface connections between water bodies, even if those connections are temporary (Nadeau and Rains 2007).

The timing and duration of wetland–stream SHC may be ecologically critical (Leibowitz et al. 2008) and are likely to determine the suitability of migratory pathways. Seasonally continuous SHC like that observed at forested Delmarva bays may provide reliable dispersal corridors for migrating biota, while more unpredictable and ephemeral connections typical of historical and restored bays are less likely to be utilized. Similarly, more continuous SHC may supply steady subsidies of carbon, nutrients, and water to downstream communities whereas short-lived connections provide a pulsed delivery of resources (see Appendix for 2010 water year wetland → stream dissolved organic carbon flux estimates). Additionally, the more ephemeral and unpredictable a stream’s flow regime, the less likely it is to provide viable habitat for aquatic organisms (Arscott et al. 2010).

In addition to potential ecological ramifications of wetland–stream SHC, connectivity patterns may be used, in part, to determine the regulatory status of an individual wetland, wetland mosaic, or a group of similarly situated wetlands. In the United States, interest in wetland–stream connectivity has been fueled largely by recent Supreme Court rulings regarding the jurisdictional scope of the Clean Water Act (CWA) and the legal protected status of geographically isolated wetlands and non-navigable streams (Leibowitz et al. 2008). These new requirements for determining wetland and non-navigable stream jurisdictional status have greatly increased the need for scientific information in support of isolated wetland and temporary stream regulatory decisions as well as future policies and legislation (Nadeau and Rains 2007, Leibowitz et al. 2008). To that end, Leibowitz et al. (2008) stress the need for inexpensive and simple approaches for evaluating SHC between wetlands and other waters and a need for case studies

quantifying SHC in a variety of regional settings. I believe this study and those by Wilcox et al. (2011) and Lang et al. (2012) provide the type of data needed to scientifically inform critical regulatory decisions and policies.

The historical wetlands I studied are considered prior-converted cropland (i.e., wetlands converted from a non-agricultural use to production of a commodity crop prior to December 23, 1985) and the hydrologically restored wetlands are enrolled in one of two Federal agricultural land retirement programs (e.g., Conservation Reserve Program and Wetland Reserve Program). As such, both the historical and restored wetlands are exempt from CWA jurisdiction. However, native forested Delmarva bays are not exempt, and SHC between them and perennial stream networks may be used, in part, to determine their jurisdictional status. Lang et al. (2012) estimated there to be 2,050 semi-natural wetlands within the Tuckahoe Creek watershed, many of which were Delmarva bays. Of the semi-natural wetlands, only 53% were physically connected to streams, and only 75% were within 80 m of the nearest stream (Lang et al. 2012). Without knowledge of the SHC patterns between forested wetlands and perennial streams, static imagery not only indicates significant geographic isolation of wetlands (Lang et al. 2012), but likely hydrologic, and in turn, ecologic isolation.

### **Impact of Cultivation and Restoration on Wetland–Stream Surface Hydrologic Connectivity**

Since European settlement there has been extensive modification within the Chesapeake Bay watershed for agriculture (Hilgartner and Brush 2006). Across the Delmarva Peninsula, Fenstermacher (2012) found 65% of Delmarva bays have been

directly impacted by agriculture, compared to an estimated 45% loss of all wetlands Maryland-wide (Tiner and Burke 1995), suggesting Delmarva bays have been disproportionately altered relative to other wetlands. Wetland drainage has significant impacts on the surface and ground water hydrology of a wetland and its hydrologic connectivity to nearby waters. Creation or modification of a channel to enhance wetland drainage decreases basin storage capacity and results in decreased ground water recharge. Additionally, active wetland infilling or infilling due to soil erosion may decrease water storage capacity in historical wetlands. Small drainage basin areas of historical and restored wetlands (Table 2.1) may indicate that cultivation and drainage have disconnected complexes of once native forested wetlands and in turn altered natural wetland–stream SHC patterns (Fig. 2.4 and Fig. 2.5). Alternatively, smaller, more disconnected Delmarva bays may have been easier to convert to agriculture and therefore preferentially selected.

Cultivation may also result in increased soil compaction and bulk density within wetlands (see Chapter 1). Soil compaction results in decreased ground water recharge and increased infiltration excess overland flow (Dunne and Leopold 1978). Together, decreased basin storage capacity and increased soil compaction lead to more frequent yet ephemeral SHC to adjacent waters. Fenstermacher (2012) found that soils within historical and recently restored depressional wetlands in Delaware, Maryland, Virginia, and North Carolina had significantly higher bulk density compared to reference wetland soils. Comparable soil compaction between historical and restored wetlands (see Chapter 1) may contribute to similarities in wetland SHC patterns marked by ephemeral, rainfall-driven connectivity events.

Within heavily agriculturally modified catchments, local ground water tables may be lowered due to artificially deep drainage ditches, ground water abstraction for irrigation, and decreased ground water recharge. Under these conditions, wetlands that once acted as discharge wetlands and maintained standing surface water at least seasonally may no longer do so. As a result, seasonal wetland–stream SHC may no longer be maintained by groundwater discharge and connections only occur during and immediately following rainfall events. Corroborating this, Sharitz and Gresham (1998) found decreased flow duration in streams fed by pocosins drained for agriculture compared to undisturbed pocosin ecosystems. I report a similar finding in cultivated Delmarva bays.

## **CONCLUSION**

Surface hydrologic connectivity (SHC) between so-called isolated wetlands and perennial streams represents an understudied but potentially significant ecological link between the two systems. Moreover, patterns of wetland–stream surface flow may be used to determine wetland jurisdictional status within the U.S. (Leibowitz et al. 2008). Patterns of SHC observed in this study reveal that Delmarva bay wetlands may not be hydrologically isolated from nearby streams. Results indicate that agricultural alteration of Delmarva bays may significantly change patterns of wetland–stream SHC, and SHC may be slow to recover post-restoration. Correlations between wetland size and wetland SHC metrics imply that restoring reference SHC patterns between Delmarva bays and adjacent streams will require restoration of larger wetland complexes rather than

individual, small basins. I propose that, when possible, multiple wetlands be restored in concert to promote reference hydrological conditions between Delmarva bays and stream networks. Understanding patterns and controls of wetland–stream SHC is but one means of assessing the link between ecosystems often studied and regulated independently.

## **Chapter 3: The impact of repeated drying and wetting on denitrification in a restored temporary stream**

### **INTRODUCTION**

Although often individually small, temporary streams collectively comprise a majority of river network length (Nadeau and Rains 2007). In mesic temperate regions, temporary streams are typically located at the headwaters of river networks (Gomi et al. 2002). Due in part to their size and landscape position, ephemeral and intermittent streams are likely to have long hydraulic retention times, high water-sediment interaction, and substantial nutrient processing capacity (Peterson et al. 2001). Yet because of their small size, large edge-to-width ratio, and intimate connection to the landscapes they drain, temporary streams are likely to be more sensitive to disturbance than larger perennial streams (Bull 1997). While there has been extensive study of the hydrology and macrofauna of temporary streams, their biogeochemistry has received little attention and their relationship to nearby wetlands even less. Recent efforts to restore wetlands that are periodically connected to temporary streams by surface flow (see Chapter 2) may have important implications for temporary streams, including their biogeochemistry.

Temporary streams are characterized by a surface hydrology alternating between dry and wet conditions. This flow regime can generate cyclical aerobic (oxidizing) and anaerobic (reducing) conditions within temporary stream sediments. Coupled oxidation and reduction has been shown to enhance biogeochemical processes such as nitrification and denitrification (Kern et al. 1996, Mitchell and Baldwin 1999). Nitrification, the

chemoautotrophic oxidation of ammonium-N ( $\text{NH}_4^+$ ) to nitrate-N ( $\text{NO}_3^-$ ), occurs under aerobic conditions, while denitrification, the largely heterotrophic reduction of nitrate-N to gaseous N ( $\text{NO}$ ,  $\text{N}_2\text{O}$ , and  $\text{N}_2$ ), is an anaerobic process. In soils and sediments undergoing cycled drying and wetting, increased ammonification (i.e., the conversion of organic N to  $\text{NH}_4^+$  by bacteria and in some cases fungi; aka *mineralization*) and nitrification may occur during dry conditions and immediately following rewetting (Groffman and Tiejde 1988, Qiu and McComb 1996, Fierer and Schimel 2002), providing a nitrate substrate for denitrifying bacteria under subsequent wet conditions (Kern et al. 1996, Mitchell and Baldwin 1999).

Given their cyclic dry – wet nature, temporary stream sediments may be biogeochemical hotspots (*sensu* McClain et al. 2003). Yet only recently have researchers investigated the influence of flow intermittency on sediment biogeochemistry, in particular nitrification and denitrification (Austin and Strauss 2011). Moreover, I am aware of no studies addressing temporary stream sediment biogeochemistry in the context of restoration. To help fill these knowledge gaps, I compared patterns of denitrification potential in restored and undisturbed forested temporary stream sediments in response to repeated drying and wetting. I hypothesized denitrification potential would increase in response to wetting and decrease upon drying. Additionally, I predicted cyclically dried - wetted sediments would show enhanced denitrification potential compared to continuously wet and continuously dry sediments as a result of coupled nitrification – denitrification. I hypothesized forested sediment denitrification potential rates would be more resistant to changes in hydrology compared to restored sediments



due to greater organic matter content and resistance to drought in forested sediments (*sensu* Hueso et al. 2011).

## **METHODS**

### **Site Descriptions**

This research was conducted using sediment cores collected from temporary streams connecting depressional wetlands to adjacent perennial streams at the headwaters of the Choptank River watershed (Delmarva Peninsula, Maryland, USA). Within the Choptank catchment and across the Delmarva Peninsula depressional wetlands known as Delmarva bays are ubiquitous (Tiner and Burke 1995). These elliptical wetlands are characterized by a sandy upland rim and have historically been considered geographically isolated from nearby surface waters (Tiner and Burke 1995, Tiner 2003). However, Delmarva bays have recently been found to be surficially connected to adjacent streams and wetlands via temporary stream flow (see Chapter 2).

For this study, one undisturbed forested and one hydrologically restored wetland–temporary stream pair were investigated (Fig. 3.1). The forested wetland–temporary stream pair exhibit a natural hydroperiod, characterized by inundation and seasonal stream outflow in winter and spring and drawdown and stream drying in summer and fall (see Chapter 2). The restored wetland–temporary stream pair was actively farmed until 2003 when it was removed from cultivation and hydrologically restored by plugging an agricultural drainage ditch with earthen fill. Since restoration, a new temporary stream



**Figure 3.1:** Forested (**left**) and restored (**right**) temporary streams connecting an undisturbed and a restored (formerly-farmed) Delmarva bay wetland to adjacent perennial reaches.

has naturally formed and episodically connects the restored wetland to an adjacent perennial stream. Table 3.1 summarizes physical attributes of each wetland–stream pair used in this study and wetland–stream surface hydrologic connectivity metrics reported in Chapter 2.

### **Sediment Sampling**

Using poly-vinyl chloride (PVC) pipe, seventy-eight cylindrical cores (7 cm deep  $\times$  5 cm internal diameter) were collected from the forested and restored temporary streams in October 2011. Cores were extracted from the middle third of each temporary stream reach as measured from the intersection of the channel and the wetland rim to the

confluence with the perennial stream. Care was taken to maintain core vertical structure. Geotextile mesh (200  $\mu\text{m}$ ) was secured on the bottom of each core to allow for better air-drying. Sediment cores were returned to the laboratory following collection. Three cores from each stream were immediately processed for physicochemical metrics and denitrification potential (see Methods) to assess field conditions. Remaining cores were air dried for 10 days at room temperature under a light / dark regime mimicking field conditions.

### **Experimental Set-Up**

Following the initial drying period, cores from each stream were randomly assigned to one of three hydrologic treatments: continuously dry (*dry*), continuously wet (*wet*), repeatedly dry / wet at a 7-day recurrence interval (*cycled*). A 7-day dry / wet recurrence interval was selected as it closely matched the observed average length of hydrologically restored wetland–perennial stream surface hydrologic connectivity (see Chapter 2; Table 1). For the dry treatment, cores were placed on a drying table for the 28-day duration of the experiment. Wet cores were placed in aerated, flow-through baths. Cycled cores were also placed in the flow-through baths following the initial drying period. After 7 days, cycled cores were removed from the flow-through baths and placed on the drying table for 7 days after which time they were returned to the baths. This cycle was repeated twice (i.e., wet  $\rightarrow$  dry  $\rightarrow$  wet  $\rightarrow$  dry).

**Table 3.1:** Wetland and temporary stream physical attributes (\*sites investigated in this study) and wetland–stream surface hydrologic connectivity data for forested wetlands ( $n = 4$ ) and restored wetlands ( $n = 3$ ) for the 2010 water year (\*\*sites investigated in Chapter 2). Values in parentheses are standard errors of the means.

<b>Wetland–Stream Type</b>	<b>Location*</b>	<b>Wetland Area (m<sup>2</sup>)*</b>	<b>Temporary Stream Length (m)*</b>	<b>Cumulative Wetland–Stream Surface Connection Duration (d)**</b>	<b># of Wetland–Stream Surface Connections**</b>	<b>Avg. Wetland–Stream Connection Duration (d)**</b>
<b>Forested</b>	39° 3'16.45"N 75°50'4.73"W	545	59	mean: 181.6 (19.2) range: 133 - 227	mean: 2.8 (1.1) range: 1 - 6	mean: 102.6 (33.2) range: 22 - 183
<b>Restored</b>	39° 2'59.29"N 75°45'14.68"W	1,513	40	mean: 73.8 (28.3) range: 20 - 115	mean: 19.7 (4.4) range: 13 - 28	mean: 4.8 (2.4) range: 1 - 9

Forested and restored cores were kept in separate flow-through baths to prevent microbial mixing between sites. Both baths were supplied with dissolved organic carbon (DOC) leachate-amended deionized water supplied by constant gravimetric head at a flow rate of approximately 15 L d<sup>-1</sup>. DOC leachate was made by amending deionized (DI) water with leaf litter from the forested site from canopy and understory species in proportion to their field abundance. The leachate consisted of litter from red maple (*Acer rubrum*), American holly (*Ilex opaca*), spicebush (*Lindera benzoin*), American sweetgum (*Liquidambar styraciflua*), sweetbay magnolia (*Magnolia virginiana*), white oak (*Quercus alba*), red oak (*Quercus rubra*), and sassafras (*Sassafras albidum*) and was maintained at a DOC concentration of 35 ± 5 mg L<sup>-1</sup> (mean, ± SE) throughout the experiment in efforts to mimic average autumn forested wetland DOC concentrations (McDonough, unpublished). DOC leachate concentration was measured daily by filtering leachate-amended DI water through pre-combusted 0.7 µm glass fiber filters (Whatman International Ltd, Maidstone, England) and processing on a Shimadzu TOC-V<sub>CPH</sub> total organic carbon analyzer (Shimadzu Scientific Instruments, Columbia, MD). Following the initial drying period, triplicate cores were randomly and destructively sampled from each treatment on days 0, 1, 7, 8, 14, 15, 21, 22, and 28. The experiment was conducted at room temperature under a light / dark regime mimicking field conditions.

### **Sediment Physicochemical Metrics**

All sediments were analyzed for pH, reduction potential (Eh), total carbon, total nitrogen, percent moisture, bulk density, and soil organic matter (SOM). Prior to analysis, sediment samples were homogenized and any rocks and roots were removed. pH was

measured by combining sediment and deionized water in a 1:1 slurry by volume (Thomas 1996) and measuring with an Accumet XL15 pH meter (Fisher Scientific, Pittsburgh, PA). Eh was measured daily in triplicate sediment cores from each treatment using a platinum electrode coupled with a calomel reference electrode calibrated with ZoBell's reference solution (Nordstrom and Wilde 2005). Sediment total carbon and total nitrogen (represented as C:N) were analyzed by dry combustion using a CHNSO elemental analyzer (ECS 4010 CHNSO Analyzer, Costech Analytical Technologies, Valencia, CA). A subsample from each core was weighed and oven-dried at 70°C to a constant mass to determine percent moisture. Bulk density was calculated as dry mass per unit volume of sediment collected ( $\text{g cm}^{-3}$ ). SOM was determined as mass loss on ignition (% LOI) at 450°C for 16 h.

### **Denitrification Potential**

Sediment denitrification potential was determined using a denitrification enzyme activity (DEA) assay (Groffman et al. 1999). DEA provides a means of quantifying the potential for denitrifying bacteria to reduce  $\text{NO}_3^-$  and is useful for measuring differences in denitrification potential for sediment subjected to different treatments (Groffman et al. 1999). The DEA assay provides denitrifying bacteria with ideal conditions for denitrification: a nitrate substrate ( $\text{KNO}_3$ ), an energy source (glucose), and anaerobic conditions. Chloramphenicol is added to sediment slurries to inhibit bacterial production without killing existing bacteria or destroying existing enzymes.

For the DEA assay, I combined 10 g (wet mass) of the sediment with 10 mL DEA media in 125 mL Erlenmeyer flasks (final slurry concentrations:  $100 \text{ mg kg}^{-1} \text{ N}$ ,

200 mg kg<sup>-1</sup> glucose, 125 mg kg<sup>-1</sup> chloramphenicol). Rubber septa seals were used to cap flasks, which were made anaerobic by repeated evacuation and subsequent flushing with N<sub>2</sub> gas. Flasks were equilibrated with atmospheric pressure after the final N<sub>2</sub> flush. Acetylene (C<sub>2</sub>H<sub>2</sub>; 5 cm<sup>3</sup>) was then added to the headspace of each flask via syringe to inhibit the reduction of nitrous oxide (N<sub>2</sub>O) to dinitrogen gas (N<sub>2</sub>). DEA assay slurries were incubated at room temperature on a shaker table (175 rpm) for 90 min. At 30 and 90 min after C<sub>2</sub>H<sub>2</sub> addition, headspace gas samples (9 mL) were taken via syringe from each vial and transferred to pre-evacuated glass serum vials. N<sub>2</sub>O concentrations within the serum vials were measured using a Shimadzu GC-14B gas chromatograph equipped with an electron capture detector and Porapak Q column (Shimadzu Scientific Instruments, Columbia, MD). Denitrification potential rates were calculated from the increase in N<sub>2</sub>O concentration between the 30 and 90 min headspace samplings. N<sub>2</sub>O dissolved in slurry water was corrected using the Bunsen equation (Groffman et al. 1999) at the incubation temperature. Denitrification potential was calculated as the hourly rate of N<sub>2</sub>O-N production per dry mass of soil within the incubation flask and is indicative of the biomass of the denitrifying enzyme pool present in each soil sample.

### **Statistical Analyses**

Two-tailed Student's t-tests were used to compare field conditions between forested and restored temporary stream sediments. Linear regression was used to assess changes in physicochemical metrics and denitrification potential independently for each stream and flow treatment over the 28-day course of the experiment. One-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) multiple

mean comparisons tests were used to assess differences in denitrification potential among treatments at each sampling day. To analyze sediment metric and denitrification potential rates of change in response to drying and wetting within cycled sediments, one-way ANOVA and pre-planned mean comparisons were employed. Pearson's product-moment correlation coefficients (*r*-values) were calculated to quantify relationships between sediment metrics and denitrification potential. A bootstrapping procedure (1,000 resamples) was used to estimate cumulative denitrification potential for each forested and restored temporary stream flow treatments over the duration of the experiment. When necessary, data were log-transformed to meet assumptions of normality. All statistical analyses were conducted using R version 2.12.2 (R Development Core Team, Vienna, Austria) with differences deemed significant at  $\alpha = 0.05$ .

## **RESULTS**

Significant differences in the field conditions of forested and restored temporary stream sediments were observed with respect to all analyzed metrics (Table 3.2). Sediment from temporary streams adjacent to forested and restored wetlands showed clear physicochemical and biogeochemical changes in response to experimentally altered hydrology. Throughout the experiment, percent moisture decreased in dry forested and dry restored temporary stream sediments (Fig. 3.2A,D). Moisture content did not change in wet forested sediments (Fig. 3.2A), but increased in wet restored sediments (Fig. 3.2D). In response to continuous wetting, I observed significant decreases in



**Table 3.2:** Comparisons of forested and restored temporary stream sediment field conditions. Values in parentheses are standard errors of the means.

<b>Metric</b>	<b>Forested</b> <i>n</i> = 3	<b>Restored</b> <i>n</i> = 3	<b>t-Test Results</b>
<b>Bulk density (g g<sup>-1</sup>)</b>	0.50 (0.04)	1.31 (0.09)	<i>t</i> (4) = 8.38, <i>p</i> < 0.01
<b>Moisture (%)</b>	51.1 (2.8)	24.4 (1.4)	<i>t</i> (4) = 8.52, <i>p</i> < 0.01
<b>SOM (%)</b>	39.5 (2.4)	5.7 (0.5)	<i>t</i> (4) = 13.92, <i>p</i> < 0.001
<b>C:N</b>	19.6 (0.3)	16.7 (0.2)	<i>t</i> (4) = 8.73, <i>p</i> < 0.001
<b>pH</b>	4.13 (0.02)	5.81 (0.03)	<i>t</i> (4) = 43.87, <i>p</i> < 0.001
<b>Eh (mV)</b>	644 (6.1)	537 (8.3)	<i>t</i> (4) = 10.47, <i>p</i> < 0.001
<b>Denit pot. (μg N<sub>2</sub>O-N g<sup>-1</sup> h<sup>-1</sup>)</b>	0.24 (0.03)	0.50 (0.02)	<i>t</i> (4) = 7.66, <i>p</i> < 0.01

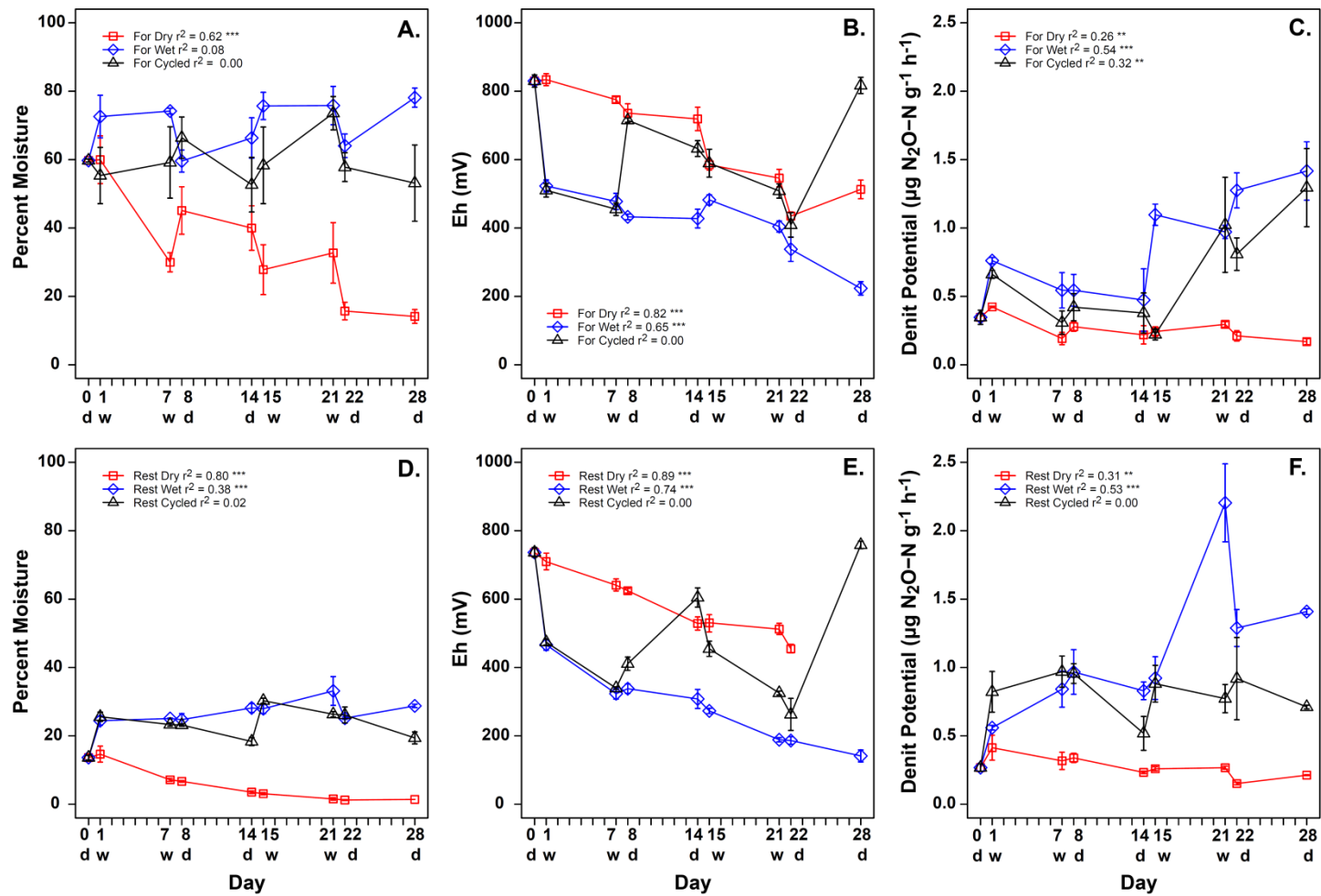
reduction potential (Eh) within both forested and restored sediments (Fig. 3.2B,E), indicating greater reducing conditions with increased wetting duration. A similar redox pattern occurred in dry forested and dry restored sediments (Fig. 3.2B,E), suggesting reducing conditions within remaining interstitial water-filled pore space as sediments dried. Denitrification potential decreased significantly in dry forested and dry restored sediments and increased in those kept continuously wet (Fig. 3.2C,F).

In cycled forested and cycled restored temporary stream sediments, no continuous change in moisture content or Eh occurred over the length of the experiment. Rather, sediments responded cyclically to changing hydrologic conditions, becoming more saturated and reducing in response to wetting and more desiccated and oxidizing in response to drying (Fig. 3.2A,B,D,E). Denitrification potential followed a similar oscillating pattern in restored sediments subjected to the cycled treatment, with increased potential for nitrate reduction in response to wetting and decreased potential when drying. Denitrification potential increased over the course of the experiment in cycled forested

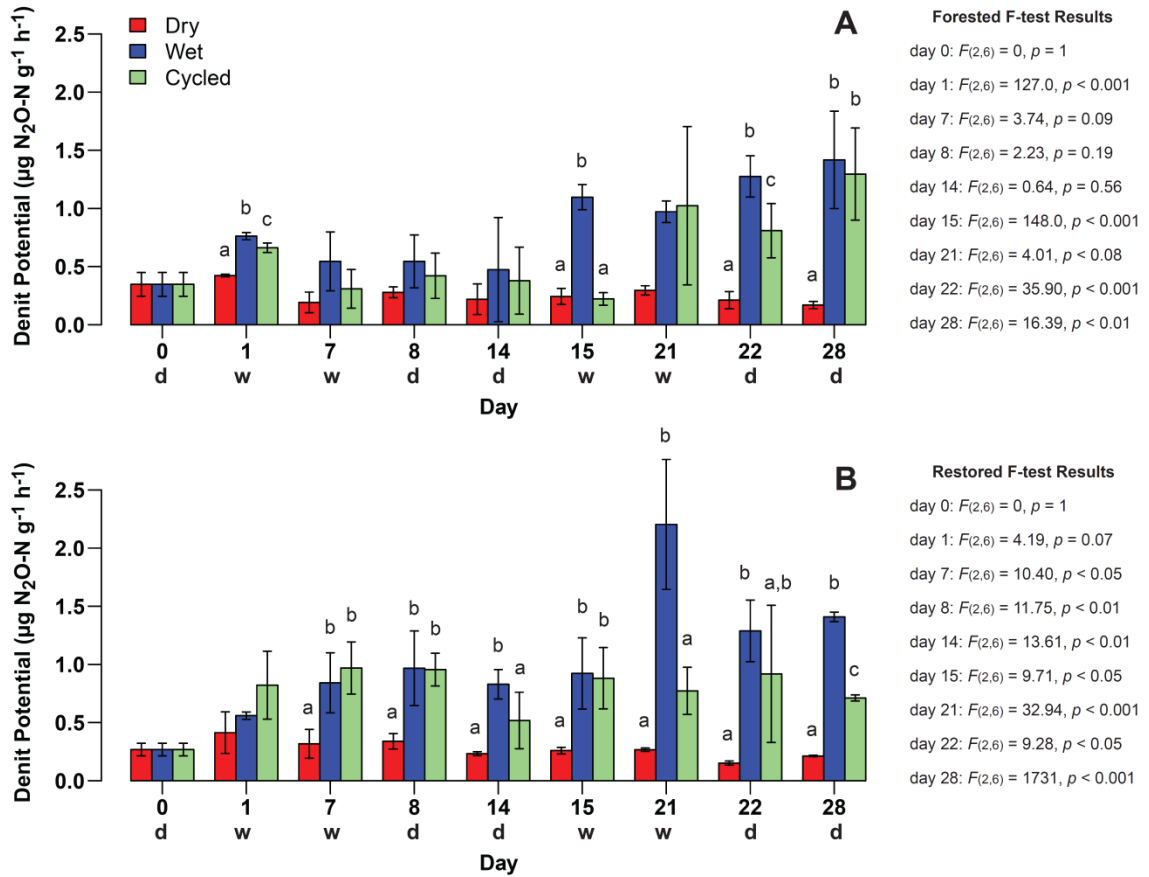
sediments (Fig. 3.2C). With the exception of an increase in pH in forested wet and cycled sediments (wet:  $r^2 = 0.55$ ,  $p < 0.001$ ; cycled:  $r^2 = 0.26$ ,  $p < 0.01$ ), no significant changes in bulk density, SOM, pH, or C:N occurred in any of the forested or restored sediments over the course of the experiment.

Barring an increase upon initial wetting, denitrification potential among dry and wet forested sediments did not differ until day 15 of the experiment (Fig. 3.3A), suggesting aerobic respiration may continue for a significant time post-wetting and a delay in nitrate reducing conditions. A 7-day delay in increased denitrifying potential was observed in wet vs. dry restored sediments (Fig. 3.3B). However, this delay may have been shorter than the sampling frequency was able to reveal.

Among cycled sediments, ANOVA F-tests followed by least-squares mean comparisons pre- and post- drying and wetting events (e.g., day 0 vs. 1 – *wetting*, day 7 vs. 8 – *drying*, day 14 vs. 15 – *wetting*, and day 21 vs. 22 – *drying*) revealed hysteresis in restored sediment moisture content, Eh, and denitrification potential whereby physicochemical metrics and denitrification potential responded rapidly to wetting but not drying (Fig. 3.4D,E,F). Within one day of initial wetting (e.g., day 0 vs. 1), moisture content nearly doubled, Eh decreased by 36%, and denitrification potential increased three-fold among restored sediments subjected to the cycle treatment. These hysteretic effects persisted upon the second rewetting and drying cycle (e.g., day 14 vs. 15 and day 21 vs. 22), albeit dampened. Hysteresis in response to wetting and drying was not observed within forested temporary stream sediments (Fig. 3.4A,B,C).



**Figure 3.2:** Changes in percent moisture, reduction potential (Eh), and denitrification potential among dry (red squares), wet (blue diamonds), and cycled (black triangles) forested (A, B, C) and restored (D, E, F) temporary stream sediments over the 28-day incubation. Error bars represent standard errors of the means. **d** = dry for cycled sediments, **w** = wet for cycled sediments. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .



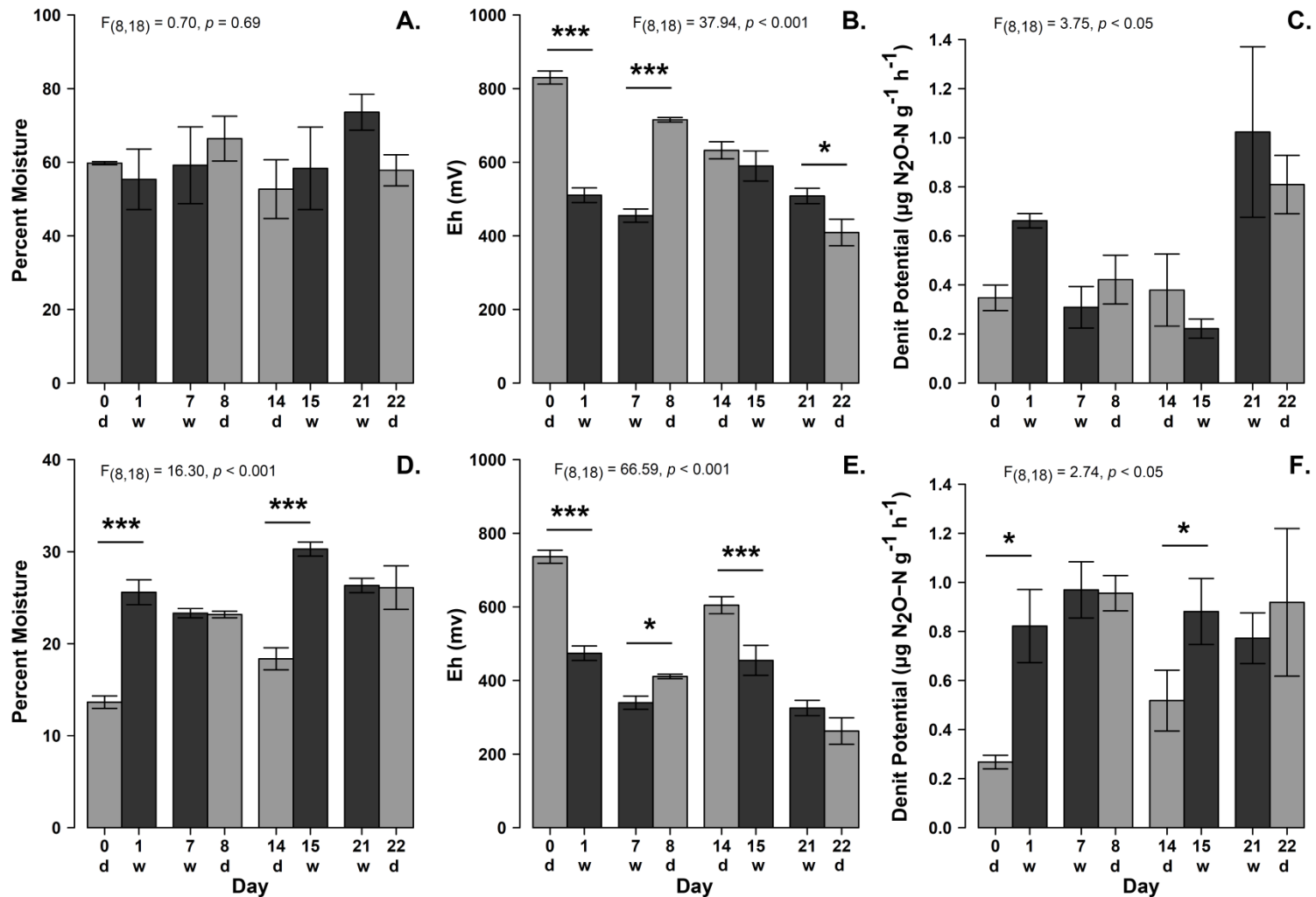
**Figure 3.3:** Mean comparisons between dry (red bars), wet (blue bars), and cycled (green bars) sediment denitrification potential rates by day for forested (A) and restored (B) temporary stream sediments. Error bars represent standard errors of the means. **d** = dry for cycled sediments. **w** = wet for cycled sediments. For a given day, means with different letters are significantly different (Tukey's HSD,  $p < 0.05$ ).

Sediment physicochemical metrics were strongly related to denitrification potential (Table 3.3). Among forested sediments, I observed positive correlations between denitrification potential and percent moisture and pH and a negative relationship between denitrification potential and bulk density and reduction potential (Eh). Similar relationships existed among restored sediments, with the addition of a positive correlation between denitrification potential and soil organic matter (SOM) content.

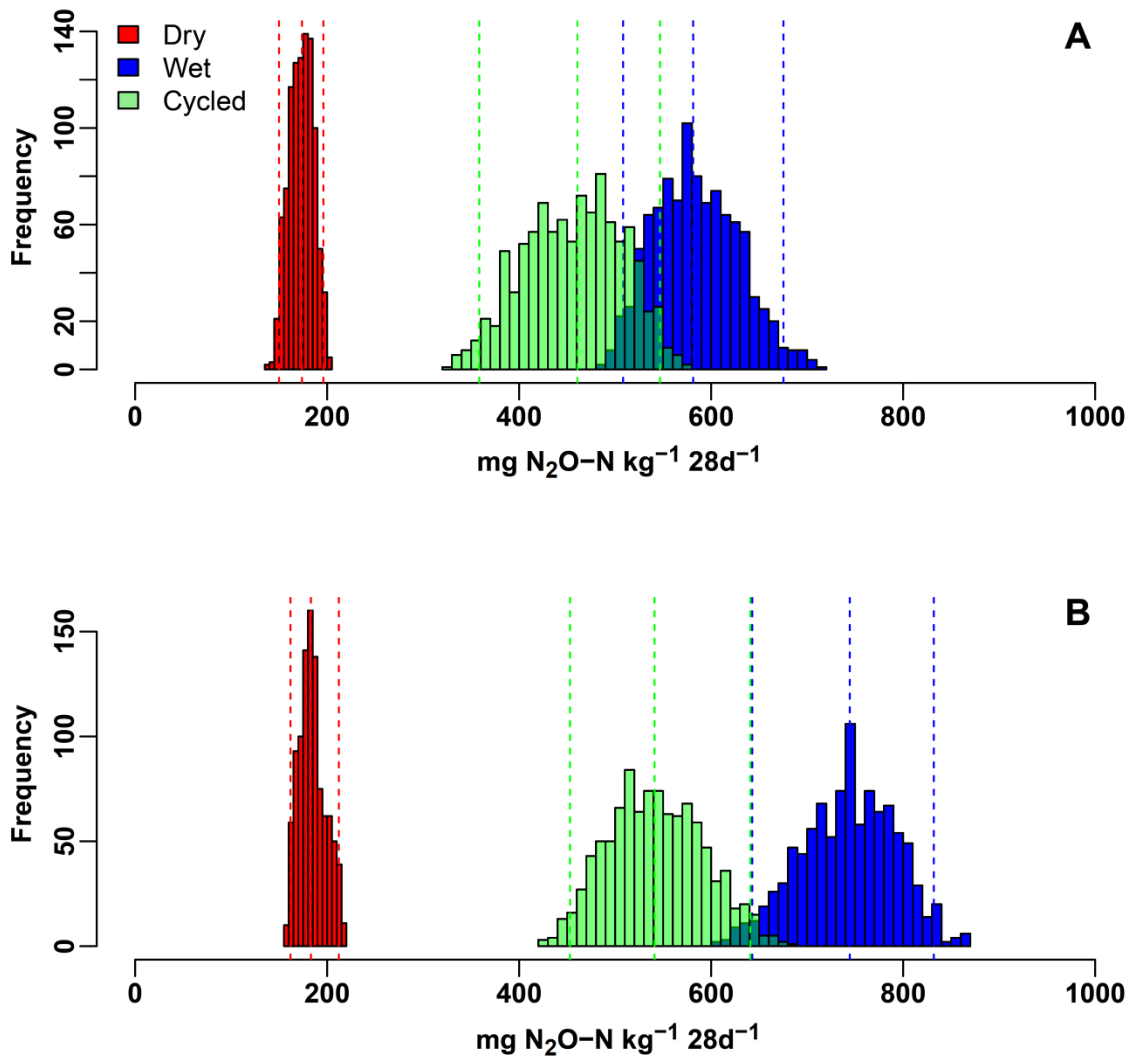
Bootstrapping results revealed greater cumulative denitrification potential estimates in wet and cycled forested temporary stream sediments compared to those kept continuously dry (Fig. 3.5A). 95% confidence intervals overlapped for wet and cycled forested sediment cumulative denitrification potential. Similar potentials were estimated for restored temporary stream sediments, albeit with less overlap between wet and cycled distributions (Fig. 3.5B).

**Table 3.3:** Pearson's product moment correlations (*r*-values) among physicochemical metrics and denitrification potential within forested and restored temporary stream sediments. Bold correlation coefficients indicate statistical significance ( $p < 0.05$ )

<b>Forested Sediments</b> ( <i>n</i> = 78)	<b>Bulk Density</b>	<b>% Moisture</b>	<b>Eh</b>	<b>pH</b>	<b>C:N</b>	<b>SOM</b>
<b>Denit. Potential</b>	<b>-0.34</b>	<b>0.66</b>	<b>-0.47</b>	<b>0.49</b>	0.17	0.23
<b>Restored Sediments</b> ( <i>n</i> = 78)						
<b>Denit. Potential</b>	<b>-0.43</b>	<b>0.75</b>	<b>-0.67</b>	<b>0.27</b>	-0.02	<b>0.33</b>



**Figure 3.4:** Mean comparisons between cycled sediment percent moisture, reduction potential (Eh), and denitrification potential during wetting phases (dry  $\rightarrow$  wet: day 0-1, day 14-15) and drying phases (wet  $\rightarrow$  dry: day 7-8, day 21-22). **A, B, C:** forested percent moisture, Eh, and denitrification potential. **D, E, F:** restored percent moisture, Eh, and denitrification potential. Error bars represent standard errors of the means. d = dry, w = wet. F-test statistics are overall one-way ANOVA results. \* =  $p < 0.05$ , \*\*\* =  $p < 0.001$ .



**Figure 3.5:** Distributions of estimated cumulative denitrification potential using bootstrapping (1,000 resamples) for dry, wet, and cycled forested (A) and restored (B) temporary stream sediments over the 28-day incubation. Dotted lines represent distribution means and 95% confidence intervals.

## DISCUSSION

By comparing the impact of drying and wetting on denitrification potential in recently restored and undisturbed forested temporary stream sediments, I found that both hydrology and land use may influence sediment denitrification potential in non-perennial reaches. Although temporary streams are ubiquitous (Nadeau and Rains 2007) and sensitive to anthropogenic disturbance (Bull 1997), non-perennial stream restoration is uncommon. Yet recent research has shown temporary stream sediments may be active zones of nitrogen processing as a result of cycled drying and wetting (Austin and Strauss 2011). Restoring biogeochemical processes in temporary streams may be an effective means to mitigate downstream nutrient loading.

Hysteresis in denitrification potential occurred among restored temporary stream sediments whereby rates changed more rapidly upon wetting than drying. This denitrification pattern corroborates changes recorded in sediment moisture and reduction potential (Fig. 3.4). Groffman and Tiedje (1988) reported similar hysteresis in upland forest soil denitrification potential under wetting and drying phases and attributed the difference to greater soil respiration and N-mineralization under wetting conditions (dry → wet) compared to drying conditions (wet → dry).

A hysteretic response, however, did not occur in sediments from the forested temporary stream. While restored sediments responded rapidly after rewetting, changes in forested sediment moisture, reduction potential, and denitrification potential were negligible. This suggests forested temporary stream sediment biogeochemistry may be more resistant to changes in hydrology and saturation relative to recently restored



sediments. The greater biogeochemical resistance observed among forested sediments is likely due in part to the high porosity, SOM content, and resistance to desiccation that characterized forested sediments but not those that had only recently been restored (e.g., Table 3.2, Fig 3.2). Corroborating this, in a study of microbial resistance to drought, Hueso et al. (2011) found soils amended with organic matter retained more moisture and supported greater microbial activity relative to unamended soils.

Ideally, ecologically successful stream restorations should be resilient to external disturbance (Palmer et al. 2005). While my experimental design did not allow me to statistically test resilience in denitrification potential in response to temporary stream sediment drying and wetting, I note that following 1 week of drying and a nearly 50% decrease in denitrification potential (day 8 – 14), restored sediment denitrification potential rates increased to pre-drying levels within 1 day of rewetting (day 14 – 15). Similarly, Austin and Strauss (2011) found denitrification potential in temporary stream sediments dried for 1 week or less recovered within 1 week of rewetting. However, they found that denitrification potential in sediments dried for > 1 week largely failed to recover even after 4 weeks of rewetting (Austin and Strauss 2011). Future work should be conducted to better assess biogeochemical resilience in response to hydrologic alterations in both natural and restored temporary streams.

The decrease in denitrification potential observed in dry sediments corroborates results reported in intermittent stream sediments in Kansas (Austin and Strauss 2011) and may have been due in part to decreased microbial abundance and activity with increasing desiccation (Kieft et al. 1987, Qiu and McComb 1996, Mitchell and Baldwin 1998).

While I expected to see increased oxidation with drying, Eh decreased continuously within dry sediments, suggesting microsites of significant reduction potential existed within the sediment matrix even after a substantial period of desiccation. Given denitrifiers are facultatively anaerobic, the increase in denitrification potential among continuously wet forested and wet restored sediments was likely largely the result of increasingly anoxic conditions. Additionally, wet soils have been shown to exhibit increased organic carbon — an energy source for heterotrophic denitrifying bacteria — relative to dry soils (Kieft et al. 1987, Groffman and Tiedje 1988). Soil wetting may also result in increased mineralization of organic N and nitrification of ammonium (Kieft et al. 1987, Groffman and Tiedje 1988, Mitchell and Baldwin 1998, Heffernan and Sponseller 2004), providing increased nitrate substrate for denitrification (Qiu and McComb 1996). The delay in increased denitrification potential rates among continuously wet sediments (forested: 15 days; restored: 7 days), however, suggests aerobic respiration may persist for a significant time post-wetting in temporary streams.

I hypothesized that denitrification would be greater in sediments subjected to repeated drying and wetting compared to continuously wet sediments as a result of coupled nitrification-denitrification. Increased denitrification has been observed as a result of cyclical drying and wetting in a variety of ecosystems (wetlands - Reddy et al. 1989; forest and grassland soils - Groffman and Tiedje 1988, Fierer and Schimel 2002; rice paddies - Reddy and Patrick 1975; lakes - Qiu and McComb 1996; river floodplains - Kern et al. 1996, Baldwin and Mitchell 2000). However, estimates of cumulative denitrification potential were greater for continuously wet vs. cycled sediments within both forested and restored temporary streams. It is possible that the 7-day drying interval

among the cycled sediments did not provide adequate time and conditions for nitrification to supply significant nitrate substrate for enhanced denitrification during subsequent periods of wetting. Alternatively, the delay observed in the onset of denitrification in wet sediments from both forested (15 days) and restored (7 days) temporary streams indicates a longer period of wetting may be necessary to generate anaerobic conditions necessary for nitrate reduction.

## **CONCLUSION**

Non-perennial stream hydrology is tightly linked to temperature and precipitation patterns, making temporary streams particularly sensitive to climatic changes (McDonough et al. 2011). With greater variability in temperature and precipitation patterns predicted under current climate change models (IPCC 2007), the frequency and intensity of drought and, in turn, stream drying are expected to increase (Lake 2000, Palmer et al. 2008, Brooks 2009). Global climate change is expected to cause increased evapotranspiration in much of North America (Schindler 1997, 2001), resulting in increased temporary stream occurrence, particularly among headwaters. Results from this study suggest that changes in temporary stream land use and flow regime may alter patterns of sediment denitrification, a primary means of mitigating downstream eutrophication (Howarth et al. 1996). Considering the ubiquity of temporary streams (Nadeau and Rains 2007) and their potential to serve as efficient nutrient processors (Peterson et al. 2001), further investigation of the role land use, restoration, and climate change play in controlling temporary stream biogeochemistry is warranted.

## **Chapter 4: Looking toward wetlands to restore stream dissolved organic matter**

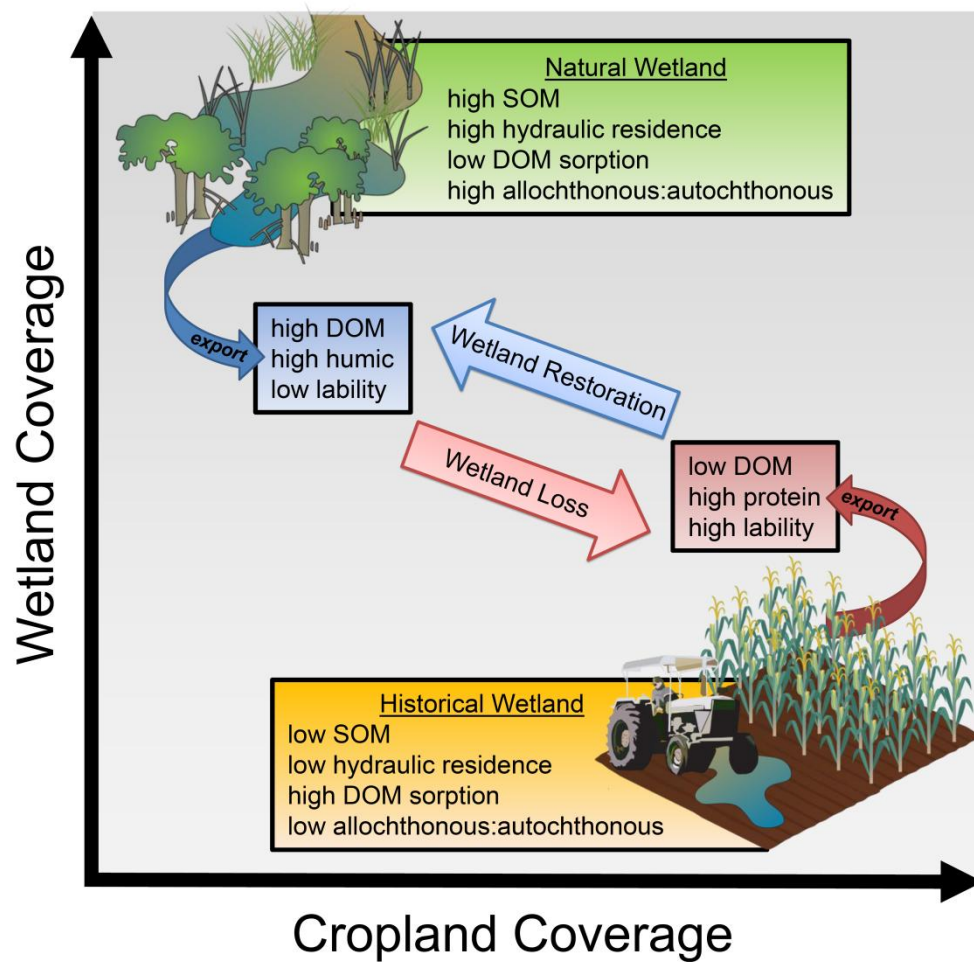
### **INTRODUCTION**

Dissolved organic matter (DOM) typically comprises the largest fraction of organic carbon in freshwaters (Wetzel 2001) and serves as an important source of energy and nutrients for heterotrophic bacteria and associated food webs (Hall and Meyer 1998). DOM affects the complexation, solubility, and transport of metals (Aitkenhead and McDowell 2000), attenuates harmful UV-B radiation in aquatic systems (Schindler and Curtis 1997), and alters stream nutrient dynamics (Bernhardt and Likens 2002). Surface water DOM is largely derived from the surrounding catchment (Aitkenhead-Peterson et al. 2003), and human land use, particularly agriculture, may alter the quantity and quality of stream DOM (Stanley et al. 2012). This may be especially common in watersheds where wetlands have been lost at the expense of cropland (Wilson and Xenopoulos 2009) (Fig. 4.1).

There is a large body of evidence showing wetland coverage within a watershed may be a strong predictor of stream DOM concentration and flux (Mulholland and Keunzler 1979, Eckhardt and Moore 1990, Dosskey and Bertsch 1994, Dillon and Molot 1997, Mulholland 1997, Gorham et al. 1998, Creed et al. 2003, Mulholland 2003, Agren et al. 2007, Andersson and Nyberg 2008, Johnston et al. 2008, Andersson and Nyberg 2009, Mattesson et al. 2009). More recently, investigators have found that wetland coverage may also influence stream DOM composition (Wilson and Xenopoulos 2009,

Williams et al. 2010). Within wetlands, anaerobic conditions reduce the rate of organic matter decomposition, which promotes accumulation of soil organic matter (Mitsch and Gosselink 2000). Water passing through organic-rich wetland soils may leach soil organic matter and transport carbon downstream as humic-rich DOM (Echardt and Moore 1990, Dosskey and Bertsch 1994). Within the continental United States, however, more than half of wetlands have been lost since European settlement (Dahl 1990), with the majority of loss ascribed to drainage for agriculture (Mitsch and Gosselink 2000). Moreover, agriculture is considered the primary cause of water quality impairment in the United States (EPA 2004). While wetland restoration has become increasingly common in agricultural landscapes (Mitsch and Gosselink 2000), information on the impact of restoration on DOM is scarce (Stanley et al. 2012).

Considering the importance of DOM in freshwater systems, it is critical that we understand controls on fluvial DOM quantity and quality and explore possible means to manage DOM in anthropogenically-modified landscapes. One such management activity worth investigating is wetland restoration (Stanley et al. 2012; Fig. 4.1). My objectives in this study were to (i) quantify the influence of cropland coverage on downstream DOM concentration, composition, and bioavailability in a once wetland-dominated landscape and (ii) test the hypothesis that agricultural wetland restoration contributes to the restoration of stream DOM. To accomplish this, two separate studies were completed: Study 1) an investigation of DOM changes in perennial streams draining watersheds along a gradient of cropland cover, and Study 2) a comparison of DOM exported from forested, historical (i.e., wetlands now in cropland, aka *prior-converted cropland*), and recently restored wetlands.



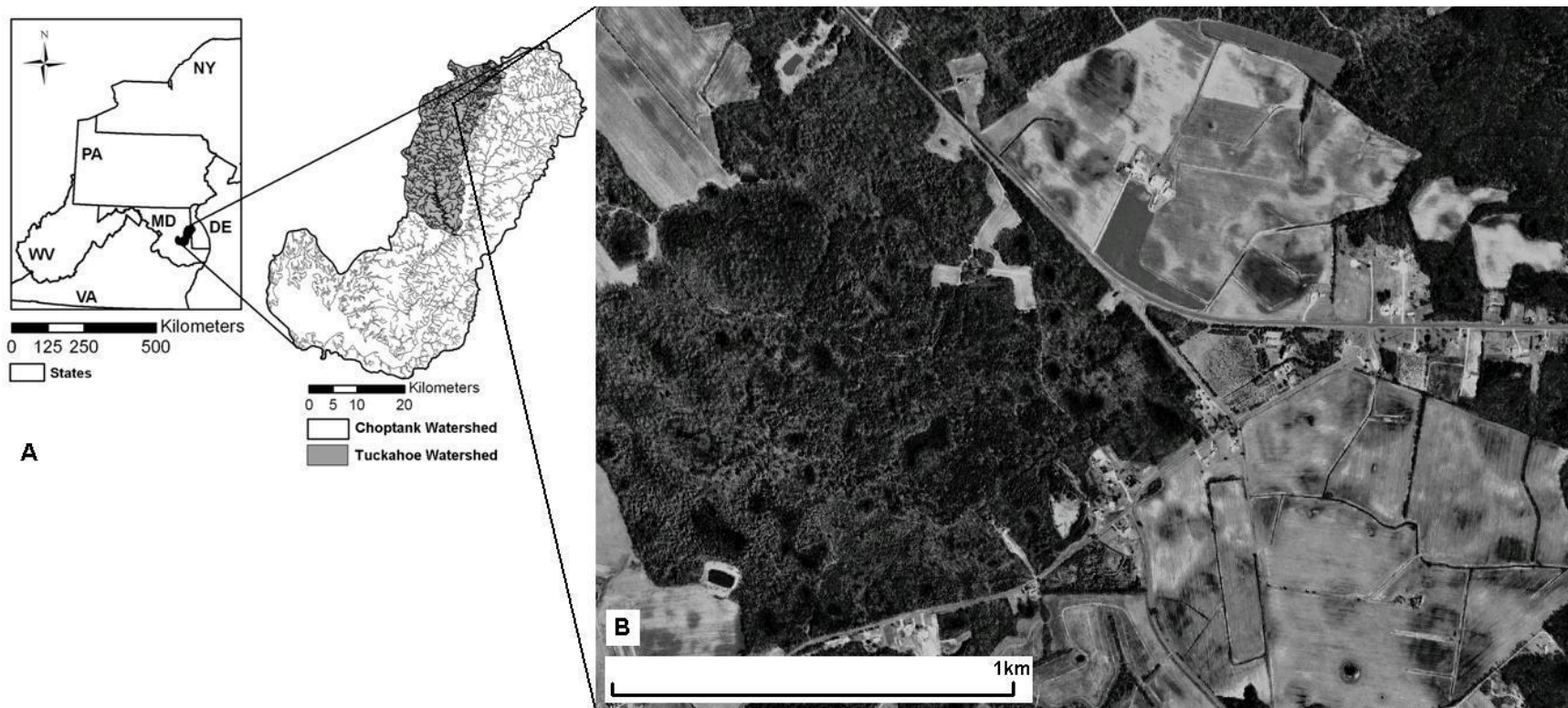
**Figure 4.1:** Wetlands have historically been lost to agriculture, fundamentally altering within-wetland controls on dissolved organic matter (DOM) export. Wetland restoration may be a viable management option to recover more natural stream DOM quantity and quality.

## **METHODS**

### **Site Description**

This research was conducted in 20 non-nested perennial headwater streams and 12 depressional wetland outflows within the Tuckahoe Creek watershed (Maryland, USA), a subbasin of the Choptank River catchment (Fig. 4.2A). The Choptank watershed is entirely situated on the Delmarva Peninsula and in the Coastal Plain physiographic province. Topographic relief is low and freshwater wetlands are ubiquitous, totaling > 20,000 ha throughout the watershed (Tiner and Burke 1995). However, much of the Delmarva Peninsula has been drained or hydrologically altered to accommodate agriculture (Fenstermacher 2012; Fig. 4.2B), and the area of natural wetlands was likely two- to three-fold greater prior to settlement (Tiner and Burke 1995). Nearly 65% of the Choptank catchment is in agricultural use, with smaller amounts of forested (26%) and urban (6%) land cover (Fisher et al. 2006).

The 20 perennial streams selected for Study 1 drain catchments spanning a cropland cover gradient from 1 – 89% of total watershed area (Table 4.1). Watersheds were delineated within ArcHydro (Environmental Systems Research Institute, Redlands, CA) using 1-meter light detection and ranging (LiDAR) derived digital elevation models. Cropland coverage was determined using the 2006 Multi-Resolution Land Characteristics Consortium (MRLC) National Land Cover Database (NLCD).



**Figure 4.2:** A) The Tuckahoe Creek watershed and stream network, a sub-basin of the Choptank River watershed in Maryland, USA. B) Depressional wetlands are common throughout the Tuckahoe watershed (near Goldsboro, MD); left side of photo shows native forested wetlands, right shows historical wetlands that have been drained for agriculture (i.e., prior-converted cropland).

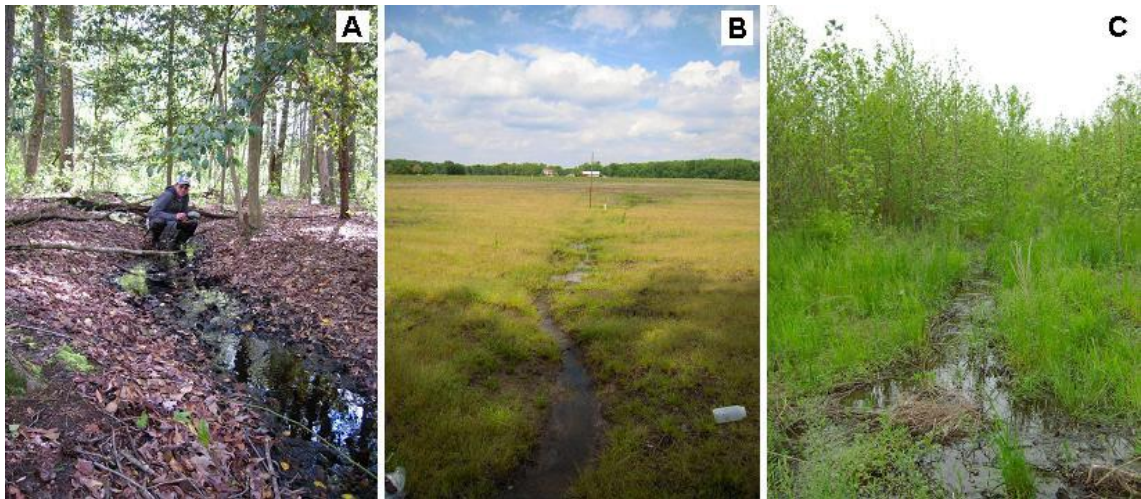


**Table 4.1:** Headwater catchments in the Tuckahoe Creek watershed vary in size, cropland coverage (% of watershed area), and baseflow discharge (March 2011).

<b>Watershed #</b>	<b>Watershed Outlet Location</b>	<b>Drainage Area (km<sup>2</sup>)</b>	<b>% Cropland Coverage</b>	<b>Discharge (L s<sup>-1</sup>)</b>
1	39°7'36.50"N, 75°54'27.38"W	1.0	1.0	0.5
2	39°7'4.13"N, 75°53'5.11"W	1.1	6.1	26.6
3	39°3'29.05"N, 75°49'44.35"W	0.6	6.9	7.4
4	39°3'3.13"N, 75°51'33.53"W	1.9	11.8	12.1
5	39°5'2.96"N, 75°49'8.16"W	2.8	22.0	24.3
6	39°4'46.45"N, 75°49'58.54"W	1.5	28.9	38.0
7	39°6'30.44"N, 75°50'5.70"W	12.6	36.5	317.7
8	38°57'41.17"N, 76° 0'19.99"W	4.1	39.2	78.3
9	38°59'7.68"N, 75°59'5.42"W	3.7	45.0	56.6
10	38°58'32.76"N, 75°58'20.65"W	3.5	45.0	47.6
11	39°2'1.77"N, 75°52'28.28"W	4.4	45.8	90.9
12	39°1'24.42"N, 75°56'57.42"W	5.5	50.3	76.4
13	38°58'35.77"N, 75°55'46.70"W	1.7	52.8	27.5
14	39°3'43.65"N, 75°53'0.20"W	3.9	53.6	48.4
15	39°4'23.26"N, 75°55'56.24"W	9.5	58.2	171.8
16	39°1'49.79"N, 75°53'45.76"W	6.5	60.1	127.1
17	39°5'17.99"N, 75°53'2.77"W	2.5	63.2	45.2
18	38°59'53.83"N, 75°55'0.54"W	10.4	75.6	204.2
19	39°6'57.10"N, 75°48'25.93"W	0.7	79.9	13.7
20	39°6'58.20"N, 75°52'35.00"W	1.0	88.7	3.0

The 12 wetlands chosen for Study 2 are classified as forested ( $n = 4$ ), historical (i.e., wetlands now in cropland;  $n = 4$ ), and hydrologically restored ( $n = 4$ ) (Table 4.2). Each wetland is considered a representative ecosystem within its type and episodically outflows via temporary stream flow to the perennial stream network (see Chapter 2; Fig. 4.3).

Forested wetlands are characterized by closed canopy palustrine forest (Cowardin et al. 1979) and exhibit an alternating hydrology, acting as discharge wetlands in winter and spring and recharge basins in summer and fall (Phillips and Shedlock 1993). Surface water is typically present within forested wetlands from late fall through late spring. Dominant tree species include *Acer rubrum*, *Liquidambar styraciflua*, *Ilex opaca*,



**Figure 4.3:** Examples of wetland outflows connecting forested (A), historical [i.e., prior-converted cropland] (B), and restored (C) wetlands to adjacent perennial streams within the Tuckahoe Creek watershed (photos: March 2011).

*Quercus rubra*, and *Prunus serotina*, and the understory is dominated by *Smilax rotundifolia*, *Lindera benzoin*, and *Leucothoe racemosa*.

Historical wetlands have been drained via ditches to enhance crop production and are characterized by brief periods of standing water following rainfall events. Crop within historical wetlands rotates annually between corn (*Zea mays*) and soybean (*Glycine max*). Prior to drainage and cultivation, historical wetlands were forested wetlands (Whigham et al. 2002).

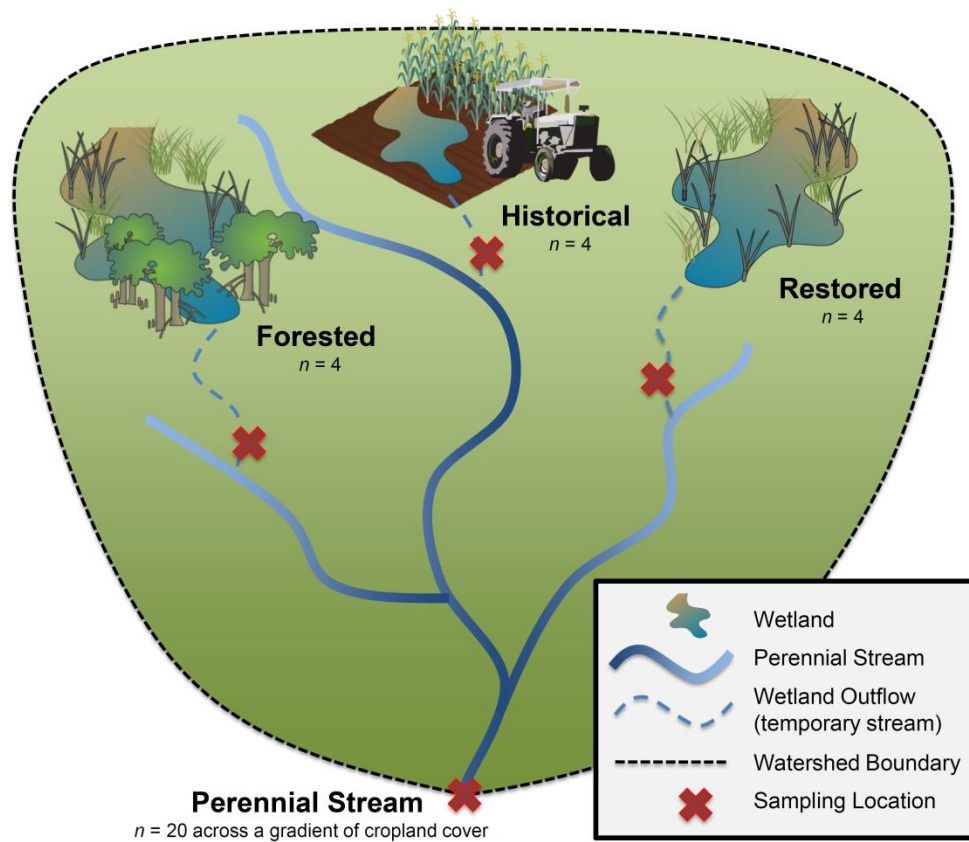
Restored wetlands were removed from cultivation 8-9 years prior to this study, and restoration efforts involved plugging drainage ditches with earthen mounds to reestablish a more natural hydroperiod. Restored wetlands in the region typically exhibit standing water from late fall to late spring. Dominant vegetation consists primarily of emergent and floating plants including *Typha latifolia*, *Phragmites australis*, *Schoenoplectus americanus*, *Carex* sp., *Scirpus cyperinus*, and *Lemna minor*. While saplings, particularly *Acer rubrum* and *Liquidambar styraciflua*, are present at two of the four restored sites, mature forested upland canopy has not developed at any of the wetlands. Prior to conversion to agriculture, restored wetlands were once forested wetlands (Whigham et al. 2002).

**Table 4.2:** Wetland classifications, physical attributes, and baseflow discharge (March 2011).  
 \* = discharge could not be measured.

Site	Wetland Type (year restored)	Location	Area (m <sup>2</sup> )	Discharge (L s <sup>-1</sup> )
F1	Forested	39° 3'24.52"N, 75°49'30.93"W	335052	46.9
F2	Forested	39° 3'28.34"N, 75°49'53.05"W	25166	3.8
F3	Forested	39° 3'16.50"N, 75°50'4.77"W	17208	3.3
F4	Forested	39° 3'25.09"N, 75°49'57.97"W	127853	12.0
R1	Restored (2002)	39° 4'8.31"N, 75°45'31.99"W	9334	*
R2	Restored (2002)	38°57'55.68"N, 75°57'55.74"W	67145	5.9
R3	Restored (2003)	39° 2'54.14"N, 75°45'19.24"W	1433	0.3
R4	Restored (2003)	39° 2'59.38"N, 75°45'14.61"W	1970	2.4
H1	Historical	39° 3'31.96"N, 75°49'44.61"W	8060	0.3
H2	Historical	39° 3'35.88"N, 75°49'47.63"W	2571	0.3
H3	Historical	39° 3'34.89"N, 75°51'4.21"W	3955	0.2
H4	Historical	39° 3'39.73"N, 75°50'56.85"W	10276	0.1

### Perennial Stream and Wetland Outflow Sampling

A stream baseflow grab sample was collected from the outlet of each perennial headwater catchment in March 2011. Within one week of perennial stream sampling, forested, historical, and restored wetland outflow grab samples were taken from the temporary streams connecting the wetlands to the perennial stream network (Fig. 4.4). All samples were collected in acid-washed high-density polyethylene bottles, kept on ice in the dark, and immediately returned to the laboratory for processing.



**Figure 4.4:** Schematic indicating dissolved organic matter sampling locations.

## Dissolved Organic Matter Analysis

Perennial stream and wetland outflow samples were filtered within 24 hours of collection using pre-combusted 0.7  $\mu\text{m}$  glass fiber filters (Whatman International Ltd, Maidstone, England). Total dissolved organic carbon (DOC) concentrations were measured using a Shimadzu TOC-V<sub>CPH</sub> total organic carbon analyzer (Shimadzu Scientific Instruments, Columbia, MD). Ultraviolet (UV) visible absorbance spectra were measured on filtered samples from 200-800 nm using a spectrophotometer with a 1 cm

path-length quartz cuvette. Specific UV-absorbance at 254 nm ( $SUVA_{254}$ ), which has been shown to correlate positively with DOM aromatic content (Weishaar et al. 2003), was calculated by standardizing UV-absorbance at 254 nm by DOC concentration. Spectral slope, defined as the ratio of UV-absorbance at 254:365 nm, was determined from the spectrophotometric scans and decreases with DOM molecular weight (De Haan 1993, Dahlen et al. 1996).

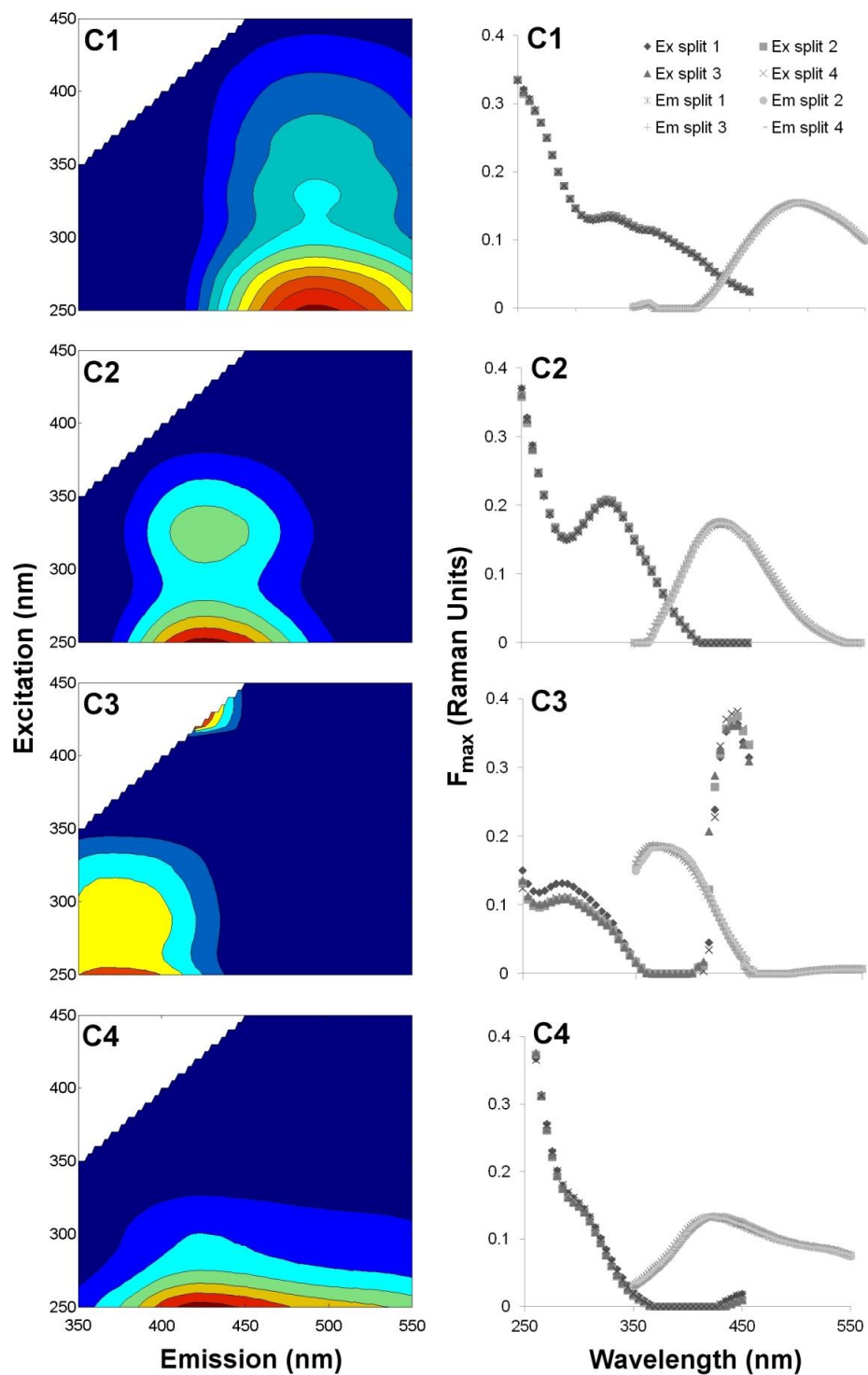
DOM fluorescence excitation-emission matrices (EEMs) were constructed for all samples using a Fluoromax-4 spectrofluorometer with a xenon lamp (Horiba Jobin Yvon, Edison, NJ). EEMs were created by measuring DOM fluorescence intensity across excitation wavelengths 240-450 nm at 5 nm increments and emission wavelengths 230-600 nm at 2 nm increments. When necessary, samples were diluted with Nanopure water (Barnstead Nanopure, Thermo Scientific, Waltham, MA) to an absorbance of  $< 0.30$  at 254 nm to avoid inner filter effects (Mobed 1996). EEMs were corrected for instrument bias and Raman-normalized by the area under a Nanopure water Raman peak measured at excitation wavelength 350 nm (Cory et al. 2010).

EEMs were used to calculate environmentally relevant fluorescence indices: humification index (HIX) and  $\beta:\alpha$  ratio. HIX was calculated from excitation at 255 nm as the ratio of the area under the curve at emissions 434-480 nm to the area under the curve at emissions 300-346 nm (Zsolnay et al. 1999). HIX is directly proportional to DOM humic content, with values  $< 3$  associated with non-humified plant matter, and values  $> 10$  associated with fulvic acids (Zsolnay et al. 1999).  $\beta:\alpha$  ratio was calculated from excitation at 310 nm as the ratio of emission intensity at 380 nm ( $\beta$ ) to the maximum

emission intensity between 420 and 434 nm ( $\alpha$ ; Parlanti et al. 2000) and is indicative of the contribution of recently produced DOM to older, degraded DOM (Wilson and Xenopoulos 2009).  $\beta:\alpha$  ratio values  $> 1.0$  are indicative of autochthonous DOM while values  $< 0.6$  indicate DOM of allochthonous origin (Huguet et al. 2009).

Parallel factor analysis (PARAFAC) modeling of complete fluorescence EEMs was conducted using MATLAB v. R2011a (MathWorks, Inc., Natick, MA) and the *DOMfluor* toolbox following the procedures described by Stedmon and Bro (2008). The PARAFAC model identified four unique DOM fluorescence components from the EEMs (Fig. 4.5 left column), and was validated via split-half analysis (Fig. 4.5 right column; Stedmon and Bro 2008). All components identified by the PARAFAC model in this study have previously been documented as part of another PARAFAC model or visual identification (Table 4.3). Components C1, C2, C3, and C4 can be described as old UVC humic-like, UVA humic-like / fulvic acid-type, protein- and tryptophan-like, and new UVC humic-like, respectively. To reduce the influence of DOC concentration on model scores, the percent relative contribution of each of the validated components within a water sample was calculated by dividing the fluorescence maximum ( $F_{max}$  in Raman units) of each individual component by the summed fluorescence maxima of all components (Williams et al. 2010):

$$\%F_{max}C_{\#} = \frac{F_{max}C_{\#}}{\sum F_{max}C_{1-4}} \times 100\% \quad \text{Equation 4.1}$$



**Figure 4.5:** Excitation-emission plots (left column) of the four fluorescent components (C1, C2, C3, C4) identified by the PARAFAC model in this study. Red and blue hues indicate higher and lower fluorescence intensities, respectively. Scatter-plots (right column) represent split-half validations of the components.



**Table 4.3.** Characteristics of the fluorescent components identified by the PARAFAC model in this study. Wavelength values in parentheses indicate secondary maxima.

Comp.	Excitation maxima (nm)	Emission maxima (nm)	Comparable components identified from previous studies	Description
C1	< 250 (330)	492	Coble (1996) - Comp. A Fellman et al. (2010) - Comp. 1 Gueguen et al. (2011) – Comp. 1 Lutz et al. (2011) - Comp. 1 Murphy et al. (2006) - Comp. 3 Stedmon and Markager (2005b) - Comp. 1	Widespread UVC humic-like fluorophore, most common in wetlands and forested streams; older origin
C2	< 250 (325)	426	Cory and McKnight (2005) - Comp. 3 Lutz et al. (2011) - Comp. 2 Murphy et al. (2006) – Comp. 2 Stedmon and Markager (2005a) - Comp. C Stedmon and Markager (2005b) - Comp. 5	UVA humic-like fluorophore, fulvic acid-type, low molecular weight
C3	440 (285)	370	Coble (1996) - Comp. T Cory and McKnight (2005) - Comp. 8 Parlanti et al. (2000) - Comp. $\delta$ Stedmon and Markager (2005a) - Comp. G Stedmon et al. (2003) - Comp. 5 Yamashita et al. (2008) - Comp. 5	Protein- and tryptophan-like, amino acids free or bound in proteins
C4	< 250	424	Cory and McKnight (2005) - Comp. 2 Ishii and Boyer (2012) - Comp. 1 Kowalczyk et al. (2009) - Comp. 2 Murphy et al. (2006) - Comp. 9 Stedmon and Markager (2005a) - Comp. A Stedmon et al. (2003) - Comp. 1	Widespread UVC humic-like fluorophore; newer origin

## **Dissolved Organic Carbon Bioavailability Assay**

A dissolved organic carbon (DOC) bioavailability assay was conducted on all perennial stream and wetland outflow samples. Briefly, 125 mL aliquots from each sample were filtered (0.2  $\mu\text{m}$ ), inoculated with 1 mL of a filtered (1  $\mu\text{m}$ ) sediment slurry (bacterial inoculate) collected from a downstream reach common to all study watersheds and wetlands, and incubated in Erlenmeyer flasks. To ensure non-limiting nutrient conditions, nitrate ( $\text{NO}_3^-$ -N) and soluble reactive phosphorus (SRP) were added to all flasks to raise ambient  $\text{NO}_3^-$ -N and SRP concentrations by 100  $\mu\text{g L}^{-1}$  and 20  $\mu\text{g L}^{-1}$ , respectively. Immediately following inoculation and nutrient addition, an initial 20 mL sample from each flask was filtered (0.2  $\mu\text{m}$ ), and DOC concentration was determined as described above. Flasks were stored in the dark at 20°C for 28 days and shaken weekly. Following the incubation period, a final 20 mL sample was filtered (0.2  $\mu\text{m}$ ) and DOC concentration was measured. Percent bioavailable dissolved organic carbon (BDOC) was calculated as the % decrease in initial DOC concentration during the 28-day incubation.

## **Statistical Analysis**

Correlations between cropland coverage and perennial stream DOM were tested using Pearson's product moment correlation coefficient,  $r$ . Differences in wetland outflow DOM among wetland types (e.g., forested, historical, restored) were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) multiple mean comparison tests. When necessary, data were log-transformed to meet the assumption of normality. Correlation analyses and ANOVA were

conducted using R version 2.12.2 (R Development Core Team, Vienna, Austria).

All statistical analyses were conducted with a significance level set at  $\alpha = 0.10$ .

To gain an understanding of the variability in perennial stream and wetland outflow DOM, I conducted principal component analysis (PCA). A PCA ordination diagram was plotted to visualize how samples cluster with axes generated using DOM concentration, fluorescence composition, and bioavailability. PCA was performed using the Vegan package in R version 2.12.2.

## **RESULTS**

### **Study 1: Perennial Stream DOM along a Cropland Cover Gradient**

Perennial stream dissolved organic carbon (DOC) concentration ranged from 2.8 - 52.0 mg L<sup>-1</sup> and decreased with increasing cropland coverage (Fig. 4.6A). I observed a negative correlation between cropland coverage and SUVA<sub>254</sub> and a positive correlation between cropland coverage and spectral slope (Fig. 4.6B,C), indicating decreased stream DOM aromaticity (Weishaar et al. 2003) and molecular weight (De Haan 1993, Dahlen et al. 1996), respectively, with increased cropland coverage. SUVA<sub>254</sub> ranged from 2.70 – 5.64 L mg C<sup>-1</sup> m<sup>-1</sup>, corresponding to a range in aromatic C content of 21 – 40% according to the linear model created by Weishaar et al. (2003). Fluorescence spectroscopic indices revealed decreased humification index (HIX) values and increased  $\beta:\alpha$  ratios with increasing cropland coverage (Fig. 4.6D,E), suggesting decreased stream DOM humic content (Zsolnay et al. 1999) and allochthonous origin

(Huguet et al. 2009), respectively, with increased cropland coverage within the watershed.

The relative contributions of PARAFAC model components C1 and C2, a UVC humic-like fluorophore of older origin and a fulvic acid-type fluorophore, respectively, were both negatively correlated with cropland coverage (Fig. 4.6F,G). Conversely, the relative contributions of components C3 and C4, a tryptophan-like fluorophore and a humic-like fluorophore of newer origin, respectively, increased with cropland coverage (Fig. 4.6H,I). The cumulative relative contribution of humic-like and fulvic acid-type fluorophores of terrestrial origin (i.e., C1 + C2 + C4), ranged from 71% – 86% and averaged  $80\% \pm 1\%$  (mean,  $\pm$  SE), corroborating the high HIX values and low  $\beta$ : $\alpha$  ratios observed across perennial stream DOM.

Percent bioavailable DOC (BDOC) ranged from 0 – 30.4% across the 20 perennial streams, and was significantly positively correlated with cropland coverage ( $r = 0.48$ ,  $p < 0.05$ ; Fig. 4.6J). Additionally, stream DOC bioavailability decreased with increasing DOM humic-like fluorescence (e.g., relative contribution of C1;  $r = -0.68$ ,  $p < 0.01$ ; Fig. 4.9A) and increased with increasing DOM protein-like fluorescence (e.g., relative contribution of C3;  $r = 0.68$ ,  $p < 0.001$ ; Fig. 4.9B).

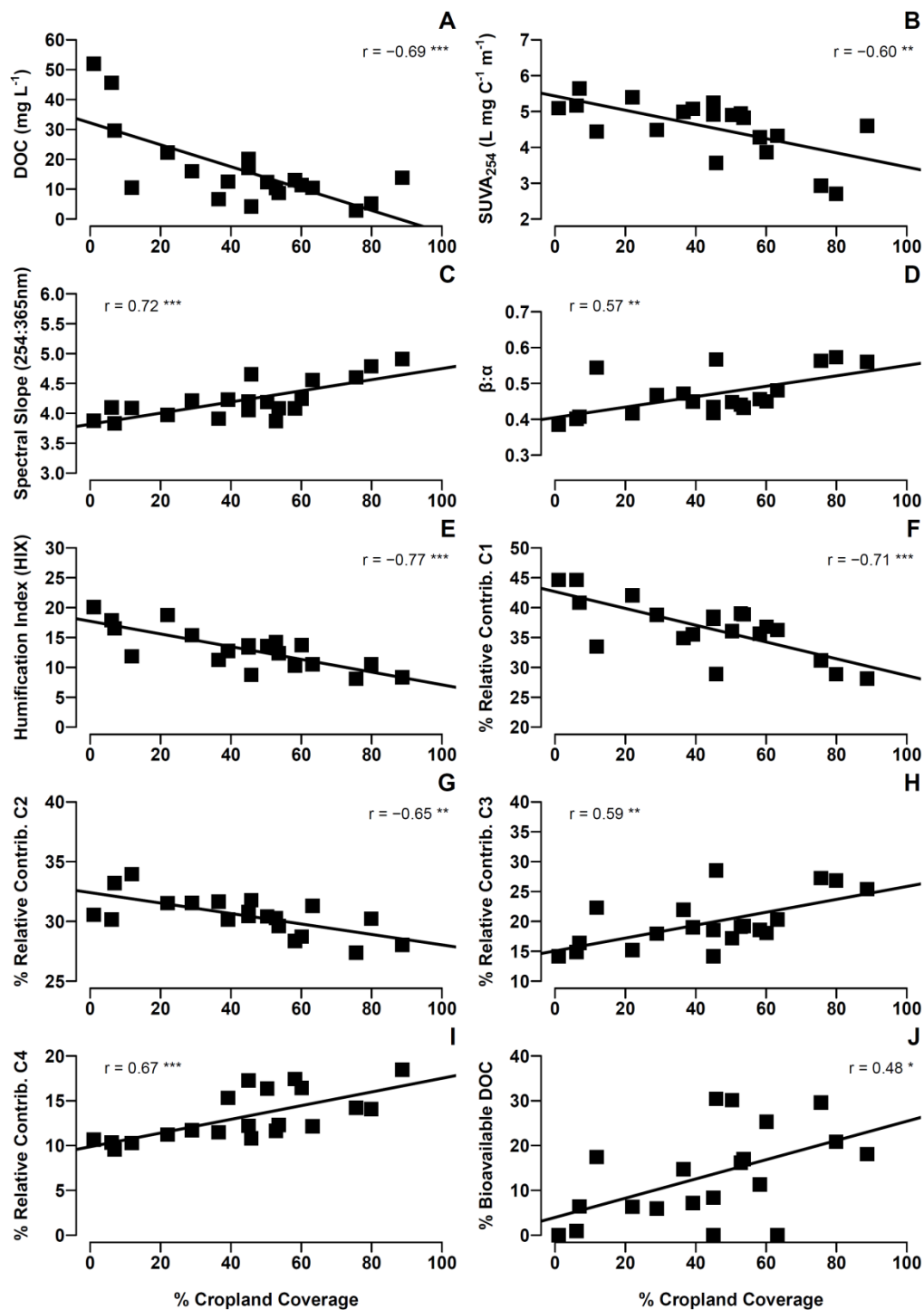
## **Study 2: Wetland Outflow DOM along an Agricultural Alteration Gradient**

DOC concentration was significantly greater in forested compared to historical and restored wetland outflows, averaging (mean,  $\pm$  SE)  $48.1 \pm 5.0$ ,  $14.2 \pm 2.4$ , and  $21.4 \pm 3.0$  mg L<sup>-1</sup>, respectively (Fig. 4.7A). DOM exported from forested wetlands was

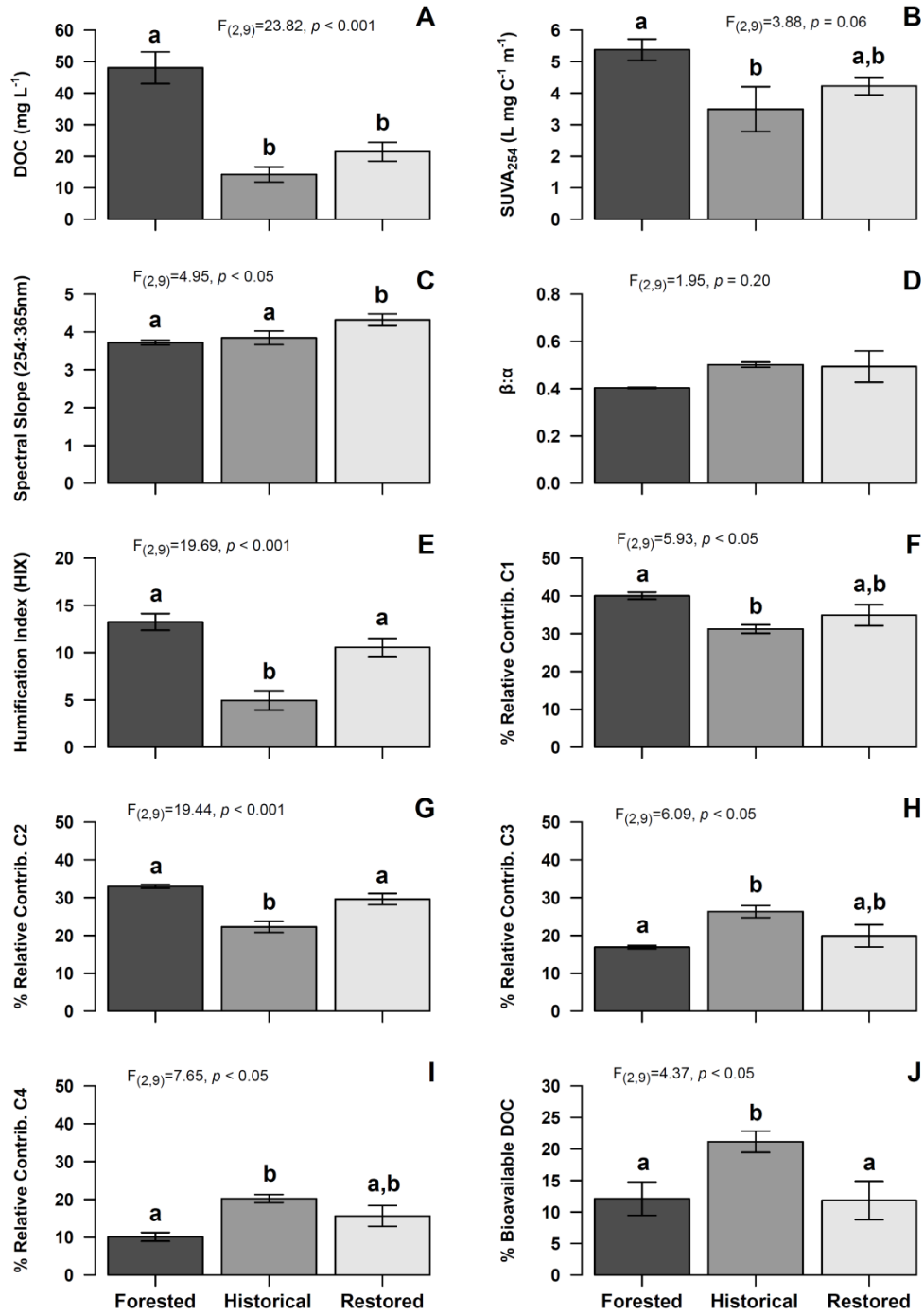
characterized by higher aromaticity, humic content, and old humic- and fulvic acid-like fluorescence relative to DOM draining historical wetlands (Fig. 4.7B,E,F,G). Conversely, DOM exported from historical wetlands had higher protein- and new humic-like fluorescence compared to forested wetlands (Fig. 4.7H,I). Historical wetland-derived DOM was also more labile (Fig. 4.7J). DOM quality was not significantly different between forested and restored wetland outflows for eight of nine calculated metrics (Fig. 4.7).

### **Study 1 + Study 2: Comparison of Perennial Stream and Wetland Outflow DOM**

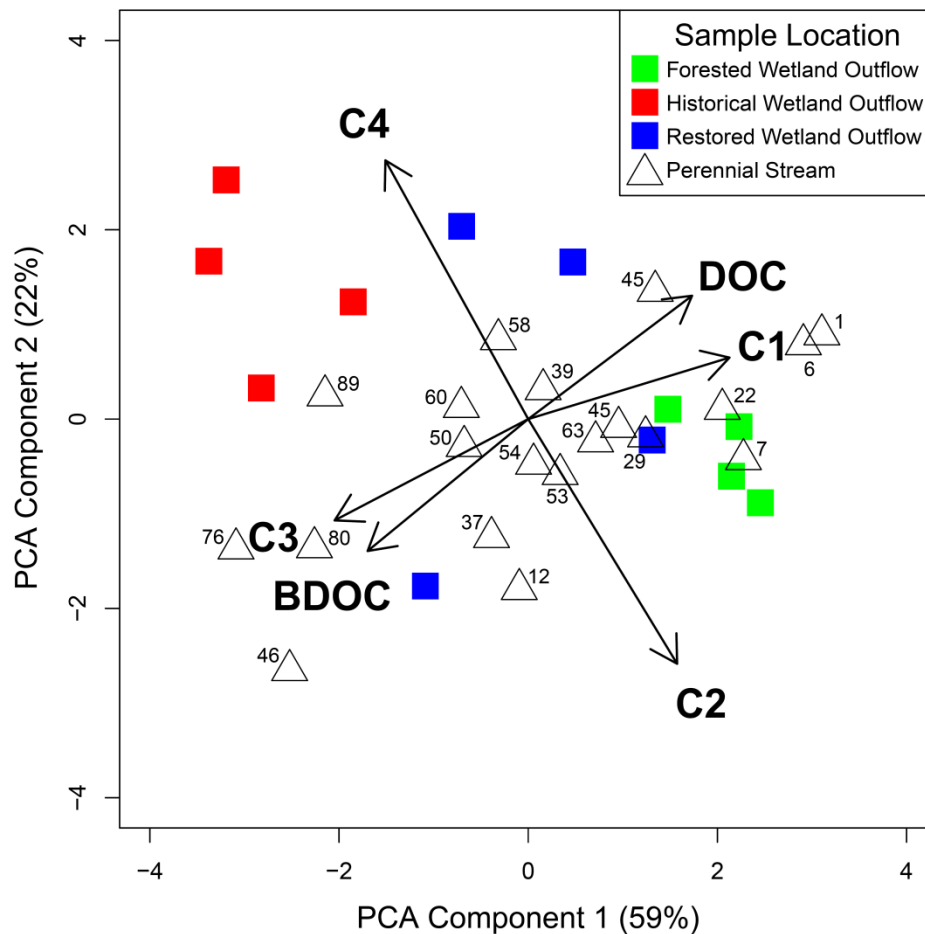
Together, the first two components of the PCA incorporating perennial stream and wetland outflow DOM concentration, composition, and bioavailability accounted for 81% of the variability in the data (Fig. 4.8). The PCA biplot indicates increased DOM concentration (DOC) and humic-like fluorescence (C1) in forested wetland outflows and perennial streams draining catchments with minimal cropland coverage. Alternatively, protein-like fluorescence (C3) and bioavailability (BDOC) appear to increase in historical wetland outflows and streams draining abundant cropland. DOM exported from restored wetlands, while variable, clusters between DOM delivered from forested and historical wetlands and compares favorably to perennial stream DOM in catchments with intermediate amounts of cropland coverage.



**Figure 4.6:** Correlations between cropland coverage in a watershed and perennial stream dissolved organic matter metrics. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ . **A)** Dissolved organic carbon concentration, **B)** specific UV-absorbance at 254nm, **C)** UV-absorbance slope at 254:365nm, **D)**  $\beta:\alpha$  fluorescence ratio, **E)** humification index, **F-D)** percent relative contributions of PARAFAC fluorescence components C1-4, respectively, and **J)** percent bioavailable dissolved organic carbon.



**Figure 4.7:** Comparison of dissolved organic matter exported from forested ( $n = 4$ ), historical ( $n = 4$ ), and restored ( $n = 4$ ) wetlands. **A)** Dissolved organic carbon concentration, **B)** specific UV-absorbance at 254nm, **C)** UV-absorbance slope at 254:365nm, **D)**  $\beta:\alpha$  fluorescence ratio, **E)** humification index, **F-I)** percent relative contributions of PARAFAC fluorescence components C1-4, respectively, and **J)** percent bioavailable dissolved organic carbon. Error bars represent standard errors of the means. Means with different letters are significantly different from one another (Tukey's HSD,  $p < 0.10$ ).



**Figure 4.8:** Principal component analysis (PCA) ordination diagram grouping perennial stream and wetland outflow samples with respect to a suite of dissolved organic matter metrics. Numbers associated with perennial stream data points indicate the percentage of cropland coverage within the watershed. (DOC = log-dissolved organic carbon concentration, C1 - C4 = percent relative contribution of PARAFAC fluorescence components C1 - C4, respectively, BDOC = percent bioavailable dissolved organic carbon). Percent values on each axis represent percent of explained variance.



## **DISCUSSION**

This research suggests that agricultural wetland restoration may be an effective means to restore dissolved organic matter (DOM) composition and bioavailability and, in turn, downstream DOM quality in cultivated watersheds. Additionally, I demonstrate — to my knowledge for the first time — a relationship between human land use and stream DOC bioavailability, whereby lability increases with cropland coverage. A suite of perennial stream DOM metrics correlated strongly with cropland coverage (Study 1), suggesting a shift has occurred from high concentration, largely humic, recalcitrant DOM under wetland-dominated conditions to a pool of lower concentration, protein-rich, more labile DOM as wetlands are lost at the expense of agriculture. These changes corroborate the findings of others (Wilson and Xenopoulos 2009, Williams et al. 2010, Petrone et al. 2011) and add to a growing recognition of DOM alteration in human-modified landscapes (Stanley et al. 2012).

The comparison of DOM exported from forested, historical, and restored wetlands to the perennial stream network (Study 2) provides insights of the role of wetland alteration on downstream DOM. Significantly lower DOC concentration observed in historical relative to forested wetland outflows implies wetland loss to cropland contributes to decreases in perennial stream DOC. Wetland coverage has long been shown to correlate positively with fluvial DOC quantity (Mulholland 2003), while croplands tend to result in low stream DOC concentration and flux (Aitkenhead and McDowell 2000, Mattsson et al. 2009). Prior to European settlement and the clearing and drainage of wetlands for cultivation, the Coastal Plain was dominated by anastomosing

streams and extensive vegetated wetlands that stored significant organic carbon (Walter and Merritts 2008). Many Coastal Plain wetlands experienced significant aggradation due to deforestation and poor agricultural practices (Jacobson and Coleman 1986). With increased forest conservation and improved farming techniques came reduced sedimentation, leading to channel incision and disconnected floodplains (Hupp 2000). Cultivated uplands may have historically delivered high DOC loads to Coastal Plain streams as a result of long flowpaths through organic-rich floodplain-dominated landscapes.

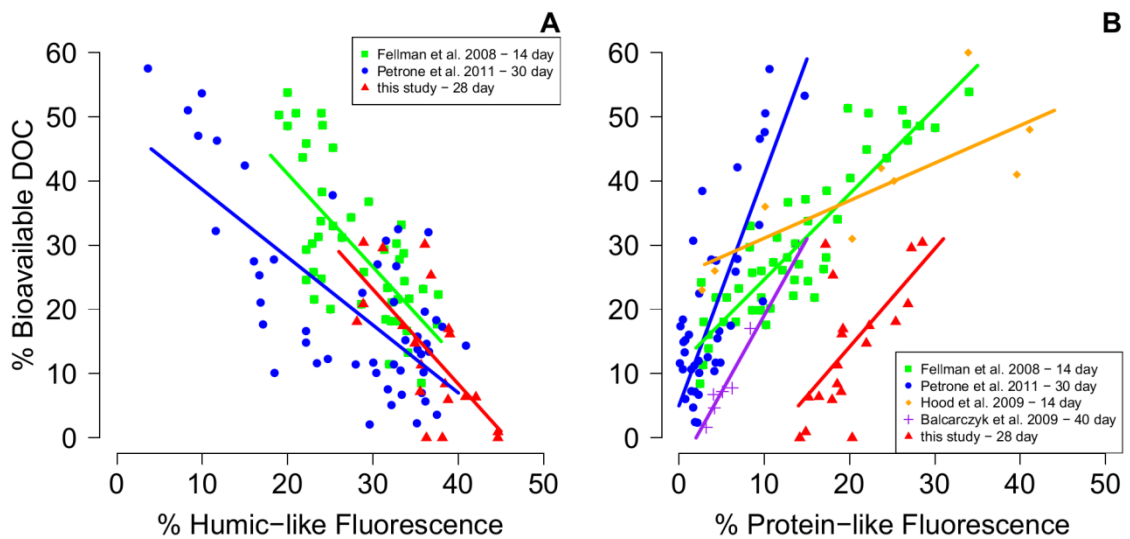
Cultivation is also associated with decreased terrestrial organic matter pools (Ogle et al. 2005, McLauchlan 2006), and wetland conversion to cropland results in particularly large losses of soil organic matter (Fenstermacher 2012). In an investigation of soils among the same wetlands in this study, I found significantly lower soil organic matter values among historical and restored wetlands compared to forested wetlands (see Chapter 1). Low restored wetland outflow DOC concentrations may be due in part to a lack of recovery of soil organic matter pools in the brief time since restoration (e.g., 8 – 9 years at the time of this study). Considering restoration of natural soil organic matter levels is generally slow, often requiring decades to centuries depending on the ecosystem (Hossler and Bouchard 2010), recovery of more natural DOC levels in restored wetlands and downstream waters may likewise take a significant amount of time.

DOM exported from restored wetlands was largely similar in composition to forested wetland-derived DOM, characterized by high aromaticity and humic content and low protein-like fluorescence.  $SUVA_{254}$  and spectral slope values indicated perennial

stream DOM with low aromaticity (Weishaar et al. 2003) and molecular weight (De Haan 1993, Dahlen et al. 1996) in cropland-dominated catchments. I found similarly low aromaticity in DOM exported from historical wetlands, corroborating observations of low DOM molecular weight and humic content in cultivated soil pore-water (Delprat et al. 1997, Kalbitz et al. 2003). The high aromaticity and molecular weight of forested wetland-derived DOM is consistent with previous observations of increased DOM humic content and structural complexity in natural wetlands (Mladenov et al. 2007, Inamdar et al. 2012). DOM aromaticity and molecular weight are known to negatively correlate with DOC bioavailability (Kalbitz et al. 2003, Roehm et al. 2009). This suggests the increase in DOC bioavailability in perennial streams and wetland outflows along a gradient of agricultural alteration may in part be governed by low DOM aromatic content and conjugation.

Fluorescence spectroscopy highlighted a shift from allochthonous, humic-like DOM to more autochthonous, protein-like DOM composition in perennial streams and wetland outflows with increased cultivation. Corroborating this, Inamdar et al. (2012) reported low protein-like fluorescence in wetland soil water samples while Wilson and Xenopoulos (2009) found stream protein-like fluorescence increased as wetlands were lost to agriculture. DOM protein-like fluorescence may increase as a result of anthropogenic runoff (Baker and Spencer 2004, Petrone et al. 2011), increased primary production (Lu et al. 2003), and decreased soil organic matter-water interaction (Hood et al. 2006), all three of which may be responsible for DOM changes among wetlands and streams in cultivated landscapes.

DOM composition was strongly related to DOC bioavailability. Similar to my findings, decreased DOC bioavailability with increased humic-like fluorescence and increased lability with increased protein-like fluorescence has been reported in a variety of aquatic ecosystems (soil water - Fellman et al. 2008; streams draining permafrost - Balcarczyk et al. 2009; glaciated rivers - Hood et al. 2009; streams in agricultural and urbanized catchments - Petrone et al. 2011; Fig. 4.9), indicating DOM fluorescence may be useful in predicting DOC lability.



**Figure 4.9:** Comparative regressions of percent bioavailable dissolved organic carbon and initial percent humic-like fluorescence (A) and initial percent protein-like fluorescence (B).

It has been hypothesized that anthropogenic alteration within watersheds (e.g., agriculture, urbanization) may increase downstream DOC bioavailability (Williams et al. 2010). Results from this study support this, but also present wetland restoration as a potential means to restore more natural stream DOC bioavailability. Increased stream

DOC bioavailability due to wetland conversion to cropland could lead to shorter DOC uptake lengths along fluvial networks. This, in turn, could reduce DOM delivery to downstream ecosystems, decreasing rates of microbial respiration and biogeochemical processing (Royer and David 2005). I observed signs of humic- and protein-like fluorescence recovery in DOM exported from restored wetlands, resulting in more natural bioavailability of DOC delivered downstream.

## **CONCLUSION**

Past work suggests that fluvial DOM is governed by large, watershed-scale processes including terrestrial accumulation of soil organic matter, catchment topography, and hydrologic flow paths (Aitkenhead-Peterson et al. 2003, Andersson and Nyberg 2009). However, the finding that DOM quality is showing signs of recovery in recently restored agricultural wetlands suggests wetland restoration may be beneficial. In their recent review, Stanley et al. (2012) state that land use change and human modification of the landscape, particularly cultivation, are “clearly altering native DOC regimes” and suggest restoration efforts are needed to better manage fluvial DOM. Results from this study underscore the alteration of freshwater DOM quantity and quality in response to cultivation and offer evidence that agricultural wetland restoration may provide a means to restore more natural downstream DOM, particularly with respect to composition and lability. Further study of wetland and riparian restoration effects on DOM quantity and quality across a range of temporal and spatial scales should be conducted in order to better manage contemporary changes in DOM.

## Summary

In this dissertation, I investigate the impact of agricultural wetland restoration on 1) within-wetland ecosystem structure and function (e.g., soil physicochemical properties, denitrification potential, sediment and nutrient accumulation [Chapter 1]) and 2) adjacent temporary and perennial stream ecosystems (e.g., patterns of surface hydrologic connectivity [Chapter 2]; temporary stream denitrification potential in response to sediment drying and wetting [Chapter 3]; changes in wetland and perennial stream dissolved organic matter quantity and quality along an agricultural alteration gradient [Chapter 4]).

While wetlands and streams have largely been studied independently, I quantify hydrological and ecological links between wetlands and adjacent streams. This work is motivated by recent U.S. Supreme Court rulings (e.g., *Solid Waste Agency of Northern Cook County v U.S. Army Corps of Engineers* 2001, *Rapanos v United States* 2006) that call into question the jurisdictional status of geographically isolated wetlands and non-perennial streams under the Clean Water Act and generate a need for basic research on wetland–stream connectivity to inform future policy and legislation (Leibowitz et al. 2008). Additionally, I seek to understand the degree to which restoration of ecosystem benefits generally attributed to wetlands in agriculturally-modified landscapes extend beyond wetlands themselves to nearby streams.

With respect to the impact of agricultural wetland restoration on wetland soils and sediments (Chapter 1), I show that recently restored Delmarva bay wetland soils are similar to historical wetland soils in terms of physicochemical properties and capacity to

denitrify. Organic-rich forested wetland soils, however, are fundamentally different from both historical and restored wetland soils. In a recent study of Delmarva bay carbon sequestration, Fenstermacher (2012) estimated it would require nearly 70 years for restored bays to achieve the level of carbon present in natural soils. While soil recovery may be slow in restored wetlands (Hossler and Bouchard 2010), I report significant net sediment and nutrient accumulation in restored Delmarva bays suggesting formerly-farmed depressional wetlands may act as sinks for carbon, nitrogen, and phosphorus. Future studies incorporating mass-balances could help determine if in fact restored Delmarva bays are mitigating downstream sediment and nutrient loads.

In Chapters 2 and 3, I move beyond wetlands to study the impact of agricultural wetland restoration on adjacent temporary stream processes. Using state data loggers, I present evidence that forested Delmarva bay wetlands, although historically considered geographically isolated from nearby surface waters (Tiner and Burke 1995, Tiner 2003), may exhibit seasonal surface hydrologic connectivity (SHC) to adjacent perennial streams via temporary stream flow when temperatures and evapotranspiration are low. Agricultural drainage appears to have altered wetland–stream SHC patterns, generating more ephemeral connections. Changes in wetland–stream SHC due to cultivation may persist post-restoration, particularly if the restoration is small in size. Alterations in wetland–stream SHC from seasonally continuous and predictable to ephemeral and sporadic are likely to limit the ability of wetlands and temporary stream channels to provide habitat, dispersal corridors, and downstream subsidies. The state data logging method I use to record wetland–stream SHC patterns could be a valuable tool to support

regulatory determinations of so-called isolated wetlands and temporary streams in a variety of settings.

With knowledge of wetland–stream SHC patterns (Chapter 2), I then explore the role of sediment drying and wetting on denitrification potential in temporary streams adjacent to a restored and a forested Delmarva bay (Chapter 3). I find that alterations in surface hydrology, a likely result of both land use change and climate change (Poff et al. 1997, Palmer et al. 2008), may alter the capacity of temporary streams to denitrify. I present evidence of hysteresis in restored temporary stream sediment denitrification potential in response to wetting vs. drying, whereas organic-rich forested temporary stream sediments appear more biogeochemically resistant to altered hydrology. Typically located at the headwaters of river networks, temporary streams may be effective nutrient processors (Peterson et al. 2001), yet particularly sensitive to anthropogenic disturbance (Bull 1997). As such, they may be ecosystems ripe for restoration in nutrient-rich landscapes (e.g., agricultural, urbanized). Yet, to my knowledge, this is the first study of a restored temporary stream in the literature. As ephemeral and intermittent streams comprise a majority of river length in most fluvial networks (Nadeau and Rains 2007) and the prevalence of non-perennial flow is predicted to increase with global climate change (Schindler 1997, 2001), understanding the physical, chemical, and biological impacts of drought and inundation on temporary streams is increasingly critical.

Finally, I investigate the role agricultural wetland restoration may play in restoring downstream dissolved organic matter (DOM) quantity and quality in an agriculturally-modified landscape (Chapter 4). Although it is now recognized that human



land use, particularly agriculture, may alter the concentration and composition of stream DOM (Wilson and Xenopoulos 2009), investigation of management options to restore fluvial DOM is rare (Stanley et al. 2012). In a two-part study, I find that 1) cropland cover within a watershed significantly alters perennial stream DOM concentration, composition, and bioavailability, and 2) agricultural wetland restoration may be a tool to recover more natural stream DOM quality.

Collectively, this research provides evidence of hydrological and ecological connectivity between so-called isolated wetlands and adjacent streams. As questions surround the definition of “waters of the United States” and the jurisdictional scope of the Clean Water Act (Leibowitz et al. 2008), this type of information is increasingly needed and provides a means for science to inform regulatory determinations and future policy. Additionally, this research suggests agricultural wetland restoration has the potential to recover the physical (e.g., surface hydrologic connectivity [Chapter 2]), chemical (e.g., DOM composition [Chapter 4]), biological (e.g., DOM bioavailability [Chapter 4]), and biogeochemical (e.g., denitrification potential [Chapter 3]) integrity of nearby running waters.

## Appendix

I estimated dissolved organic carbon (DOC) export via surface flow from the wetland–stream pairs studied in Chapter 2 for the 2010 water year (1 Oct 2009 – 30 Sept 2010).

Wetland–stream pairs were visited monthly from Oct 2009 to Sept 2010. If present, baseflow discharge ( $\text{L s}^{-1}$ ) in temporary streams connecting the study wetlands to adjacent perennial streams was measured using either a 1L graduated cylinder and stopwatch or the cross-sectional area method (Gordon et al. 2004; Table A.1). A grab sample was collected when surface water was present within each wetland. A baseflow grab sample was collected when temporary streams were flowing (see Fig. 4.4). All samples were collected in acid-washed high-density polyethylene bottles, kept on ice in the dark, and immediately returned to the laboratory for processing. Wetland and temporary stream samples were filtered within 24 hours of collection using pre-combusted  $0.7 \mu\text{m}$  glass fiber filters (Whatman International Ltd, Maidstone, England). Total dissolved organic carbon (DOC) concentrations were measured using a Shimadzu TOC-V<sub>CPH</sub> total organic carbon analyzer (Shimadzu Scientific Instruments, Columbia, MD; Table A.2).

Lower and upper bounds of DOC exported from each wetland were estimated (Table A.3) by multiplying the lowest and highest observed discharge by the lowest and highest measured DOC concentration, respectively. These values were multiplied by the total duration of wetland–stream surface hydrologic connectivity (SHC; see Table 2.2) and standardized by wetland area (see Table 2.1).

**Table A.1:** 2010 water year Monthly baseflow discharge measurements at each study site. F = forested, R = restored, H = historical, \* = no measurement taken, — = dry / no discharge.

Site	Monthly Baseflow Discharge ( $L s^{-1}$ )											
	Oct '09	Nov '09	Dec '09	Jan '10	Feb '10 *	Mar '10	Apr '10	May '10	Jun '10	Jul '10	Aug '10	Sep '10
<b>F1</b>	11.68	9.06	*	54.19	*	33.9	15.25	—	—	—	—	—
<b>F2</b>	—	0.45	*	2.68	*	1.03	0.8	—	—	—	—	—
<b>F3</b>	—	0.19	*	1.16	*	1.1	0.55	—	—	—	—	—
<b>F4</b>	1.84	0.6	*	1.74	*	1.81	1.42	—	—	—	—	—
<b>R1</b>	—	*	*	*	*	*	—	—	—	—	—	—
<b>R2</b>	—	22.54	*	6.04	*	18.21	—	—	—	—	—	—
<b>R3</b>	—	1.1	*	0.12	*	0.03	0.1	—	—	—	—	—
<b>H1</b>	0.1	0.03	*	0.16	*	0.13	0.09	—	—	—	—	—
<b>H2</b>	—	0.75	*	—	*	0.24	—	—	—	—	—	—
<b>H3</b>	—	0.27	*	0.05	*	—	—	—	—	—	—	—
<b>H4</b>	—	0.44	*	—	*	0.38	—	—	—	—	—	—

**Table A.2:** 2010 water year monthly dissolved organic matter concentrations at each wetland and adjacent temporary stream. F = Forested, R = Restored, H = Historical, \* = no measurement taken, — = dry

Site	Sample Location	Oct '09	Nov '09	Dec '09	Jan '10	Feb '10	Mar '10	Apr '10	May '10	Jun '10	Jul '10	Aug '10	Sep '10
F1	wetland	67.2	61.2	*	13.6	*	18.5	34.1	37.4	50.6	—	—	—
	temp stream	64.6	62.1	*	15.1	*	18.0	33.7	—	—	—	—	—
F2	wetland	77.4	62.9	*	18.6	*	23.2	37.4	39.2	—	—	—	—
	temp stream	—	60.2	*	23.7	*	18.7	35.5	—	—	—	—	—
F3	wetland	57.3	63.0	*	21.8	*	18.7	30.9	32.4	53.3	—	—	—
	temp stream	—	62.9	*	23.7	*	18.7	31.5	—	—	—	—	—
F4	wetland	62.1	54.7	*	17.5	*	18.2	33.3	27.4	—	—	—	—
	temp stream	60.1	58.2	*	18.9	*	22.3	33.9	—	—	—	—	—
R1	wetland	13.4	12.3	*	7.7	*	7.6	12.2	16.6	—	—	15.7	17.6
	temp stream	—	13.4	*	7.8	*	9.4	—	—	—	—	—	—
R2	wetland	21.5	21.7	*	24.5	*	13.9	20.5	28.5	61.3	—	18.9	26.7
	temp stream	—	27.2	*	23.6	*	20.6	—	—	—	—	—	—
R3	wetland	22.2	22.0	*	12.3	*	18.4	21.9	29.2	—	—	—	—
	temp stream	—	24.9	*	14.7	*	21.0	25.8	—	—	—	—	—
H1	wetland	17.4	21.4	*	10.1	*	12.7	22.4	—	—	—	—	—
	temp stream	20.3	19.8	*	11.2	*	15.8	26.0	—	—	—	—	—
H2	wetland	—	20.3	*	10.9	*	9.7	23.3	—	—	—	—	—
	temp stream	—	17.1	*	—	*	9.5	—	—	—	—	—	—
H3	wetland	48.6	15.2	*	15.7	*	18.5	17.5	—	—	—	—	—
	temp stream	—	15.2	*	15.6	*	—	—	—	—	—	—	—
H4	wetland	—	16.3	*	13.7	*	25.6	—	—	—	—	—	—
	temp stream	—	17.5	*	—	*	23.4	—	—	—	—	—	—

**Table A.3:** Estimated dissolved organic carbon (DOC) export from wetlands to adjacent streams via temporary stream flow during the 2010 water year. F = forested, R = restored, H = historical.

<b>Site</b>	<b>Estimated DOC Export (kg ha<sup>-1</sup>)</b>
<b>F1</b>	76 – 2092
<b>F2</b>	43 – 754
<b>F3</b>	33 – 671
<b>F4</b>	13 – 139
<b>R1</b>	(unable to estimate)
<b>R2</b>	116 – 612
<b>R3</b>	5 – 312
<b>H1</b>	1 – 13
<b>H2</b>	7 – 51
<b>H3</b>	1 – 4
<b>H4</b>	6 – 13

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