

# **Describing Nitrogen Dynamics in Eastern North Carolina Waterfowl Impoundments by Combining Field-Scale and Laboratory-Scale Based Approaches**

by

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## **Abstract**

Waterfowl impoundments are wetlands that are hydrologically managed to provide food and habitat for migratory bird populations. Certain impoundment types have recently been found to export nitrogen (N) loads during seasonally prescribed drawdowns. A recent study observed these high N loads in moist-soil managed (MSM) waterfowl impoundments, while knowledge of N export in other impoundment types, such as seasonally flooded agricultural fields (Ag), is lacking. Literature describing the N cycling properties of impoundment soils at the laboratory scale is also scarce. This study focused on (1) identifying the effects of storms and drawdowns on N dynamics at the field scale, (2) comparing seasonal N cycling differences between and within MSM and Ag impoundment soils, and (3) evaluating the potential for their soils to act as treatment wetlands. I monitored N dynamics at an Ag and MSM impoundment in the Lake Mattamuskeet (Hyde County, NC) watershed for 17-months. Storm events were identified during this period and their influence on N dynamics was examined. N concentrations were monitored at a frequency of 30-minutes using *in situ* spectroscopy. Twelve soil cores were collected at each site at three time points to represent Summer, Fall, and Winter conditions. Soil cores were assayed for nitrification, denitrification, and N-mineralization potential rates, and these rates were compared to soil physiochemical properties (soil moisture, pH, nitrate, ammonium, total

soil carbon and nitrogen). Mesocosms were collected from each of the coring locations at each site, subjected to monthly N-amended simulated rainfall for 6 months and assessed for changes in N concentrations at 24 and 72 hours post rainfall. At the field scale, I observed substantial differences in N dynamics between the Ag and MSM systems when the Ag impoundment was not flooded, while similar N dynamics were observed in both systems during the fall and winter flooding period. I found that seasonal flooding from early October through late March resulted in greatly increased ambient  $\text{NH}_4\text{-N}$  conditions. Storm events stimulated coupled nitrification-denitrification processes in these open water systems. At the laboratory scale, I found substantial differences of seasonal N cycling dynamics between and within the Ag and MSM sites. Hydrologic management was identified to be the main driver of variability in N cycling dynamics at the Ag site, and a combination of low soil pH and hydrologic management drove variability in MSM N cycling dynamics. I identified nitrification and denitrification processes to be strongly coupled in both systems, resulting in low denitrification potential in both sites. In addition, soils from both sites showed limited potential to act as treatment wetlands under experimental mesocosm conditions. This study demonstrates that N dynamics of different waterfowl impoundment types can function similarly during seasonal flooding periods. As such, timing seasonal drawdowns of waterfowl impoundments shortly after storm events can be a strategy to enhance coupled nitrification-denitrification processes that result in less inorganic N being exported to downstream systems. I suggest changes in Ag and MSM hydrologic management, as well as efforts to increase soil pH at MSM, to stimulate nitrification potential to promote N removal via denitrification year-round.



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Combining Field-Scale and Laboratory-Scale Based Approaches**

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Brian Hinckley  
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## **Chapter 1**

### **Introduction**

Eutrophication and increasing hypoxic conditions of coastal waters have drastically increased due to increased anthropogenic nutrient loading (Smith et al. 2003; Paerl 2010; Rabalais et al. 2002; Liu et al. 2010). The estimated annual costs of eutrophication in aquatic systems is around \$2.2 billion in the U.S. alone (Dodds et al. 2008). Prolonged eutrophication in lakes has the potential to promote shifts from macrophyte to phytoplankton dominated states (Smith 2003). Such state shifts are detrimental to wildlife populations and aquatic vegetation (Lefcheck et al. 2017; Moorman et al. 2017) as increased algal blooms in lakes may decrease light attenuation through the water column and impair growth of submerged aquatic vegetation (SAV) (Moorman et al. 2017; Waters et al. 2009; Waters et al. 2010).

Coastal systems such as shallow lakes and estuaries often receive nutrient-laden drainage from upstream lands including urban and agricultural areas (Skaggs et al. 1980; Woltemade 2000; Gilbert et al. 2006; Trejo-Gaytan et al. 2006). Lakes are strongly affected by eutrophication due to long recovery periods of important lake qualities such as water clarity, submerged aquatic vegetation (SAV) density, and invertebrate populations. For example, a recent meta-analysis modeled scenarios with complete reductions of anthropogenic nutrient loadings to lakes and found water clarity recovery periods ranged from less than 5 to greater than 50 years, while SAV recovery periods ranged from less than 5 to greater than 90 years (McCrackin et al. 2016). The sensitivity of lakes to eutrophication highlights the necessity of improved nutrient management to protect and restore these systems (Vicente et al. 2006; Papastergiadou et al. 2007; Waters et al. 2009; Waters et al. 2010).

Wetlands are traditionally considered nutrient sinks, especially regarding inorganic forms of nitrogen (N) (Mitsch & Gosselink 2015). Wetlands facilitate N transformations, with N inputs being incorporated into biomass through assimilation by vegetative and microbial uptake (temporary) (Jordan et al. 2011) and microbial denitrification to gaseous forms (permanent) (Jordan et al. 2011; Racchetti et al. 2011). Heavy precipitation and flow events mobilize N from the soil profile and decrease residence time of wetland water, potentially shifting these N sinks to sources (Jordan et al. 2003, Etheridge et al. 2017). Wetlands whose water levels are managed to promote certain vegetation or wildlife populations (Nelms et al. 2007) have the potential to further increase N export. These wetlands may represent substantial unaccounted sources of N to coastal systems. For example, past research identified that waterfowl impoundments can export N loads at approximately the same magnitude as agricultural fields (Winton et al. 2016, Maul & Cooper 2000).

Waterfowl impoundments are wetlands that are hydrologically managed to create habitats for migratory bird populations (Nelms et al. 2007). Impoundments often play an important economic role in the local community, as they are the focus of activities such as bird hunting and viewing (Santos 2011). There are multiple types of waterfowl impoundments, including moist soil managed (MSM) and improvised agricultural waterfowl impoundments (Ag). Impoundment water levels are typically managed through water control mechanisms such as dikes, flashboard risers, and pumps (Nelms et al. 2007). MSM impoundments undergo prescribed drawdowns, intended to promote growth of specific vegetation to provide as much food as possible for migratory bird populations. The timing and magnitudes of MSM drawdowns are highly variable depending upon location and target vegetation species, but generally occur between March and July (Nelms et al. 2007). Further physical measures, such as disking and mowing, may be

implemented to control or remove undesirable vegetation (Nelms et al. 2007). Flooding of MSM impoundments typically occurs in the fall and persists through winter seasons, which is intended to coincide with waterfowl arrival (Nelms et al. 2007). In contrast, Ag impoundments are managed as agricultural fields that use standard methods to grow and harvest crops (Maul & Cooper 2000). Additionally, Ag impoundments may require drainage systems to maximize crop yields (Smith et al. 2011). Agricultural drainage systems, such as tiling used in the Midwestern U.S., greatly increase the rate at which nutrient-laden water is exported from agricultural fields and have been shown to increase N export (Woli et al. 2010). Post growing season, crops are partially harvested or not harvested, and the fields are flooded to facilitate waterfowl habitat (Maul & Cooper 2000).

A deeper understanding of the drivers of N dynamics and export in impoundments is required to improve nutrient management strategies. Fluctuating water levels strongly impact N cycling dynamics, with waterlogged conditions stimulating denitrification, the anoxic process where microorganisms convert nitrate ( $\text{NO}_3^-$ ) to nitrous oxide ( $\text{N}_2\text{O}$ ) or dinitrogen gas ( $\text{N}_2$ ) (Racchetti et al. 2011). Drainage activities aerate soils and can stimulate nitrification, the microbial conversion of ammonium ( $\text{NH}_4^+$ ) to  $\text{NO}_3^-$  under oxic conditions (Peralta et al. 2013). Anoxic or oxic N-mineralization, the conversion of organic N (ON) to  $\text{NH}_4^+$ , provides a  $\text{NH}_4^+$  source to fuel the nitrification processes (Geisseler et al. 2010). Assimilation processes, the vegetative and microbial uptake and conversion of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  to ON, also affect N balances (Nizzoli et al. 2014). Additionally, organic matter represents an important microbial energy source, and its availability has been identified to potentially be the most important limiting factor in N cycling processes (Castaldelli et al. 2013). Therefore, soil organic matter and dissolved

organic carbon (DOC) availability is a factor that may strongly affect N cycling processes in waterfowl impoundments.

Storms have potential to enhance N mobilization and export in several different manners. Storms can suspend sediments and organic matter, potentially stimulating N mineralization processes (Xiang et al. 2008) and mobilizing N in aquatic systems (Inamdar et al. 2015). Dissolved oxygen (DO) concentrations fluctuate greatly during storms (Wehmeyer and Wagner 2011), and aerobic or anaerobic soil conditions dictate nitrification or denitrification rates. Storms also alter DOC concentrations (Chow et al. 2013), affecting microbial N processing rates (Castaldelli et al. 2013). Understanding the effects of storms on N mobilization and export within waterfowl impoundments has the potential to improve nutrient management. For example, coinciding drawdowns with minimum N conditions can reduce N export substantially (Winton et al. 2016).

Typical water quality sampling frequencies range from weekly to monthly in previous studies of N in waterfowl impoundments (Winton et al. 2016, Maul & Cooper 2000). This has potential to generate uncertainty of N concentrations and loadings, due to the exclusion of many storm events (Birgand et al. 2011; Etheridge et al. 2014). Thus, nutrient dynamics can be described more accurately when the sampling frequency is increased to the hourly or more frequent scales (Birgand et al. 2016; Etheridge et al. 2015; Etheridge et al. 2017). Monitoring using *in situ* sensors greatly improve descriptions of N dynamics and quantification of N export from waterfowl impoundments.

Lake Mattamuskeet (Hyde County) is the largest natural lake (16,228 ha) in North Carolina. The lake drains a coastal watershed where the most prominent industries are agriculture and waterfowl hunting and viewing. Hyde County has flat topography, high water

tables and low elevations (~ 1m above MSL), necessitating the use of pumping for water management in this watershed. Since the early 1990's, the lake has experienced a consistent decline of historically dense beds of SAV, largely attributed to a decrease in light attenuation and increased nutrient concentrations, pH, and phytoplankton growth (Moorman et al. 2017; Waters et al. 2010; Waters et al. 2009). The work by Winton et al. (2016) highlighted the need, not only for a better understanding of N dynamics in waterfowl impoundments, but also a need to reduce the N export from waterfowl impoundments while preserving their crucial functions.

### **Objectives/Hypotheses**

The chapter 1 objective was to examine the effects of storms on dissolved N processing and export at one Ag and one MSM impoundment using field-collected nutrient measurements. I hypothesizes that storms will stimulate N mineralization processes and mobilize  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N at both sites.  $\text{NO}_3^-$ -N concentrations will be greater at Ag than MSM due to more aerated soils, while the opposite will occur with  $\text{NH}_4^+$ -N concentrations. Drawdown will stimulate N processing and export in both systems, with  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N being the main N species exported at respective Ag and MSM sites. *The goal of this chapter was to characterize field-scale N dynamics within Ag and MSM waterfowl impoundments during pumping and storm events using in situ collected physical and chemical data.*

### **Materials & Methods**

**Site Description.** Two waterfowl impoundments in the Lake Mattamuskeet watershed, a 66.5 ha agricultural field (Ag) on the western side of the lake and a 170 ha moist soil management (MSM) impoundment on the eastern side of the lake, were monitored from July 2016 through January 2018. Both sites are hydrologically isolated, and drainage of the impoundments is

controlled by pumping stations. The Ag site is separated into 3 fields (Figure 1B) with fields 1 and 3 used to grow cotton and corn, respectively. Field 2 had plots of corn and cotton that were separated by parallel drainage ditches. The cotton was harvested at all locations. The corn in field 3 and a portion of the corn in field 2 was not harvested and was left in place to provide food and habitat for waterfowl. Standard production methods are used to grow the corn and cotton. The MSM site is managed by the U.S. Fish and Wildlife Service (USFWS). Vegetation present at MSM includes *Juncus spp.*, *Spartina spp.*, *Phragmites spp.*, among others.

**Water Level Monitoring.** Water level was measured every 15 minutes using pressure transducers (HOBO U20) at the pump inlet (impoundment side) of each site. Rainfall was measured using a tipping bucket (Rain Collector II Davis) with logger (HOBO Pendant) and a manual backup gauge (All Weather Rain Gauge Productive Alternatives). Precipitation data from USGS Station # 352936076125245 (Latitude 35°29'35.65", Longitude 76°12'52.39") was used when there were equipment failures of the on-site MSM monitoring equipment. This USGS station was located just off of the road that roughly divides the lake in half, which is approximately 13 miles from the MSM impoundment (see Supplemental Data).

**Water Quality Monitoring.** Water quality was monitored every 30 minutes throughout the monitoring period at the pump inlets of both sites (Figure 1). Measurements were made using multi-parameter probes (Eureka Water Probes Manta2) that measured pH, temperature, turbidity, and dissolved oxygen (DO), and UV-Visible spectrometers (Scan Spectrolyser) measured absorbance ranging from 200-750 nm at 2.5 nm increments. Instruments were mounted approximately 0.3 m below the minimum expected water level at the pumping inlet. Automated water samplers (AS950 Hach Sigma) collected samples for laboratory analyses at times coinciding with absorption spectra measurement immediately prior to my arrival for equipment

servicing. Samples were collected from the samplers and stored on ice before being transported to the lab for analysis. Standard laboratory techniques were used to measure dissolved constituents ( $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N) following filtration using 0.45  $\mu\text{m}$  filters (APHA 2012). Total dissolved N (TDN) and DOC were measured in filtered water samples following Kjeldahl digestions (APHA 2012). Discrete samples were used to facilitate calibration of the spectrometers to measure  $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N, TDN, and DOC.

Field spectra measurements were related to parameters of lab measured samples using partial least squares regression (PLSR) as outlined by Etheridge et al. (2014). Parameter predictions were compared to lab-measured parameters and assessed for goodness of fit using coefficient of determination ( $R^2$ ) values (Table 1). Site-specific PLSR calibrations were developed for TDN and  $\text{NH}_4^+$ -N parameters. I applied a single DOC PLSR calibration to both sites to increase the predictive accuracy for the lower end of the concentration range. This was justified because PLSR calibrations can more accurately describe nutrient concentrations when lab samples with a wider range of values are included, and the Ag site had a low range of concentrations measured in the lab. All PLSR calibrations were developed using the pls package (Mevik et al., 2011) in the R environment (R v3.3.2, R Core Development Team 2015).  $\text{NO}_3^-$ -N PLSR calibrations were not applied to spectra due to a low maximum concentration from lab-analyzed samples (1.5  $\text{mg L}^{-1}$ ). Therefore  $\text{NO}_3^-$ -N was accounted for using the s::can  $\text{NO}_3^-$ -N output, and calibrated using lab-measured  $\text{NO}_3^-$ -N data ( $R^2 = 0.91$ ) from both sites (See Supplemental Data).  $\text{NO}_3^-$ -N calibrations were developed using Microsoft Excel (Microsoft Office 2013). Dissolved organic N (DON) was computed by subtracting  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N from TDN values and used for all subsequent analysis.

### **Statistical/Data Analyses**

**Field Storm Event Analysis.** For the purpose of this study, storm events were defined as precipitation depths of greater than 1.3 cm occurring in a 24-hr period. This was done to establish criteria to capture storm events used to test the hypothesis stated in the “Objectives/Hypothesis” section above. The storm period was started when the first recorded precipitation occurred and ended when N parameter dynamics stabilized post-storm event. Multiple linear regression analysis was conducted to compare storm DON,  $\text{NO}_3^-$ -N, and  $\text{NH}_4^+$ -N parameters to cumulative rainfall, average water level, water level range, average temperature, average pH, average DOC, and average DO parameters. Due to the potential for storms to both increase and dilute N concentrations, I computed individual storm vectors (assigned magnitude and direction) for DON,  $\text{NO}_3^-$ -N, and  $\text{NH}_4^+$ -N concentrations to power the regression models. I justify the use of averages for explanatory variables, instead of vectors, because I was interested in how average environmental conditions affected N dynamics during storms. For example, cumulative rainfall would have less of a concentrating or diluting effect on N concentrations if average water levels were high, compared to lower average water levels. Storm DON,  $\text{NO}_3^-$ -N, and  $\text{NH}_4^+$ -N vectors were computed by taking the greatest deviation from initial storm DON,  $\text{NO}_3^-$ -N, and  $\text{NH}_4^+$ -N concentrations using equations 1 & 2.

$$(1) \text{ N concentration} = \text{Max N} - \text{Initial N}$$

$$(2) \text{ N dilution} = \text{Min N} - \text{Initial N}$$

For example, if initial storm DON concentrations were  $2.00 \text{ mg L}^{-1}$ , with respective minimum and maximum values of  $1.50 \text{ mg L}^{-1}$  and  $3.00 \text{ mg L}^{-1}$ , DON was assigned  $1.00 \text{ mg L}^{-1}$  for the hypothetical storm. Twenty-two and 23 individual storms were identified at Ag and MSM sites, respectively, to power regression models. The best multiple regression models were determined by adding or removing explanatory variables to result in the highest model adjusted

$R^2$  value. This is justified because adjusted  $R^2$  is a more robust model performance indicator than multiple  $R^2$ , due to penalizing values for inclusion of additional parameters (i.e., average DOC) that do not explain additional variability in the predictor variable (i.e.,  $\text{NH}_4^+$ -N vector).

## **Results & Discussion**

### **Multiple Linear Regression Analysis**

**Dissolved Organic Nitrogen.** Ag site DON concentrations were significantly influenced by cumulative rainfall, pH, and average water level (Table 2). Cumulative rainfall was negatively associated with DON, while pH and average water level were positively associated. This suggests that rainfall did not typically mobilize DON and dilute concentrations. Higher average water levels were also present during impoundment conditions (no pumping) compared to non-impoundment conditions (pumping). During impoundment conditions, migratory bird ON inputs were highest, potentially further increasing DON concentrations. Anoxic conditions also increased during impoundment conditions, prompting a shift from aerobic to anaerobic N-mineralization processes. Aerobic microbial processes are more efficient than anaerobic processes (Oehmen et al. 2010), which may have also increased DON concentrations at lower water levels. The pH may have been most important during impoundment conditions, due to the increased inputs of animal waste from migratory birds. Manure applications have been previously shown to raise soil pH (Haynes & Judge 2008), which indicates they may also increase surface water pH at Ag. This increase in pH would coincide with increases in DON stemming from bird waste and explain why pH positively associated with DON concentrations at Ag. DON dynamics at MSM were far more stable than Ag, and little fluctuations in DON at MSM were observed throughout most storm periods. MSM DON dynamics were most

influenced by cumulative rainfall (Table 2), and the inclusion of other variables resulted in decreased model adjusted  $R^2$  values. Cumulative rainfall was negatively associated with DON concentrations. This relationship of rainfall diluting DON signals was consistent at both sites, but the dilution effect was far more pronounced at Ag than MSM (slope coefficient of -0.258 vs -0.069).

**Ammonium.** Significant drivers of Ag site  $\text{NH}_4^+$ -N dynamics included water level range, temperature, pH, DO, and DOC (Table 2), and notably excluded cumulative rainfall. Water level range was negatively associated with  $\text{NH}_4^+$ -N. This was expected since greater water level ranges during storms indicated drawdown was occurring, and the soil profile was sufficiently aerated for nitrification to persist. During impoundment conditions, storm water level ranges were much smaller, and  $\text{NH}_4^+$ -N was observed to gradually increase overtime. DO was found to be significant likely due to water level range, along with drawdown further aerating the soil profile at Ag. Both temperature and pH were positively associated with Ag  $\text{NH}_4^+$ -N and may have been most important under impoundment conditions. Coinciding increases in temperature and microbial metabolism are well established (Karhu et al. 2014) and suggest that N-mineralization processes were enhanced, while nitrification was inhibited when water levels were above the soil profile during impoundment conditions. I suggest that pH was found to be significant for the same reasons as in DON Ag models, while DOC was negatively associated with  $\text{NH}_4^+$ -N potentially due to DOC being more sensitive to dilutions during storms than N parameters.

Significant regressions for MSM  $\text{NH}_4^+$ -N included cumulative rainfall and DOC (Table 2). Cumulative rainfall was positively associated with MSM  $\text{NH}_4^+$ -N, indicating that storm rainfall and winds may promote internal water movement throughout the otherwise stagnant

MSM impoundment waters and stimulate N-mineralization processes. I suggest that DOC was found to be negatively associated with MSM  $\text{NH}_4^+$ -N for the same reasons at Ag (i.e., dilutions). Overall, long-term monitored data (See Supplemental Data) confirmed my hypothesis that  $\text{NH}_4^+$ -N concentrations were significantly greater at MSM than Ag sites (Mann-Whitney  $W = 184640000$ ,  $p = 2.2e^{-16}$ ).

**Nitrate.** No significant multiple regression models were found at either site for  $\text{NO}_3^-$ -N. The strongest associated variables in the multiple regressions were average water level, pH, DO, and DOC at the Ag site, and only cumulative rainfall at the MSM site (Table 2). Notably, observed  $\text{NO}_3^-$ -N concentrations were lower than in many other agricultural systems (Wang et al. 2017;2016, Schilling et al. 2006, Royer et al. 2004), presumably due to high water tables promoting denitrification processes at Ag, while they were typically low ( $< 0.5 \text{ mg L}^{-1}$ ) throughout the entire monitoring period at MSM. These factors may have had a confounding effect and resulted in non-significant  $\text{NO}_3^-$ -N linear models. While no statistically significant associations between explanatory variables and  $\text{NO}_3^-$ -N were found, commonly recurring trends were observed and described in detail using storm chemographs (see “N Dynamics at Agricultural Impoundments” & “Nitrogen Dynamics at Moist-Soil Managed Impoundments” sections). Overall, my hypothesis that  $\text{NO}_3^-$ -N concentrations were significantly greater at Ag than MSM sites (Mann-Whitney  $W = 284730000$ ,  $p = 2.2e^{-16}$ ) was supported from long-term monitored data.

### **Nitrogen Dynamics at Agricultural Impoundments**

Storm chemographs demonstrated that DON is the main N species exported at Ag. Therefore, I reject my hypothesis that  $\text{NO}_3^-$ -N would be the primary species exported (Figures 2 & 3, supplemental data). Overall, N dynamics functioned distinctly different at Ag when

comparing non-impoundment and impoundment conditions. Under non-impoundment conditions, rainfall mobilized  $\text{NO}_3^-$ -N (Figure 2B on 10/7/16 & 10/8/16, 3B on 3/17/17 & 3/18/17). In the event of Hurricane Matthew (Figure 2B on mid 10/8/17), I also observed the potential for large loads of DON to be mobilized. Drawdown appeared to consistently increase DON concentrations, suggesting DON is present in high concentrations in the subsurface water that is mobilized via drawdowns (Figures 2A on 10/7/16 & 10/8/16, 3A on mid 3/18/17 - 3/19/17). Increases in DON and  $\text{NO}_3^-$ -N were also observed to coincide with increases in water level following drawdown past  $\sim 0.3$  m in the drainage ditch (Figures 2A 10/7/16 & 10/8/16, 3A on mid 3/17/17 - 3/19/17). This increase in water level appeared to mobilize  $\text{NH}_4^+$ -N if rainfall was not occurring (Figure 3A on mid 3/18/17 – 3/19/17). This is attributed to changes in crop field subsurface flow dynamics, whereby rainfall presumably infiltrated through dry top-soils with high  $\text{NO}_3^-$ -N concentrations (confirmed in chapter 2 & See  $\text{NO}_3^-$ -N peaks in Figures 2A 10/7/16 & 10/8/16, 3A on mid 3/17/17 - 3/19/17). During this period, drawdown promoted horizontal flow from the soil subsurface into the drainage ditch.  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N were visually observed to be negatively associated during non-impoundment conditions, corroborating high nitrification rates (See Chapter 2) and low  $\text{NH}_4^+$ -N concentrations observed. Storms resulted in the highest N export, especially DON, immediately after impoundment conditions. All N species during this period were present in high ambient conditions ( $\geq 1.0 \text{ mg L}^{-1}$ ) (Figure 3). Ambient  $\text{NH}_4^+$ -N concentrations remained consistently high ( $\sim 1.0 \text{ mg L}^{-1}$ ) during storm events following impoundment conditions throughout April of 2017 (See Supplemental Data), potentially due to mineral fertilizer application.

These non-impoundment trends were less apparent during 2017 storms compared to 2016 storms, potentially due to the substantially greater precipitation rates of 2016. In 2017,  $\text{NO}_3^-$ -N

was less responsive to rainfall, but DON concentration responses were still observed during drawdown events (See supplemental data). Another potential reason to explain lower 2017 storm N responses may be attributed to multiple storms occurring within a few days of each other. This likely resulted in dissolved N having been exported and diminished observed N responses during subsequent storm events. Overall, my hypothesis of N processing and export being stimulated by drawdown was supported.

Under impoundment conditions, storms influence N dynamics differently. I observed gradual increases in ambient  $\text{NH}_4^+$ -N and decreases in  $\text{NO}_3^-$ -N (See Supplemental Data). This was attributed to decreases in nitrification rates (see Chapter 2) as water levels rise past the soil surface and create anoxic conditions. During this time,  $\text{NO}_3^-$ -N was presumed to be gradually removed from the system via denitrification while ON pools mineralized into  $\text{NH}_4^+$ -N. DON was observed to sharply increase in response to rainfall under impoundment conditions (Figure 4B on 1/22/17), suggesting that ON inputs from migratory bird waste were substantial and readily mobilized. In contrast,  $\text{NO}_3^-$ -N was not typically mobilized during storm events under impoundment conditions, potentially due to increased denitrification potential. In Figure 4 on mid 1/22/17, a sharp decrease in  $\text{NH}_4^+$ -N coincided with a less prominent increase in  $\text{NO}_3^-$ -N. This suggested that nitrification was stimulated by intense rainfall oxygenating the soil-water interface, and the newly converted  $\text{NO}_3^-$ -N was removed via denitrification when rainfall ceases and soils return to anoxic conditions. Environments necessary for storms to facilitate nitrification in anoxic systems such as this are not well characterized and warrant further study. These results supported my hypothesis of storms stimulating N-mineralization processes under impoundment conditions but not under non-impoundment conditions. My hypothesis of storms mobilizing

$\text{NO}_3^-$ -N (and  $\text{NH}_4^+$ -N if drawdown was not occurring) was supported under non-impoundment conditions but rejected under impoundment conditions

### **Nitrogen Dynamics at Moist Soil Managed Impoundments**

Storms effects on MSM N dynamics were less clear than the Ag site, but I was still able to identify trends in N dynamics that were driven by seasonal differences in water levels. Water movement within the impoundment was driven by wind, rainfall, and drawdown events throughout this study. Reduced water circulation throughout the impoundment resulted in lagging water quality changes relative to observed rainfall and water level fluctuations at the monitoring site. Dominant N species monitored in the impoundment were DON and  $\text{NH}_4^+$ -N, with  $\text{NO}_3^-$ -N concentrations typically being below  $0.5 \text{ mg L}^{-1}$  throughout the study. Storm events typically resulted in lagging increases in  $\text{NH}_4^+$ -N concentrations, while  $\text{NO}_3^-$ -N remained below  $0.5 \text{ mg L}^{-1}$  and DON remained stable in the impoundment when water levels were at the lowest in April through October (rainfall on 4/5/17 in Figure 5). I attribute these observed N dynamics to saturated soil conditions throughout the impoundment inhibiting nitrification processes. Gradually increasing  $\text{NH}_4^+$ -N concentrations were observed throughout late fall flooding until early spring drawdown of the impoundment (See supplemental materials), and this effect was more prominent at MSM compared to Ag. I also observed the potential of prolonged rainfall to stimulate nitrification processes, but this effect was not typically coupled with denitrification when water levels were low (Figure 6A on 5/4/17).

When MSM was seasonally flooded from early October through late April, storms affected N dynamics differently. Rainfall during seasonal flooding periods typically did not increase  $\text{NH}_4^+$ -N or DON concentrations. Interestingly, rainfall at MSM during seasonal flooding periods appeared to stimulate coupled nitrification-denitrification processes that were not

observed with lower water levels (Figure 6B on 3/31/17). This phenomenon was observed most frequently in January 2017 storms, and notably did not occur in December 2017, despite observing several storms with high ambient  $\text{NH}_4^+$ -N conditions. The potential for storms to stimulate nitrification during seasonal flooding periods was much more pronounced at MSM than Ag (e.g., Figures 6B and 4B). For example, the observed decreases in  $\text{NH}_4^+$ -N were  $0.7 \text{ mg L}^{-1}$  in Ag (Figure 4B on mid 1/22/17) compared to  $2.2 \text{ mg L}^{-1}$  at MSM (Figure 6B on 3/31/17). This may be due to the higher  $\text{NH}_4^+$ -N concentrations at MSM and discrepancies in microbial community function between sites. Storms coinciding with drawdown resulted in  $\text{NH}_4^+$ -N export (Figure 7A on 8/8/16 – mid 8/9/16), and were most prominent in early spring (Figure 7B on mid 4/25/17) when water levels were drawn down below 1.2 m. During drawdowns of this magnitude, water is removed from the impoundment soil surface, resulting in soil subsurface waters high in DON and  $\text{NH}_4^+$ -N being mobilized. These results supported my hypothesis that drawdown results in increased N processing and export, but also showed that DON, and not  $\text{NH}_4^+$ -N, was the dominant N species exported from MSM.

### **Management Suggestions for Agricultural Impoundments**

Drawdown magnified N processing and export compared to periods of no drawdown. DON residing in the drainage ditch subsurface water was mobilized by drawdown, and drawdown past 0.3 m seemed to result in additional DON and  $\text{NO}_3^-$ -N in the crop field subsurface being mobilized and exported. There are a few potential management solutions to reduce these mechanisms of N export. An option to reduce the  $\text{NO}_3^-$ -N exported from the drainage ditch subsurface water would be implementing treatment wetlands (Etheridge et al. 2017) between the pumping outlet and the main canal draining into the lake (See Methods Map). Reducing the DON and  $\text{NO}_3^-$ -N exported from the crop field subsurface without impacting

agricultural operations will be challenging. A solution to reduce crop field subsurface N export would be to change the way drawdown is currently implemented.

Drawdown is currently regulated via a float-switch that automatically begins pumping when water levels reach a certain depth. Increasing the depth that this switch is currently set at would reduce subsurface flow into the drainage ditch, and likely result in less crop field subsurface DON and  $\text{NO}_3^-$ -N mobilization and export. The most crucial time to reduce N export will be during initial dewatering of the fields after being used as an impoundment when all N species concentrations are greater than  $1.0 \text{ mg L}^{-1}$  (beginning of Figure 3B). This period of dewatering should be examined beyond the storm events that this study captured to fully understand the effect of drawdown on N dynamics and export. A possible solution to reduce N export during this period is to dewater the fields earlier in the year, but this option would result in negative economic impacts to farmers who allow hunting on their fields during impoundment conditions. However, this would allow less time for the substantial ON pools to mineralize into  $\text{NH}_4^+$ -N and have the added effects of cooler temperatures inhibiting mineralization rates and decreasing N solubility, which can lead to reduced N mobilization and export.

### **Management Suggestions for Moist Soil Managed Impoundments**

A greater impact was seen from management (drawdown times and duration) than storms on MSM N dynamics. Storms did not appear to substantially increase N concentrations without subsequent drawdown as defined in this study, potentially due to a combination of reasons (see MSM Dynamics Section). Periods of N export were observed when drawdown past 1.0 m depths occurred shortly following storms (Figure 7B on mid 4/25/17), resulting in increased  $\text{NH}_4^+$ -N and DON. This work corroborates the findings of Winton et al. (2016) that MSM impoundment conditions inhibit nitrification and result in anoxic mineralization of large pools of ON into

$\text{NH}_4^+\text{-N}$ . A potential solution to reduce N exported from this impoundment would be to strategically aerate portions of the impoundment to promote coupled nitrification-denitrification (Figure 6 on 3/31/17 - 4/1/17).

Modifying drawdown rates and duration may further reduce N export. Similar to the suggestion for reducing Ag N export, drawdowns occurring earlier than mid-March would benefit from two aspects: less of the substantial ON pool would be mineralized into  $\text{NH}_4^+\text{-N}$  and cooler temperatures would inhibit mineralization rates and N solubility. Earlier drawdowns may have a detrimental effect on desired vegetation growth but may be worthwhile to reduce  $\text{NH}_4^+\text{-N}$  export which has been linked to increased toxic algal blooms in the lake (Moorman et al. 2017). Other MSM impoundments should take note of these conditions and monitor  $\text{NH}_4^+\text{-N}$  concentrations before early spring dewatering. Another potential, and less certain, solution to reduce N export but maintain similar early-spring drawdown times would be to time drawdowns based on storm events when  $\text{NH}_4^+\text{-N}$  concentrations greatly decrease due to coupled nitrification-denitrification observed in this study. For example, in Figure 6B drawdown occurring under initial N concentrations on 3/31/17 would export significantly more  $\text{NH}_4^+\text{-N}$  than if the drawdown occurred 24 hours post rainfall on mid 4/1/17. Potential solutions to reduce DON export are less clear, as DON was relatively high ( $> 1.0$  mg/L) throughout most of the monitoring period and was less affected by drawdown and storm events. Reducing this DON export is of less concern than  $\text{NH}_4^+\text{-N}$  export, as inorganic N sources typically have more potential to cause eutrophication in downstream systems (Anderson et al. 2008).

### **Benefits of High Temporal Resolution Water Quality Data**

This analysis would not have been possible without the breakthrough  $\text{NH}_4^+\text{-N}$  PLSR calibration. Accurately accounting for all 3 forms of dissolved N at a high resolution allowed us

to fully visualize the N cycle at both sites and assess when N processes were inhibited or stimulated. These N cycling mechanisms are very important but happen on temporal scales missed by traditional, low temporal resolution sampling methods. This study demonstrates that utilization of *in situ* spectroscopy can further the understanding of biogeochemical processes in field studies. This study provided the opportunity to identify that rainfall stimulates coupled nitrification-denitrification processes in anoxic systems and that drawdowns effect on Ag DON concentrations. There are many other systems that could benefit from this type of monitoring to provide more informed decisions about reducing nutrient export.

## **Conclusion**

This study demonstrates that prescribed drawdowns of both Ag and MSM waterfowl impoundments export dissolved organic and inorganic N. I have shown that waterfowl ON inputs from waste have potential to be easily mobilized and mineralized into  $\text{NH}_4^+\text{-N}$ , which adversely impacts downstream systems during prescribed drawdown periods. This research builds on previous suggestions to alter drawdown timings to avoid periods of maximum surface water N concentrations and shows that drawdowns timed after storm events have potential to stimulate coupled nitrification-denitrification processes. This may remove the most biologically available N species from surface waters prior to being exported. This phenomenon of coupled nitrification-denitrification in saturated, anoxic systems would not have been discovered if this study used traditional bi-weekly or monthly water quality sampling methods in place of *in-situ* spectroscopy. Therefore, I suggest that future studies should strive to use similar water quality sampling frequencies to better characterize conditions that lead to this observed nitrification-denitrification coupling. Utilizing this coupling represents a novel, low-cost, and energy-efficient

method to reduce N concentrations in waterfowl impoundments prior to drawdown and improve the quality downstream ecosystems.

## Chapter 2

### Introduction

Hydrology is a strong ecological filter that determines microbial community composition and function in many different environments (Menning et al. 2018, Foulquier et al. 2013, Danczak et al. 2013, Peralta et al. 2013). Hydrologic changes, both natural and anthropogenic, create gradients in soil redox potential that strongly influence microbial metabolism (Sporer et al. 2017). Under flooded, saturated conditions, soil redox potential decreases ( $E_H$  becomes negative) due to low  $O_2$  concentrations, shifting microbial metabolism from aerobic to anaerobic microbial respiration, where alternative terminal electron acceptors (TEA) such as nitrate or iron are used. (Zhao et al. 2013). In contrasting dry conditions, soils are well aerated, redox potential increases ( $E_H$  becomes positive), and aerobic microbial metabolism persists (Sporer et al. 2017, Peralta et al. 2014). Varying hydrologic regimes of different areas within the same watershed can produce distinct differences in microbial nitrogen (N) cycling functions and rates (Racchetti et al. 2017:2016, Peralta et al. 2013). Dominant microbial N cycling processes and rates, such as nitrification ( $NH_4^+ \rightarrow NO_3^-$ ) or denitrification ( $NO_3^- \rightarrow N_2O \rightarrow N_2$ ), are particularly affected by these redox gradients, and have potential to be enhanced or inhibited by hydrologic changes (Wolf et al. 2011, Wolf et al. 2013). Understanding how hydrology may influence N cycling dynamics is especially important in systems that export high nutrient loads. For example, high N concentrations have been observed in surface waters of waterfowl impoundments during seasonally prescribed drawdown periods (Winton et al. 2016). It is currently unclear how ongoing hydrologic manipulation in support of waterfowl habitat influences microbial N cycling processes. Understanding the impacts of intensive hydrologic management on microbial N cycling processes has potential to reduce N export to downstream ecosystems.

Waterfowl impoundments are wetlands that are hydrologically managed to serve as waterfowl habitat (Nelms et al. 2007). Hydrologic management practices can vary across impoundment types depending on climate and desired vegetation in order to promote growth of vegetative food sources that attract migratory waterfowl populations to the impoundment. Two prominent types of waterfowl impoundments are improvised agricultural (Ag) and moist-soil managed (MSM) waterfowl impoundments. Management of MSM impoundments typically involves prescribed drawdowns in early March through April to promote desired vegetation growth, and prescribed flooding during the waterfowl migration period (from October through February in North Carolina) (Nelms et al. 2007). Fertilizers are not applied, but vegetation is disked during some drawdown periods to further promote desired vegetation growth via controlled disturbances. Ag impoundments typically time the start of flooding regimes similar to MSM impoundments but apply drawdowns earlier in the year (Maul & Cooper 2000). Hydrologic management distinctly differs between Ag and MSM impoundments in post flooding periods. Standard agricultural methods are used to grow and produce crops, and drainage systems may be required in agricultural systems to dewater fields and increase crop production (Smith et al. 2011). Agricultural fields are partially or not harvested, and the remaining crops in combination with prescribed flooding conditions create waterfowl habitat. In both MSM and Ag impoundments, these intense hydrologic management practices may disrupt microbial N cycling processes in ways that lead to N export to downstream aquatic ecosystems. For example, in my high-resolution water quality monitoring (Ch. 1), I observed that the prescribed flooding period (October to February) resulted in  $\text{NH}_4^+\text{-N}$  gradually accumulating in surface waters in both impoundment types, possibly due to nitrification inhibition. Therefore, waterfowl impoundment

management has the potential to change N cycling processes, but the link between hydrology, impoundment type, soil physiochemical environment, and seasonality requires further study.

Hydrologic management can affect waterfowl impoundment N cycling dynamics in several ways. As mentioned above, seasonal flooding periods create anaerobic conditions, which can inhibit aerobic processes such as nitrification. In addition, ON inputs from vegetation and migratory bird feces (Yoshitake et al. 2014) may be stimulating anaerobic N-mineralization (Organic N (ON)  $\rightarrow$   $\text{NH}_4^+$ ), further increasing surface water  $\text{NH}_4^+$ -N concentrations during flooding periods at impoundments. These anaerobic conditions may also accelerate denitrification rates until  $\text{NO}_3^-$ -N substrates are exhausted due to low nitrification rates. During spring and summer months (April - July), MSM drawdowns aerate some areas within the impoundment and potentially increase soil nitrification. These drawdowns would presumably inhibit denitrification, but it is unknown how they influence N-mineralization rates. Flooding periods may affect N cycling dynamics at Ag impoundments similar to MSM impoundments. Ag impoundments require drawing down water levels to well below the crop field soil surface during the cropping season (March - September). This drawdown can lead to high nitrification rates as observed in other agricultural systems (e.g., Balaine et al. 2015, Subbarao et al. 2006, Booth et al. 2005, Burger et al. 2003), and potentially decreased N-mineralization and denitrification rates. In these agriculturally managed systems, short-term N-cycling can be altered at certain points during grow seasons, depending on the type and amount of fertilizer applied (Shang et al 2015). For example, fertilizers with high  $\text{NH}_4^+$ -N contents applied to dry top soils would be expected to stimulate short-term nitrification processes.

Interactions between hydrology and environmental factors (e.g., vegetation, soil organic matter, inorganic N concentrations, temperature, soil pH) also impact N cycling dynamics

(Peralta et al. 2016, 2013). Yearly N assimilation ( $\text{NH}_4^+$  &  $\text{NO}_3^- \rightarrow \text{ON}$ ) dynamics at both impoundment types would be highly variable depending on the type and amount of vegetation present, hydrology, and nutrient conditions (Fang et al. 2016). For example, N assimilation at Ag would presumably be much lower in September post-harvest due to minimal vegetative N uptake, and N-mineralization may be stimulated by fresh plant litter inputs, while N assimilation in September would be relatively high at MSM due to more permanent vegetation. Seasonal temperature variability has been shown to have significant effects on all N cycling processes (Booth et al. 2005), with all rates generally being positively associated with increasing temperatures. Organic matter availability also strongly affects N cycling dynamics by providing an energy source for microbial N processing to persist over time (Castaldelli et al. 2013, Booth et al. 2005). For example, previous literature has found nitrate removal in groundwater incubations to be proportional to carbon amendments (Israel et al. 2009). Soil pH is another potent environmental filter of microbial communities and inhibits certain chemical processes, as highly acidic soils have been shown to inhibit  $\text{NH}_4^+$  oxidation to  $\text{NO}_3^-$  compared to more alkaline soils, further influencing N cycling processes (Zhang et al. 2013, Zhao et al. 2007).

Nitrogen export contributes to downstream eutrophication and highlights the necessity for increased nutrient management of waterfowl impoundments (Moorman et al. 2017; Waters et al. 2009; Waters et al. 2010). Previous studies demonstrate that current hydrologic management of waterfowl impoundments results in export of N to downstream systems (Ch. 1, Winton et al. 2016). The intensive hydrologic management of waterfowl impoundments presents several potential management targets to reduce N export. However, more research is required to better understand seasonal waterfowl impoundment N cycling dynamics at the laboratory scale to inform nutrient management strategies. For example, a more holistic understanding of seasonal

N cycling dynamics would inform management to better time drawdowns and optimize N removal via denitrification to reduce N export (Racchetti et al. 2011). However, it is unlikely that optimizing drawdown times and denitrification rates alone will be sufficient due to the dominant N species monitored in Ag (DON) and MSM ( $\text{NH}_4^+$ , DON) impoundments (Ch. 1). Therefore, additional nutrient management strategies that promote N transformations to  $\text{NO}_3^-$ , in addition to optimizing denitrification processes, should be explored. One potential option to facilitate N processing and further reduce N export is implementing treatment wetlands between the impoundments and downstream waters to potentially reduce N loadings. Implementing these systems would potentially reduce nutrient export via sedimentation, increased vegetative uptake, and microbial processing (Barszczewski et al. 2012, Wang et al. 2014). Similar techniques have been successful in treating drainage from urban and agricultural environments (Wang et al. 2014; Woltemade 2000), but there is uncertainty about ability of waterfowl impoundment soils to efficiently act as treatment wetlands.

Lake Mattamuskeet (Hyde County, NC) is the largest natural lake (16,190 ha) in North Carolina. The watershed area is roughly 27,590 ha and contains over 1000 ha of United States Fish and Wildlife Service (USFWS) managed MSM impoundments, and roughly 4090 ha of agricultural fields. There is uncertainty about the proportion of agricultural fields that double as waterfowl impoundments. Hyde County has flat topography, high-water tables and low elevations (~ 1m above MSL). These county-wide conditions necessitate intensive hydrologic management via pumping, rather than traditional gravity drainage, to facilitate these land uses. The lake has experienced a consistent decline of historically dense beds of submerged aquatic vegetation (SAV) since the 1990's. This SAV loss has been linked to decreased light attenuation and increased nutrient concentrations, pH, and phytoplankton growth (Moorman et al. 2017;

Waters et al. 2009; Waters et al. 2010). To combat SAV losses, we need a better understanding of N dynamics in waterfowl impoundments to begin developing strategies to reduce the N export from waterfowl impoundments while preserving natural (i.e., Lake Mattamuskeet) and managed (i.e., impoundments) waterfowl habitat.

### **Objectives/hypothesis**

The specific objectives of this study were to (1) examine how N processing rates (N-mineralization, nitrification, denitrification) differed seasonally between and within Ag and MSM waterfowl impoundments through conducting biogeochemical assays on field collected soils; and (2) determine the potential of the soils to remove N if managed as a treatment wetland using a mesocosm based approach. I hypothesized that all N processing rates will be highly variable due to differences in microbial response to the soil environment and hydrologic conditions. Denitrification processes will be higher at MSM than Ag due to more anoxic conditions. Nitrification processes will be significantly higher at Ag than MSM due to more oxygenated soils from longer drawdown periods during the growing season. N-mineralization processes will be higher at MSM due to more diverse and permanent vegetation compared to Ag soils. In addition, N concentrations will decrease with increasing water residence times in Ag and MSM mesocosms simulating treatment wetlands. *The goal of this study was to characterize N processing within Ag and MSM waterfowl impoundments at the laboratory scale, examine how changes in soil physiochemical environment affect N cycling rates, and determine the effectiveness of Ag and MSM soils to process N when used as a treatment wetland.*

### **Materials and Methods**

**Site Description.** A privately-owned Ag impoundment and a USFWS managed MSM impoundment within the Lake Mattamuskeet watershed were selected as study sites (Fig. 1). Both sites are hydrologically isolated, and water levels are managed using pumping stations (stars in Fig. 1). MSM pumping stations are manually controlled to manage water levels during seasonally prescribed drawdown and flooding periods. Ag pumping stations are automatically controlled via a float-switch mechanism that begins drawdown when water level depth reaches a pre-determined level in the main canal. This float-switch is turned off during the fall to allow water levels to rise. Precipitation is the primary water source input into both systems. The MSM soils were classified as Weeksville loam (loam marine deposits, mixed, hydric), and Ag soils were classified as Portsmouth mucky sandy loam (Loamy fluviomarine deposits, mixed, hydric) (NRCS Online Soil Database). For a more thorough description of vegetation present and area measurements reference chapter 1.

**Soil Sampling Description.** I designed soil sampling schemes to examine the effects of distinct hydrologic management practices on microbial N processing within and between respective Ag and MSM sites. Sampling schemes were designed to capture plots with high seasonal water table variability driven by management practices. I sampled the MSM perimeter along areas inundated year-round (n=6) and areas only inundated during prescribed flooding periods (n=6) (Fig. 1A). At the Ag site, I established two transects that ran parallel to the drainage ditch at 20 m and 40 m into the agricultural field, and perpendicular to the drainage ditch at 5 m and 25 m, to collect soil cores (n=12, Fig. 1B). Only Field 2 was sampled to minimize vegetation variability among Ag plots. Field 2 was used to grow both corn-cotton throughout the study period, but only corn portions were sampled (Fig. 1B) since vegetation impacts microbial community composition and function (e.g., Morris et al. 2017).

I collected soil samples from respective MSM and Ag plots at 3 time points in 2017 (July, October, December) to assess the effects of seasonal impoundment management practices on short-term microbial N processing. I sampled in July when water levels were low; September during prescribed flooding; and December after prolonged flooding and arrival of migratory bird populations. I collected 6 soil cores (3 cm diameter) to a depth of 15 cm at each plot to represent a composite soil sample. Soils were transported to the laboratory on ice and stored at 4°C until processing. Prior to laboratory analyses, I passed soils through a 6-mm sieve to remove plant and rocky materials and homogenized the sample. For each sample, I subsampled soils to be air-dried for soil pH, soil % C, and % N analyses, and used field-moist soils for gravimetric moisture, KCl extractions to measure extractable  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , and N cycling assay analyses.

**Soil Physical and Chemical Analysis.** I measured gravimetric soil moisture by drying ~25 g of field-moist soil in at 105°C overnight. I reweighed the dried soils and computed sample moisture and dry mass (DM) percentages using the following equations:

$$(1) \text{ Dry Soil (g) / Field-Moist Soil (g) = \% DM (wt)}$$

$$(2) 1 - \text{DM} = \% \text{ Moisture (wt)}$$

Soil pH was determined with a soil solution (1:1 soil:water) and a pH probe (Genemate-Bioexpress; Kaysville, Utah; USA). I measured soil pH values in triplicate, and values were averaged and recorded. KCl extractable  $\text{NH}_4^+$  and  $\text{NO}_3^-$  ions were measured colorimetrically (Autoanalyzer Lachat Quikchem 8000; Lachat Instruments/Hach Company; Loveland, Colorado; USA) (Robertson et al., 1999; Ma et al., 2005). Soil organic matter was determined by combusting finely ground samples using an elemental analyzer (2400 CHNS Analyzer; Perkin Elmer; Waltham, Massachusetts; USA). I used monthly mean air temperature of each sampling date as a proxy for relative seasonal temperature changes in soils. The Environmental and

Agricultural Testing Services (EATS) laboratory of North Carolina State University conducted the colorimetric analyses of inorganic N species and elemental analysis of soil organic matter.

**Denitrification Activity.** I measured denitrification potential of fresh soils using the acetylene inhibition method (Tiedje et al. 1989, Peralta et al. 2013). Experimental (A+) and control groups (A-), along with control bottles (no soil added) for each group, were prepared for each sample. In 125-mL Wheaton bottles, I combined 75 mL of 1 mM KNO<sub>3</sub> solution and 1.3 mL of chloramphenicol (100 mg mL<sup>-1</sup>) with ~25 g of fresh soil. I sealed the bottles with septa-centered caps, shook them, purged with He for 5 min, and vented the bottles with a needle prior to starting the assay. Prior to collecting the 0 h sample, I shook the bottles to release N<sub>2</sub>O in sediment and aqueous phases and allowed system equilibration for 5 min, and 15 mL of headspace gas was replaced with 15 mL of acetylene in A+ bottles. I collected gas samples (10 mL) in exetainer vials from the bottle headspace with a syringe at 0, 1, 2, and 3 h during the assay, and replaced removed headspace gas with 10 mL of 1:10 acetylene/He mix (A+) or 10 mL of He (A-). I analyzed the headspace gas samples for N<sub>2</sub>O using a gas chromatograph (GC) with electron capture detector (ECD) and single manual injection port (Shimadzu 2014 GC, Shimadzu Scientific Instruments, Durham, NC, USA). I generated a gas standard calibration curves (0.0 - 100.0 ppm-v) from 1.0 ppm and 100.0 ppm N<sub>2</sub>O standards (Matheson Tri-Gas; Basking Ridge, NJ; United States), and analyzed sample N<sub>2</sub>O ppm values using GC Solution software (version 5.81 SPI Shimadzu Corporation). I determined potential denitrification rates (ng N<sub>2</sub>O g<sup>-1</sup> DM h<sup>-1</sup>) by regressing N<sub>2</sub>O concentrations/DM of each sample against assay time (0, 1, 2, 3 h). The slope of each samples regression line was interpreted as the potential denitrification rate. Samples were corrected for non-linearity by removing 1 of the 4 time points if necessary (See

Supplemental Data). DM values were determined from fresh soils based on gravimetric moisture measurements (See Soil Physical and Chemical Analysis above).

**Nitrification Activity.** I measured potential nitrification activity using 5-h soil incubations (Kandeler 1996, Peralta et al. 2013). I weighed 3 replicates (2 experimental, 1 control) of 5 g fresh soil into 125-mL flasks and added 20 mL of 1 mM  $(\text{NH}_4)_2\text{SO}_4$  and 0.1 mL of 2 M  $\text{NaClO}_3$  to each flask. Experimental flasks were incubated and shaken at ~130 rpm for ~5 h at room temperature. During the incubation period, the control sample was stored at  $-20^\circ\text{C}$ . I collected and analyzed filtrate from experimental and control flasks colorimetrically for  $\text{NO}_2^-$ -N concentrations. I developed calibration standards for 0, 0.2, 0.4, 0.8, and 1.0  $\mu\text{g NO}_2\text{-N mL}^{-1}$  (Kandeler 1996) and analyzed standards and samples using a spectrophotometer (UV-1800 Spectrophotometer; Shimadzu Scientific Instruments; Kyoto, Kyoto Prefecture; Japan). I averaged experimental  $\text{NO}_2\text{-N}$  values and computed the potential nitrification rates using equation 3.

$$(3) ((\text{Inc. ng N / g DM}) - (\text{Con. ng N / g DM})) / \text{time} = \text{nitrification rate (ng N g}^{-1} \text{ DM h}^{-1})$$

DM of fresh soils was determined based on gravimetric moisture measurements (See Soil Physical and Chemical Analysis above), and rates were adjusted for samples that deviated from 5 h incubation times.

**Nitrogen Mineralization.** I measured potentially mineralizable N (PMN) of soils using a 7-day incubation (Drinkwater et al., 1996). For each sample, I weighed 2 replicates (Experimental and control) of 8.0 g fresh soil into 50 mL centrifugal tubes. I added 40 mL of 2.0 M KCl to the control tube and shook samples at ~150 rpm at room temperature for 1 hour. After shaking, I collected control filtrate and stored at  $-20^\circ\text{C}$  until  $\text{NH}_4^+$ -N determination. I added 10 mL of deionized water to the experimental tube, and incubated samples anaerobically at  $37^\circ\text{C}$  in dark

conditions for exactly 7 days. Post incubation, I added 30 mL of 2.67 M KCl to each sample to extract exchangeable  $\text{NH}_4^+\text{-N}$ , collected the filtrate, and stored the filtrate at  $-20^\circ\text{C}$ . To measure  $\text{NH}_4^+\text{-N}$ , colorimetric analysis was used (See Soil Physical and Chemical Analysis). I calculated PMN rates using equation 4. DM of fresh soils was determined based on gravimetric moisture measurements (See Soil Physical and Chemical Analysis above).

$$(4) ((\mu\text{g Inc. N / g DM}) - (\mu\text{g Con. N / g DM})) / \text{Days Inc.} = \text{PMN } (\mu\text{g PMN g}^{-1} \text{ DM Day}^{-1})$$

**Mesocosm Experiment.** A monthly mesocosm experiment was conducted based on a  $2 \times 2$  factorial design (Fig. 2B) from August 2017 through January 2018. The influence of management (MSM vs. Ag) and retention time (24 h vs. 72 h) post simulated rainfall on water quality was examined. Mesocosms were designed to simulate treatment wetlands as possible management strategies to improve water quality by increasing retention time to promote denitrification. Individual soil mesocosms at Ag ( $n=12$ ) and MSM ( $n=12$ ) sites were collected in July 2017 at previously described soil plots (Fig. 1). I collected cores to a depth of 20 cm using a 7.62 cm diameter PVC tubes (Fig. 2A). Each soil core represented a mesocosm and the experiment was conducted in an open hoop house to account for day/night temperature fluctuations. Cores had drainage control valves installed to facilitate ponding within the system. Valves were closed, cores were saturated with 0.01 M  $\text{CaCl}_2$  (artificial rainwater) (Revitt et al., 2013), and then subjected to 10.2 cm of N adjusted artificial rainwater (Fig. 2A). Eight experimental cores for each site received  $\text{NO}_3^-\text{-N}$  and  $\text{NH}_4^+\text{-N}$  adjusted rain via  $\text{KNO}_3$  and  $\text{NH}_4\text{Cl}$  additions (Piehler et al. 2010). The  $\text{NO}_3^-\text{-N}$  and  $\text{NH}_4^+\text{-N}$  concentrations were adjusted to match the mean value of  $\text{NO}_3^-\text{-N}$  or  $\text{NH}_4^+\text{-N}$  at the respective Ag or MSM site 30 days prior to each rain addition (see supplemental materials for exact dates). Four control mesocosms received artificial rain adjusted for the molar equivalent of  $\text{Cl}^-$  and  $\text{K}^+$  ions added to the experimental

samples. For each sampling event, 96 mL of ponded surface water was collected from the mesocosm top using a manual pipette bulb and 25 mL serological pipettes at 24 h and 72 h post rainfall. After 72 h sampling, valves were opened to drain each mesocosm. Water samples were stored in a cooler and transported to the lab to be filtered (0.45  $\mu\text{m}$  pore size) and frozen at  $-20^{\circ}\text{C}$  until analysis. Samples were analyzed for  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$ , and total Kjeldahl N (TKN) colorimetrically on an autoanalyzer (Seal Autoanalyzer III system; SEAL Analytical; Mequon, Wisconsin; USA). Mesocosm dissolved organic N (DON) values were computed by subtracting  $\text{NH}_4\text{-N}$  concentrations from TKN concentrations. All mesocosm water quality analysis were conducted at the NCSU Environmental Analysis Laboratory (EAL).

### **Statistical Analyses**

**Biogeochemical Rate Analysis.** I used multiple linear regression analysis to examine the relationship between soil physiochemical parameters (soil pH, temperature, soil moisture, soil  $\text{NH}_4^+\text{-N}$ , soil  $\text{NO}_3^-\text{-N}$ , soil organic C %, soil N %, and C/N ratio) and biogeochemical rates separately for Ag and MSM. I determined the best model by performing model selection through adding or removing parameters to produce the highest adjusted  $R^2$  model value. Additionally, I plotted rates against soil moisture and pH gradients to examine their individual impacts on biogeochemical functions within each site.

**Mesocosm Analysis.** I used analysis of variance (ANOVA) to examine the main effects (Site vs Retention Time) on mesocosm DON,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations. Monthly 24 h and 72 h DON,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  values were averaged for respective Ag and MSM experimental and control groups. I determined between-group differences in DON,  $\text{NO}_3^-\text{-N}$  and  $\text{NH}_4^+\text{-N}$  concentrations in experimental mesocosms using Tukey's HSD test. Additionally, I plotted 24 h and 72 h N

concentrations to visually track N transformations throughout the retention period for experimental and control groups.

## **Results**

### **Patterns in soil physical and chemical factors between agricultural and moist-soil managed waterfowl impoundments**

Respective Ag and MSM sites showed substantial differences in the average ( $5.80 \pm 0.16$  vs  $5.23 \pm 0.36$ ) and range (0.65 vs 1.46) of soil pH (Table 1). Average soil moisture ( $18.46 \pm 5.54$  %) and range (17.19 %) at the Ag site was notably lower compared to MSM average soil moisture ( $41.45 \pm 7.33$  %) and range (27.01 %) (Table 1). Average soil  $\text{NH}_4^+\text{-N}$  was over 3x higher at MSM compared to Ag ( $7.19 \pm 5.54 \mu\text{g NH}_4^+\text{-N g}^{-1}\text{ DM}$  vs  $2.38 \pm 2.54 \mu\text{g NH}_4^+\text{-N g}^{-1}\text{ DM}$ ), while average soil  $\text{NO}_3^-\text{-N}$  was over 7x higher at Ag ( $5.34 \pm 10.18 \mu\text{g NO}_3^-\text{-N g}^{-1}\text{ DM}$  vs  $0.73 \pm 0.45 \mu\text{g NO}_3^-\text{-N g}^{-1}\text{ DM}$ ) compared to MSM soils (Table 1). Additionally, large standard deviations in MSM soil  $\text{NH}_4^+\text{-N}$  and Ag soil  $\text{NO}_3^-\text{-N}$  indicate large ranges of values that did not center around the mean were observed for both parameters. Average soil organic carbon was higher and more variable at MSM compared to Ag ( $4.45 \pm 1.39$  % vs  $2.95 \pm 0.84$  %) soils, while average total soil nitrogen concentrations were also higher and more variable at MSM compared to Ag ( $0.24 \pm 0.12$  % vs  $0.16 \pm 0.04$  %) (Table 1). Average soil C/N ratios were higher at MSM compared to Ag soils ( $20.24 \pm 4.45$  vs  $18.95 \pm 2.59$ ) (Table 1). Overall standard deviations were higher across all MSM soil parameters, except soil  $\text{NO}_3^-\text{-N}$ , compared to Ag soils. Thus, more variability in soil physiochemical environment was present throughout the MSM impoundment.

### **Seasonal variability in nitrification, denitrification, and N-mineralization rates at Ag and MSM impoundments**

Average summer denitrification rates were over 2x higher at MSM compared to Ag ( $113.71 \pm 143.76 \text{ ng N}_2\text{O g}^{-1} \text{ DM h}^{-1}$  vs  $54.67 \pm 57.85 \text{ ng N}_2\text{O g}^{-1} \text{ DM h}^{-1}$ ) sites, while average fall ( $755.00 \pm 270.75 \text{ ng N}_2\text{O g}^{-1} \text{ DM h}^{-1}$  vs  $589.58 \pm 368.23 \text{ ng N}_2\text{O g}^{-1} \text{ DM h}^{-1}$ ) and winter ( $722.01 \pm 384.67 \text{ ng N}_2\text{O g}^{-1} \text{ DM h}^{-1}$  vs  $351.15 \pm 227.07 \text{ ng N}_2\text{O g}^{-1} \text{ DM h}^{-1}$ ) denitrification rates were greater at Ag compared to MSM sites (Table 2). Overall, average denitrification rates at both sites were low ( $< 1 \text{ } \mu\text{g N}_2\text{O g DM}^{-1} \text{ h}^{-1}$ ) during all seasons sampled, compared to other saturated systems (Jahangir et al. 2012, Song et al. 2010) (Table 2). Average nitrification rates were over 10x higher during summer ( $5.68 \pm 1.43 \text{ ng NO}_2^{-}\text{-N g}^{-1} \text{ DM h}^{-1}$  vs  $0.48 \pm 0.55 \text{ ng NO}_2^{-}\text{-N g}^{-1} \text{ DM h}^{-1}$ ), fall ( $6.14 \pm 1.26 \text{ ng NO}_2^{-}\text{-N g}^{-1} \text{ DM h}^{-1}$  vs  $0.42 \pm 0.43 \text{ ng NO}_2^{-}\text{-N g}^{-1} \text{ DM h}^{-1}$ ), and winter ( $3.27 \pm 1.56 \text{ ng NO}_2^{-}\text{-N g}^{-1} \text{ DM h}^{-1}$  vs  $0.05 \pm 0.22 \text{ ng NO}_2^{-}\text{-N g}^{-1} \text{ DM h}^{-1}$ ) months at Ag compared to MSM sites (Table 2). In addition, Ag nitrification rates were similar in summer and fall, but sharply decreased in the winter, while MSM nitrification rates steadily decreased from summer through winter seasons (Table 2). Average summer N-mineralization rates at MSM were about 2x higher than Ag rates ( $1.39 \pm 1.42 \text{ } \mu\text{g NH}_4^{+}\text{-N g}^{-1} \text{ DM day}^{-1}$  vs  $0.78 \pm 0.54 \text{ } \mu\text{g NH}_4^{+}\text{-N g}^{-1} \text{ DM day}^{-1}$ ), and maximum values were 2.57x higher at MSM compared to Ag sites ( $4.75 \text{ } \mu\text{g NH}_4^{+}\text{-N g}^{-1} \text{ DM day}^{-1}$  vs  $1.85 \text{ } \mu\text{g NH}_4^{+}\text{-N g}^{-1} \text{ DM day}^{-1}$ ) (Table 2). Average fall N-mineralization rates were about 7x higher at Ag compared to MSM sites ( $0.69 \pm 0.36 \text{ } \mu\text{g NH}_4^{+}\text{-N g}^{-1} \text{ DM day}^{-1}$  vs  $0.10 \pm 0.32 \text{ } \mu\text{g NH}_4^{+}\text{-N g}^{-1} \text{ DM day}^{-1}$ ) (Table 2). Average winter N-mineralization rates were comparable between MSM and Ag sites ( $2.02 \pm 1.09 \text{ } \mu\text{g NH}_4^{+}\text{-N g}^{-1} \text{ DM day}^{-1}$  vs  $1.75 \pm 0.60 \text{ } \mu\text{g NH}_4^{+}\text{-N g}^{-1} \text{ DM day}^{-1}$ ) (Table 2). High standard deviations in N-mineralization rate averages were observed in all sampling seasons at MSM compared to Ag sites (Table 2). Ranges of N-mineralization rates in all sampling seasons were greater at MSM compared to Ag (summer =  $5.25 \text{ } \mu\text{g NH}_4^{+}\text{-N g}^{-1} \text{ DM day}^{-1}$  vs  $1.76 \text{ } \mu\text{g NH}_4^{+}\text{-N g}^{-1} \text{ DM day}^{-1}$ , fall =  $1.20 \text{ } \mu\text{g NH}_4^{+}\text{-N g}^{-1} \text{ DM}$

day<sup>-1</sup> vs 1.05 μg NH<sub>4</sub><sup>+</sup>-N g<sup>-1</sup> DM day<sup>-1</sup>, winter = 2.93 μg NH<sub>4</sub><sup>+</sup>-N g<sup>-1</sup> DM day<sup>-1</sup> vs 2.36 μg NH<sub>4</sub><sup>+</sup>-N g<sup>-1</sup> DM day<sup>-1</sup>), and MSM soils showed potential to be an NH<sub>4</sub><sup>+</sup> sink during the summer (minimum = -0.50 μg NH<sub>4</sub><sup>+</sup>-N g<sup>-1</sup> DM day<sup>-1</sup>) and fall (minimum = -0.51 μg NH<sub>4</sub><sup>+</sup>-N g<sup>-1</sup> DM day<sup>-1</sup>) months (Table 2).

### **Relationship between soil environment and N cycling transformations**

**Denitrification.** Denitrification rates at the Ag site were significantly predicted by soil moisture, soil C/N ratio, and soil pH parameters ( $p = 2.1e^{-12}$ ,  $R^2 = 0.82$ ), while MSM rates were significantly predicted by soil moisture, temperature, and soil NH<sub>4</sub><sup>+</sup>-N ( $p = 0.03$ ,  $R^2 = 0.17$ ) (Table 3). Multiple linear regression models described over 4x the variability in Ag denitrification rates compared to MSM rates ( $R^2 = 0.82$  vs 0.17). At the Ag site, soil moisture content was lowest in the summer and coincided with the lowest measured denitrification rates, compared to fall and winter where ranges of soil moisture and denitrification rates were similar (Table 2, Fig. 3). MSM denitrification rates were also lowest in the summer and coincided with lower soil moisture content, while denitrification rates in fall and winter showed strong positive associations with soil moisture (Table 2, Fig. 3). In addition, soil moisture content and summer denitrification rates were negatively associated when water levels were lowest at both Ag and MSM sites, indicating drawdowns may affect denitrification rates in both sites.

**Nitrification.** Soil moisture, temperature, soil C/N ratio, and soil pH significantly predicted potential nitrification rates at both Ag ( $p = 4.4e^{-7}$ ) and MSM ( $p = 2.1e^{-3}$ ) sites (Table 3). Multiple linear regression predicted nitrification rates more accurately for the Ag compared to the MSM site ( $R^2 = 0.62$  vs 0.33). Lower soil moisture content was associated with higher nitrification rates at Ag compared to MSM sites (Fig. 4). Within the Ag site, soil moisture content was higher in fall compared to summer and resulted in increased nitrification rates. However, there was no

relationship between soil moisture and nitrification rates at the MSM site across all sampling seasons (Fig. 4, Table 2). In addition, low nitrification rates at the MSM site were associated with low soil pH (Fig. 5).

**N-mineralization.** N-mineralization at Ag was significantly predicted by soil % C, soil  $\text{NO}_3^-$ -N, and soil  $\text{NH}_4^+$ -N parameters ( $p = 2.5e^{-8}$ ), while MSM rates were significantly predicted by soil moisture, temperature, soil C/N ratio, and soil  $\text{NO}_3^-$ -N ( $p = 4.0e^{-5}$ ) (Table 3). There was less of a discrepancy in N-mineralization Ag and MSM model performances ( $R^2 = 0.66$  vs  $0.50$ ) compared to nitrification and denitrification models, but Ag models still performed slightly better than MSM models. N-mineralization rates in general were positively associated with increasing soil moisture content at both sites, but this effect was much more pronounced at MSM (Fig. 6). This positive association was not observed in Ag July soils due to 2 outliers with low moisture but high N-mineralization rates (Fig. 6).

**Mesocosm Study.** No significant interactions between main effects of field site and retention time were observed for all N species over the six-month experiment (Table 4). Mesocosm  $\text{NH}_4^+$ -N concentrations were significantly higher at MSM ( $0.88 \pm 0.56 \text{ mg NH}_4\text{-N L}^{-1}$ ) compared to Ag ( $0.35 \pm 0.16 \text{ mg NH}_4\text{-N L}^{-1}$ ) soil ( $F_{1,21}=9.523$ ,  $p = 0.006$ ) (Table 4). However, interpretations of this significant effect should take into consideration that N concentrations in simulated rainfall differed slightly from Ag and MSM sites (For monthly simulated rainfall N concentrations see Supplemental Data). Mesocosm  $\text{NO}_3^-$ -N and DON concentrations were similar across site and retention time in both sites (Table 4). However, MSM DON concentrations were observed to visually decrease as  $\text{NH}_4^+$ -N increased over the course of the experiment (Fig.7), but this effect was not apparent in Ag mesocosms (Fig. 7). Overall there was little difference between control

and treatment mesocosms, with treatment mesocosm N concentrations sometimes exceeding experimental mesocosm concentrations (Fig. 7).

## **Discussion**

### **Soil physiochemical environment drives N cycling differences between Ag and MSM sites**

Nitrification rates were substantially higher at Ag compared to MSM in all sampling seasons, even during winter when water levels were above the soil profile in both sites. This indicates factors beyond changes in redox conditions are inhibiting MSM nitrification rates. Based on this study, a combination of low soil pH and poor year-round soil aeration may be driving low MSM nitrification rates, compared to Ag where soils are managed for higher pH, and the soil profile aerated due to tillage and plant growth during growing seasons. Greater ranges of N-mineralization and denitrification rates were measured at MSM compared to Ag sites. This was likely driven by the substantial spatial variability in soil physiochemical environment at the MSM site, evidenced by larger ranges being observed in all measured soil parameters except for soil  $\text{NO}_3^-$ -N (Table 1). These differences in soil physiochemical environment and N cycling rates could be a consequence of greater spatial variability and more diverse vegetation at the MSM impoundment compared to Ag site. Additionally, the conventional row crop management (corn-cotton rotation), fertilizer application, and soil management (such as liming soil to increase pH levels above 5.5) methods have been shown to environmentally filter soil microbial communities such that N cycling functions were more similar across the Ag site (Feckler et al. 2018). This environmental filtering effect on the microbial communities at the Ag site may have contributed to decreased ranges of N-mineralization and denitrification being observed in this study.

## Soil pH and redox conditions inhibit impoundment nitrification rates

Higher nitrification rates were associated with drier soil conditions and soil pH greater than 5.5 at the Ag sites. This field study supported the hypothesis that potential nitrification rates are greater at Ag compared to MSM sites. Ag nitrification rates showed strong seasonal variability, presumably due to changes in hydrologic management. Ag nitrification rates were high in the summer (non-impoundment conditions) and fall (initial-impoundment conditions) compared to the winter (late-impoundment conditions) rates (Table 2, Fig. 4). The high Ag nitrification rates during the fall season coincided with soil sampling occurring soon after impoundment conditions had been invoked and water levels were still below the soil surface. In contrast, Ag winter nitrification rates observed when water levels rose above the soil surface resulted in inhibited nitrification rates (Table 2, Fig. 4). MSM nitrification rates showed little seasonal variability and were very low year-round compared to Ag. This indicates that MSM nitrification could be inhibited by factors beyond hydrology. Soil pH was notably lower at MSM compared to Ag and potentially inhibiting MSM nitrification rates. Previous literature shows that nitrification potential of highly acidic soils is typically low (Zhang et al. 2013, Zhao et al. 2007) for two key reasons; low soil pH suppresses  $\text{NH}_4^+$  oxidation and volatilization. The suppression of  $\text{NH}_4^+$  oxidation directly inhibits nitrification rates, and the low  $\text{NH}_4^+$  volatilization substantially decreases soil  $\text{NH}_4^+$ -N loss, which has been connected to  $\text{NH}_4^+$  becoming the dominant inorganic N species in acidic soils (Zhang et al. 2013). In the present study, low pH levels were measured at the MSM site (Table 1) and are contributing, in part, to low  $\text{NH}_4^+$  oxidation and volatilization, as evidenced by the high  $\text{NH}_4^+$ -N levels and low nitrification rates.

Low nitrification potential at MSM impoundments is contributing to high surface water  $\text{NH}_4^+$ -N concentrations, which was suggested in a past field study (Winton et al. 2016). This low

nitrification potential at MSM is due to a combination of low soil pH and saturated soil conditions, which indicates that management strategies solely focused around modifying MSM hydrology to aerate portions of the impoundment soils will not increase nitrification potential. Ag site nitrification potential was also observed to decrease in both the field (See Ch. 1) and laboratory scales (Table 2, Fig. 4), whereby increased water levels/soil moisture resulted in increased  $\text{NH}_4^+$ -N concentrations. Based on these results, changes to hydrologic management to aerate portions of the soil profile can be effective at increasing nitrification potential during seasonal flooding at Ag sites, while similar hydrologic management changes, as well as efforts to increase soil pH, will be required to increase MSM nitrification potential. However, increasing soil pH at MSM will be difficult and require innovative strategies, as I found no studies that involved managing soil pH in restored and created wetland systems.

### **Soil moisture and nitrification limit impoundment denitrification potential**

Interestingly, higher average denitrification rates were observed at Ag than MSM sites and does not support my initial hypothesis that MSM soils support greater denitrification potential. This may have been due to soil  $\text{NO}_3^-$ -N concentrations being over 7x higher at the Ag site compared to MSM (Table 1), which provided substantially greater N substrate to fuel denitrification processes. Strong seasonal differences in denitrification rates were observed at both sites and were presumably driven by changes in managed hydrology, generally evidenced by strong positive associations in denitrification rates and soil moisture in both systems (Table 3, Fig. 3). These results are consistent with prior literature and demonstrate denitrification is generally enhanced with increasing soil moisture content creating anaerobic conditions (Eickenscheidt et al. 2014, Peralta et al. 2013, Jayakumar et al. 2009). Soil C/N ratio and low soil pH (seasonal averages < 5.80) appeared to limit denitrification potential in the Ag site (Table 3),

while temperature and soil  $\text{NH}_4^+$ -N limited denitrification potential in the MSM site (Table 3). Soil C/N ratio was significant at Ag but not MSM perhaps due to C/N ratios being slightly lower at Ag compared to MSM, with higher ratios being shown to inhibit denitrification potential (Toma & Hatano 2007). Another potential reason for Ag denitrification potential being greater than MSM is due to differences in quality of carbon within each system (Rocher et al. 2015). MSM soil organic carbon sources were likely composed of woody and high cellulose plant and seed residues (more recalcitrant C), whereas Ag soil organic carbon was likely composed of dried corn and cotton stalk residues (more labile C). These Ag soil organic C sources would also be subject to greater aerobic breakdown, rendering their C content even more labile compared to MSM. Together, the lower C/N ratio at Ag, along with potentially more labile soil C fractions, may have further contributed to the higher Ag denitrification rates. Soil  $\text{NH}_4^+$ -N was negatively associated with MSM denitrification, and I suggest this was found to be significant due to being a proxy for decreased nitrification (Zhang et al. 2013) and increased N-mineralization in response to changes in hydrology, which led to low soil nitrate levels.

MSM site denitrification was lower than expected and was much lower than previous studies observed in created wetland systems (Song et al. 2010). This is potentially due to low MSM nitrification potential, as nitrification processes provide the  $\text{NO}_3^-$ -N substrate to fuel denitrification and suggests that the MSM microbial communities are using other TEAs, evidenced by high methane emissions previously measured at another MSM impoundment (Winton et al. 2017). This effect of highly reducing conditions limiting denitrification potential of soils has been shown in other wetland systems (Seo & DeLaune 2010). If MSM microbial communities are using other TEA sources, aerating the impoundment will not immediately increase denitrification potential due to microbial communities requiring time to adapt to the

environmental changes, such as availability of TEAs that would occur (Oosterkamp et al. 2013). Winter denitrification rates at the Ag site were also lower than expected when compared to restored agricultural wetlands (Ballantine et al. 2017), which may also be attributed to decreased winter nitrification potential (Table 2, Fig. 4). This suggests that nitrification and denitrification may be strongly coupled, meaning nitrification processes are the main source of  $\text{NO}_3^-$  to fuel denitrification processes, in both sites (Kessler et al. 2013, Racchetti et al. 2011).

Both Ag and MSM sites showed increased denitrification potential with increased water levels/soil moisture when comparing summer and fall rates within sites. However, both sites also showed decreases in denitrification potential when comparing fall and winter rates within sites, presumably due to exhausted  $\text{NO}_3^-$ -N pools along with lower temperatures suppressing denitrification rates (Table 2). This observed nitrification-denitrification coupling and lower denitrification potential is common in hydrologically isolated systems (Racchetti et al. 2011), such as Ag and MSM waterfowl impoundments. In this study, water level drawdown may inhibit denitrification potential at both sites since negative associations between denitrification and soil moisture occurred during the summer. This drawdown may have artificially aerated the soil-water interface and exported water may have washed away excess soil  $\text{NO}_3^-$ -N unable to be used as substrate for denitrification processes. This further demonstrates the dependence of denitrification potential on nitrification processes in hydrologically isolated systems and shows that more research is needed to understand denitrification processes in systems where pumping is prevalent.

### **Hydrology, soil $\text{NO}_3^-$ -N, and waterfowl waste affects N-mineralization rates**

Average N-mineralization rates were higher and much more variable at MSM compared to Ag soils and support my initial hypothesis of more permanent and diverse vegetation results in

more dynamic N-mineralization rates. Both sites showed steady increases in N-mineralization rates as water levels rose from summer to winter months in both the field (Ch. 1 supplemental data) and laboratory scale (Fig. 6), indicating that  $\text{NH}_4^+$ -N becomes a more important N substrate for microbial and vegetative communities than  $\text{NO}_3^-$ -N as nitrification rates decrease with increasing anoxic conditions (Fig. 4). This aligns with previous literature, indicating that microbial and vegetative communities prefer inorganic N sources over organic N due to being more energetically efficient (Hill et al. 2011). This overall seasonal variability of N-mineralization was clearer at both sites compared to seasonal nitrification and denitrification rates (Table 3, Fig. 3 & 4), and further demonstrates the importance of  $\text{NH}_4^+$ -N as an N source at both sites when water levels rise above the soil surface. This effect of wetting/drying cycles has also been shown to stimulate N mineralization processes in previous literature (Borken et al. 2009, Xiang et al. 2008). These wetting/drying cycles also coincide with the presence of migratory bird populations in December, along with increased bird waste ON inputs, which may further contribute to increased winter N-mineralization rates at both sites (Table 2).

These results indicate that N-mineralization rates at both sites were strongly affected by hydrology/soil moisture, despite the soil moisture factor being absent in the Ag N-mineralization regression models (Table 3). N-mineralization rates were also shown to increase with increasing ON inputs from migratory bird waste, which was demonstrated to have potential to be easily mobilized and converted to  $\text{NH}_4^+$ -N (Ch. 1). This may explain why soil  $\text{NO}_3^-$ -N was shown to significantly influence N-mineralization rates at both sites (Table 3), as soil  $\text{NO}_3^-$ -N decreased from summer through winter. Previous studies have also found this to occur in scenarios where nitrification is limited (Zhang et al. 2013). This decrease in soil  $\text{NO}_3^-$ -N may have prompted microbial communities in both sites to increase N-mineralization of newly deposited bird waste,

resulting in  $\text{NH}_4^+$ -N an N source. Future research on waterfowl impoundments should investigate this trend of increasing N-mineralization rates from July through December and expand studies to include early spring sampling prior to impoundment dewatering. Doing so would confirm if this trend of increasing N-mineralization persists. Better characterization of N-mineralization trends would yield crucial information to develop more robust N management strategies that promote complete N-cycling year-round to reduce high  $\text{NH}_4^+$ -N concentrations during early spring dewatering of waterfowl impoundments (See Ch. 1).

### **Potential for hydrologic management to enhance N retention**

Gradual decreases in mesocosm DON (Fig. 7) could be indicative of assimilation processes gradually depleting mesocosm soil  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N, prompting microbial communities to mineralize soil ON to  $\text{NH}_4^+$ -N as an N source. This corroborates the trend of increasing  $\text{NH}_4^+$ -N observed in MSM mesocosms over time, but this was less clear in Ag mesocosms (Fig. 7). This supports the hypothesis that N-mineralization potential is greater at MSM than Ag sites. Overall, residence time did not impact mesocosm N concentrations, and in some cases, longer residence time increased N concentrations in the ponded mesocosm water (Fig. 7). These results demonstrate that Ag and MSM soils have limited potential to perform as treatment wetlands under these experimental conditions since water column N concentrations were similar with increased mesocosm residence time.

### **N cycling management recommendations**

Agricultural N management strategies traditionally focus on managing fertilizer application and plant growth to enhance productivity and limit N runoff. To further reduce N runoff from fields, management of hydrology by enhancing anoxic conditions can promote N

removal via denitrification pathways (Arce et al. 2013, Kellogg et al. 2010). However, promoting complete nitrogen removal from fields via denitrification can only occur if organic carbon and nitrate do not limit microbial metabolism. Therefore, when water levels rise above the soil surface from October through February (Ag) or March (MSM), nitrification rates are inhibited, and denitrification is decoupled from nitrification in both systems (See Fall and Winter differences in Fig. 3 & 4). Based on my previous water quality study (Ch. 1) and the current N processing study, I observed that N cycling is predominately controlled by hydrology at the Ag site. Ag nitrification and denitrification processes were enhanced in late September when there was no active drawdown applied and water levels were at or slightly below the soil surface. This may imply that managing Ag system hydrology to hold conditions consistent with late September water levels, compared to December conditions when they were ~ 6 cm above the soil profile and nitrification was inhibited, is a viable strategy to reduce N export. Having waterlogged portions adjacent to slightly elevated, aerated soils (in this case the main drainage ditch adjacent to the cropping fields) may result in enhanced N removal via coupled nitrification-denitrification pathways (Wolf et al. 2011) in October through early February when Ag is treated as an impoundment.

MSM N management strategies should focus on enhancing the nitrification potential of the impoundment soils to reduce N export. Similar conclusions were drawn from Winton et al. (2016). Experimental data suggests that solely altering hydrology to aerate portions of the impoundment, as suggested for Ag, may be ineffective at increasing MSM nitrification rates. This is evidenced by stable MSM year-round nitrification rates and surface water  $\text{NO}_3^-$ -N concentrations despite drastic changes in impoundment water levels/soil moisture content (Ch. 1, Fig. 3). Therefore, MSM N management strategies should focus on permanently aerating

portions of the impoundment adjacent to waterlogged portions. Aerating portions to emphasize movement of water through soils, rather than over them, before export may promote coupled nitrification-denitrification to remove N from surface waters. These efforts should coincide with seeking strategies to slightly raise soil pH, such as agricultural liming or manure amendments (Goulding et al. 2016, Walker et al. 2004), throughout the impoundment. Before management efforts are made to increase MSM soil pH, a more comprehensive study should be conducted to identify what areas have soil pH values below 5.0 and efforts should be made to increase the soil pH in those areas. Efforts to increase soil pH should manage for pH values slightly below 5.6, as greater soil pH values have been shown to decrease seed persistence (Basto et al. 2015). It should be stated that increasing MSM soil pH may not result in an immediate increase in nitrification rates, as microbial community function develops temporally in response to changes in environmental filters (Lauber et al. 2013, Bell et al. 2008). However, low soil pH and persisting waterlogged conditions have been shown to result in a decreased species numbers in grassland systems (Michalcová et al. 2011). Therefore, strategically aerating portions and carefully increasing soil pH may benefit the plant communities and reduce the N exported from the MSM impoundment.

## **Conclusion**

In this study, seasonal hydrologic management practices and the soil physiochemical environment greatly affected N cycling in Ag and MSM impoundments. I showed that conventional agricultural land management led to decreased soil physiochemical environment variability, which decreased variability in N cycling rates at Ag and compared to MSM impoundments. Seasonal flooding greatly impaired nitrification processes in both impoundment types, which was shown to be strongly coupled to denitrification processes. Low soil pH was

also shown to inhibit nitrification at the MSM impoundment. This inhibition of nitrification resulted in exhausted soil  $\text{NO}_3^-$ -N during prolonged flooding periods, which may have prompted microbial communities to mineralize bird waste derived ON to  $\text{NH}_4^+$ -N to provide an N source in both systems. This builds on a previous study that observed high  $\text{NH}_4^+$ -N concentrations during prescribed drawdowns and shows that previous recommendations of modifying drawdown times and magnitudes alone will be insufficient to reduce N exported. I suggest that both impoundments should seek to aerate portions during seasonal flooding periods, along with seeking methods to increase soil pH at MSM impoundments. Doing so would restore the coupled nitrification-denitrification processes and greatly reduce inorganic N concentrations exported to downstream systems.

## References

Anderson, D. M., Burkholder, J. M., Cochlan, W. P., Glibert, P. M., Gobler, C. J., Heil, C. A., Vargo, G. A. (2008). Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the united states. *Harmful Algae*, 8(1), 39-53.

doi:10.1016/j.hal.2008.08.017

APHA (2012) American Public Health Association. Standard methods for the examination of water and wastewater. 22nd edition. American Water Works Association.

Arce, M. I., Gómez, R., Suárez, M. L., & Vidal-Abarca, M. R. (2013). Denitrification rates and controlling factors in two agriculturally influenced temporary Mediterranean saline streams.

*Hydrobiologia*, 700(1), 169-185. doi:10.1007/s10750-012-1228-4

Balaine, N., Clough, T. J., Kelliher, F. M., & Kotev, C. v. (2015). Soil aeration affects the degradation rate of the nitrification inhibitor dicyandiamide. *Soil Research*, 53(2), 137-143.

doi:10.1071/SR14162

Ballantine, K. A., Anderson, T. R., Pierce, E. A., & Groffman, P. M. (2017). Restoration of denitrification in agricultural wetlands. *Ecological Engineering*, 106, 570.

Barszczewski, J., & Kaca, E. (2012). Water retention in ponds and the improvement of its quality during carp production. *Journal of Water and Land Development*, 17(1), 31-38.

Basto, S., Thompson, K., & Rees, M. (2015). The effect of soil pH on persistence of seeds of grassland species in soil. *Plant Ecology*, 216(8), 1163-1175. doi:10.1007/s11258-015-0499-z

Bell, C., McIntyre, N., Cox, S., Tissue, D., & Zak, J. (2008). Soil Microbial Responses to Temporal Variations of Moisture and Temperature in a Chihuahuan Desert Grassland. *Microbial Ecology*, 56(1), 153-167. Retrieved from <http://www.jstor.org/stable/40343355>

Birgand, F., Appelboom, T. W., Chescheir, G. M., & Skaggs, R. W. (2011). Estimating nitrogen, phosphorus, and carbon fluxes in forested and mixed-use watersheds of the lower coastal plain of north carolina: Uncertainties associated with infrequent sampling. *Transactions of the ASABE*, 54(6), 2099-2110. doi:10.13031/2013.40668

Birgand, F., Aveni-Deforge, K., Smith, B., Maxwell, B., Horstman, M., Gerling, A. B., & Carey, C. C. (2016). First report of a novel multiplexer pumping system coupled to a water quality probe to collect high temporal frequency in situ water chemistry measurements at multiple sites: High-resolution water chemistry in time and space. *Limnology and Oceanography: Methods*, 14(12), 767-783. doi:10.1002/lom3.10122

Booth, M. S., Stark, J. M., & Rastetter, E. (2005). Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. *Ecological Monographs*, 75(2), 139-157. doi:10.1890/04-0988

Borken, W., & Matzner, E. (2009). Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. *Global Change Biology*, 15(4), 808-824. doi:10.1111/j.1365-2486.2008.01681.x

Burger, M., & Jackson, L. E. (2003). Microbial immobilization of ammonium and nitrate in relation to ammonification and nitrification rates in organic and conventional cropping systems. *Soil Biology and Biochemistry*, 35(1), 29-36. doi:10.1016/S0038-0717(02)00233-X

Castaldelli, G., Colombani, N., Vincenzi, F., & Mastrocicco, M. (2013). Linking dissolved organic carbon, acetate and denitrification in agricultural soils. *Environmental Earth Sciences*, 68(4), 939-945. doi:10.1007/s12665-012-1796-7

Chow, A. T., Dai, J., Conner, W. H., Hitchcock, D. R., & Wang, J. (2013). Dissolved organic matter and nutrient dynamics of a coastal freshwater forested wetland in winyah bay, south carolina. *Biogeochemistry*, 112(1), 571-587. doi:10.1007/s10533-012-9750-z

Danczak, R. E., Sawyer, A. H., Williams, K. H., Stegen, J. C., Hobson, C., & Wilkins, M. J. (2016). Seasonal hyporheic dynamics control coupled microbiology and geochemistry in colorado river sediments. *Journal of Geophysical Research: Biogeosciences*, 121(12), 2976-2987. 10.1002/2016JG003527

Dodds, W. K., Bouska, W. W., Eitzmann, J. L., Pilger, T. J., Pitts, K. L., Riley, A. J., Thornbrugh, D. J. (2009). Eutrophication of U.S. freshwaters: Analysis of potential economic damages. *Environmental Science & Technology*, 43(1), 12-19. doi:10.1021/es801217q

Drinkwater, L., C.A. Cambardella, J.D. Reeder, and C.W. Rice. 1996. "Potentially Mineralizable Nitrogen as an Indicator of Biologically Active Soil Nitrogen." In *Methods for Assessing Soil Quality*, 217–29. Madison, WI: SSSA.

Eickenscheidt, T., Heinichen, J., Augustin, J., Freibauer, A., & Drösler, M. (2014). Nitrogen mineralization and gaseous nitrogen losses from waterlogged and drained organic soils in a black alder (*alnus glutinosa* (L.) gaertn.) forest. *Biogeosciences*, 11(11), 2961-2976. doi:10.5194/bg-11-2961-2014

Etheridge, J. R., Birgand, F., Osborne, J. A., Osburn, C. L., Burchell, M. R., & Irving, J. (2014). Using in situ ultraviolet-visual spectroscopy to measure nitrogen, carbon, phosphorus, and

suspended solids concentrations at a high frequency in a brackish tidal marsh. *Limnology and Oceanography: Methods*, 12(1), 10–22. <https://doi.org/10.4319/lom.2014.12.10>

Etheridge, J. R., Birgand, F., & Burchell, M. R. (2015). Quantifying nutrient and suspended solids fluxes in a constructed tidal marsh following rainfall: The value of capturing the rapid changes in flow and concentrations. *Ecological Engineering*, 78, 41–52.

<https://doi.org/10.1016/j.ecoleng.2014.05.021>

Etheridge, J. R., Burchell II, M. R., & Birgand, F. (2017). Can created tidal marshes reduce nitrate export to downstream estuaries? *Ecological Engineering*, 105, 314–324.

<https://doi.org/10.1016/j.ecoleng.2017.05.009>

Fang, Y., Singh, B. P., Badgery, W., & He, X. (2016). In situ assessment of new carbon and nitrogen assimilation and allocation in contrastingly managed dryland wheat crop-soil systems. *Agriculture, Ecosystems & Environment*, 235, 80-90. [10.1016/j.agee.2016.10.010](https://doi.org/10.1016/j.agee.2016.10.010)

Feckler, A., Goedkoop, W., Korschak, M., Bundschuh, R., Kenngott, K. G. J., Schulz, R., . . . Bundschuh, M. (2018). History matters: Heterotrophic microbial community structure and function adapt to multiple stressors. *Global Change Biology*, 24(2), e402-e415.

[doi:10.1111/gcb.13859](https://doi.org/10.1111/gcb.13859)

F. Schinner, R. Öhlinger, E. Kandeler, R. Margesin (Eds.), *Methods in Soil Biology*, Springer, Berlin (1996), pp. 146-148

Foulquier, A., Volat, B., Neyra, M., Bornette, G., & Montuelle, B. (2013). Long-term impact of hydrological regime on structure and functions of microbial communities in riverine wetland sediments. *FEMS Microbiology Ecology*, 85(2), 211-226. [10.1111/1574-6941.12112](https://doi.org/10.1111/1574-6941.12112)

Geisseler, D., Horwath, W. R., Joergensen, R. G., & Ludwig, B. (2010). Pathways of nitrogen utilization by soil microorganisms – A review. *Soil Biology and Biochemistry*, 42(12), 2058–2067. <https://doi.org/10.1016/j.soilbio.2010.08.021>

Goulding, K. W. T., & Varennes, A. (2016). Soil acidification and the importance of liming agricultural soils with particular reference to the United Kingdom. *Soil use and Management*, 32(3), 390-399. doi:10.1111/sum.12270

Glibert, P. M., Harrison, J., Heil, C., & Seitzinger, S. (2006). Escalating worldwide use of urea: A global change contributing to coastal eutrophication. *Biogeochemistry*, 77(3), 441-463. doi:10.1007/s10533-005-3070-5

Haynes, R. J., & Judge, A. (2008). Influence of surface-applied poultry manure on topsoil and subsoil acidity and salinity: A leaching column study. *Journal of Plant Nutrition and Soil Science*, 171(3), 370-377. doi:10.1002/jpln.200700167

Hill, P. W., Farrar, J., Roberts, P., Farrell, M., Grant, H., Newsham, K. K., Jones, D. L. (2011). Vascular plant success in a warming antarctic may be due to efficient nitrogen acquisition. *Nature Climate Change*, 1(1), 50-53. doi:10.1038/nclimate1060

Inamdar, S., Dhillon, G., Singh, S., Parr, T., & Qin, Z. (2015). Particulate nitrogen exports in stream runoff exceed dissolved nitrogen forms during large tropical storms in a temperate, headwater, forested watershed: TROPICAL STORMS AND PARTICULATE NITROGEN. *Journal of Geophysical Research: Biogeosciences*, 120(8), 1548-1566. doi:10.1002/2015JG002909

- Israel, S., Engelbrecht, P., Tredoux, G., & Fey, M. V. (2009). In situ batch denitrification of nitrate-rich groundwater using sawdust as a carbon source-marydale, south africa. *Water, Air, and Soil Pollution*, 204(1-4), 177-194. doi:10.1007/s11270-009-0036-6
- Jayakumar, A., O'Mullan, G. D., S. W. A. Naqvi, & Ward, B. B. (2009). Denitrifying bacterial community composition changes associated with stages of denitrification in oxygen minimum zones. *Microbial Ecology*, 58(2), 350-362. doi:10.1007/s00248-009-9487-y
- Jordan, T. E., Whigham, D. F., Hofmockel, K. H., & Pittek, M. A. (2003). Nutrient and sediment removal by a restored wetland receiving agricultural runoff. *Journal of Environmental Quality*, 32(4), 1534. doi:10.2134/jeq2003.1534
- Jordan, S. J., Stoffer, J., & Nestlerode, J. A. (2011). Wetlands as Sinks for Reactive Nitrogen at Continental and Global Scales: A Meta-Analysis. *Ecosystems*, 14(1), 144–155.
- Karhu, K., Auffret, M. D., Dungait, J. A. J., Hopkins, D. W., Prosser, J. I., Singh, B. K., Sveriges lantbruksuniversitet. (2014). Temperature sensitivity of soil respiration rates enhanced by microbial community response. *Nature*, 513(7516), 81-84. doi:10.1038/nature13604
- Kellogg, D. Q., Gold, A. J., Cox, S., Addy, K., & August, P. V. (2010). A geospatial approach for assessing denitrification sinks within lower-order catchments. *Ecological Engineering*, 36(11), 1596-1606. doi:10.1016/j.ecoleng.2010.02.006
- Kessler, A. J., Glud, R. N., Cardenas, M. B., & Cook, P. L. M. (2013). Transport Zonation Limits Coupled Nitrification-Denitrification in Permeable Sediments. *Environmental Science & Technology*, 47(23), 13404–13411. <https://doi.org/10.1021/es403318x>

Lauber, C. L., Ramirez, K. S., Aanderud, Z., Lennon, J., & Fierer, N. (2013). Temporal variability in soil microbial communities across land-use types. *The ISME Journal*, 7(8), 1641-50. doi:<http://dx.doi.org.jproxy.lib.ecu.edu/10.1038/ismej.2013.50>

Lefcheck, J. S., Wilcox, D. J., Murphy, R. R., Marion, S. R., & Orth, R. J. (2017). Multiple stressors threaten the imperiled coastal foundation species eelgrass (*Zostera marina*) in Chesapeake Bay, USA. *Global Change Biology*, 23(9), 3474–3483. <https://doi.org/10.1111/gcb.13623>

Liu, W., Zhang, Q., & Liu, G. (2010). Lake eutrophication associated with geographic location, lake morphology and climate in china. *Hydrobiologia*, 644(1), 289-299. doi:10.1007/s10750-010-0151-9

Ma, BL, Ying, J and Balchin, D 2005 Impact of sample preservation methods on the extraction of inorganic nitrogen by potassium chloride. *J Plant Nutr* 28(5): 785–796. DOI: 10.1081/PLN-200055536

Marchant, H. K., Holtappels, M., Lavik, G., Ahmerkamp, S., Winter, C., & Kuypers, M. (2016). Coupled nitrification–denitrification leads to extensive N loss in subtidal permeable sediments. *Limnology and Oceanography*, 61(3), 1033–1048. <https://doi.org/10.1002/lno.10271>

Maul, J. D., & Cooper, C. M. (2000). Water quality of seasonally flooded agricultural fields in mississippi, USA. *Agriculture, Ecosystems and Environment*, 81(3), 171-178. doi:10.1016/S0167-8809(00)00157-2

McCrackin, M. L., Jones, H. P., Jones, P. C., & Moreno-Mateos, D. (2017). Recovery of lakes and coastal marine ecosystems from eutrophication: A global meta-analysis. *Limnology and Oceanography*, 62(2), 507–518. <https://doi.org/10.1002/lno.10441>

Menning, D. M., Carraher-Stross, W. A., Graham, E. D., Thomas, D. N., Phillips, A. R., Scharping, R. J., & Garey, J. R. (2018). Aquifer discharge drives microbial community change in karst estuaries. *Estuaries and Coasts*, 41(2), 430-443. 10.1007/s12237-017-0281-7

Mevik, B., R. Wehrens, and K. H. Liland. 2011. pls: Partial least squares and principal component regression. R package 2.3-0. <<http://CRAN.R-project.org/package=pls>>

Michalcová, D., Gilbert, J. C., Lawson, C. S., David J. G. Gowing, & Marrs, R. H. (2011). The combined effect of waterlogging, extractable P and soil pH on  $\alpha$ -diversity: A case study on mesotrophic grasslands in the UK. *Plant Ecology*, 212(5), 879-888. doi:10.1007/s11258-010-9871-1

Moorman, M. C., Augspurger, T., Stanton, J. D., & Smith, A. (2017). Where's the grass? disappearing submerged aquatic vegetation and declining water quality in lake mattamuskeet. *Journal of Fish and Wildlife Management*, 8(2), 404. 10.3996/082016-JFWM-068

Morris, D., Basiliko, N., Hazlett, P., Caspersen, J., Webster, K., & Smenderovac, E. E. (2017). Does intensified boreal forest harvesting impact soil microbial community structure and function? *Canadian Journal of Forest Research*, 47(7), 916-925. doi:10.1139/cjfr-2016-0468

Nelms, K., Ballinger, B., Boyles, A. (2007). *Wetland Management for Waterfowl Handbook*. National Resources Conservation Service.

Nizzoli, D., Welsh, D. T., Longhi, D., & Viaroli, P. (2014). Influence of *potamogeton pectinatus* and microphytobenthos on benthic metabolism, nutrient fluxes and denitrification in a freshwater littoral sediment in an agricultural landscape: N assimilation versus N removal. *Hydrobiologia*, 737(1), 183-200. doi:10.1007/s10750-013-1688-1

Oehmen, A., Lopez-Vazquez, C. M., Carvalho, G., Reis, M. A. M., & van Loosdrecht, M. C. M. (2010). Modelling the population dynamics and metabolic diversity of organisms relevant in anaerobic/anoxic/aerobic enhanced biological phosphorus removal processes. *Water Research*, 44(15), 4473-4486. doi:10.1016/j.watres.2010.06.017

Oosterkamp, M. J., Boeren, S., Plugge, C. M., Schaap, P. J., & Stams, A. J. M. (2013). Metabolic response of *alicycliphilus denitrificans* strain BC toward electron acceptor variation. *Proteomics*, 13(18-19), 2886-2894. doi:10.1002/pmic.201200571

Paerl, H. W., & Scott, J. T. (2010). Throwing fuel on the fire: Synergistic effects of excessive nitrogen inputs and global warming on harmful algal blooms. *Environmental Science & Technology*, 44(20), 7756.

Papastergiadou, E. S., Retalis, A., Kalliris, P., & Georgiadis, T. (2007). Land use changes and associated environmental impacts on the Mediterranean shallow lake Stymfalia, Greece. *Hydrobiologia*, 584(1), 361-372. doi:10.1007/s10750-007-0606-9

Peralta, A. L., Johnston, E. R., Matthews, J. W., & Kent, A. D. (2016). Abiotic correlates of microbial community structure and nitrogen cycling functions vary within wetlands. *Freshwater Science*, 35(2), 573-588. doi:10.1086/685688

- Peralta, A. L., Ludmer, S., & Kent, A. D. (2013). Hydrologic history influences microbial community composition and nitrogen cycling under experimental drying/wetting treatments. *Soil Biology and Biochemistry*, 66, 29–37. <https://doi.org/10.1016/j.soilbio.2013.06.019>
- Peralta, A. L., Ludmer, S., Matthews, J. W., & Kent, A. D. (2014). Bacterial community response to changes in soil redox potential along a moisture gradient in restored wetlands. *Ecological Engineering*, 73, 246-253. [10.1016/j.ecoleng.2014.09.047](https://doi.org/10.1016/j.ecoleng.2014.09.047)
- Piehlner, M. F., Currin, C. A., & Hall, N. S. (2010). Estuarine intertidal sandflat benthic microalgal responses to in situ and mesocosm nitrogen additions. *Journal of Experimental Marine Biology and Ecology*, 390(2), 99-105. [doi:10.1016/j.jembe.2010.05.012](https://doi.org/10.1016/j.jembe.2010.05.012)
- Rabalais, N. N., Turner, R. E., Dortch, Q., Justic, D., Bierman Jr, V. J., & Wiseman Jr, W. J. (2002). Nutrient-enhanced productivity in the northern gulf of mexico: Past, present and future. *Hydrobiologia*, 475(1), 39-63. [doi:10.1023/A:1020388503274](https://doi.org/10.1023/A:1020388503274)
- Racchetti, E., Bartoli, M., Soana, E., Longhi, D., Christian, R. R., Pinardi, M., & Viaroli, P. (2011). Influence of hydrological connectivity of riverine wetlands on nitrogen removal via denitrification. *Biogeochemistry*, 103(1/3), 335-354. [doi:10.1007/s10533-010-9477-7](https://doi.org/10.1007/s10533-010-9477-7)
- Racchetti, E., Longhi, D., Ribaud, C., Soana, E., & Bartoli, M. (2017;2016;). Nitrogen uptake and coupled nitrification-denitrification in riverine sediments with benthic microalgae and rooted macrophytes. *Aquatic Sciences*, 79(3), 487-19. [10.1007/s00027-016-0512-1](https://doi.org/10.1007/s00027-016-0512-1)
- Revitt, D. Michael, Tamas Balogh, and Huw Jones. (2014). “Soil Mobility of Surface Applied Polyaromatic Hydrocarbons in Response to Simulated Rainfall.” *Environmental Science and Pollution Research* 21 (6): 4209–19. [doi:10.1007/s11356-013-2231-7](https://doi.org/10.1007/s11356-013-2231-7).

Robertson, GP, Sollins, P, Ellis, BG and Lajtha, K. (1999) Exchangeable ions, pH, and cation exchange capacity. In: Standard Soil Methods for Long-Term Ecological Research, 106–114. New York: Oxford University Press.

Rocher, V., Laverman, A. M., Gasperi, J., Azimi, S., Guérin, S., Mottelet, S., Pauss, A. (2015). Nitrite accumulation during denitrification depends on the carbon quality and quantity in wastewater treatment with biofilters. *Environmental Science and Pollution Research*, 22(13), 10179-10188. doi:10.1007/s11356-015-4196-1

Royer, T. V., Tank, J. L., & David, M. B. (2004). Transport and fate of nitrate in headwater agricultural streams in illinois. *Journal of Environmental Quality*, 33(4), 1296-1304. doi:10.2134/jeq2004.1296

Schilling, K. E., & Spooner, J. (2006). Effects of watershed-scale land use change on stream nitrate concentrations. *Journal of Environmental Quality*, 35(6), 2132-2145. doi:10.2134/jeq2006.0157

Seo, D. C., & DeLaune, R. D. (2010). Fungal and bacterial mediated denitrification in wetlands: Influence of sediment redox condition. *Water Research*, 44(8), 2441-2450. doi:10.1016/j.watres.2010.01.006

Shang, F., Ren, S., Yang, P., Li, C., & Ma, N. (2015). Effects of different fertilizer and irrigation water types, and dissolved organic matter on soil C and N mineralization in crop rotation farmland. *Water, Air, & Soil Pollution*, 226(12), 1-25. 10.1007/s11270-015-2667-0

Skaggs, R.W., Gilliam, J.W., Sheets, T.J., & Barnes, J.S. (1980). Effect of agricultural land development on drainage waters in the North Carolina Tidewater region. Raleigh: North Carolina Water Resources Research Institute of the University of North Carolina.

Smith, E. L., & Kellman, L. M. (2011). Nitrate loading and isotopic signatures in subsurface agricultural drainage systems. *Journal of Environmental Quality*, 40(4), 1257.

doi:10.2134/jeq2010.0489

Smith, V. H. (2003). Eutrophication of freshwater and coastal marine ecosystems a global problem. *Environmental Science and Pollution Research*, 10(2), 126-139.

doi:10.1065/espr2002.12.142

Song, K., Lee, S., Mitsch, W. J., & Kang, H. (2010). Different responses of denitrification rates and denitrifying bacterial communities to hydrologic pulsing in created wetlands. *Soil Biology and Biochemistry*, 42(10), 1721-1727. doi:10.1016/j.soilbio.2010.06.007

Sporer, A. J., Kahl, L. J., Price-Whelan, A., & Dietrich, L. E. P. (2017). Redox-based regulation of bacterial development and behavior. *Annual Review of Biochemistry*, 86, 777-797.

doi:10.1146/annurev-biochem-061516-044453

Subbarao, G. V., Ito, O., Sahrawat, K. L., Berry, W. L., Nakahara, K., Ishikawa, T., Rao, I. M. (2006). Scope and strategies for regulation of nitrification in agricultural systems-challenges and opportunities. *Critical Reviews in Plant Sciences*, 25(4), 303-335. 10.1080/07352680600794232

Tiedje, J. M., S. Simkins, and P. M. Groffman. 1989. Perspectives on measurement of denitrification in the field including recommended protocols for acetylene based methods. *Plant and Soil* 115:261–284

Toma, Y., & Hatano, R. (2007). Effect of crop residue C:N ratio on N<sub>2</sub>O emissions from gray lowland soil in mikasa, hokkaido, japan. *Soil Science & Plant Nutrition*, 53(2), 198-205.

doi:10.1111/j.1747-0765.2007.00125.x

Trejo-Gaytan, J., Bachand, P., & Darby, J. (2006). Treatment of urban runoff at lake tahoe: Low-intensity chemical dosing. *Water Environment Research*, 78(13), 2487-2500.

doi:10.2175/106143006X102042

Vicente, I. d., Moreno-Ostos, E., Amores, V., Rueda, F., & Cruz-Pizarro, L. (2006). low predictability in the dynamics of shallow lakes: Implications for their management and restoration. *Wetlands*, 26(4), 928-938. doi:10.1672/0277-5212(2006)26[928:LPITDO]2.0.CO;2

Walker, D. J., Clemente, R., & Bernal, M. P. (2004). Contrasting effects of manure and compost on soil pH, heavy metal availability and growth of *chenopodium album* L. in a soil contaminated by pyritic mine waste. *Chemosphere*, 57(3), 215-224. doi:10.1016/j.chemosphere.2004.05.020

Wang, L., & Burke, S. P. (2017;2016;). A catchment-scale method to simulating the impact of historical nitrate loading from agricultural land on the nitrate-concentration trends in the sandstone aquifers in the eden valley, UK. *Science of the Total Environment*, 579, 133-148.

doi:10.1016/j.scitotenv.2016.10.235

Wang, C., & Sample, D. J. (2014). Assessment of the nutrient removal effectiveness of floating treatment wetlands applied to urban retention ponds. *Journal of Environmental Management*, 137, 23-35. doi:10.1016/j.jenvman.2014.02.008

Waters, M. N., Piehler, M. F., Rodriguez, A. B., Smoak, J. M., & Bianchi, T. S. (2009). Shallow lake trophic status linked to late holocene climate and human impacts. *Journal of Paleolimnology*, 42(1), 51-64. doi:10.1007/s10933-008-9247-x

Waters, M. N., Piehler, M. F., Smoak, J. M., & Martens, C. S. (2010). The development and persistence of alternative ecosystem states in a large, shallow lake. *Freshwater Biology*, 55(6), 1249-1261. doi:10.1111/j.1365-2427.2009.02349.x

Wehmeyer, Loren & Wagner, Chad (2011). Relation between Flows and Dissolved Oxygen in the Roanoke River between Roanoke Rapids Dam and Jamesville, North Carolina, 2005–2009.

USGS

Wetlands (5th edition) 2016 023 William J. Mitsch, and James C. Gosselink wetlands (5th edition) Hoboken, NJ wiley 2015 vii + 736 pp. 9781118676820(print); 9781119019787(e-book) £85 \$125 (print); £76.99 \$100.99 (e-book). (2016). Reference Reviews, 30(1), 30-31.

doi:10.1108/RR-09-2015-0230

Winton, R. S., & Richardson, C. J. (2017). Top-down control of methane emission and nitrogen cycling by waterfowl. *Ecology*, 98(1), 265-277. doi:10.1002/ecy.1640

Winton, R. S., Moorman, M., & Richardson, C. J. (2016). Waterfowl Impoundments as Sources of Nitrogen Pollution. *Water, Air, & Soil Pollution*, 227(10), 390.

<https://doi.org/10.1007/s11270-016-3082-x>

Wolf, K. L., Ahn, C., & Noe, G. B. (2011). Microtopography enhances nitrogen cycling and removal in created mitigation wetlands. *Ecological Engineering*, 37(9), 1398-1406.

10.1016/j.ecoleng.2011.03.013

Wolf, K. L., Noe, G. B., & Ahn, C. (2013). Hydrologic connectivity to streams increases nitrogen and phosphorus inputs and cycling in soils of created and natural floodplain wetlands.

*Journal of Environmental Quality*, 42(4), 1245-1255. 10.2134/jeq2012.0466

Woli, K. P., David, M. B., Cooke, R. A., McIsaac, G. F., & Mitchell, C. A. (2010). Nitrogen balance in and export from agricultural fields associated with controlled drainage systems and denitrifying bioreactors. *Ecological Engineering*, 36(11), 1558-1566.

doi:10.1016/j.ecoleng.2010.04.024

Woltemade, C. J. (2000). Ability of restored wetlands to reduce nitrogen and phosphorus concentrations in agricultural drainage water. *Journal of Soil and Water Conservation*, 55(3), 303-309

Xiang, S., Doyle, A., Holden, P. A., & Schimel, J. P. (2008). Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface california grassland soils. *Soil Biology and Biochemistry*, 40(9), 2281-2289. doi:10.1016/j.soilbio.2008.05.004

Yoshitake, S., Soutome, H., & Koizumi, H. (2014). Deposition and decomposition of cattle dung and its impact on soil properties and plant growth in a cool-temperate pasture. *Ecological Research*, 29(4), 673-684. 10.1007/s11284-014-1153-2

Zhang, J., Cai, Z., Zhu, T., Yang, W., & Müller, C. (2013). Mechanisms for the retention of inorganic N in acidic forest soils of southern china. *Scientific Reports*, 3, 2342. doi:10.1038/srep02342

Zhao, W., Cai, Z., & Xu, Z. (2007). Does ammonium-based N addition influence nitrification and acidification in humid subtropical soils of china? *Plant and Soil*, 297(1/2), 213-221. doi:10.1007/s11104-007-9334-1

## Tables Section

**Chapter 1, Table 1.** Partial least squares regression (PLSR) modeled parameters and associated  $R^2$  values using spectra data as the predictor variables.

<b>Nutrient</b>	<b>Site</b>	<b><math>R^2</math></b>
<b>TDN</b>	Ag	0.913
	MSM	0.809
<b>NH<sub>4</sub>-N</b>	Ag	0.804
	MSM	0.852
<b>DOC</b>	Ag & MSM	0.950

**Chapter 1, Table 2:** Summary of Multiple Linear Regression Analysis on N Parameters

reporting best models according to adjusted R<sup>2</sup> value. Significant models (p < 0.05) are bolded.

Variables are coded as: CR = Cumulative Rainfall, AWL = Average Water Level, WLR = Water

Level Range, T = Temperature

<b>Nutrient</b>	<b>Site</b>	<b>Best Model</b>	<b>Adjusted R<sup>2</sup></b>	<b>Model p-Value</b>
<b>DON</b>	<b>AG</b>	<b>~ CR + pH + AWL</b>	<b>0.398</b>	<b>0.017</b>
	<b>MSM</b>	<b>~ CR</b>	<b>0.453</b>	<b>2.62 * 10<sup>-5</sup></b>
<b>NH<sub>4</sub>-N</b>	<b>AG</b>	<b>~ T + pH + DO + DOC + WLR</b>	<b>0.467</b>	<b>0.030</b>
	<b>MSM</b>	<b>~ CR + DOC</b>	<b>0.207</b>	<b>0.049</b>
<b>NO<sub>3</sub>-N</b>	<b>AG</b>	<b>~ pH + DO + DOC + AWL</b>	0.200	0.159
	<b>MSM</b>	<b>~ CR</b>	0.112	0.065

**Chapter 2, Table 1.** Summary of soil physiochemical properties over all sampling seasons at agricultural (Ag) and moist-soil managed (MSM) waterfowl impoundments.

<b>Variable</b>	<b>Site</b>	<b>Minimum</b>	<b>Maximum</b>	<b>Mean <math>\pm</math> SD</b>
<b>pH</b>	Ag	5.49	6.14	5.80 $\pm$ 0.16
	MSM	4.40	5.86	5.23 $\pm$ 0.36
<b>Moisture (%)</b>	Ag	11.01	28.20	18.46 $\pm$ 5.54
	MSM	30.03	57.04	41.45 $\pm$ 7.33
<b>NH<sub>4</sub>-N (<math>\mu\text{g NH}_4\text{-N g}^{-1}</math> DM)</b>	Ag	0.52	9.28	2.38 $\pm$ 2.54
	MSM	1.50	22.91	7.19 $\pm$ 5.54
<b>NO<sub>3</sub>-N (<math>\mu\text{g NO}_3\text{-N g}^{-1}</math> DM)</b>	Ag	0.44	44.65	5.34 $\pm$ 10.18
	MSM	0.17	2.11	0.73 $\pm$ 0.45
<b>Total Organic Carbon (%)</b>	Ag	1.46	5.02	2.95 $\pm$ 0.84
	MSM	2.48	8.61	4.45 $\pm$ 1.39
<b>Total Nitrogen (%)</b>	Ag	0.08	0.25	0.16 $\pm$ 0.04
	MSM	0.09	0.61	0.24 $\pm$ 0.12
<b>Soil C/N Ratio (wt/wt)</b>	Ag	14.57	26.69	18.95 $\pm$ 2.59
	MSM	12.79	30.33	20.24 $\pm$ 4.45

**Chapter 2, Table 2.** Summary of laboratory-measured nitrogen cycling rates over all sampling seasons at agricultural (Ag) and moist-soil managed (MSM) waterfowl impoundments.

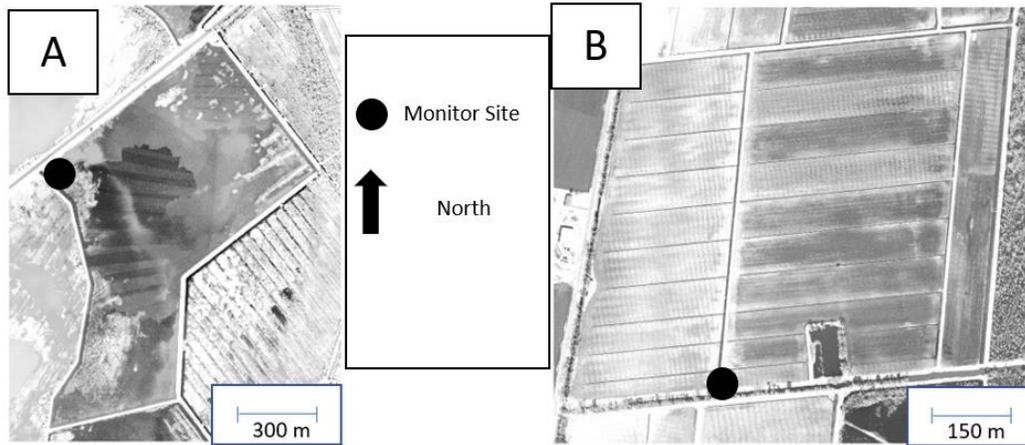
<b>N-Rate</b>	<b>Season</b>	<b>Site</b>	<b>Minimum</b>	<b>Maximum</b>	<b>Mean <math>\pm</math> SD</b>
<b>Denitrification</b> (ng N <sub>2</sub> O g <sup>-1</sup> DM h <sup>-1</sup> )	Summer	Ag	-0.34	181.13	54.67 $\pm$ 57.85
		MSM	19.28	540.08	113.71 $\pm$ 143.76
	Fall	Ag	421.63	1215.78	755.00 $\pm$ 270.75
		MSM	286.58	1429.32	589.58 $\pm$ 368.23
	Winter	Ag	105.48	1299.08	722.01 $\pm$ 384.67
		MSM	-27.07	718.44	351.15 $\pm$ 227.07
<b>Nitrification</b> (ng NO <sub>2</sub> -N g <sup>-1</sup> DM h <sup>-1</sup> )	Summer	Ag	4.25	9.51	5.68 $\pm$ 1.43
		MSM	-0.16	1.42	0.48 $\pm$ 0.55
	Fall	Ag	4.81	9.65	6.14 $\pm$ 1.26
		MSM	-0.06	1.13	0.42 $\pm$ 0.43
	Winter	Ag	1.13	5.09	3.27 $\pm$ 1.56
		MSM	-0.26	0.46	0.05 $\pm$ 0.22
<b>Nitrogen Mineralization</b> ( $\mu$ g NH <sub>4</sub> -N g <sup>-1</sup> DM day <sup>-1</sup> )	Summer	Ag	0.09	1.85	0.78 $\pm$ 0.54
		MSM	-0.50	4.75	1.39 $\pm$ 1.42
	Fall	Ag	0.17	1.22	0.69 $\pm$ 0.36
		MSM	-0.51	0.69	0.10 $\pm$ 0.32
	Winter	Ag	0.58	2.94	1.75 $\pm$ 0.60
		MSM	0.50	3.43	2.02 $\pm$ 1.09

**Chapter 2, Table 3.** Summary of multiple linear regression used to test the relationship between soil physicochemical factors and N cycling processes. Significant model terms are bolded.

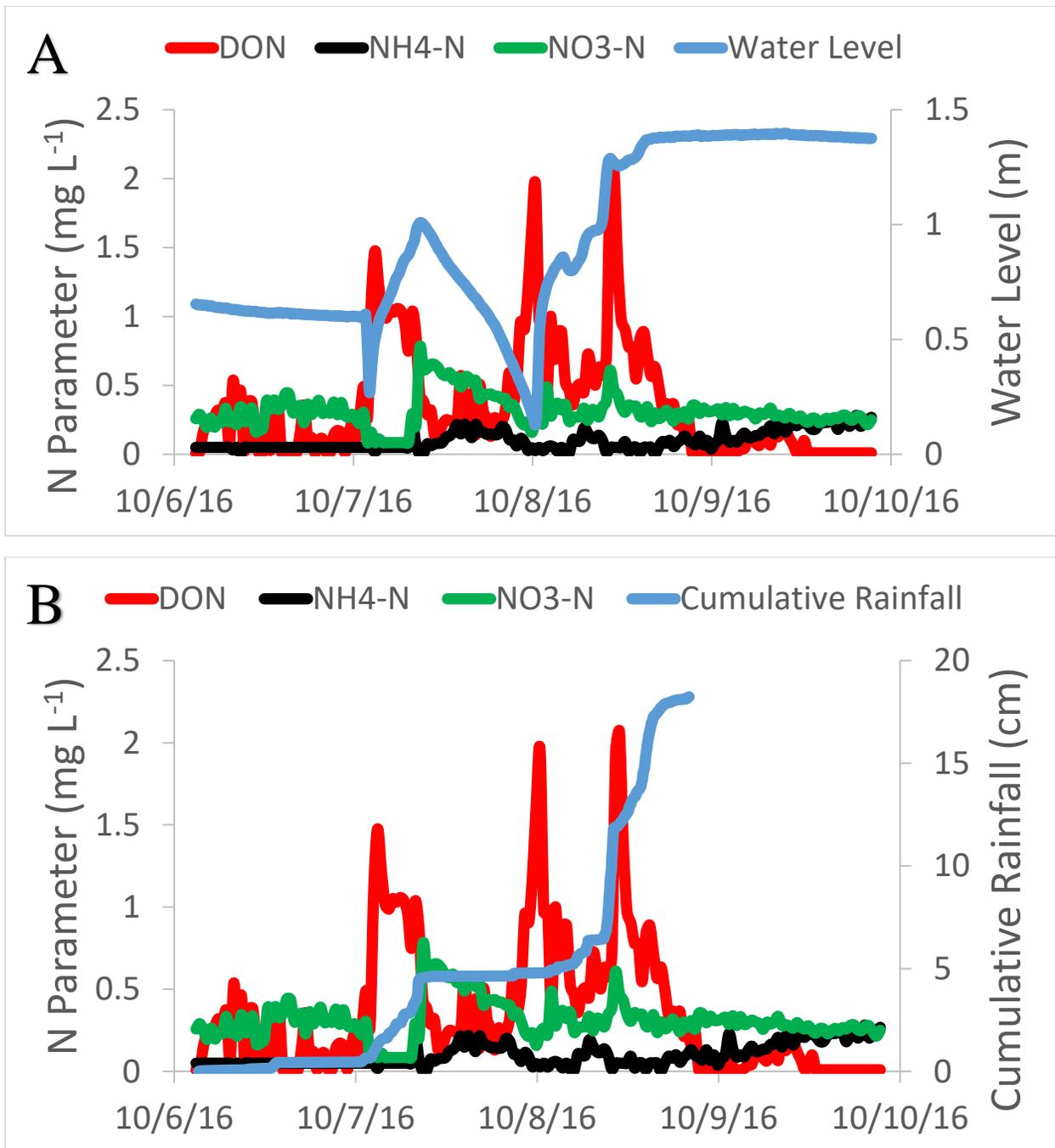
Variables are coded as: M = soil moisture, T = Air Temperature (°C), SC = soil organic C, SN = soil N, CN = soil C/N ratio, pH = soil pH, NO = soil NO<sub>3</sub>-N, NH= soil NH<sub>4</sub>-N.

<b>N- Rate</b>	<b>Site</b>	<b>Best Model</b>	<b>Adjusted R<sup>2</sup></b>	<b>F-Statistic, Model p</b>
<b>Denitrification</b>	Ag	~ <b>M</b> + <b>CN</b> + pH	0.82	F <sub>3,31</sub> = 53.93, p = 2.1e <sup>-12</sup>
	MSM	~ <b>M</b> + T + NH	0.17	F <sub>3,32</sub> = 3.45, p = 0.03
<b>Nitrification</b>	Ag	~ <b>M</b> + <b>T</b> + <b>CN</b> + pH	0.62	F <sub>4,31</sub> = 15.57, p = 4.4e <sup>-7</sup>
	MSM	~ M + <b>T</b> + <b>CN</b> + pH	0.33	F <sub>3,32</sub> = 5.38, p = 2.1e <sup>-3</sup>
<b>N-Mineralization</b>	Ag	~ <b>SC</b> + <b>NO</b> + <b>NH</b>	0.66	F <sub>4,31</sub> = 24.04, p = 2.5e <sup>-8</sup>
	MSM	~ M + <b>T</b> + <b>CN</b> + NO	0.50	F <sub>4,30</sub> = 9.62, p = 4.0e <sup>-5</sup>

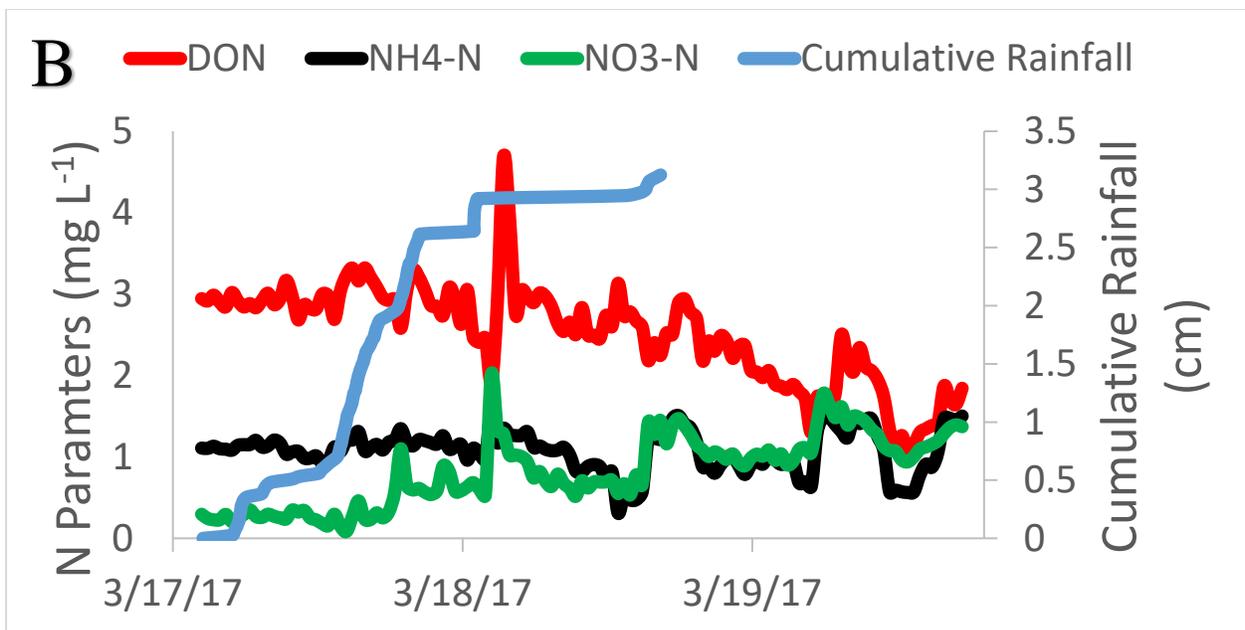
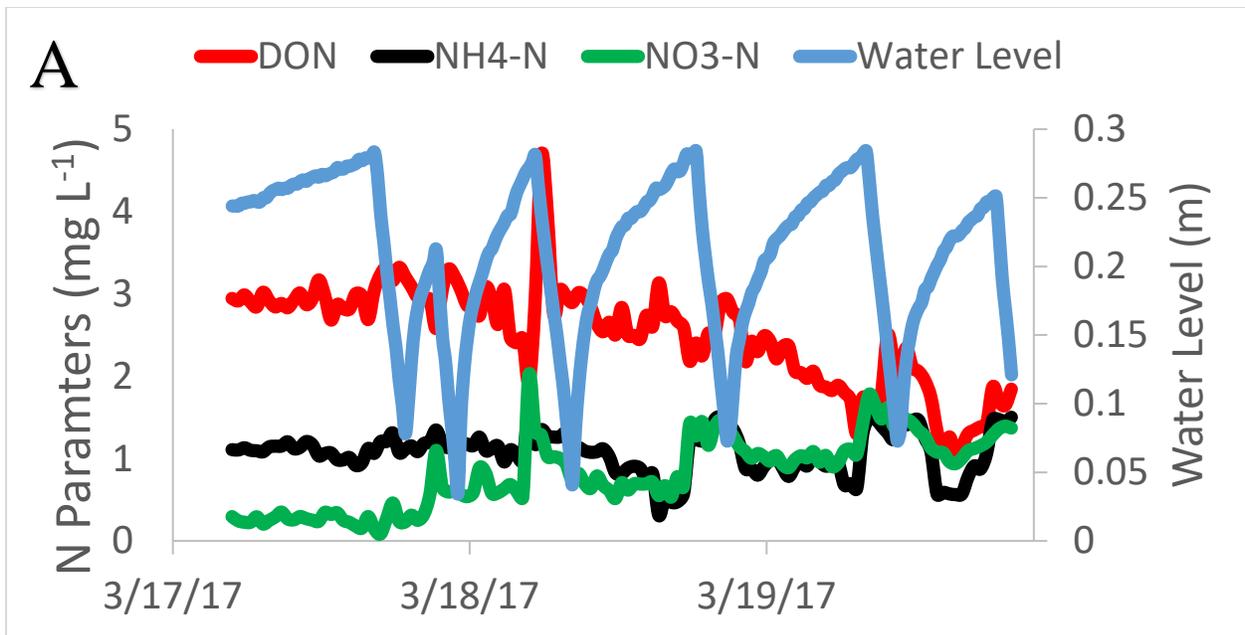
## Figures Section



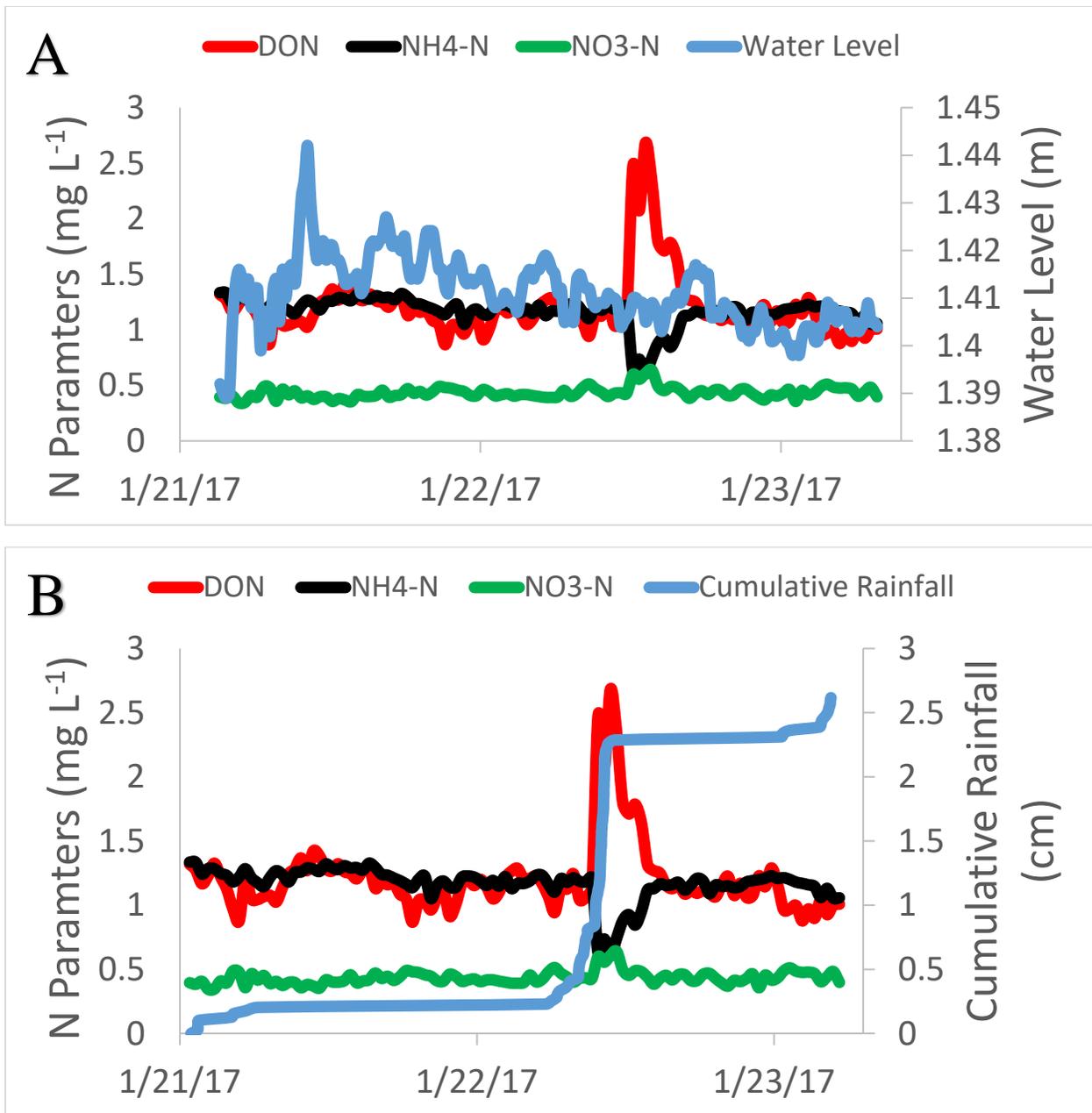
**Chapter 1, Figure 1** Aerial view of study area showing MSM impoundment map (A) and Ag site map (B).



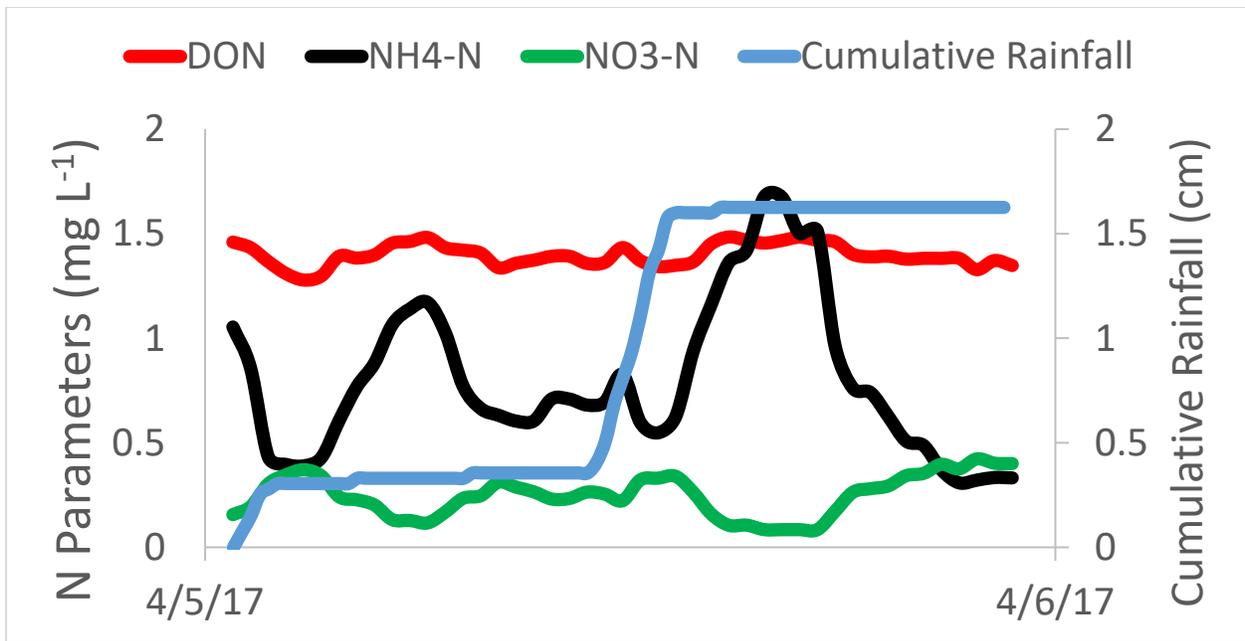
**Chapter 1, Figure 2:** Ag N dynamics during Hurricane Matthew. N parameters are plotted on the left y axis, and explanatory variables (blue) on the 2nd y axis. Storm N parameter dynamics are compared to water level (A) and cumulative rainfall (B).



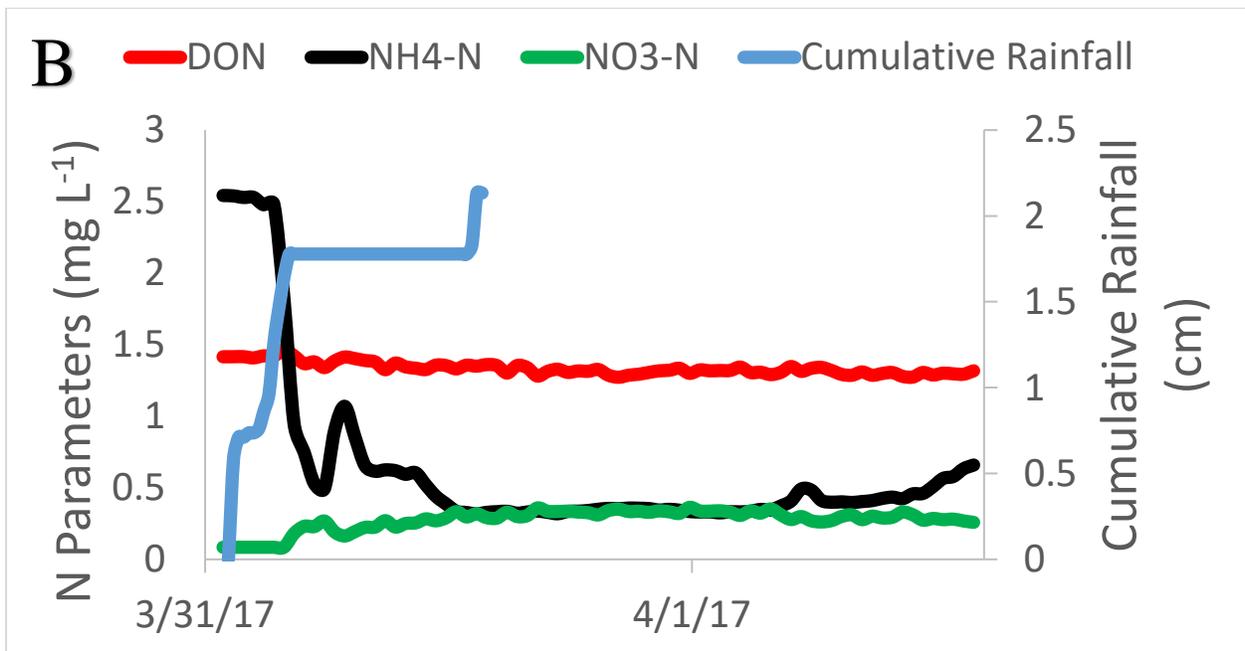
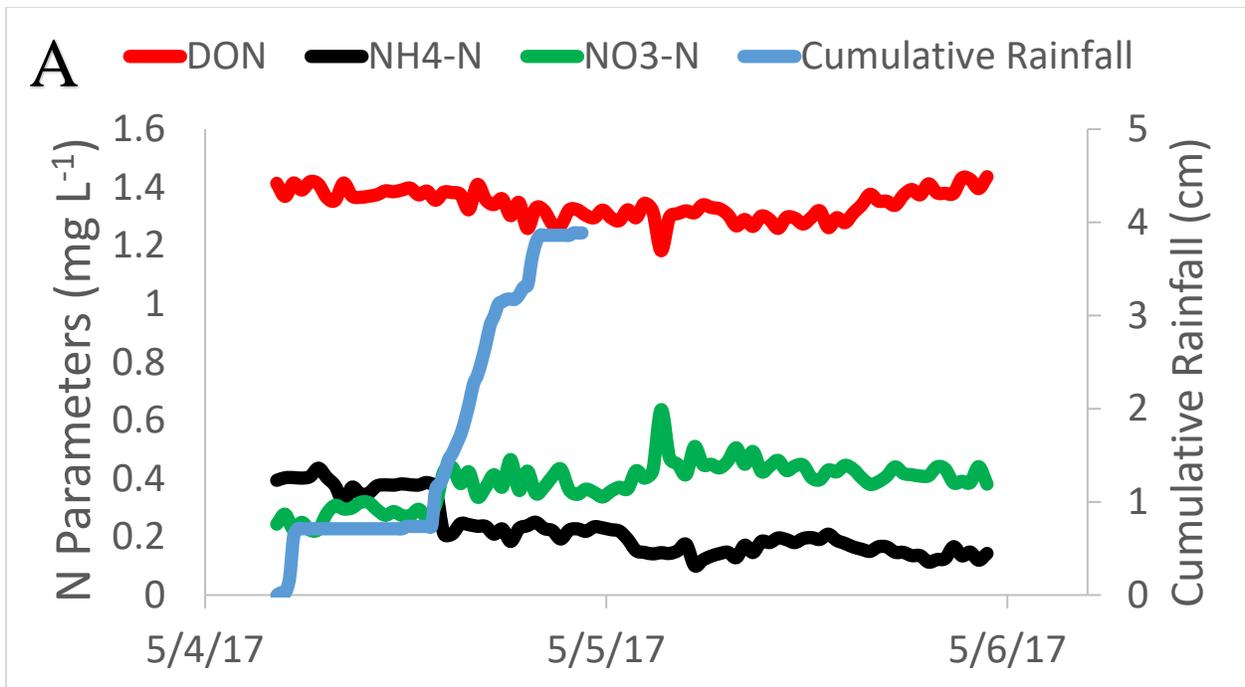
**Chapter 1, Figure 3:** Drawdown is occurring to dewater the field (A) following a storm event (B) in post-impoundment conditions. N parameters are plotted on the left y axis, and explanatory variables (blue) on the 2nd y axis. Storm N parameter dynamics are compared to water level (A) and cumulative rainfall (B).



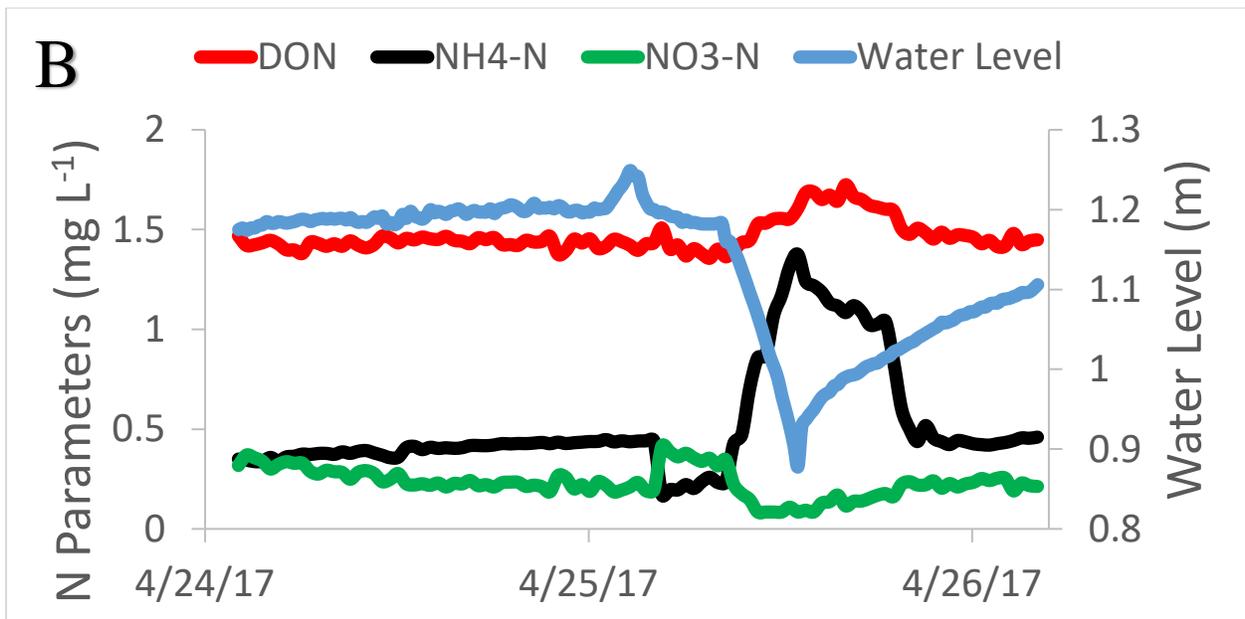
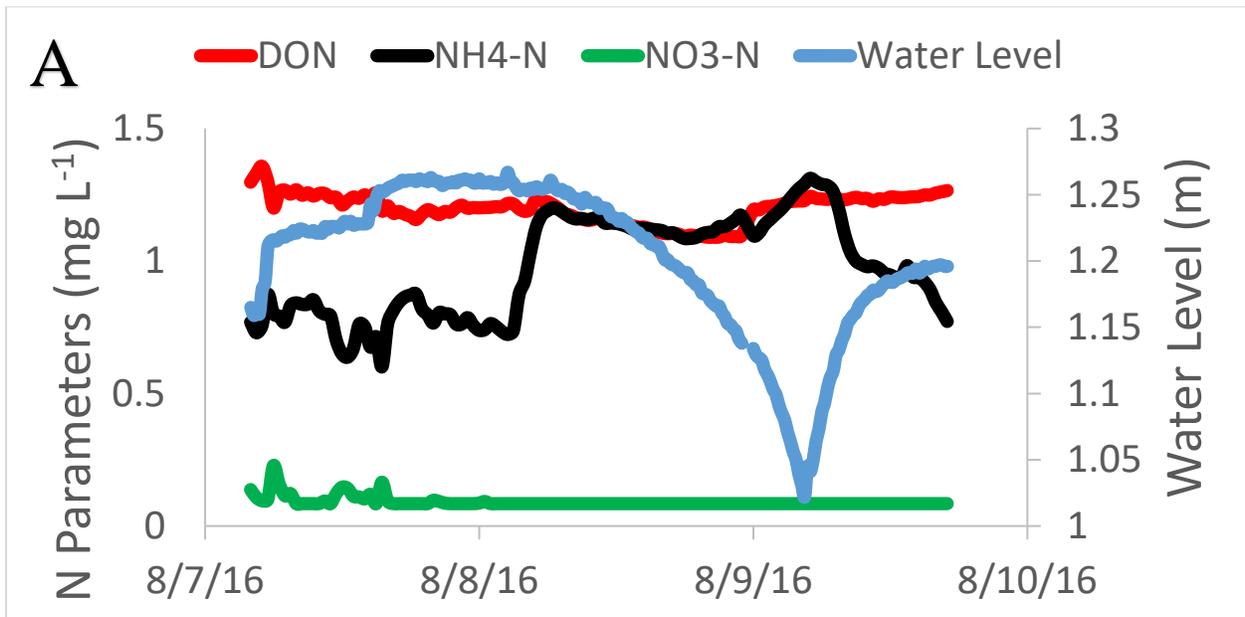
**Chapter 1, Figure 4:** Ag site N dynamics observed during a storm event when the field was under impoundment conditions. N parameters are plotted on the left y axis, and explanatory variables (blue) on the 2nd y axis. Storm N parameter dynamics are compared to water level (A) and cumulative rainfall (B).



**Chapter 1, Figure 5:** An observed storm at MSM resulted lagging increases in NH<sub>4</sub>-N relative to time of rainfall occurring. This effect may be proportional to amount of rainfall received.



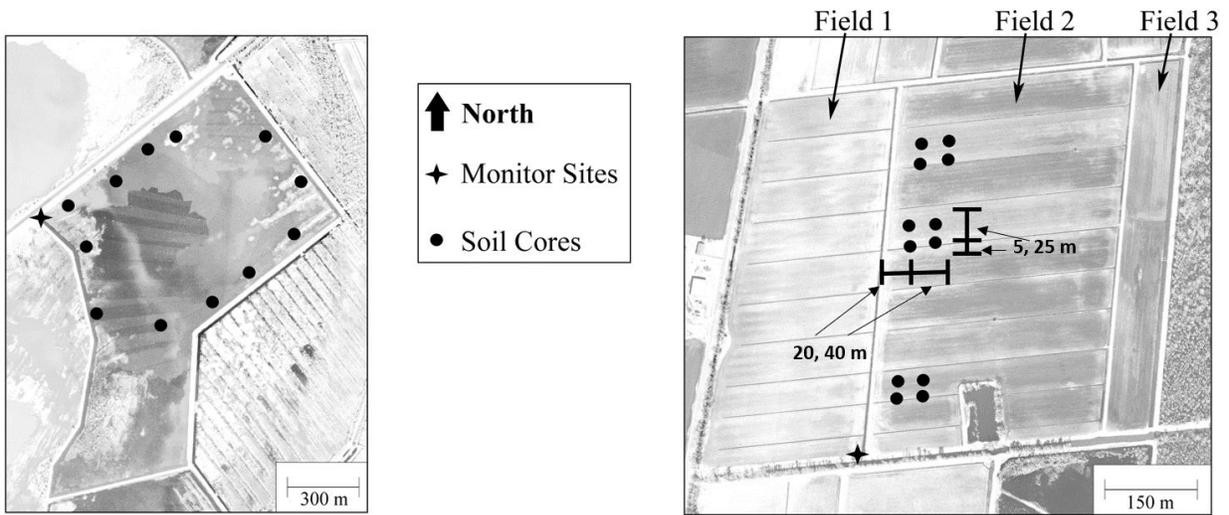
**Chapter 1, Figure 6:** N dynamics observed during periods of high water levels preceding early-Spring drawdown in the MSM site. N parameters are plotted on the left y axis, and explanatory variables (blue) on the 2nd y axis. Storm N parameter dynamics are compared to water level (A) and cumulative rainfall (B).



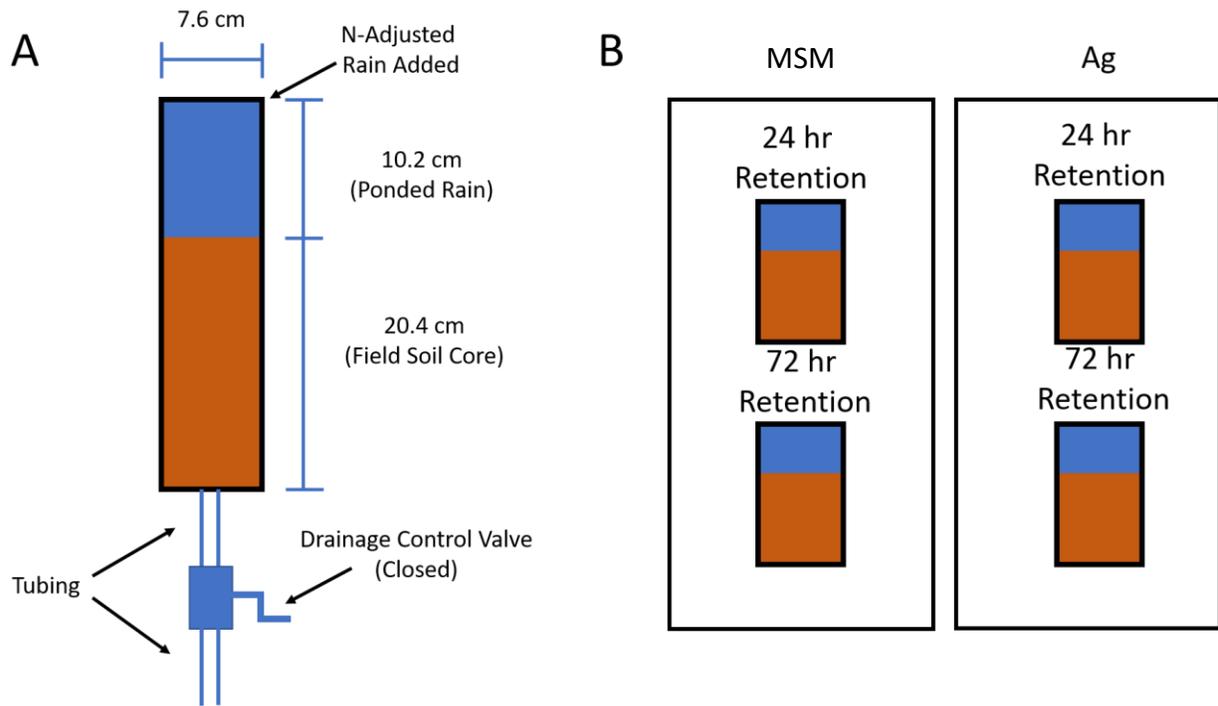
**Chapter 1, Figure 7:** N dynamics observed during MSM drawdown events during storm events.

N parameters are plotted on the left y axis, and explanatory variables (blue) on the 2nd y axis.

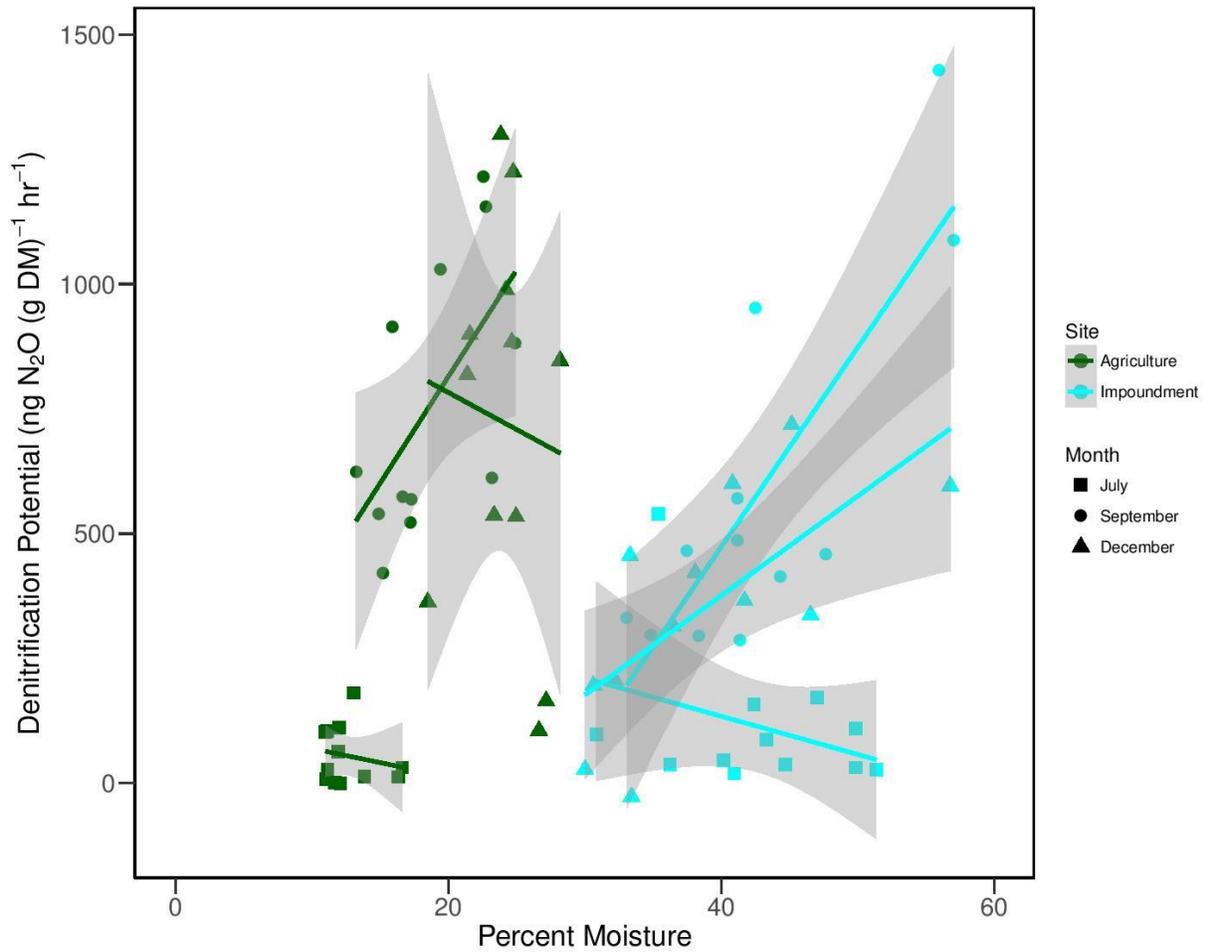
Storm N parameter dynamics are compared to water level (A) and cumulative rainfall (B).



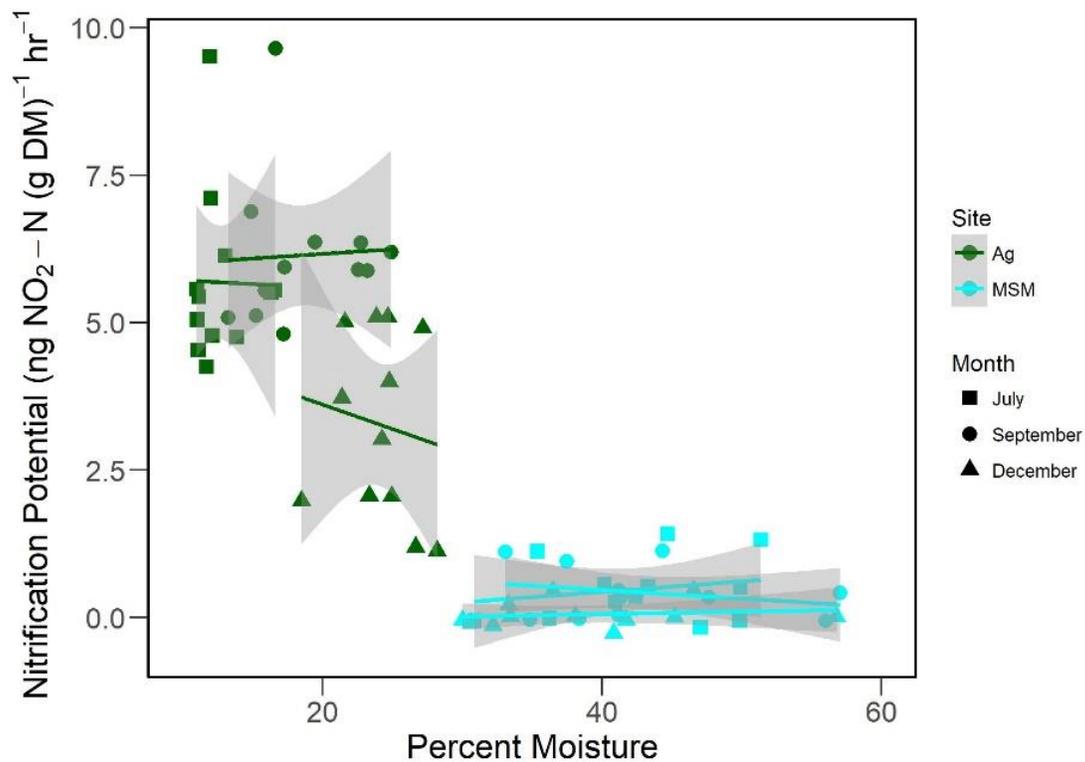
**Chapter 2, Figure 1.** Map of study sites. N cycling functions were measured on soil cores collected at the MSM impoundment managed by USFWS (A) and a privately managed agricultural (Ag) field that doubles as a waterfowl impoundment (B). Distances between coring sites in the Ag field may not be to scale.



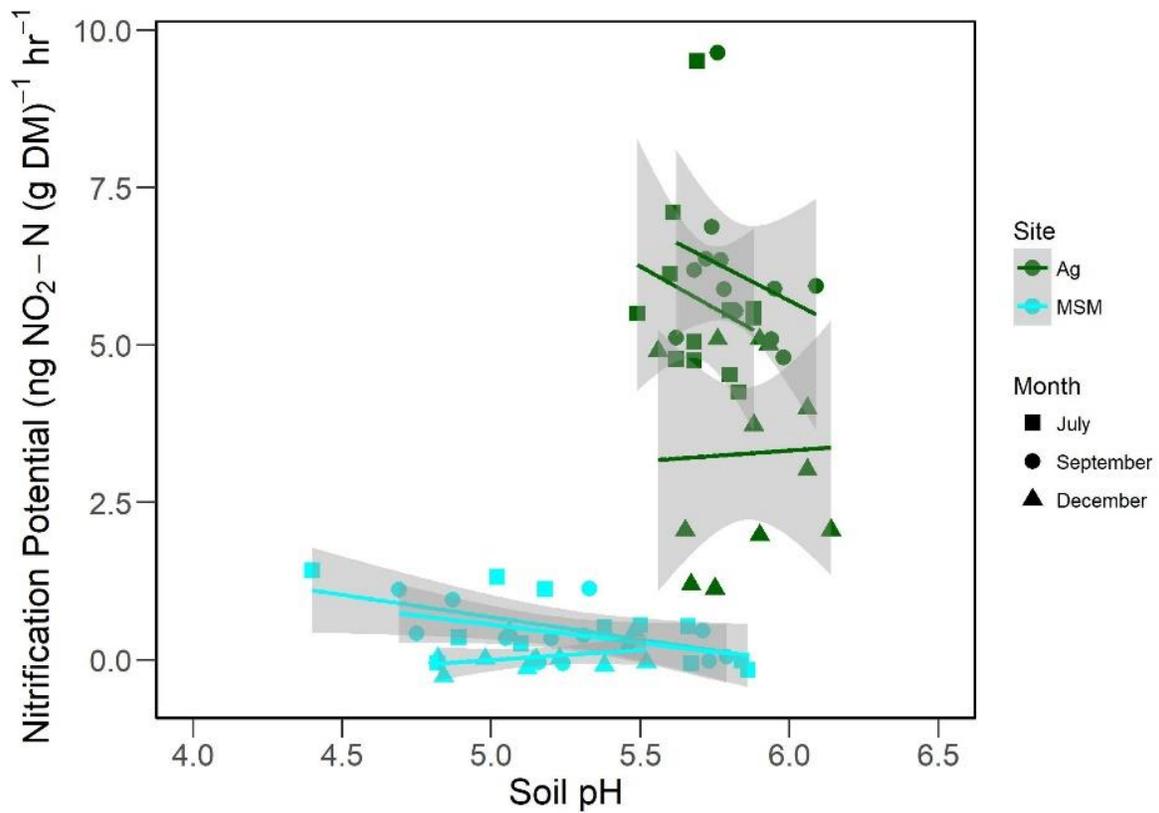
**Chapter 2, Figure 2.** Mesocosm experimental design. Drainage systems and modified caps facilitated sample collection (A). The 2x2 factorial design was used to measure the effects of site and retention time on water N quality (B).



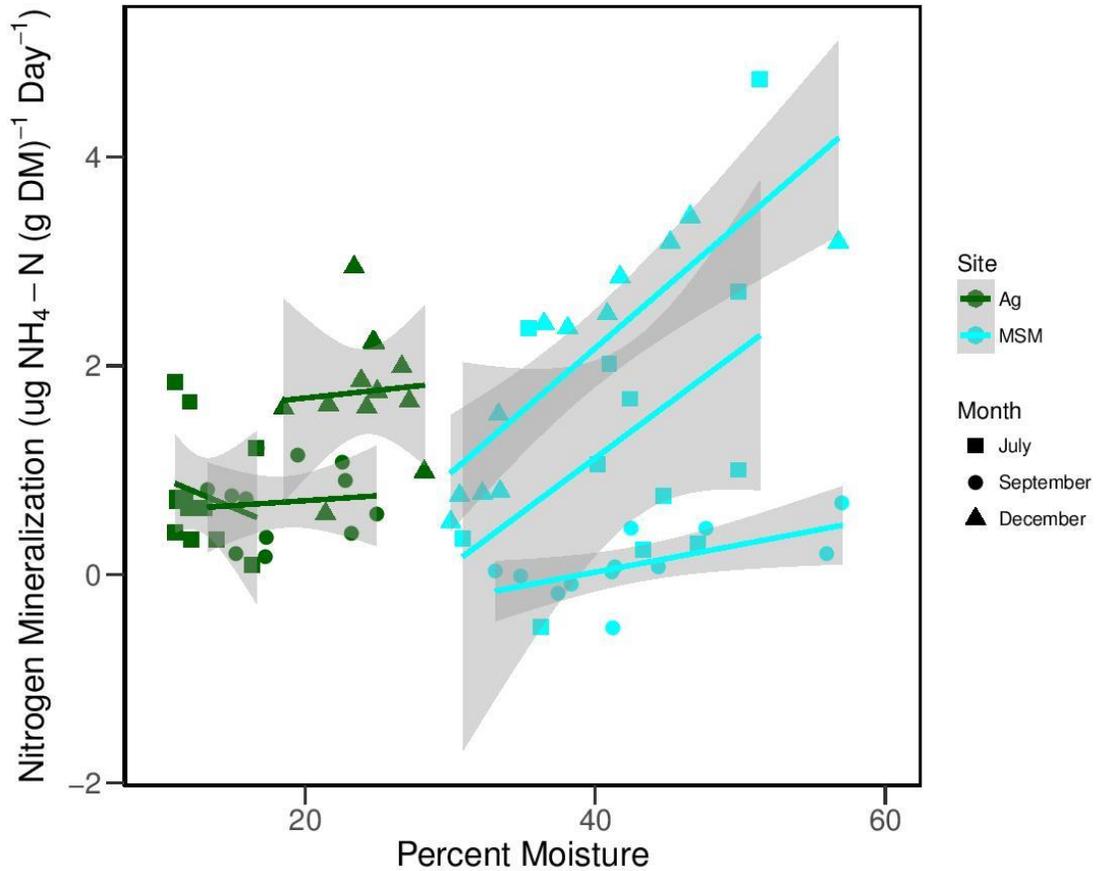
**Chapter 2, Figure 3.** Relationship between potential denitrification rates and soil moisture at Ag (green) and MSM (blue) sites. Potential nitrification rates were measured in July (square), September (circle), and December (triangle) 2017. The gray confidence bands around the points represent the 95% confidence interval for predictions based on a linear model.



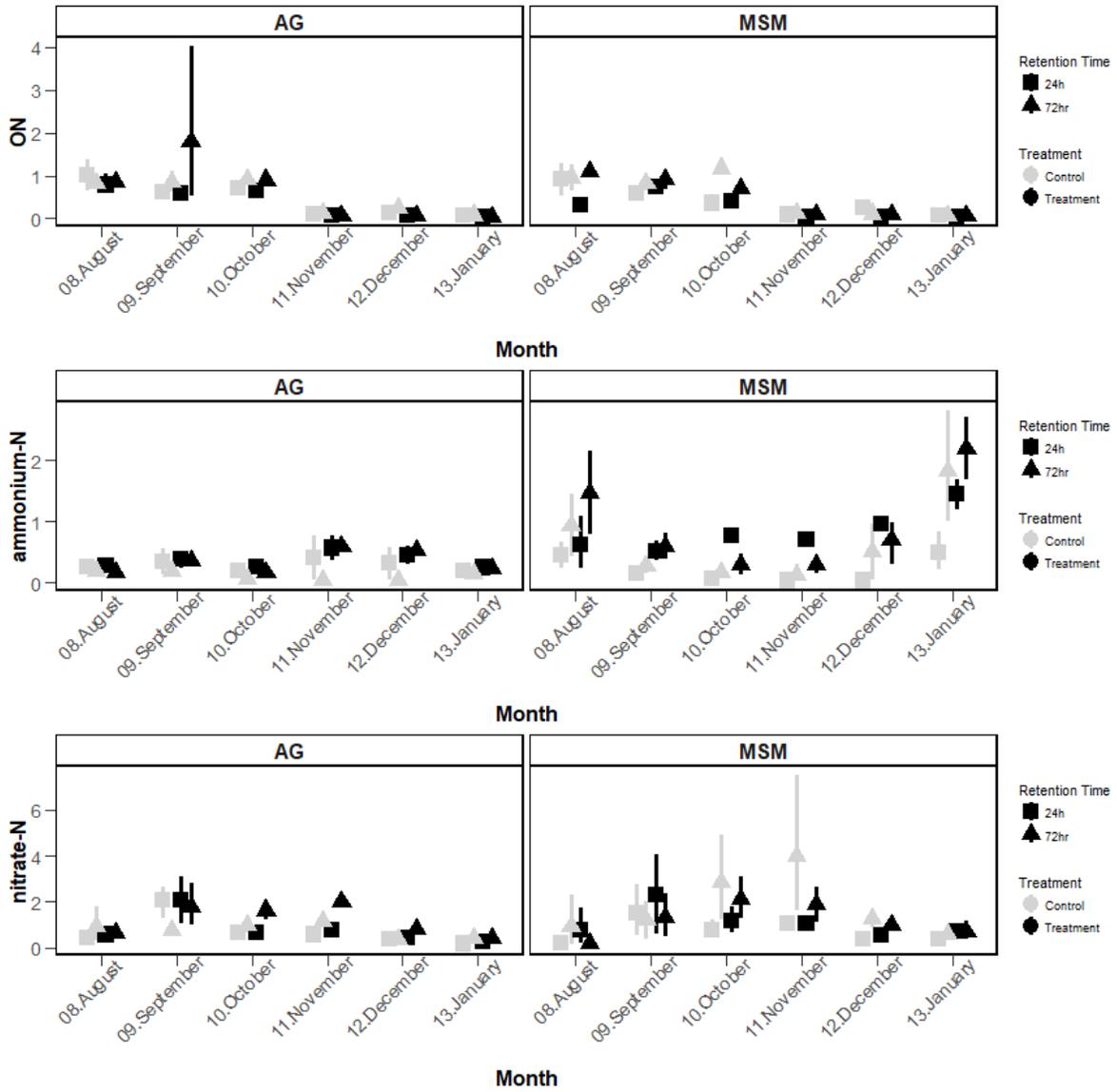
**Chapter 2, Figure 4.** Relationship between potential nitrification rates and soil moisture at Ag (green) and MSM (blue) sites. Potential nitrification rates were measured in July (square), September (circle), and December (triangle) 2017. The gray confidence bands around the points represent the 95% confidence interval for predictions based on a linear model.



**Chapter 2, Figure 5.** Relationship between potential nitrification rates and soil pH at Ag (green) and MSM (blue) sites. Potential nitrification rates were measured in July (square), September (circle), and December (triangle) 2017. The gray confidence bands around the points represent the 95% confidence interval for predictions based on a linear model.



**Chapter 2, Figure 6.** Relationship between potential N-mineralization rates and soil moisture at Ag (green) and MSM (blue) sites. Potential nitrification rates were measured in July (square), September (circle), and December (triangle) 2017. The gray confidence bands around the points represent the 95% confidence interval for predictions based on a linear model.

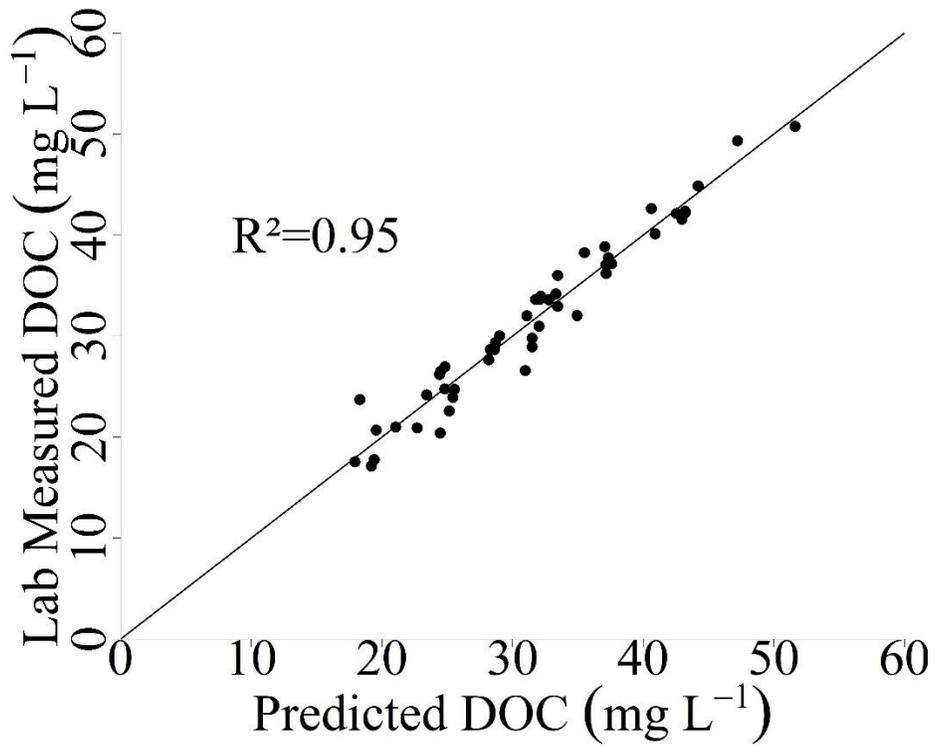


**Chapter 2, Figure 7.** Average monthly control (no N added) and treatment (N added) mesocosm DON, NH<sub>4</sub>-N, and NO<sub>3</sub>-N concentrations between Ag and MSM sites after 24 and 72-hour retention treatment.

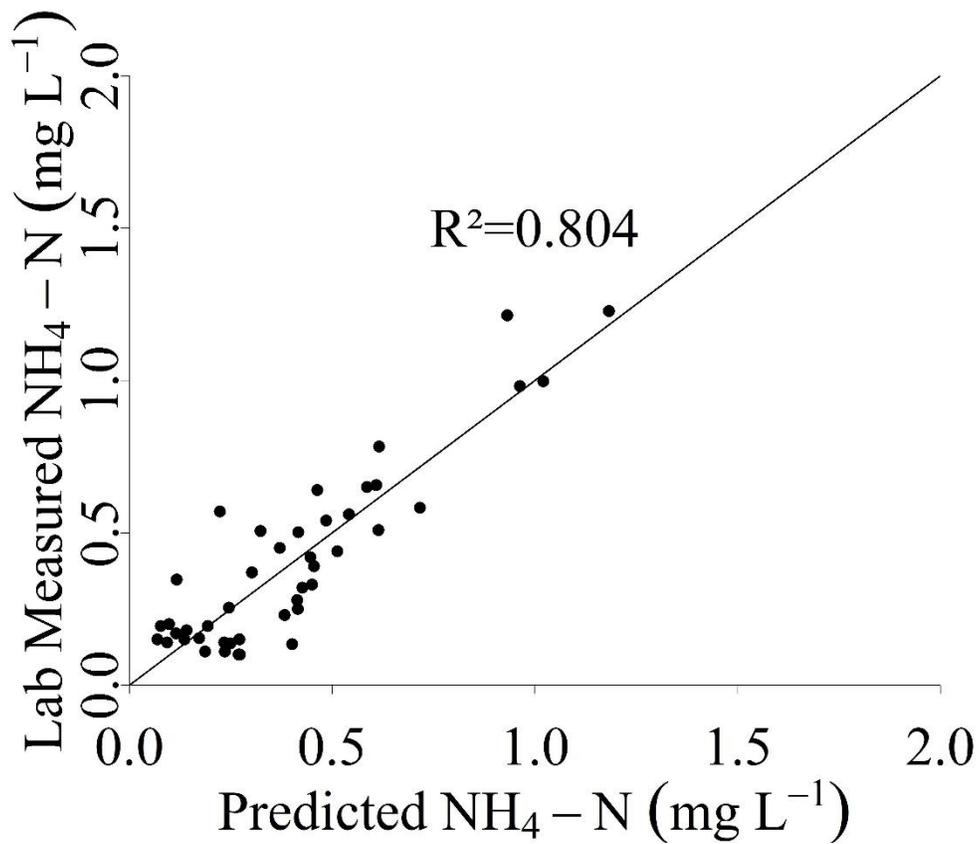
## Appendix A: Chapter 1

**Supplemental Table C1S1:** Table with the root mean square error of prediction (RMSEP) for all PLSR predicted parameters.

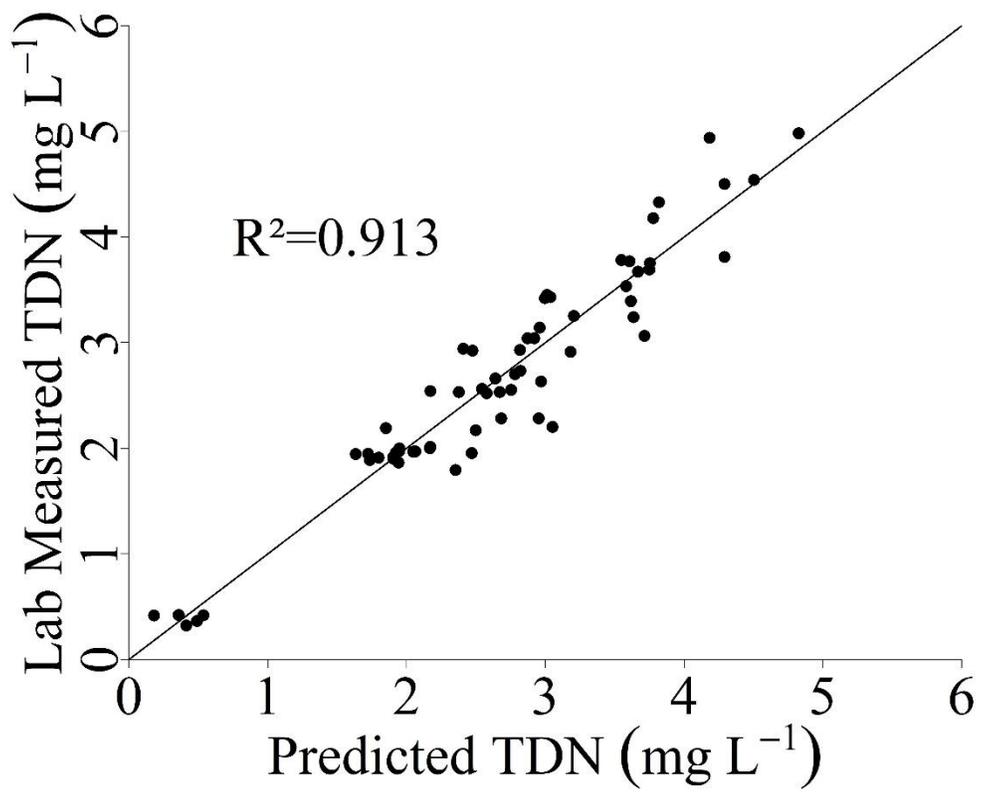
<b>Parameter</b>	<b>Site</b>	<b>RMSEP (mg L<sup>-1</sup>)</b>
<b>TDN</b>	Ag	0.43
	MSM	0.33
<b>NH<sub>4</sub>-N</b>	Ag	0.24
	MSM	0.25
<b>DOC</b>	Combined Site	2.51



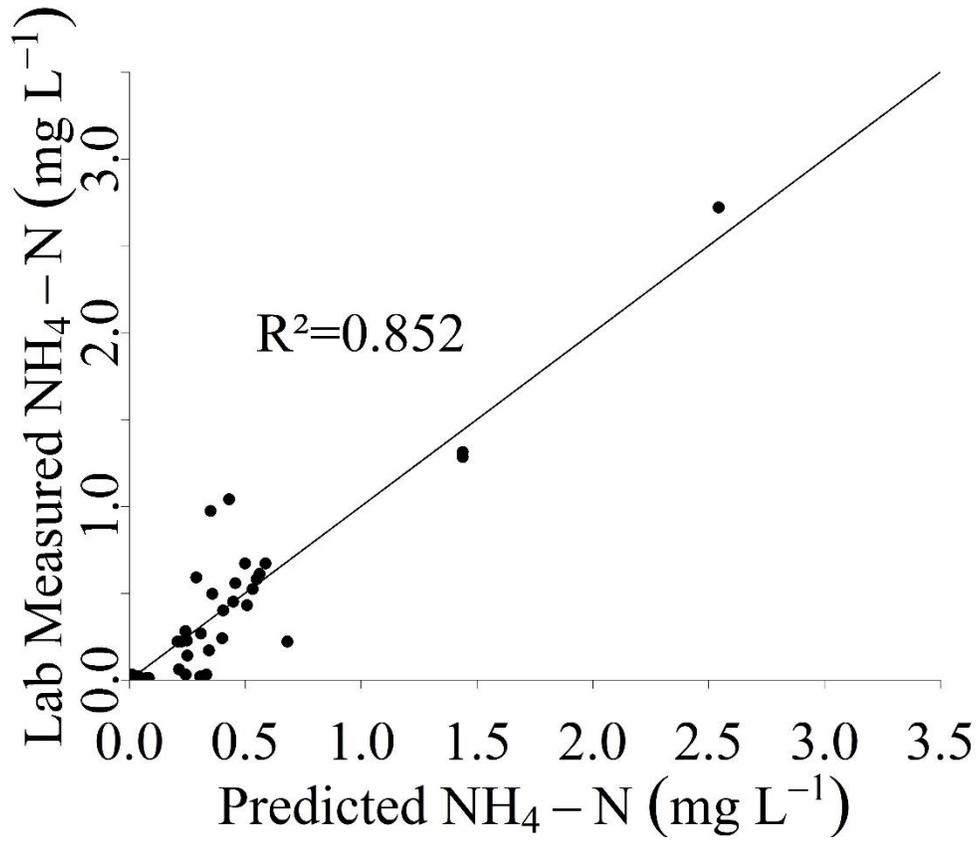
**Supplemental figure C1S1:** Combined Ag and MSM DOC calibration used.



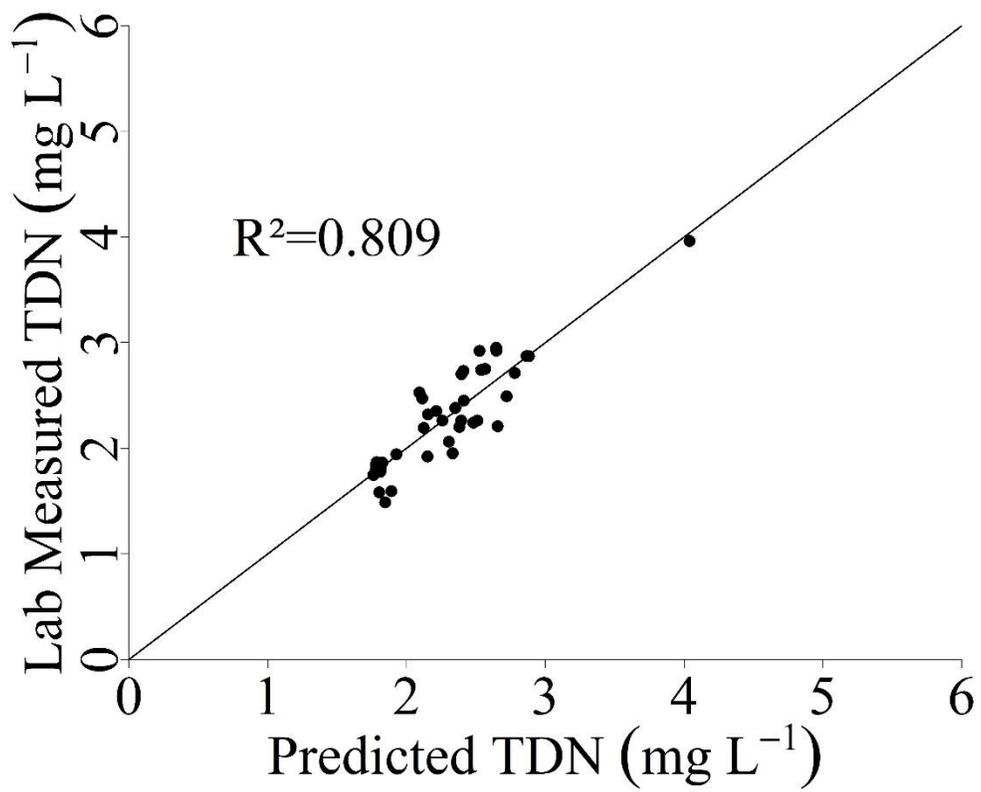
Supplemental figure C1S2: Ag  $\text{NH}_4\text{-N}$  calibration used.



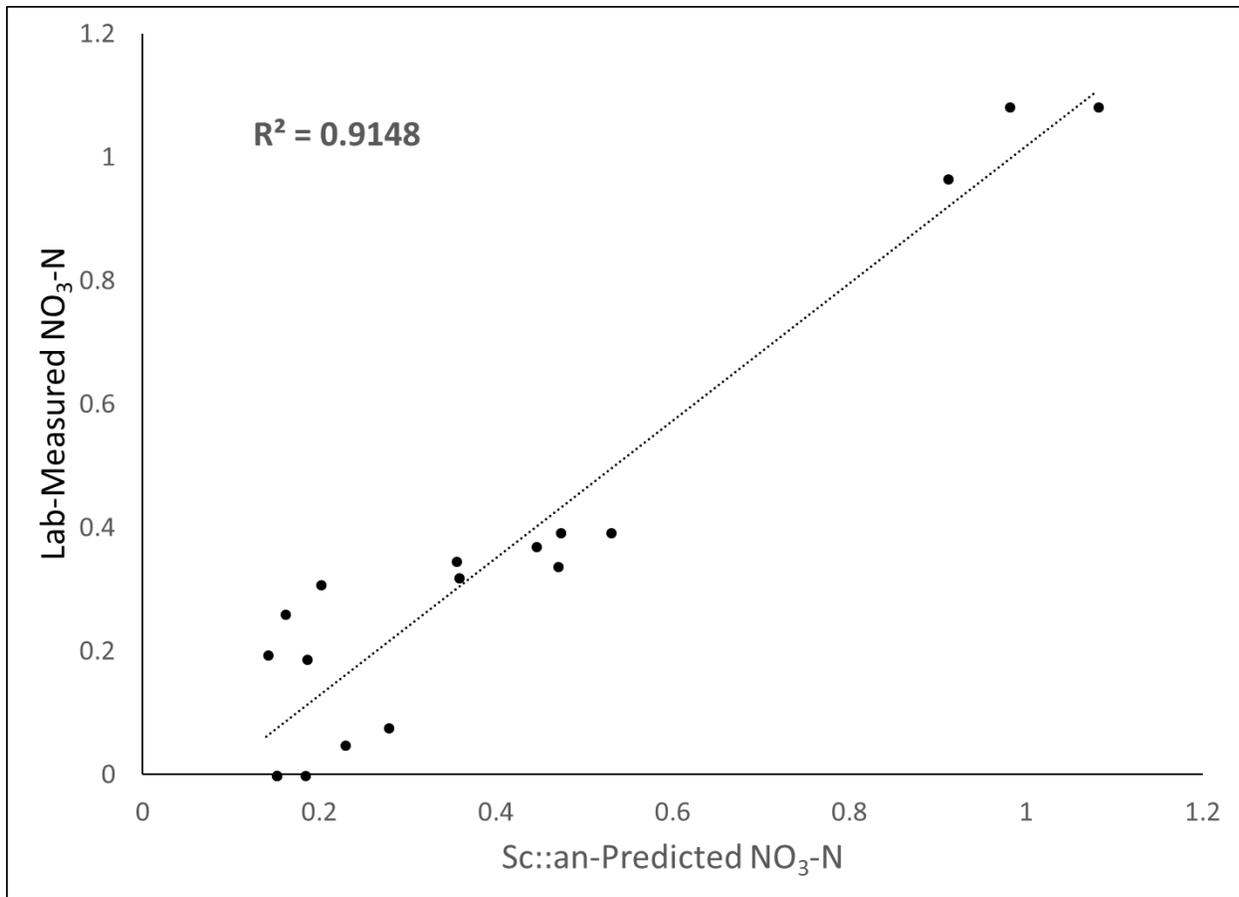
**Supplemental figure C1S3:** Ag TDN calibration used.



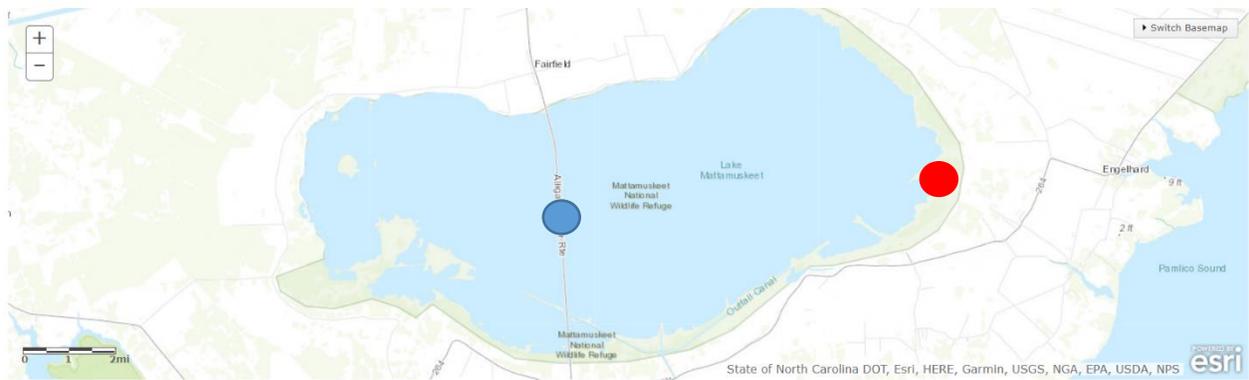
Supplemental figure C1S4: MSM NH<sub>4</sub>-N calibration used.



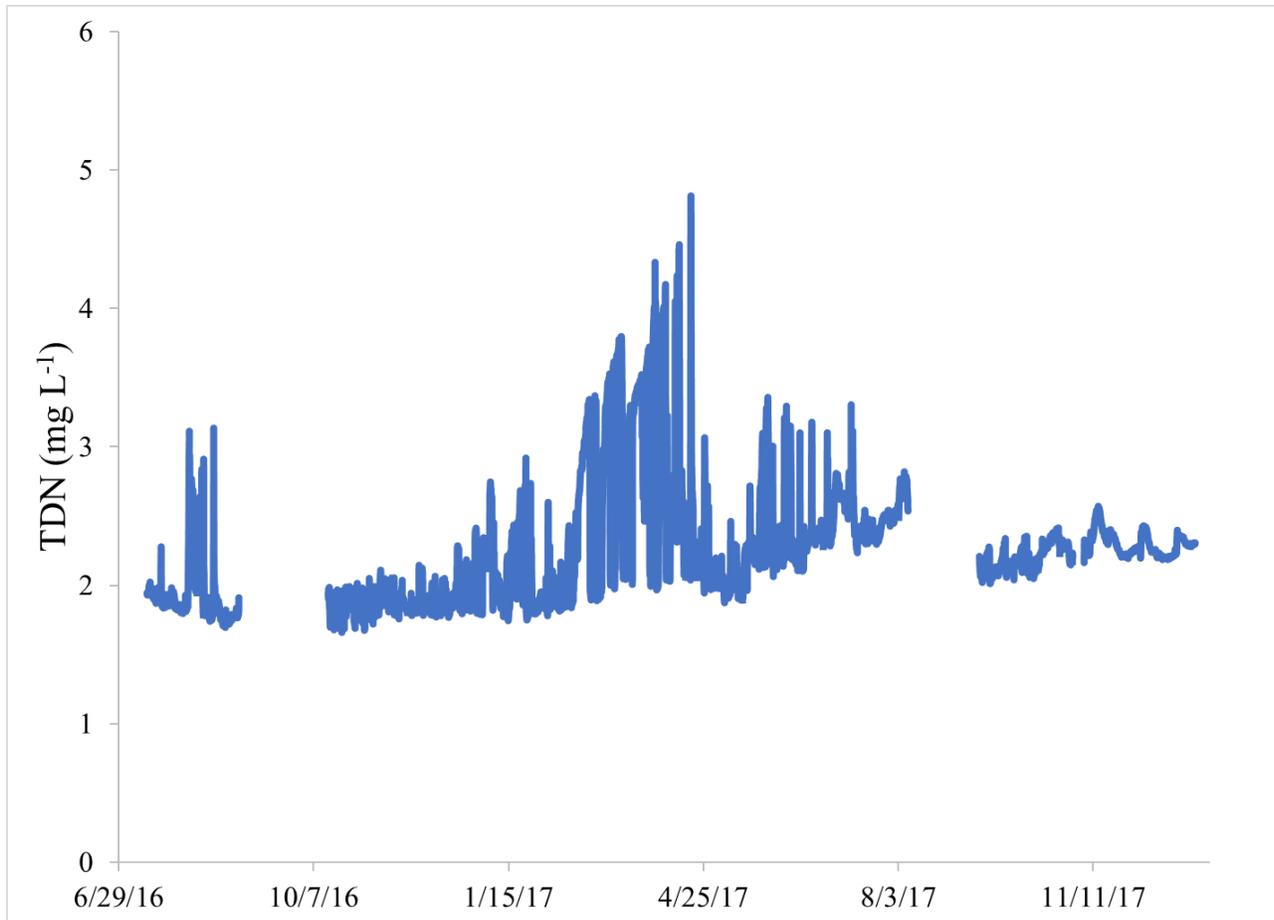
**Supplemental figure C1S4:** MSM TDN calibration used.



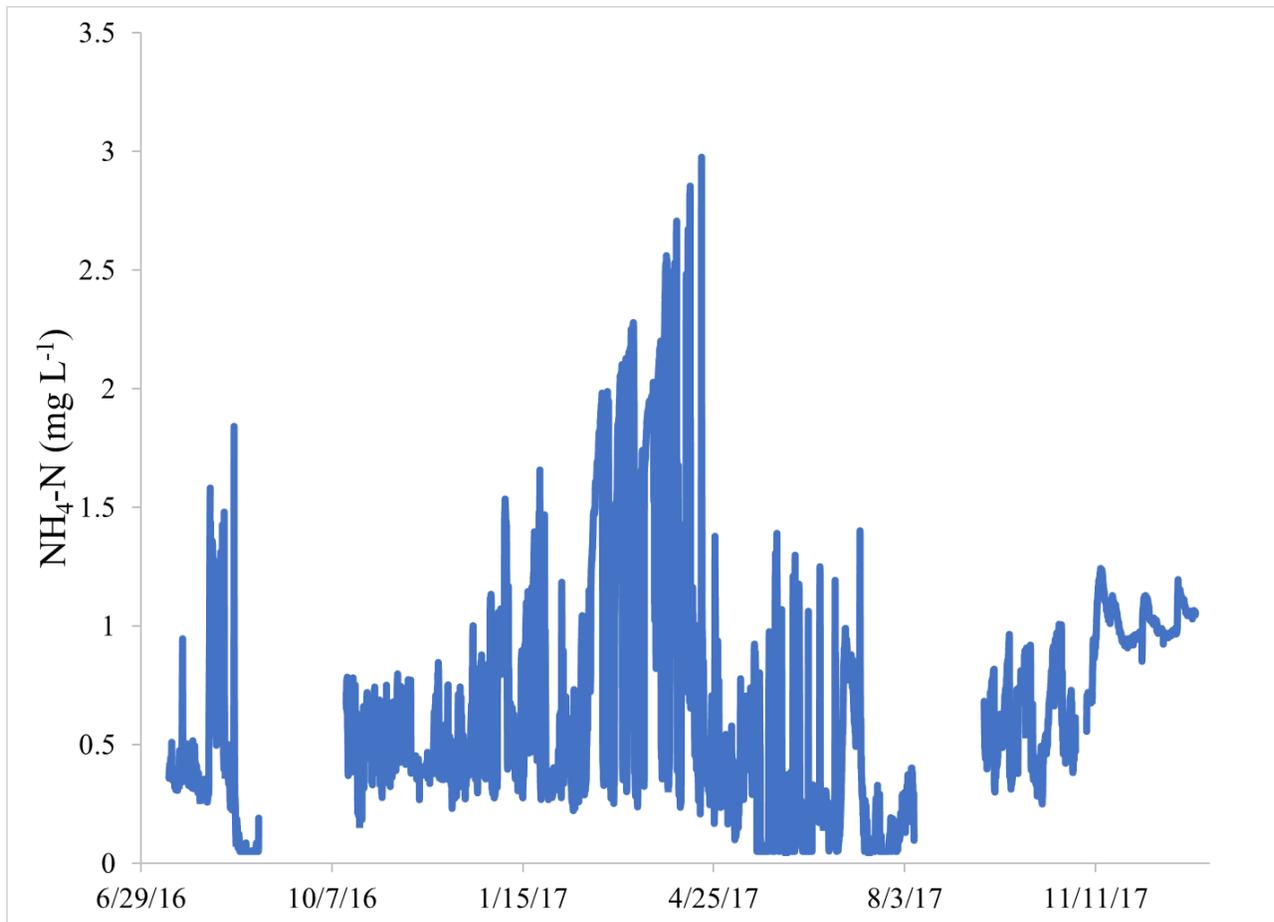
**Supplemental figure C1S5:** NO<sub>3</sub>-N calibration used to adjust default Sc::an NO<sub>3</sub>-N concentration data at Ag and MSM sites.



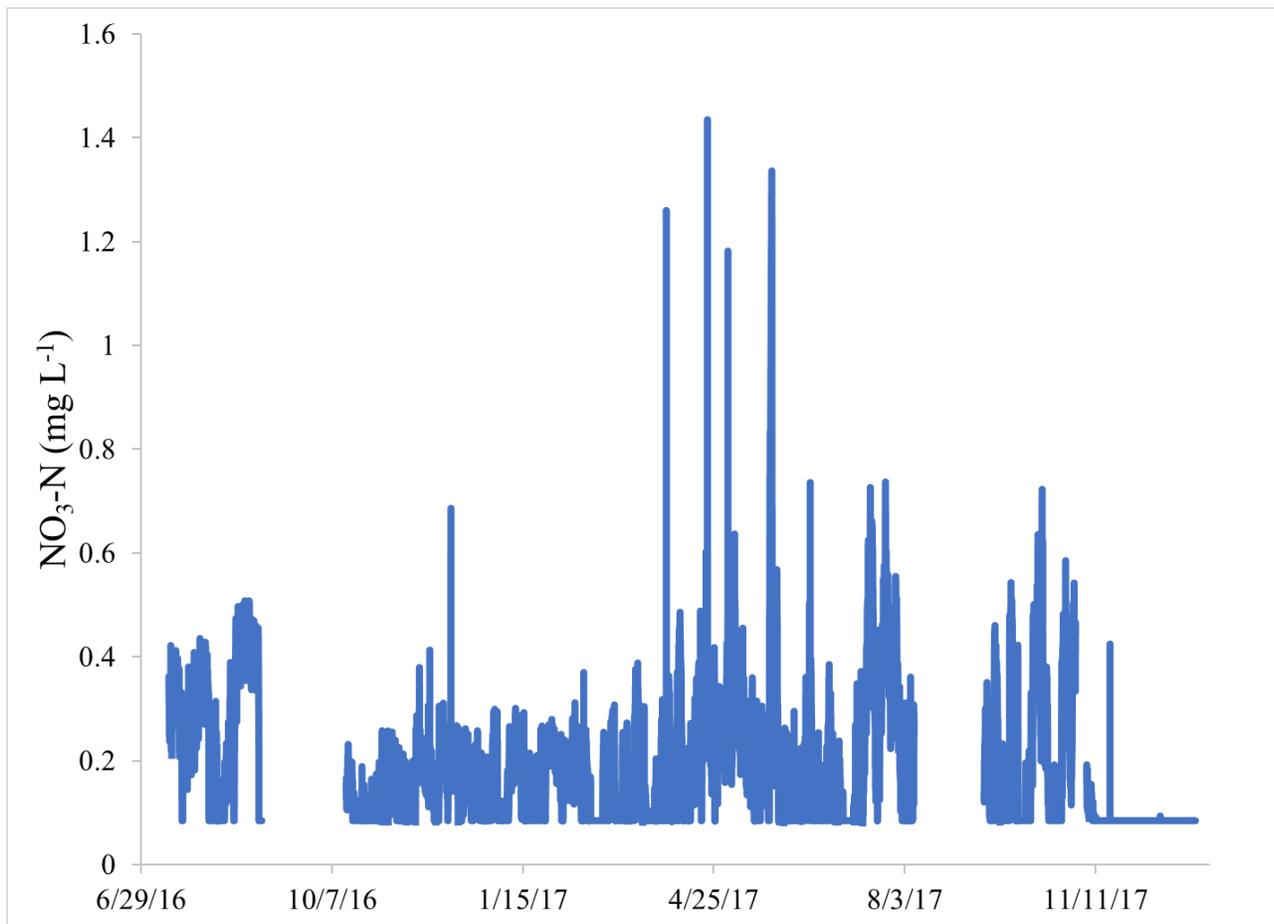
**Supplemental figure C1S6:** A map taken from the USGS website showing the rain gauge location (blue dot) relative to the MSM site (red dot).



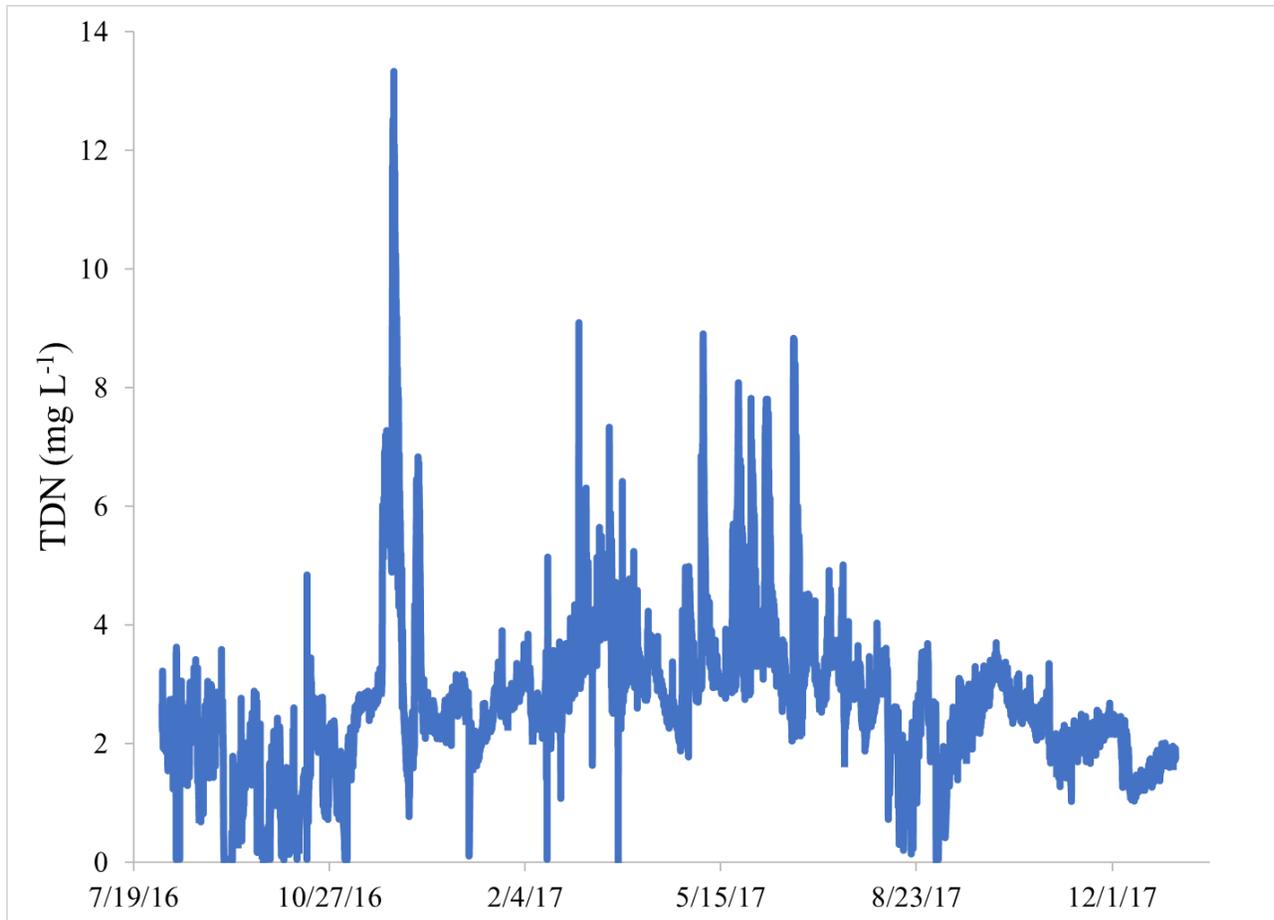
**Supplemental figure C1S7:** Full 30-minute time TDN time series data used for the MSM site.



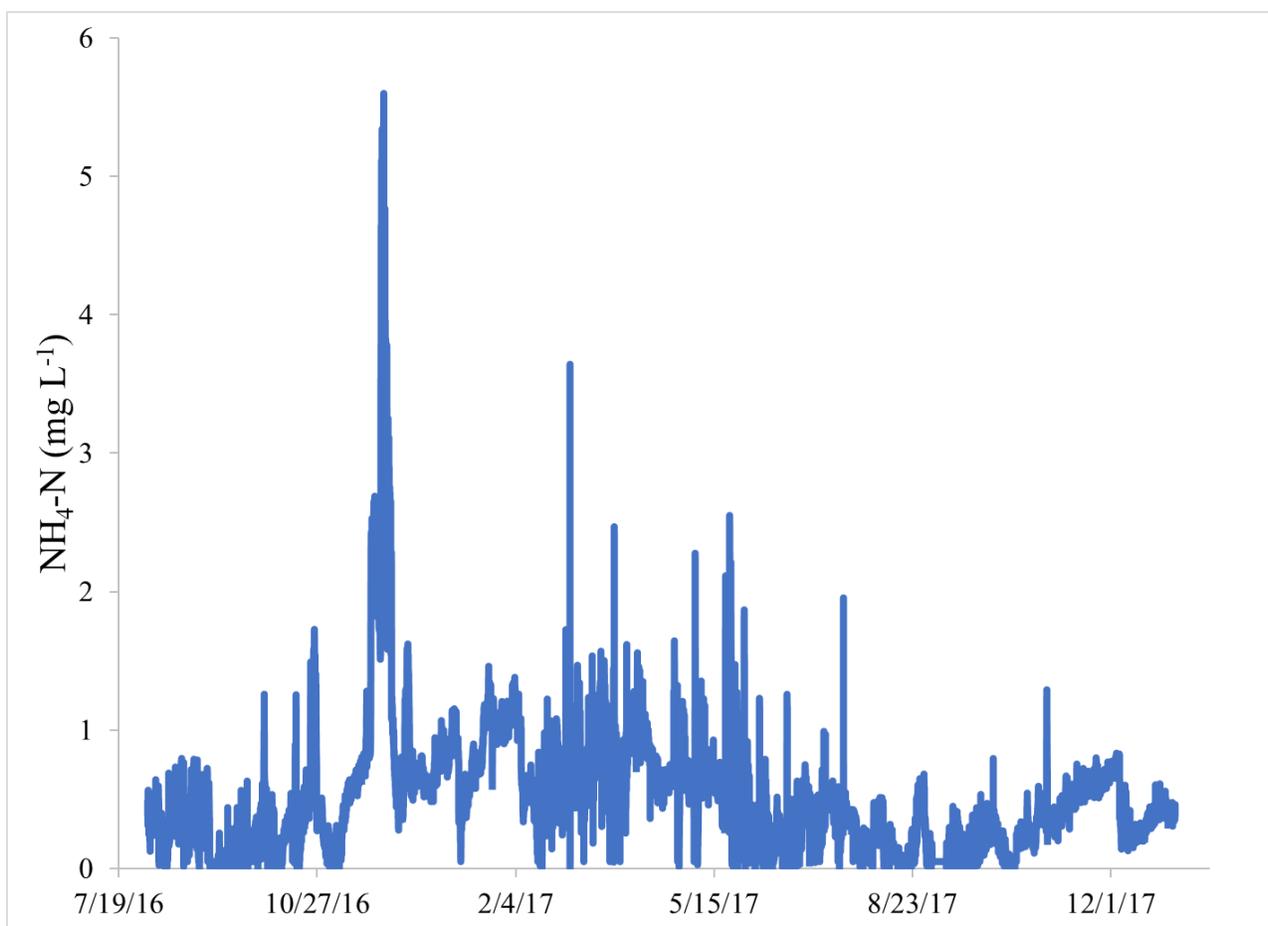
**Supplemental figure C1S8:** Full 30-minute time NH<sub>4</sub>-N time series data used for the MSM site.



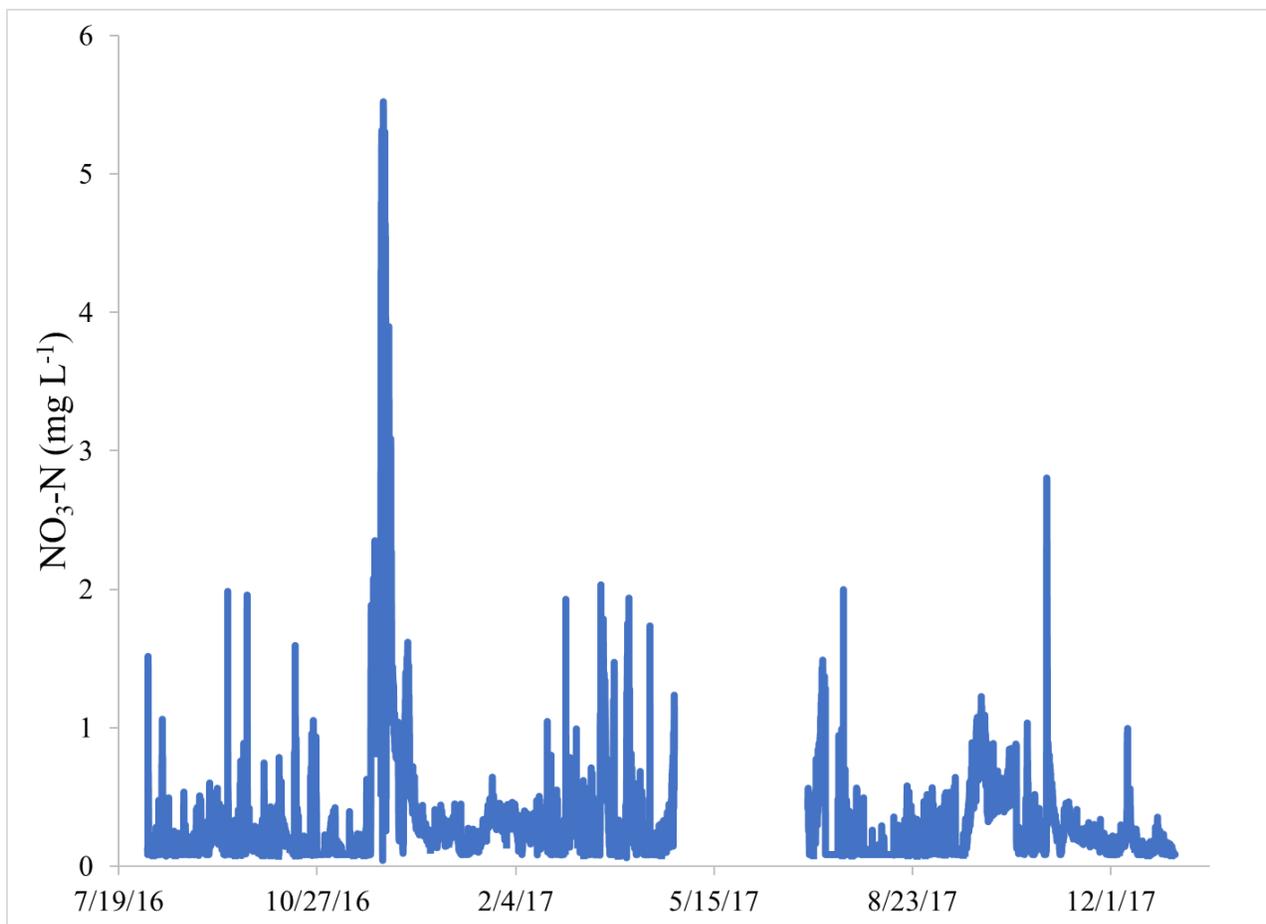
**Supplemental figure C1S9:** Full 30-minute time NO<sub>3</sub>-N time series data used for the MSM site.



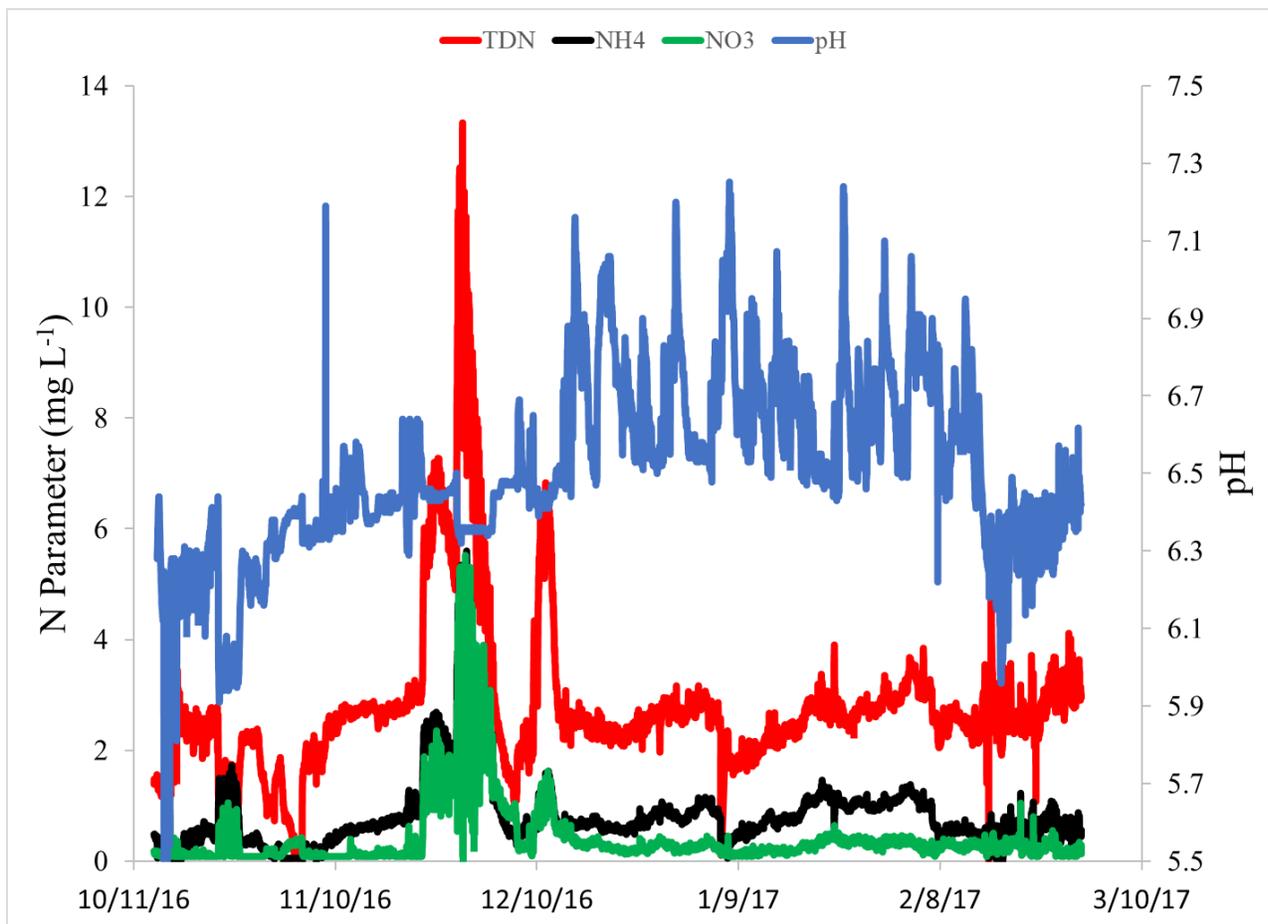
**Supplemental figure C1S10:** Full 30-minute time TDN time series data used for the Ag site.



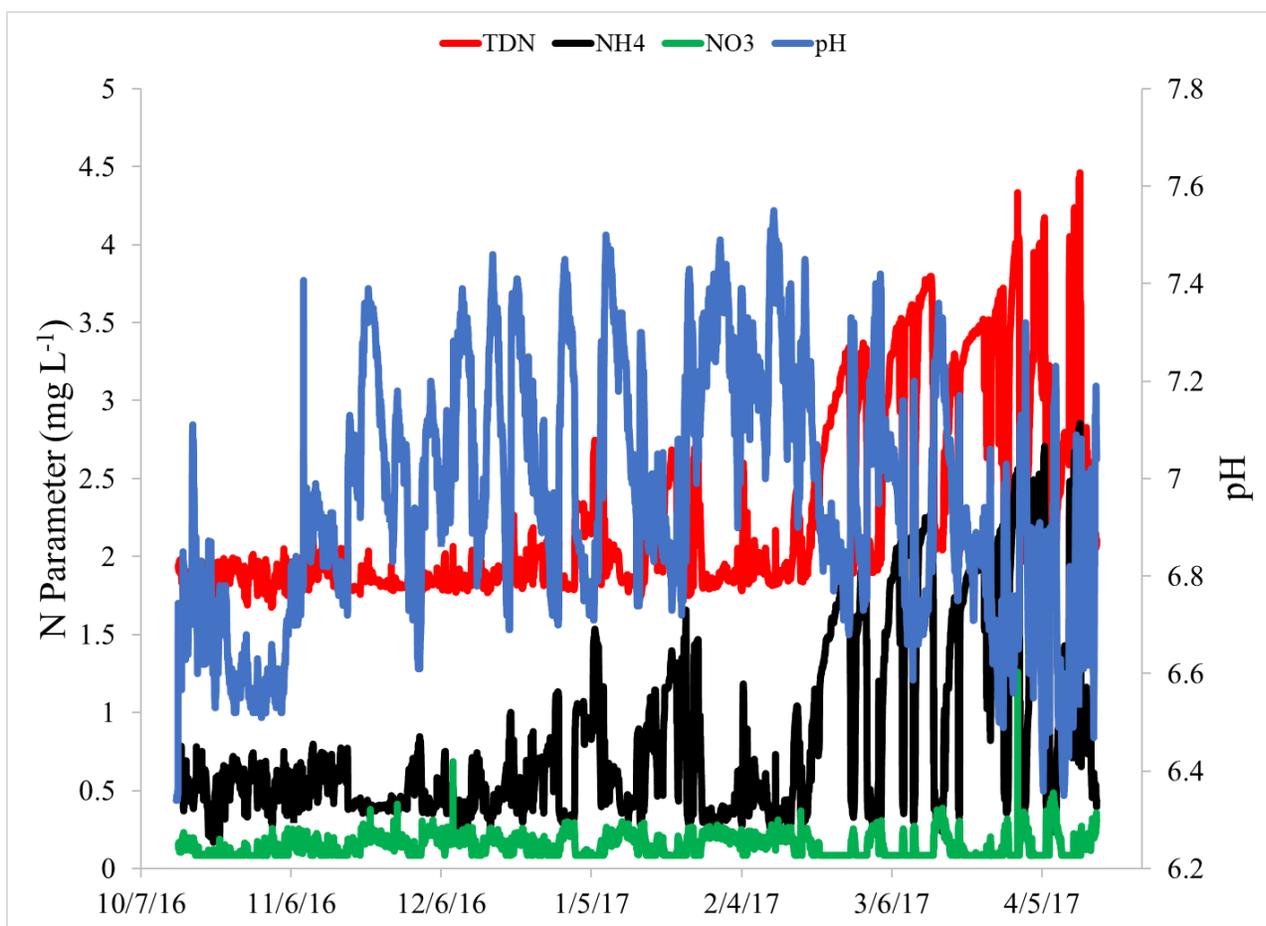
**Supplemental figure C1S11:** Full 30-minute time NH<sub>4</sub>-N time series data used for the Ag site.



**Supplemental figure C1S12:** Full 30-minute time NO<sub>3</sub>-N time series data used for the Ag site.



**Supplemental figure C1S13:** N parameters compared to pH during the seasonal flooding period at the Ag site.



**Supplemental figure C1S14:** N parameters compared to pH during the seasonal flooding period at the MSM site.

## Appendix B: Chapter 2

**Supplemental Table C2S1:** Table showing monthly mesocosm N-adjusted rain.

<b>Month</b>	<b>Site</b>	<b>NH<sub>4</sub><sup>+</sup>-N (mg L<sup>-1</sup>)</b>	<b>NO<sub>3</sub><sup>-</sup>-N (mg L<sup>-1</sup>)</b>
<b>August</b>	Ag	0.64	0.28
	MSM	0.20	0.21
<b>September</b>	Ag	0.43	0.20
	MSM	0.59	0.21
<b>October</b>	Ag	0.40	0.51
	MSM	0.72	0.20
<b>November</b>	Ag	1.05	0.53
	MSM	0.85	0.20
<b>December</b>	Ag	0.62	0.20
	MSM	1.11	0.20
<b>January</b>	Ag	0.26	0.20
	MSM	1.08	0.20

