

USING NUTRIENTS AND MICROBIOTA BIOASSESSMENT TO INVESTIGATE POTENTIAL BENEFITS  
OF AGRICULTURAL WETLANDS

by

CODY THOMAS MATTESON

(Under the Direction of Charles Rhett Jackson and Susan Bennett Wilde)

ABSTRACT

Here we evaluate field-to-stream water quality gradients across a forested alluvial swamp and through a ditched wetland swale on the same working farm. During 2016 and 2017, soil, surface water, and shallow groundwater samples were collected on this farm and analyzed for nitrate + nitrite (nitrate), total phosphorus (TP), and microbial species richness and biovolume. Significant nitrate and TP reduction occurred as shallow groundwater and surface water moved from the farm through an alluvial swamp. Conversely, a ditched depressional wetland swale with cropping on the margins did not significantly alter nutrient concentrations between where water enters the wetland and its discharge point. While pollutant additions were similar into both types of floodplain wetlands, water quality improvements were distinct in the alluvial swamp. Cyanobacteria algal abundance and biovolume correlated positively with phosphorus levels in both nutrient rich agricultural wetlands, and negatively with light intensity.

INDEX WORDS: wetlands, agriculture, nitrate, phosphorus, microbiota

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## DEDICATION

I would like to dedicate this thesis to my family and friends, and to everyone who made this project possible.

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## CHAPTER 1

### INTRODUCTION AND OBJECTIVES

#### Introduction

Wetlands are vital landscape features that provide benefits such as sediment trapping, water quality improvement, flood water storage, fish and wildlife habitat, stabilization of stream banks, and recreation opportunities (Jackson et al. 2014b). Wetlands reduce downstream nutrient loading by several processes including denitrification, plant uptake, and dilution (Cey et al. 1999). Denitrification is the main driver and releases ammonium and nitrate in soil and water, into the atmosphere as  $N_2O$  and  $N_2$  (Cey et al. 1999; Saunders and Kalff 2001; Rivett et al. 2008; Bastviken et al. 2009; Anderson et al. 2014a; Anderson et al. 2014b). Phosphorus is delivered to water bodies largely by sediment, phosphorus sorbs strongly to clay particles (Dupas et al. 2015) which make up much of the upland soil at the study site. Riparian zones have shown to be important in achieving acceptable water quality standards in areas affected by non-point sources of pollution (Secoges et al. 2013), wetlands in riparian zones may be more efficient in processing pollutants in water. By allowing water to flow through a forested floodplain or an alluvial swamp (Conner et al. 2001), pollutants may be trapped or transformed in the soil and water before being discharged into water flowing downstream.

Nutrients from farm runoff cause eutrophication in downstream estuaries (Cheschair et al. 1991). Eutrophication comes from large amounts of nutrients, such as nitrogen and phosphorus, running off into a waterway (Jahangir et al. 2013). These excess nutrients cause large algal blooms in downstream estuaries (Conley et al. 2009). Dense blooms of harmful algae shade out other beneficial algal species before dying and sinking to the bottom of the waterbody. When algal cells senesce and start to break down, decomposition uses up oxygen in the water and creates a dead zone where fish and other organisms cannot survive (Conley et al. 2009). Algae aid in the cycling of nutrients, so water quality indications can be made by microbiological species presence, biovolume, and diversity in a sample (Liang et al. 2003; Bellinger and Sigee 2010a). Varying wetland types generally support different microbiological communities; community composition may be used as an inexpensive and more spatially and temporally holistic method for assessing water quality.

Floodplains located adjacent to active farms offer the opportunity to mitigate agricultural runoff before it runs off into sensitive river and downstream estuary habitat. While the importance of these systems has been realized, additional legislation protecting them is unlikely (Cole and Somerville 2014). Providing justification to preserve and construct additional agricultural wetlands directly to farmers is essential in mitigating non-point discharges. Establishing differences in microbiota species richness and biovolume, along with assessing nutrient changes in wetlands from agriculture, will aid future studies assessing how wetlands process nutrients.

## Site Description

This study evaluated hydrologic connections and spatial water quality variation between a working farm and adjacent wetlands. Crops during the study period included soybeans, cotton, rye grass, and corn. This assessment took place at the University of Georgia Iron Horse Farm and attempted to characterize nutrient regimes within agricultural wetlands on a piedmont farm in Northeast Georgia (Figure 1.1). The study site in Greene County, Georgia is located to the southwest of the confluence of the Oconee River and Rose Creek. Iron Horse Farm is a 300 ha active agricultural research site located off route 15 between Watkinsville, and Greensboro Georgia. Iron Horse Farm was acquired by the University of Georgia from L.C. Curtis in 2013 (Adapted from, Chammoun 2008). The study site is named after an abstract modern art sculpture that sits off Highway 15 on the farm. A large (17 ha) alluvial swamp and depressional wetland (4 ha) at the northern and southern edge of the farm will be the focus of this study. The Oconee National Forest will serve as the studies reference site because it's a forested hillslope discharging into a floodplain wetland.

Rose Creek intersects with the Oconee River after it passes by a large forested alluvial swamp complex that receives water from Iron Horse Farm. The forested alluvial swamp is heavily affected by beaver activity, permanently flooded areas of the swamp are dominated by shrubs and large woody debris. During periods of excess precipitation, Rose Creek overtakes the levee dividing the swamp from the creek, and the alluvial swamp flows like a secondary channel. A ditched wetland swale drains the southern side of the farm and will be assessed for the same criterion as the forested wetland. This wetland is a low area of the Oconee River

floodplain, which has been ditched to allow attempted row-crop production. The ditch draining the wetland swale is evident on a plat of the farm from 1875, so is a well-established feature on the study site (Coulter 1964). The Oconee National Forest, which is serving as a reference site, was intensively managed farmland and has now been converted to a national forest. Erosion resulting from cotton era farming in piedmont Georgia resulted in massive amounts of sediments leaving the farms by gully erosion and entering the surrounding streams and rivers (Jackson et al. 2005). Farms experiencing exceptional amounts of erosion were converted to national forests to restore the eroded landscape. The Oconee National Forest being used as a reference site is representative of current forested land in piedmont Georgia that was previously agriculture.

The Scull Shoals historic site, located across the Oconee River from Iron Horse Farm, has seen intense floods since the early 1800s (Ferguson 1997). Similar flood and sediment regimes have historically taken place at low areas on Iron Horse Farm. A bridge and dam once spanned across the Oconee River near the mouth of Rose Creek. Sunken wagon highways are still evident in the upland forested area of Iron Horse Farm. Rose Creek was once diverted to intersect the Oconee downstream of the dam, this channel is also still evident in the alluvial swamp, and cuts through the upland forested area (Coulter 1964). The area around Scull Shoals was a supporting agricultural hub, this area included the current study site (Coulter 1964).

Before sediment largely filled in the Oconee River, the river was navigated for shipping goods up and down stream (Coulter 1964). Present day farms do not benefit from the shipping

convenience of being located along the river and are contributing to pollution and eutrophication of downstream Lake Oconee (Burt et al. 2013). The watershed of Lake Oconee, which Iron Horse Farm falls within, is dominated largely by cattle and poultry production (approximately 60,2000 meat cows, 22,100 dairy cows, and 3,767,800 chickens). Research has linked large poultry and cattle operations to increased nutrient pollution and harmful algal blooms within these watersheds (Burt et al. 2013). Research on nutrient mitigation provided by agricultural wetlands in southeastern piedmont watersheds is lacking. Iron Horse Farm offers the opportunity to observe pollution levels from a long-established farm, with a retired cattle feedlot and current row-crop production.

#### Objectives and Hypotheses

Iron Horse Farm and Oconee National Forest floodplain wetlands were assessed using standard water sampling protocol (USEPA 1983) for nutrients. Soil sampling and analysis (UGA Extension 2016) were conducted to assess nutrient source areas on the farm, and microbial community analysis in surface water was conducted to help us understand the effects of nutrient enrichment on aquatic systems. Storms may mobilize sediments and nutrients from the fields, so storm samples were measured to compare with concentrations during non-storm periods. Assessment of contributions to the Oconee River were undertaken with sampling of a long stretch of the river.

Research objectives relating to soil and water chemistry include:

1. Examination of the amount of available nitrate + nitrite and phosphorus held by soils in uplands and lowlands.

2. Assessment of spatial differences in nutrient levels discharging into the forested alluvial swamp to those in the ditched wetland swale.
3. Observe nutrient and water quality changes in the Oconee River from the Barnett Shoals Dam to Iron Horse Farm, and nutrient regimes during summer and fall.
4. Observe water quality changes in Rose Creek and the Oconee River.
5. Investigate nutrient input from upland farms to lowland catchments during storm events.

Research objectives relating to microbiological organisms include:

6. Observe microbiological species richness, cell count, and species population biovolume in Georgia Piedmont agricultural wetlands over a year.
7. Assess microbiological biovolume in wetlands based on light intensity and nutrient regimes.
8. Examine microbiological species richness changes in the Oconee River from the Barnett Shoals Dam to Iron Horse Farm during summer and fall.
9. Observe microbiological changes from Rose Creek to the Oconee River as water quality changes.
10. Investigate how microbiological species richness changes during storm events.

Hypotheses for the above goals and objectives are listed below.

1. Deposition of nitrate + nitrite and phosphorus in topographically low wetland soils will exceed that of soils in upland agricultural production.

2. Levels of dissolved nitrate + nitrite and phosphorus in surface water and groundwater will be lower in the forested alluvial swamp than the ditched wetland swale.
3. Nutrients in the Oconee River will be greater upstream and reduce as the Oconee River flows downstream. The Oconee River will contain more nutrients in the spring when fertilizers are added to agricultural fields.
4. Rose Creek will contain statistically lower levels of nutrients than the Oconee River. Due in part to the fact that Rose Creek flows through wetlands and agriculture, while the Oconee River flows through urban areas.
5. Lower levels of nitrate + nitrite and higher levels of phosphorus will be found in water samples from large storm events than from routine dry weather sampling events.
6. Species richness will be higher in the forested alluvial swamp while species population biovolume and cell count will be higher in the ditched wetland swale.
7. Cyanobacteria species population biovolume will be greater in a sunlit wetland with high nitrate + nitrite. Overall species population biovolume will be greater in more sunlit and nutrient rich waters, while species richness will be greater in the shaded wetland.
8. Microbiota species richness will increase as nutrients lower downstream of Barnett Shoals Dam, there will be more species richness during the summer survey.
9. Microbiota species richness will be greater in Rose Creek than in the Oconee River.
10. During storm events, microbiological communities in wetlands will shift toward more riverine communities. This will include more diatoms and less cyanobacteria.



Figure 1.1: Location of Iron Horse Farm and Reference Site.

## CHAPTER 2

### LITERATURE REVIEW

#### *Wetland Hydrology*

Wetlands catch sediment before it runs off into rivers and creeks, process nutrients, serve as a home for wildlife, prevent stream erosion, lower water temperature, and are aesthetically pleasing (Jackson et al. 2014b). Many of these benefits to the surrounding ecosystem are dictated by the hydropattern of the wetland, which is the series of water levels over the year (Jackson et al. 2014b). Hydropatterns affect the status of the entire wetland, including soils, biogeochemistry, and biology and should be measured using the average water level, amount of fluctuation, and cycling of the water level (Jackson et al. 2014b).

Hillslopes are composed of specific segments from the top of the hill known as the summit, and the hillslope composed of the shoulder, backslope, and footslope, and toeslope at the bottom (Jackson et al. 2014b). Water moves from areas of high head to areas of low head (Dunne and Leopold 1978), however runoff flows can flow underneath downslope landscapes or unseen soil characteristics may be completely different than surface topography (Lowrance et al. 1997; Winter 1999; Winter et al. 2001; Denver et al. 2014). Depth of bedrock often enables long, deep connections between uplands and lowlands (Lowrance et al. 1997; Cey et al. 1999; Winter 1999). Wetlands are the foundation of hydrology (Jackson et al. 2014b), and can function to mitigate excess nutrient levels. Effectiveness of nonpoint pollution control depends

on the type of pollutant and hydrologic connections of landscapes involved (Lowrance et al. 1997; Winter 1999). Determining flow paths of upland pollution into wetlands could help determine sensitive downslope areas which settle sediments by breaking up surface flow (Dupas et al. 2015; Griffiths et al. 2017). Climate and hydrology also significantly affect hydrologic linkages and water chemistry (Winter et al. 2001). Particles, depth, and porosity of the soil all can alter direction and speed of flows, climate of a site may further dictate how an assessment site functions (Winter 1999). Rivers also carry nutrients downstream at elevated water levels and store them in the banks of the river. As water levels fall, it releases the nutrient laden water from the banks at a later time than they were input (Winter 1999). Flow paths display short travel times adjacent to a wetland, and longer travel times upslope (Cey et al. 1999; Jackson et al. 2014a; Musolff et al. 2016; Griffiths et al. 2017). Horton Overland Flow is an uneven sheet of water moving down a hillslope after the soil cannot hold more water (Dunne and Leopold 1978). This occurs more often on manipulated sites when precipitation outpaces infiltration capacity and depression storage (Dunne and Leopold 1978).

### *Wetland Ecology*

Wetland classification considers both ecological and hydrologic factors which dictate plants and animals living there (Euliss et al. 2004). Attempts have been made to establish a way to categorize these systems, which is called the wetland continuum concept (Euliss et al. 2004). The continuum allows for managers to classify a system even if the type of wetland shifts during a wet or dry year. Bringing together ecological and hydrological classifications allows us to better understand the biology of wetland systems (Euliss et al. 2004). Another classification

model involves floodplain wetlands and their differences along a river channel (Batzer et al. 2018). Classification of these systems is important because the functional importance of floodplain wetlands changes from headwaters to coastal areas. Headwater creeks will rely on allochthonous (outside stream) additions of carbon and nutrients inputs from floodplain wetlands, while autochthonous processes contribute more to floodplain and river productivity downstream. Piedmont and coastal floodplain wetland systems functionality comes from inputs within the wetland such as plants, animals, and biogeochemistry (Batzer et al. 2018). This concept demonstrates that different wetland landscapes are affected by a gradient of external and internal loading and cannot all be managed the same.

Forested wetlands are prominent features in the southern United States, though agriculture through the 1960s and 1970s caused rapid decline (Conner et al. 2001). Many forested wetlands occur on river floodplains and are referred to as alluvial swamps (Conner et al. 2001). Floodplain alluvial swamps often are located adjacent to production crop fields, as prices of the crop go up, motivation for swamp preservation goes down. At the study site, land use has been agriculture for a long time (Coulter 1964) and current wetlands are well established. Aerial imagery shows the study wetlands going back 23 years, mitigation benefits should be substantial in this unshifting landscape (Yates and Sheridan 1982).

### *Buffer Areas*

The hydrologic study taking place at Iron Horse Farm is located upstream of the 7,709 ha Lake Oconee (Burt et al. 2013). Nutrients and toxic algal blooms from excess agricultural nutrients threaten the effective use of Lake Oconee (Burt et al. 2013). A promising method of

mitigating nutrient pollutants is to allow farm runoff to be filtered through riparian buffers (Burt et al. 2013) and floodplain wetlands which can reduce cattle and crop loss (Peltzer et al. 2008). Each site should be observed independently with regard to the size of its buffer area (Secoges et al. 2013). Vegetated buffer areas of 15.2 m are sufficient for filtering fertilizer out of the water, decreasing water temperature, stabilizing streambanks, and reducing sediment input (Secoges et al. 2013). New York City and Munich have seen improved water quality since they have begun paying farmers for the utilization of best management practices (Grolleau and McCann 2012). Effective BMPs for non-point source pollution includes riparian zones and floodplain wetlands. Natural water quality filtration has reduced the cost of water treatment by preventing water purification plant construction in New York City and Munich (Grolleau and McCann 2012).

#### *Nitrogen and phosphorus in Agricultural Wetlands*

Nitrogen travel decreases with increased flow, described as a dilution relationship; while phosphorus does not show a relationship with water velocity (Dunne and Leopold 1978; Lewis and Grant 1979; Dupas et al. 2015). Stored nitrogen in agricultural areas has resulted in a nitrogen concentration pattern instead of its typical dilution relationship with flow (Wriedt et al. 2007; van der Velde et al. 2010; Musolff et al. 2016). Phosphorus is transported into rivers by sorbing to clay particles and running off in overland flow (Dupas et al. 2015). Clay particles may become saturated in wastewater (Cheschair et al. 1991). Histosols do not hold onto phosphorus as well as other soils (Cheschair et al. 1991; Murkin et al. 1991) which could make

wetland retention difficult. Phosphorus also does not have an atmospheric phase which complicates management strategies (Schindler 2010).

### *Nitrogen Cycle*

Nitrogen makes up 79% of the earth's atmosphere (Groffman et al. 2006; Funk and Wagnalls 2017). Nitrogen goes through mineralization, immobilization, volatilization, fixation, nitrification, and denitrification (Galloway et al. 2003; Anderson et al. 2014a). Mineralization or ammonification is the oxidation of dead organic nitrogen to inorganic nitrogen, and immobilization is the reverse process (Vymazal 1995; Funk and Wagnalls 2017). Ammonium accumulates quickly the first two weeks after a flood event and leaves the site up through plants or down into groundwater (Vymazal 1995). Volatilization converts ammonium to ammonia, while fixation converts nitrogen gas to ammonia (Vymazal 1995). Nitrification happens as ammonia is converted to nitrite then nitrate; denitrification is a microbial process converting nitrite or nitrate to nitrogen gases (Vymazal 1995; Funk and Wagnalls 2017). During denitrification, oxides act as terminal electron acceptors for electron transport, 5 electrons are required for denitrification and 8 for nitrate ammonification (Vymazal 1995).

Nitrate ammonification is favored in systems with low levels of nitrogen and processed by *Bacillus*, *Citrobacteria*, *Aeromonas*, all in the *Enterobacteriaceae* family, as well as *Clostridium*. Chemolithotrophic bacteria fix ammonium to hydroxylamine, followed by nitroxyl, then nitro hydroxylamine, and finally to nitrite; and *Nitrosomonas* convert ammonium in soils (Vymazal 1995). Faculative chemolithotrophic bacteria also convert nitrite to nitrate but can do so in the absence of oxygen, such as soils in a wetland.

Nitrate is highest in the winter (Anderson et al. 2014b; Griffiths et al. 2017), and shallow groundwater contains the most pollution (Griffiths et al. 2017). Specific conductivity can be positively or negatively correlated with nitrogen, tracking conductivity levels can serve as a surrogate for nitrogen levels in groundwater (Griffiths et al. 2017). Temperature and dissolved oxygen is often correlated with ammonium in the water, which displays an inverse relationship with nitrate (Griffiths et al. 2017).

### *Denitrification*

Many papers cite denitrification as the most prominent method of nitrogen attenuation in a wetland system (Cey et al. 1999; Saunders and Kalff 2001; Rivett et al. 2008; Bastviken et al. 2009; Anderson et al. 2014a; Anderson et al. 2014b). Denitrification in saturated soil accounts for half of the total denitrification in riparian zones (Anderson et al. 2014a). Plant uptake may not be the main component of nitrogen loss, but is still a significant contributor (Bastviken et al. 2009). Denitrification levels in unmanaged landscapes have shown seasonal differences opposite of those in a managed landscape (Anderson et al. 2014b). Studies documenting higher levels of nitrogen processing in the fall may indicate that uptake by plants has a larger effect than previously thought (Bastviken et al. 2009).

High atmospheric nitrogen levels make quantifying rates of denitrification difficult (Anderson et al. 2014b). Agricultural studies assessing soil denitrification have predominately taken place in laboratory trials (Anderson et al. 2014b). Deep groundwater contains lower levels of dissolved oxygen, and increased anoxic conditions promote denitrification (Musolff et al. 2016). Denitrification is most effective when dissolved oxygen falls below 2 mg/l (Mariotti et

al. 1988; Cey et al. 1999, Rivett et al. 2008, Musolff et al. 2016). High nitrate levels could be attributed to high levels of denitrification, or denitrification could be using up the nitrate, thus displaying low nitrate levels (Starr and Gillham; Mariotti et al. 1988; Cey et al. 1999).

Nitrate runs off of a farm landscape into an anaerobic zone where microbes can convert nitrate to the inactive  $N_2$  form of nitrogen (Seitzinger et al. 2006; Anderson et al. 2014a). Up to 44% of the nitrogen that was missing from a mass balance model can be attributed to areas where the water table is near the surface and periodically rises and falls (Anderson et al. 2014a). Denitrification converts nitrogen oxides, nitrate ( $NO_3$ ), and nitrite ( $NO_2$ ) in water or soil to nitric oxide (NO), nitrous oxide ( $N_2O$ ), and dinitrogen ( $N_2$ ) (Groffman et al. 2006). Studies of denitrification are difficult to perform accurately, but over the years various methods have been developed, thereby slowly advancing the science of denitrification (Groffman et al. 2006). Direct dinitrogen to argon ( $N_2:Ar$ ) ratio measurements are useful for measuring gases produced by denitrification, mostly utilized in water assessments (Groffman et al. 2006). Measuring gas exchange is typically done with a membrane-introduction mass spectrometer (MIMS) analysis for  $N_2$ -Ar (Groffman et al. 2006). The method of denitrification analysis being used in this study assessed  $N_2O$  and  $N_2$ , but the main product of denitrification is likely  $N_2$  (Anderson et al. 2014b). Assessing rates of denitrification using this method compares  $N_2$  to Ar in the atmosphere and provides a precision of 0.03% (Groffman et al. 2006).

### *Eutrophication*

Agriculture is known to contribute nutrients into rivers flowing into estuaries which can cause eutrophication (Lavoie et al. 2004). Nitrogen is the most limiting nutrient in saltwater

bodies (Dodson 2005), while phosphorus is the most limiting nutrient in freshwater bodies (Dodson 2005; Schindler 2010; Dupas et al. 2015). In 1981 The Coastal Water Management Taskforce recommended that nutrient rich water be filtered through wetlands to reduce the eutrophication effects in downstream estuaries (Cheschair et al. 1991). Quality of inland water bodies has large impacts on the quality of water in downstream marine environments, and pollutants entering estuaries are magnified by the size of the system (Abboud-Abi Saab and Hassoun 2017). Eutrophication from farm runoff and large population centers (Grolleau and McCann 2012) can cause large algal blooms to form (Smith et al. 1987; Diaz and Rosenberg 2008). Eutrophication results from large quantities of nutrients running off into a waterbody, which leads to an environment ideal for algal growth. As algae die, they sink to the bottom and decompose, using up dissolved oxygen in the process. If dead zones caused by decomposition proliferate seasonally, organic accumulation may lead to anoxia in a body of water with dissolved oxygen below 2 mg/l (Diaz and Rosenberg 2008).

### *Microbiota*

Microalgae can be classified into diatoms, green algae, blue-green algae, and golden algae (Demirbas 2011). Diatoms are algae with distinctive cell walls resembling a glass dome (Spaulding et al.). Green Algae are protists that form thick green mats on the water's surface, and Golden Algae can tint water a golden-brown color and are protected by siliceous plates or spines (Dodson 2005). Algae and periphyton can be indicators of water quality (Dodson 2005), algae are the bottom of the food chain (Biggs and Kilroy 2000), and dictate the composition of the ecosystem.

Slimy coatings on rocks in streams is attached algae or Periphyton which responds to relative nutrient levels. Periphyton, comprised of algae, fungi, and bacteria, can indicate water conditions even during drought, making them ideal indicators (Biggs and Kilroy 2000).

Benthic organisms process ~15% of nitrogen in waterbodies which can be a large amount when coming from agriculture (Saunders and Kalff 2001). Intense light increases the size of the organism while not increasing nutrients in the algae. This lowers the benefit predators receive by eating the algae, and will lower the species diversity and volume (Lies and Kahlert 2009; Boon et al. 2014). Algae can make up as much as 30% of primary producers, so a primary food source lacking nutrients can be an issue for animals that rely on them (Vymazal 1995; Boon et al. 2014).

Potentially toxic cyanobacterial taxa include *Oscillatoria*, *Anabaena*, *Microcystis*, *Nostoc*, *Phormidium*, and *Leptolyngbya*. A toxic cyanobacterial species (*Aetokthonos hydrillicola*) can attach to aquatic plants consumed by water birds and cause mortality in coots and their predators (Wilde et al. 2005). This disease, called Avian Vacuolar Myelinopathy, results in characteristic brain lesions and only occurs in lakes where the toxic epiphytic cyanobacteria is dominant on the submerged aquatic plants. In lakes where this disease does not occur, the epiphytic community is primarily comprised of diatoms in the winter months and green algae in the summer/fall (Wilde et al 2005; Wilde et al 2014). Excess cyanobacterial blooms also provide a challenge for water purifiers, and clog turbines (Falconer and Humpage).

### *Microbiota Bioassay*

Anthropogenic inputs originate from society's increasing need for buildings, food, power, and mining (Smucker 2014). Runoff from animal production areas will typically contain excess nutrients which shifts the microbiotic community (Burt et al. 2013). For example, nitrogen fixers such as *Anabaena* and *Aphanizomenom* can indicate high phosphorus loading. The Everglades in southern Florida have a very distinct open water cyanobacterial mat which breaks up in the presence of increased phosphorus (Rader and Richardson 1992). This provides evidence of cyanobacteria's ability to outcompete beneficial species under low phosphorus conditions because of its ability to fix its own nitrogen from atmospheric N<sub>2</sub>. When both nitrogen and phosphorus are high, green algae will proliferate as they can take up both nutrients rapidly and dominant the biomass (Rader and Richardson 1992, Biggs and Kilroy 2000).

### *Species Counts and Biovolume Conversion*

Calculating biovolume allows for the determination of relative productivity of algal groups using standardization of geometric equations to obtain comparable results (Hillebrand et al. 1999). Cell dimension measurements are entered into various shape specific equations to obtain the mean unit biovolume (Bellinger and Sigee 2010b). Mean unit biovolume is derived from the median of 10 measurements of each species, 10 replications of a species measurement will minimize error and reduce variability (Hillebrand et al. 1999). Multiplication of biovolume by the number of cells in a sample provides the species population biovolume

(Bellinger and Sigee 2010b). Benthic water is often mixed with sediment and must be counted by hand (Hillebrand et al. 1999).

Errors occur when performing light microscopy; light peeking around the sides of the cell makes them look larger. Errors of this sort are typically less than 1  $\mu\text{m}$ , which is lower than errors attributed to cell counting. It is sometimes difficult to determine individual cells of filamentous algae, so length measurement of single filaments can be related to cells/filament. Variations within cell stages occur between seasons, so comparisons may be skewed due to life histories (Hillebrand et al. 1999). Another issue measuring cells lies in the difficulty of obtaining the depth of the cell, some papers conclude that depth may be skipped (Hillebrand et al. 1999), or width can be substituted as an assumption of symmetry for depth (Verity et al. 1992). Depth may also already be included in measured samples because all cells are not turned the same direction. Lugols solution reduces the size of cells by 25% and many non-spherical cells become increasingly spherical (Hillebrand et al. 1999). Photosynthetically active radiation (PAR) was assessed at and just below the surface (LI-COR 2017) to determine light levels for microbiota.

## CHAPTER 3

### NITRATE AND PHOSPHORUS GRADIENTS FROM FARM THROUGH WETLANDS TO STREAM

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<sup>1</sup>Matteson, C. T., C.R. Jackson, D.B. Batzer, S.B. Wilde, J.L. Shelton, and J.B. Jeffers. To be submitted to *Wetlands*.

## Abstract

Wetlands can sequester and transform nonpoint source pollutants moving from farms to receiving waters. This study evaluated water quality benefits of agricultural wetlands by tracking nutrient concentrations and other water quality parameters along hydrologic pathways from a working farm through a forested alluvial swamp and a ditched wetland swale down to their receiving waters. Soils were collected from row-crop fields, a retired feedlot, and wetlands; and were analyzed for nitrate, ammonium, and phosphorus. Water samples were collected from shallow piezometers, surface waters (overland flow, ditch flow, and a beaver-modified alluvial swamp), and the receiving creek and river and analyzed for nitrate + nitrite (nitrate), and total phosphorus (TP). Nutrient concentrations, particularly nitrate, in groundwater and surface water in the alluvial swamp bordering the northern side of the study farm, were much lower than concentrations measured in flows from the farmed uplands. Nitrogen in water running off the farm was dominated by nitrate, but nitrogen in the alluvial swamp soil occurred as ammonium; phosphorus is low in wetland soils, but is mitigated in water samples as it moves through the alluvial swamp. The forested alluvial swamp provided an especially effective environment for water quality mitigation. Across the farm, the ditched wetland swale used in row-crop production did not significantly reduce nitrate but appears to provide some phosphorus mitigation. High nitrate levels were seen in the entire ditched complex, possibly due to oxic conditions where ammonium was converted to nitrate.

INDEX WORDS: wetlands, agriculture, nitrate, phosphorus, soils, water

## Introduction

Depending on hydrology and landscape position, depressional wetlands are effective in nitrate mitigation (Denver et al. 2014) for land use activities that mobilize subsidized nutrients. However, ditching depressional wetlands for crop production reduces some of that mitigation potential due to lower residence times and greater oxygenation. Wetland draining is justified by the financial incentive of additional croplands (Kramer and Shabman 1993), but incurs environmental costs by allowing nutrients, sediments, and other pollution to be discharged directly to streams and rivers.

Filtering of agricultural runoff through wetlands has been recommended in legislation since 1981 (Cheschair et al. 1991). Wetlands are particularly effective in nutrient mitigation (Saunders and Kalff 2001), and here we will evaluate a hydrologically undisturbed forested alluvial swamp. Alluvial wetlands are naturally eutrophic, so added nutrients from agriculture are unlikely to have adverse effects on the system (Hefting et al. 2013). Many nutrients passing through agricultural in wetlands today are from fertilizer or manure applied decades prior, due to long groundwater flow times (Hefting et al. 2013).

Excess nitrogen discharges from agriculture are a major source of pollution and downstream eutrophication (Jahangir et al. 2013), and methods for mitigating these inputs has been long debated (Yates and Sheridan 1982). Nitrogen inputs from farms can accumulate in downslope wetland areas (Meals et al. 2010; Van Meter and Basu 2015; Musolff et al. 2016), processing of these storages is important for downstream water quality. Wetlands can be efficient mitigation sites for nitrogen processing (Lowrance et al. 1997; Burt et al. 2013)

especially nitrate (Bastviken et al. 2009). Mitigation of nitrate concentrations can occur due to denitrification, plant uptake, and dilution (Cey et al. 1999; Saunders and Kalff 2001; Jahangir et al. 2013; Secoges et al. 2013; Denver et al. 2014). Plant uptake increases as plants age, and does not depend on the amount of nitrogen present (Bastviken et al. 2009). Biological uptake is slower during colder periods (Cey et al. 1999; Anderson et al. 2014b). Dilution of nutrients in a large waterbody can reduce concentrations (Cey et al. 1999), but is not always a significant influence (Yates and Sheridan 1982).

Phosphorus, which sorbs strongly to clay particles (Cheschair et al. 1991), is trapped in standing water, broken down by microbes, and taken up by plants (Biggs and Kilroy 2000); phosphorus may settle out in agricultural wetlands (Cheschair et al. 1991). Storm events can flush agricultural wetlands of their nutrients, but wetland complexes may process up to 70% of these nutrients (Cheschair et al. 1991). Phosphorus is mitigated in agricultural wetlands by reducing flow velocities and allowing phosphorus to settle out (Cheschair et al. 1991; Lowrance et al. 1997; Dupas et al. 2015).

To evaluate the water quality mitigation provided by both undisturbed and ditched wetlands adjacent to a working farm, we monitored soil, shallow groundwater, and surface waters along hydrologic flow paths moving from the farm fields through both intact and ditched wetlands, and into receiving waters. Spatial gradients of nutrients in soils, shallow groundwater, and surface waters will aid in understanding if and to what extent agricultural wetlands mitigate nutrient concentrations before being discharged downstream. The differences between Rose Creek and the Oconee River may provide insight into the primary

pollution inputs in piedmont Georgia creeks and rivers, and if the study site is discharging directly into these waterbodies during dry weather and storm events.

## Methods

### *Site Description*

The study farm is a 300 ha active agriculture research farm, known as the Iron Horse Farm and operated by the University of Georgia, located in the Georgia Piedmont on route 15 in Greene County, GA; just northwest of where route 15 crosses the Oconee River (Figure 3.1). Iron Horse Farm offers a unique hydrologic opportunity to observe ground water, surface water, storm runoff movements, and conditions in agricultural wetlands adjacent to a creek and river. Annual average rainfall is 1240 mm per year, distributed relatively uniformly through the year, and the mean annual temperature is 16.5°C (Cowell 1998). Iron Horse Farm received 1344 mm of rain and the average temperature was 18 °C.

The portion of the farm studied has two hillslopes, one to the north and a second to the south and east (Figure 3.2). The hill top consists of a large row-crop field and a complex of farm buildings. In 2016, the crop in the field was soybean, with some plots of cotton, and in 2017, it was planted with corn. The slope to the north passes through grassy pasturelands, which historically were used as a cattle feedlot. However, the grassland no longer is used for livestock, and beyond mowing was minimally managed over the study period. The base of this hillslope discharges onto the floodplain of Rose Creek, via drainways and toeslope seeps. The floodplain contains an extensive alluvial swamp (17 ha), with two large beaver wetlands, named

the upper and lower alluvial swamps (Figure 3.2). The floodplain and its wetlands discharge into Rose Creek, which flows into the Oconee River.

The slope to the south and east consists of row-crop fields (soybean in 2016 and corn in 2017) that discharges onto the floodplain of the Oconee River through drainways and groundwater seeps, primarily into a 4 ha depressional wetland (Figure 3.2). The rest of the floodplain supports row-crop agriculture, with the crop matching those planted in the upland field. The water from the depressional wetland discharges towards the Oconee River through a maintained agricultural ditch. Row-crop agriculture and ditching of this floodplain may have been initiated as early as 1875, and has been maintained continually since that time (Coulter 1964). The history of fertilization and manure application is not documented, but the study site has been managed for crop and livestock production for over 100 years.

In 2016, potassium and phosphorus were added before soybean planting in April. Hill top cotton plots were planted in May. In 2017, glyphosate herbicide was applied to the row-crop fields in March, followed by potassium and phosphorus applications in April. Corn was planted in April, lime was added in April, and liquid nitrogen fertilizer in May.

The Oconee National Forest, across the Oconee River from the Iron Horse farm, served as a natural forested (pine) reference hillslope for this study. For this, a 0.80 hectare reference swamp, located along Sandy Creek, 2.5 km from the Oconee River was used. Like the agricultural landscapes, we sampled along the upland toeslope seep, the floodplain wetland, and the creek.

This farm setting allowed us to monitor soil conditions and water movements from upland agricultural lands (pasture to the north, and row-crops to the south and east), to discharge drainways and seeps onto the floodplain (alluvial beaver swamp to the north and a depressional wetland, floodplain agricultural field and ditch network to the south and east), and finally into receiving waterways (Rose Creek and the Oconee River to the north, and the Oconee River to the south and east). Further, by sampling in Rose Creek and the Oconee River, above, at, and below the farm, we could further assess the impacts of the farm on water quality. Finally, the existence of a natural reference area adjacent to the farm, the Sandy Creek watershed in the Oconee National Forest, allowed us to frame patterns in the agricultural influenced areas to a comparable natural area.

### *Soils*

A soil map of Iron Horse Farm was previously created for farm management purposes (Iron Horse Plant Sciences Farm 2014), and Oconee National Forest soils were identified using the NRCS Web Soil Survey. The alluvial swamp consisted of Toccoa, Cartecay, Roanoke, and State soil series, all alluvial entisols and inceptisols. The ditched wetland swale soils included Cartecay, Roanoke, and Dogue series. The retired feedlot soils include a mix of upland and alluvial soils including the Masada, Wickham, Hiwassee, and Starr series. Cropped fields also included both upland and alluvial soils including Masada, Madison, Hiwassee, Wickham, Starr, Buncombe, Dogue, Riverview, Congaree, and Toccoa. The upland soils were Ultisols characterized by sandy loam or loamy sand topsoils and a sandy clay loam to clay loam argillic

layer. The reference swamp included Chewacla and Congaree floodplain soils, while the hillslope soils were Cecils.

#### *Soil Sampling and Analysis*

Composite soil sampling within each major soil/land cover combination at Iron Horse Farm took place during the summer and fall of 2017. Within each polygon of similar soils and farm use, soil plugs were taken by walking transects of the sample area. Plugs were mixed in a bucket and a homogenized sub-sample (fill line of soil sample bag) was taken from the bucket. A total of 242 plugs were taken and stratified into 19 samples (Appendix 3.1, Figure 3.2), based on soil types within the forested alluvial swamp, the retired feedlot, the upper row-crop field, the ditched wetland swale, the lower floodplain row-crop field, and the reference forest and swamp (Appendix 3.1, Figure 3.1.2). Soil samples were analyzed for phosphorus, ammonium, and nitrate (UGA Extension 2016). To assess variation within sample units, distributions of nutrients across previously grouped landscapes and soil types were categorized into high, medium and low levels, with respect to phosphorus, nitrate, and ammonium. Individual soil cores were then taken from across selected locations to be analyzed for their respective nutrient contents (Appendix 3.1, Figure 3.1.3), and provide a measure of natural variation within soil type categories.

#### *Shallow Groundwater and Surface Water Sampling and Analyses*

Shallow piezometers were installed on toeslopes, within the ditch system, within the alluvial swamp complex, and within the reference wetland system. Groundwater samples were collected from 24 piezometers in the agricultural areas, and 3 in the forested reference swamp;

stratified to sample drainways and seep discharge off of uplands, and movements across the Rose Creek alluvial swamp, the Oconee River floodplain depression wetland swale and ditch complex, and the reference Sandy Creek floodplain (Figure 3.2). Piezometers were installed after visual inspection of the field site, in low spots on the landscape and where groundwater was visibly seen daylighting from a hillslope. Samples were collected bimonthly in 125 ml HDPE Polyethylene bottles from 75 cm or 1.5 m deep piezometers. Piezometers were made of PVC pipe; the lower section was cut into a screen and a fabric sock was placed over it to prevent sediment from entering the piezometer. Piezometers were installed using bucket augers that bored 8 cm diameter holes, into which the piezometer was dropped. To anchor the piezometer, sand was poured into the augured hole until the PVC pipe was stabilized. Bentonite was used as a cap to hold the piezometer and sand in place, and reduced direct surface water infiltration. A few piezometers were initially disturbed by feral pig activity, and these were cut so less than a meter of pipe remained above the soil surface, which solved that problem.

Piezometers were purged prior to sampling to reduce exposure of the sample to the atmosphere. Purging took place 24 hours before sampling using 1 l Teflon bailers. A 200 ml ECOPVC-73 bailer was used to collect a 125 ml sample. Collection sequences included three rinses of the bailer and bottle with water from the piezometer, prior to filling the sample bottle. Once groundwater samples were extracted, they were placed on ice or icepacks until delivery to the analytical laboratory (within 24 hrs). Basic water quality was characterized using a YSI Professional Plus Quatro Cable (YSI), which includes sensors for oxidation reduction potential

(ORP) (mV), dissolved oxygen (DO) (% and mg/l), potential of hydrogen (pH), temperature (°C), and specific conductivity ( $\mu\text{S}/\text{cm}$ ). Depth was also recorded for each measurement.

Odyssey Capacitance Water Level Loggers were installed on 6 piezometers at Iron Horse Farm and 1 piezometer at the Oconee National Forest. Each Odyssey was calibrated in the lab and programmed to collect data at hourly intervals, data was downloaded at each sampling event.

Surface water was collected bimonthly from 25 surface water and river/creek sites, with an additional 2 in the forested reference swamp and creek (Figure 3.2), situated from the upland edges, across the floodplains, and to the respective receiving waterbodies. Surface water samples were chosen after visual inspection of the field site, based on low spots on the landscape and the proximity of installed piezometers. Collection bottles were rinsed three times in the sample water before being filled to minimize contamination. An extendible sampling arm was used to place the bottle at the surface of the water and allow water to trickle in. After samples were taken, they were then placed on ice until delivery to the analytical laboratory. The YSI probe was then submerged in the waterbody for water quality, and depth was recorded with a staff gauge.

All samples collected from piezometers and surface water sites were sent to the UGA Agricultural & Environmental Services Laboratory-Soil, Plant and Water Lab (*UGACAES*). Samples were analyzed for total phosphorus and nitrate + nitrite ( $\text{NO}_3+\text{NO}_2=\text{N}$ ) (UGA Extension 2016). Water samples were kept at 4°C until analysis could take place the following day (UGA Extension 2016). Cadmium reduction coils in a Perstorp Analytical Enviroflow Continuous Flow

Analyzer were used to analyze samples colorimetrically for  $\text{NO}_3+\text{NO}_2=\text{N}$ . To assess water samples for TP, a Spectro Arcos FHE was used, which runs Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES) for metals, chemicals, and other materials. Before analysis, samples were filtered only if sediment was visible in the sample.

Samples in August 2017 were analyzed for ammonium, nitrate, and total nitrogen at the UGA Stable Isotope Ecology Laboratory (UGAIEL). Chemistry samples were separated into three separate analysis bottles. One was unfiltered and analyzed for total nitrogen, another was filtered and measured for nitrate and ammonium, the third was placed in an amber bottle, 2 drops of hydrochloric acid (HCl) were added, and the sample was analyzed for dissolved organic carbon (DOC). Filtered, unfiltered, and preserved samples were analyzed for nitrate, ammonium, and total nitrogen using colorimetry; while DOC was analyzed using a Shimadzu TOC-5000A Total Organic Carbon Analyzer and Shimadzu TOC-Vcsh.

#### *Oconee River Survey*

Two shoreline-based water-quality surveys were conducted on the reach of the Oconee River adjacent to Iron Horse Farm using the YSI probe. Additionally, water quality in the Oconee River was assessed by canoe from Barnett Shoals Dam in Oconee County, 15 km upstream of the farm, to a site about 1 km downstream from the farm, a stretch totaling about 20 km. Water measurements and samples were collected with the YSI from 8 sample locations in the river, 5 upstream of the farm, 2 adjacent to the farm, and 1 downstream of the farm. YSI measurements were taken along each bank and the middle of the river, and water samples from the middle of the river channel were collected, as above, in bottles for laboratory analyses

(Appendix 3.3, Figure 3.3.1). Historic and extant nutrient data in the Oconee River, at the United States Geological Survey (USGS) Penfield River Gauge (02218300) immediately downstream from the Iron Horse Farm study area, were downloaded as a reference.

#### *Storm Sampling and Continuous Logging*

Sampling during an active storm event took place during April of 2017. Eight samples were collected from areas receiving runoff from uplands, including the Rose Creek forested alluvial swamp toeslope, the ditched depressional wetland on the Oconee River floodplain, and the Oconee National Forest reference swamp. Besides this event, storms coincided with routine monthly samplings in May and June 2017, providing additional storm related data.

#### *Statistics*

The study design permitted an analyses of soil conditions and water quality from uplands to wetlands to receiving water bodies across three distinct and independent hillslopes: 1) from a row-crop upland, pastureland and former feedlot, to the alluvial forested swamp and beaver wetlands of Rose Creek, to Rose Creek itself; 2) from a row-crop agriculture upland, to the cultivated-depressional wetland-ditch complex floodplain of the Oconee River, to the Oconee River itself; and 3) from a natural forested upland, to a natural floodplain, to the largely un-impacted Sandy Creek reference area. Further we could assess water quality in the Oconee River, above, at, and below the farm, to potentially detect the influence of the farm on the river. Data were tested for normality of variances using a Bartlett test, followed by a Shapiro Test on the normality of the residuals. One-way ANOVA with a block for water type was run, followed by a post hoc Tukey Test on data which meets the assumptions of ANOVA (Dowdy et

al. 2003; Dalgaard 2008). If data did not meet assumptions of ANOVA, a non-parametric Kruskal-Wallis test was used. Following a Kruskal-Wallis test on non-parametric data, a Dunn test was run in the place of Tukey. Individual surface water and groundwater samples at Iron Horse Farm were grouped into landscapes for assessment (*white lines of Figures 3.6, and 3.7*). Correlations between water quality and nutrients were assessed, correlation bins include r values from +-1 and +-0.5 indicating a strong relationship, medium between +-0.49 and +-0.3, and weak between +-0.29 to +-0.1.

## Results

### *North Slope*

The northern gradient of the study site incorporated the upper row-crop field, retired feedlot, alluvial swamp toeslope, upper alluvial swamp, beaver dam, lower alluvial swamp, and Rose Creek (Figure 3.2, 3.6, and 3.7). Soil nitrate concentrations within the northern portion of the farmed area varied from 6.99 to 0.36 mg/Kg. The highest and lowest nitrate concentration on the north side of the farm came from soil in the alluvial swamp toeslope. Nitrate levels in the soil do not significantly change from the upper row-crop field, median values include 4.67 mg/kg, to the retired feedlot at 3.14 mg/kg (Figure 3.4, Figure 3.6, and appendix 3.1). Nitrate in the soil is also not significantly different through the alluvial swamp, the toeslope median value was 1.18 mg/kg while soil in the body of the alluvial swamp was 2.78 mg/kg. Nitrate levels in groundwater (GW) and surface water (SW) entering the alluvial swamp, and water within the alluvial swamp did show significant differences ( $\chi^2(13) = 128.37, P < 0.001$ ). The highest nitrate levels in the water on the north side of the farm included the toeslope of the alluvial swamp at

39.13 mg/l, and the lowest were in the body of the alluvial swamp at 0.10 mg/l (the limit of quantification (LOQ) for nitrate, LOQ was 0.04 for samples analyzed at the UGACAES and 0.01 for August sampling analyzed at UGAIEL. Median levels of nitrate in water entering the forested alluvial swamp at the toeslope was 5.71 GW/1.94 SW mg/l, and two piezometers averaged 28.59 mg/l and 32.27 mg/l over the sampling period. Median values in surface water and groundwater in the alluvial swamp were 0.01 GW/0.18 SW mg/l in the upper alluvial swamp, 0.24 GW/0.04 SW mg/l at the beaver dam, and 0.09 GW/0.24 SW mg/l in the lower alluvial swamp. Rose Creeks median was 0.82 mg/l of nitrate over the sampling period (Figure 3.5, Figure 3.6, and appendix 3.2).

Certain water quality measurements can be used to infer nitrate and phosphorus loadings in place of somewhat expensive nutrient analysis. Figure 3.9 shows correlations between physical parameters at Iron Horse Farm and water quality measurements, Figure 3.8 shows water quality down the same gradients used for nutrients analysis.

Significant differences were not detected between landscapes assessed for ammonium in the soil. Ammonium varied from 42.89 mg/kg in the forested alluvial swamp to 1.41 mg/kg in the upper row-crop field. Median ammonium in the upper row-crop field was 2.10 mg/kg, and 5.95 mg/kg in the retired feedlot. Median levels of ammonium in soils of the alluvial swamp toeslope were 6.23 mg/kg, 12.63 mg/kg in the upper alluvial swamp, 18.76 mg/kg at the beaver dam, and 26.69 mg/kg in the soil of the lower alluvial swamp (Figure 3.4, and appendix 3.1). Ammonium accumulation in the soil of the forested alluvial swamp is apparent. Ammonium levels in groundwater and surface water entering the alluvial swamp, and water

within the alluvial swamp, did not show significant differences. Water samples down the northern gradient were only analyzed for ammonium during the sampling in August, and only in shallow groundwater. The highest level was in the upper alluvial swamp at 17.98 mg/l, and the lowest at the alluvial swamp beaver dam and toeslope at 0.02 mg/l (LOQ for ammonium). Median levels of ammonium in water entering the alluvial swamp was 0.02 mg/l, and increased to 11.80 mg/l in the upper alluvial swamp, 0.74 mg/l at the beaver dam, and 1.15 mg/l in the lower alluvial swamp (Appendix 3.2, Figure 3.2.4).

Phosphorus analyzed from soil samples yielded significant differences between landscapes ( $\chi^2(6) = 32.87, P < 0.001$ ). Phosphorus varied from 746.85 mg/kg in the retired feedlot, to 2.07 mg/kg at the toeslope of the forested alluvial swamp. Statistical differences exist between phosphorus in the soil of the alluvial swamp toeslope, and the retired feedlot. Median phosphorus levels in the soil of the upper row-crop field were 54.10 mg/kg, and 396.30 mg/kg in the retired feedlot. Median soil values in the toeslope of the alluvial swamp were 4.44 mg/kg, and 14.10 mg/kg in the body of the alluvial swamp (Figure 3.4, Figure 3.7, and appendix 3.1). Phosphorus levels in surface water and groundwater entering the forested alluvial swamp are significantly different than water from samples in the body of the forested alluvial swamp ( $\chi^2(13) = 54.13, P < 0.001$ ). The highest phosphorus in the water on the north side of the farm was taken from the alluvial swamp toeslope at 1838.00  $\mu\text{g/l}$ , and the lowest located in the body of the alluvial swamp at 10.00  $\mu\text{g/l}$  (LOQ for phosphorus). Median levels of phosphorus entering the alluvial swamp in water were 10.00 GW/1820.00 SW  $\mu\text{g/l}$ , 42.00 GW/ 125.00 SW

µg/l in the upper alluvial swamp, 10.00 GW/129.00 µg/l on the beaver dam, and 10.00 GW/94.00 SW µg/l in the lower alluvial swamp (Figure 3.5, Figure 3.7, and appendix 3.2).

### *South Slope*

The southern hillslope gradient included the upper row-crop field, the lower row-crop field, the groundwater seep, depressional wetland, center ditch, west ditch, combined ditch, and the Oconee River (Figure 3.2). Nitrate in the soil across the southern slope varies between 15.07 mg/kg in the groundwater seep, to 6.50 mg/kg in the upper row-crop field. Nitrate levels in the soil do not significantly change from the upper row-crop field which showed a median of 4.67 mg/kg, to the depressional wetland swale and ditches at 6.92 mg/kg, and lower row-crop field median at 7.21 mg/kg (Figure 3.4, Figure 3.6, and appendix 3.1). Nitrate levels in groundwater and surface water entering the ditched wetland swale, and water within the depressional wetland and ditches did not show significant differences. The highest nitrate levels in the water on the south side of the farm included the groundwater seep at 14.82 mg/l, and the lowest were in the center ditch at 0.01 mg/l. Nitrate median levels in water entering the ditched wetland swale were 1.47 GW/0.08 SW mg/l in the toeslope, 1.65 SW mg/l in the depression, and 1.34 GW/1.26 SW mg/l, 1.91 GW/2.88 SW mg/l, and 0.40 GW/0.81 SW mg/l in the ditch complex. The Oconee River median value was 1.02 mg/l of nitrate over the sampling period (Figure 3.5, Figure 3.6, and appendix 3.2).

Significant differences in ammonium levels were not observed in the soil on the south side of the farm. Ammonium in soil varied from 8.34 mg/kg in the ditched wetland seep to 1.41 mg/kg in the upper row-crop field; and medians were 2.12 mg/kg in the upper row-crop field,

7.72 mg/kg in the ditched wetland seep, and 3.28 mg/kg in the lower row-crop field (Figure 3.4 and appendix 3.1). Ammonium levels in groundwater and surface water entering the ditched wetland swale, and water in the depression and ditched complex did not show significant differences. Water samples on the south gradient were only analyzed for ammonium during sampling in August, and only from shallow groundwater. Ammonium in water peaked in the groundwater seep at 19.73 mg/l, and was the lowest in the center ditch at 0.02 mg/l. Median ammonium in groundwater entering the ditched wetland was 3.24 mg/l, and reduced to 0.06 mg/l in the center ditch, 0.19 mg/l in the west ditch, and 4.29 mg/l in the combined ditch (Appendix 3.2, Figure 3.2.4).

Between phosphorus in soil, significant differences do not exist along the southern gradient of the study site. Phosphorus varied from 95.24 mg/kg in the upper row-crop field, to 7.08 mg/kg in the lower row-crop field. Median phosphorus levels in the soil of the upper row-crop field were 54.10 mg/kg, 15.45 mg/kg in the ditched wetland swale, and 17.08 mg/kg in the lower row-crop field (Figure 3.4, Figure 3.7, and appendix 3.1). Phosphorus levels in surface water and groundwater entering the ditched wetland swale are significantly different than water from samples in the depressional wetland and ditch complex ( $\chi^2(13) = 54.13, P < 0.001$ ). The maximum phosphorus value in water on the south side of the farm was in the combined ditch at 1326.00  $\mu\text{g/l}$  and the lowest at 10.00  $\mu\text{g/l}$ , found everywhere at some point in the sampling period except the depressional wetland. Median levels of phosphorus entering the ditched wetland swale at the groundwater seep were 41.00 GW/138.00 SW  $\mu\text{g/l}$  and increased to 332.00 SW  $\mu\text{g/l}$  in the depressional wetland, 30.50 GW/64.00 SW  $\mu\text{g/l}$  in the center ditch,

10.00 GW/16.00 SW  $\mu\text{g/l}$  in the west ditch, and 10.00 GW/38.00 SW  $\mu\text{g/l}$  in the combined ditch. The median in the Oconee River was 30.00  $\mu\text{g/l}$  of phosphorus in its water over the sampling period (Figure 3.5, Figure 3.7, and appendix 3.2).

### *Reference Slope*

The reference hillslope in the Oconee National Forest consisted of a toeslope, swamp area, and Sandy Creek. Nitrate in the soil across the reference gradient varies between 6.39 mg/kg in the reference swamp, to 0.53 mg/kg on the reference hillslope. Nitrate levels within the soil at the reference site do not significantly change from the reference hillslope at a median of 0.62 mg/kg, to the reference swamp at 3.78 mg/kg (Figure 3.4, and appendix 3.1). Nitrate levels in groundwater and surface water entering the reference swamp at the toeslope, and water within the reference swamp did not show significant differences. The highest nitrate levels in the water along the reference gradient was found in Sandy Creek 0.83 mg/l, and the lowest were in the toeslope and swamp area at 0.01 mg/l. Median nitrate levels in water entering the reference swamp were 0.04 GW mg/l, and 0.04 GW/0.20 SW mg/l within the reference swamp. The median value of Sandy Creek was 0.25 mg/l of nitrate over the sampling period (Figure 3.5, and appendix 3.2).

Significant differences in ammonium levels were not observed in the soil along the reference gradient. Ammonium in soil varied from 5.01 mg/kg on the reference hillslope to 4.08 mg/kg in the reference swamp. Median soil ammonium levels were 4.82 mg/kg on the reference hillslope, and 4.48 mg/kg in the reference swamp (Figure 3.4 and appendix 3.1). Ammonium levels in groundwater entering the reference swamp, the reference swamp itself,

and Sandy Creek, did not show significant differences. Water samples at the reference site were only analyzed for ammonium during denitrification sampling in August, and only shallow groundwater was assessed. Ammonium in water peaked in the reference toeslope at 0.37 mg/l, and was also lowest in the toeslope at 0.07 mg/l. Median ammonium in groundwater entering the reference swamp at the toeslope was 0.22 mg/l and reduced to 0.18 mg/l in the body of the reference swamp complex (Appendix 3.2, Figure 3.2.4).

Between phosphorus levels in soil, significant differences do not exist along the reference gradient of the Oconee National Forest. Phosphorus varied from a maximum of 16.56 mg/kg on the reference sites hillslope, to a minimum of 2.38 mg/kg within the soil of the reference swamp. Median phosphorus values in the soil of the reference hillslope were 13.57 mg/kg, and reduced to 4.41 mg/kg in the reference swamp (Figure 3.4, and appendix 3.1). Phosphorus levels in surface water and groundwater at the toeslope and in the swamp of the reference site do not show significant differences. The maximum phosphorus value in water on the reference gradient was in Sandy Creek at 525.00 µg/l and the lowest at 10.00 µg/l, found in the reference swamp and toeslope at some point in the sampling period. Median values of phosphorus entering the reference site at the toeslope were 20.00 GW µg/l and decreased to 10.00 GW/44.00 SW µg/l in the reference swamp. The median value of Sandy Creek was 69.50 µg/l of phosphorus in its water over the sampling period (Figure 3.5, and appendix 3.2).

#### *Storm Events*

Phosphorus values by month at the study site differed significantly between those months sampled during storms and those during dry periods ( $\chi^2(7) = 43.20, P < 0.001$ ) (Figure

3.3, Figure 3.10, and Table 3.1). Nitrate also differed significantly between months sampled during storms and those during dry weather. But there were also significant differences in nitrate levels between two months where sampling took place during dry weather ( $\chi^2(8) = 46.94, P < 0.001$ ).

#### *Oconee River Experiment*

During wading and canoe surveys of the Oconee River, no significant differences were detected along the gradient of the Oconee River from Barnett Shoals Dam to Redland Sand. There was also no significant difference between water quality measurements taken on different banks of the Oconee River (Figure 3.11, and Appendix 3.3). Phosphorus levels are significantly lowered between data sampled at the USGS Penfield River Gauge and phosphorus measurements conducted during this study ( $\chi^2(2) = 16.17, P = 0.0003$ ) (Figure 3.12). Median values included 90.00  $\mu\text{g/l}$  in 1996, 110.00  $\mu\text{g/l}$  in 1999, and 22.00  $\mu\text{g/l}$  in 2016/2017.

### Discussion

#### *Nutrient processing in the forested alluvial swamp*

On the north side of the farm, soil nitrate concentrations in the former feedlot and pasture were high. Shallow groundwater and surface water nitrate concentrations at the toeslope on the edge of the wetland complex were also high. However, nitrate concentrations were very low within the wetland complex, while ammonium was quite high, indicating denitrification occurring within the wetland complex (Starr and Gillham; Vymazal 1995; Cey et al. 1999; Simon 2002; Boon et al. 2014; Funk and Wagnalls 2017) (Figure 3.5, and Figure 3.6, Appendix 3.2, Figure 3.2.4). Nitrate in surface waters diminished through the alluvial swamp

complex. In the upper swamp, surface water nitrate was highly variable, with some locations comparable to the farm toeslope, and some much lower, indicating variability in hydrologic pathways. Nitrate concentrations in the lower alluvial swamp were uniformly low.

Total phosphorus also diminished from the upper alluvial swamp through the alluvial swamp complex, but concentrations in the upper alluvial swamp were generally higher than those observed in the farm toeslope. Indicating that the farm was probably not the total phosphorus source for the upper portion of the swamp. Phosphorus concentrations in the feedlot soils were very high. Taken together, these data indicate that phosphorus mobilization from the former feedlot was low. Phosphorus is generally mobilized by surface runoff and soil erosion, while nitrate moves readily in groundwater.

#### *Nutrient processing in the ditched wetland swale*

Nitrate in the soil along the southern hillslope gradient indicates deposition of nitrate in the ditched wetland swale and lower row-crop field. Nitrate levels are lower in the upper row-crop field, so deposition of nitrate seems to be coming from other areas, or from previous potentially large nitrate additions to row-crop fields. Nitrate in the groundwater and surface water is reduced slightly after leaving the groundwater seep. But the ditches that drain the depression wetland do not further mitigate nitrate as the levels are not reduced significantly. Nitrate reduction would likely increase if the ditches draining the depression wetland were filled in. Restored hydrology would allow for the possibility of denitrification hotspots to return to the system. Ammonium in water is low in the ditches and depression, and higher in the groundwater seep, while soil ammonium in the ditched wetland swale is higher than the

surrounding row-crop fields. Ammonium accumulates in the soils except where water is typically present in the groundwater seep. Here ammonium is released into the water, which then flows out of the seep and is converted to nitrate in the depressional wetland and ditches.

The ditches are not mitigating phosphorus runoff as significant differences are only detected in the west ditch which receives surface water runoff directly from the upper row-crop field and groundwater upwelling as indicated by the fact that the piezometer in the ditch showed water levels higher than the ground surface (Figure 3.3, *West Ditch*). Phosphorus mitigation would likely increase if ditches draining the depression wetland were filled in. The increase in mitigation would likely come from keeping water on the site longer during storm events. Lower phosphorus during this study than in 1996/1999 in the Oconee River at the USGS Penfield Gauge are most likely a result of the location the sample was taken or possible pollutant improvements upstream (Figure 3.12, and Appendix 3.3). The USGS location is unknown, but our samples were taken in the Oconee River at the outflow of the ditched wetland swale.

### Conclusions

Elevated levels of nitrate at the toeslope of the forested alluvial swamp, and lower levels in the swamp itself, indicate a hotspot of denitrification. In the ditched wetland swale, nitrate is not mitigated, as levels do not statistically change from the groundwater seep to the end of the ditches.

The forested alluvial swamp is also efficient in mitigating phosphorus, the mechanism being less clear because of low phosphorus values in the soil. The ditched complex does not

reduce levels of phosphorus before runoff discharges into the Oconee River. A lot of the nitrate and phosphorus which releases from agricultural wetlands does so during storm events which produce enough precipitation to wash them in.

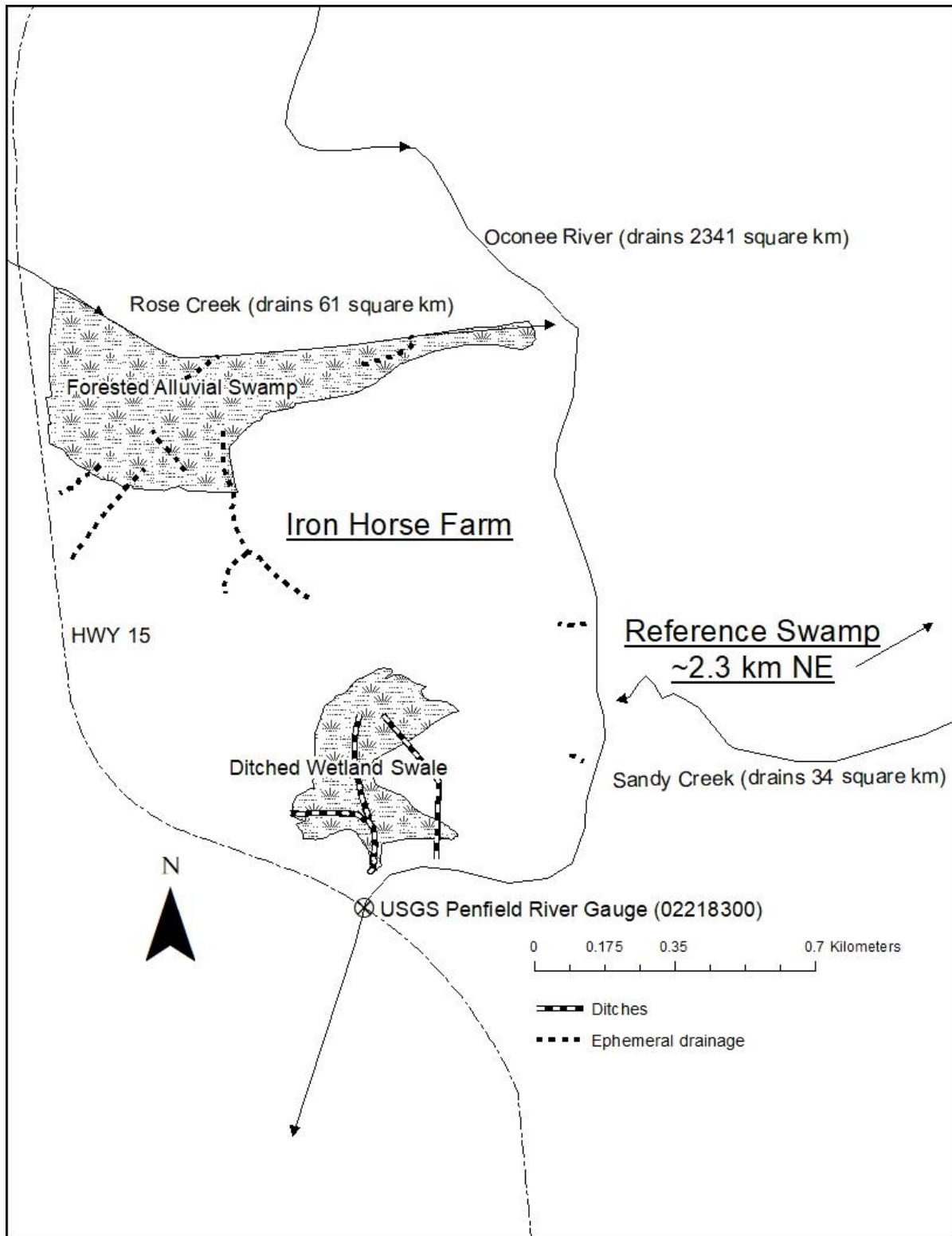


Figure 3.1: Iron Horse Farm and reference site landscape position along Highway 15, the Oconee River, and Rose Creek.

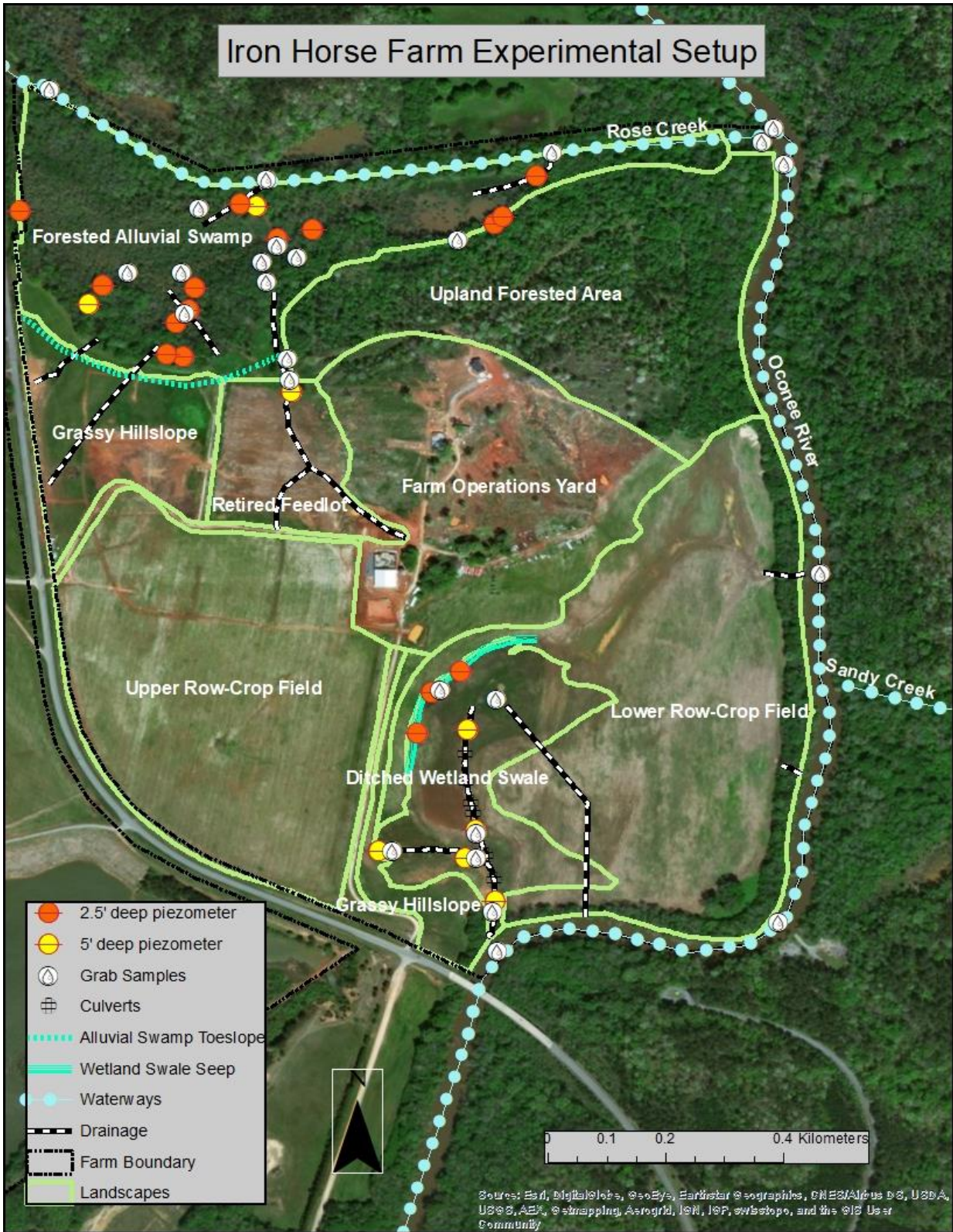


Figure 3.2: Experimental setup of Iron Horse Farm.

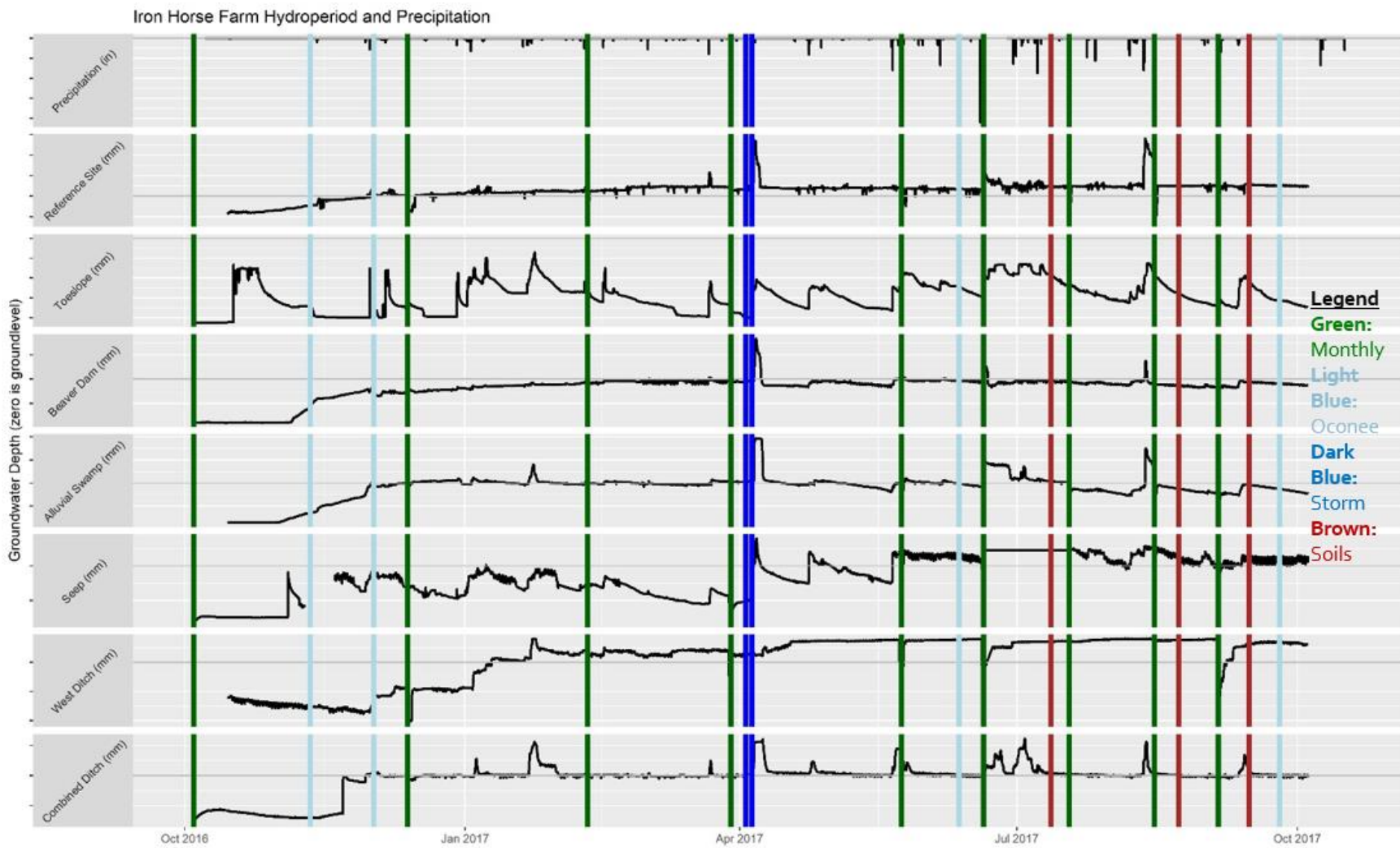


Figure 3.3: Iron Horse Farm hydroperiod and precipitation over the sampling period. Lines correspond to sampling events.

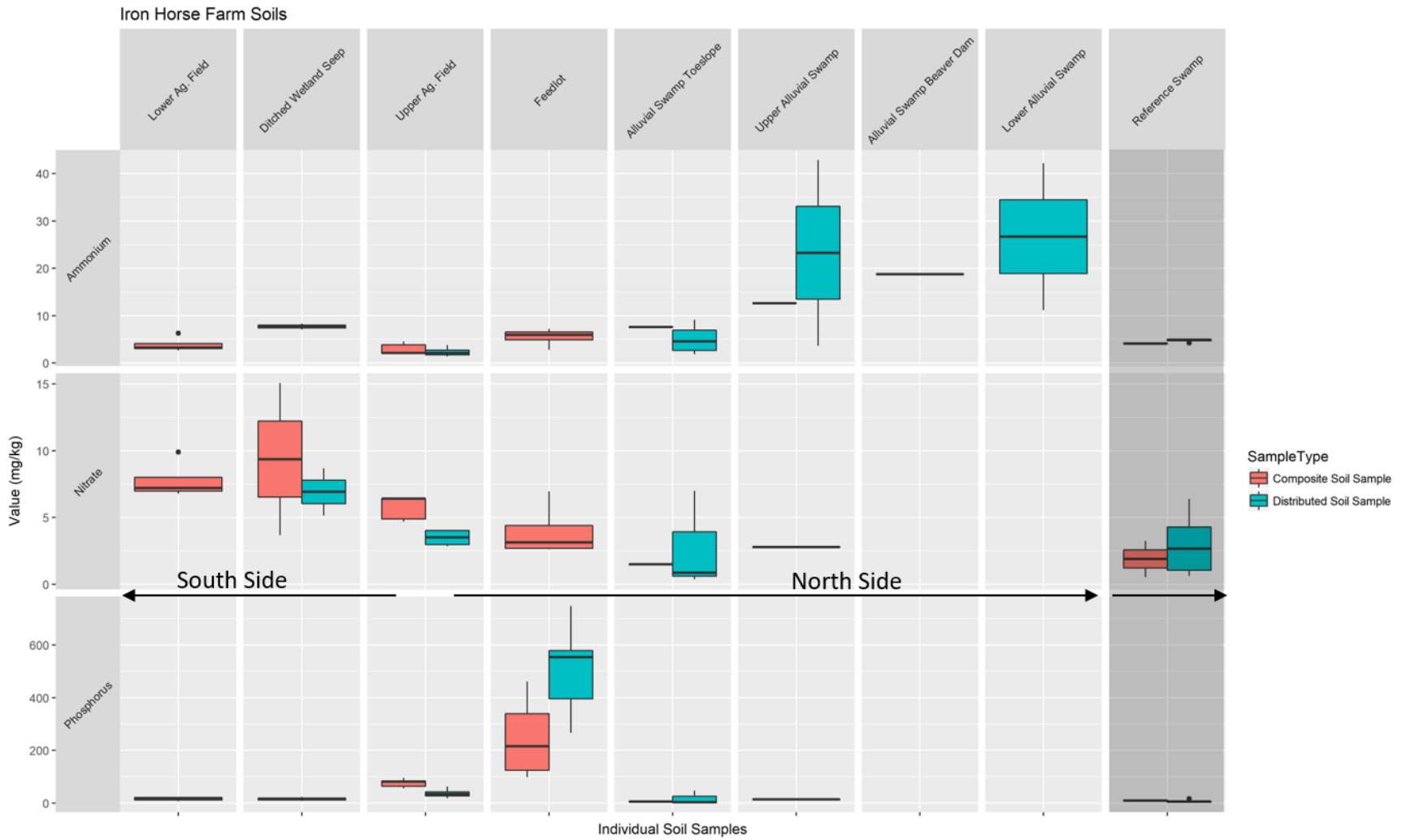


Figure 3.4: Soil nitrate, ammonium, and phosphorus between landscapes, arrows indicate the direction of elevation drop (slope position) on each side of the farm. Blank boxes indicate no sample taken.



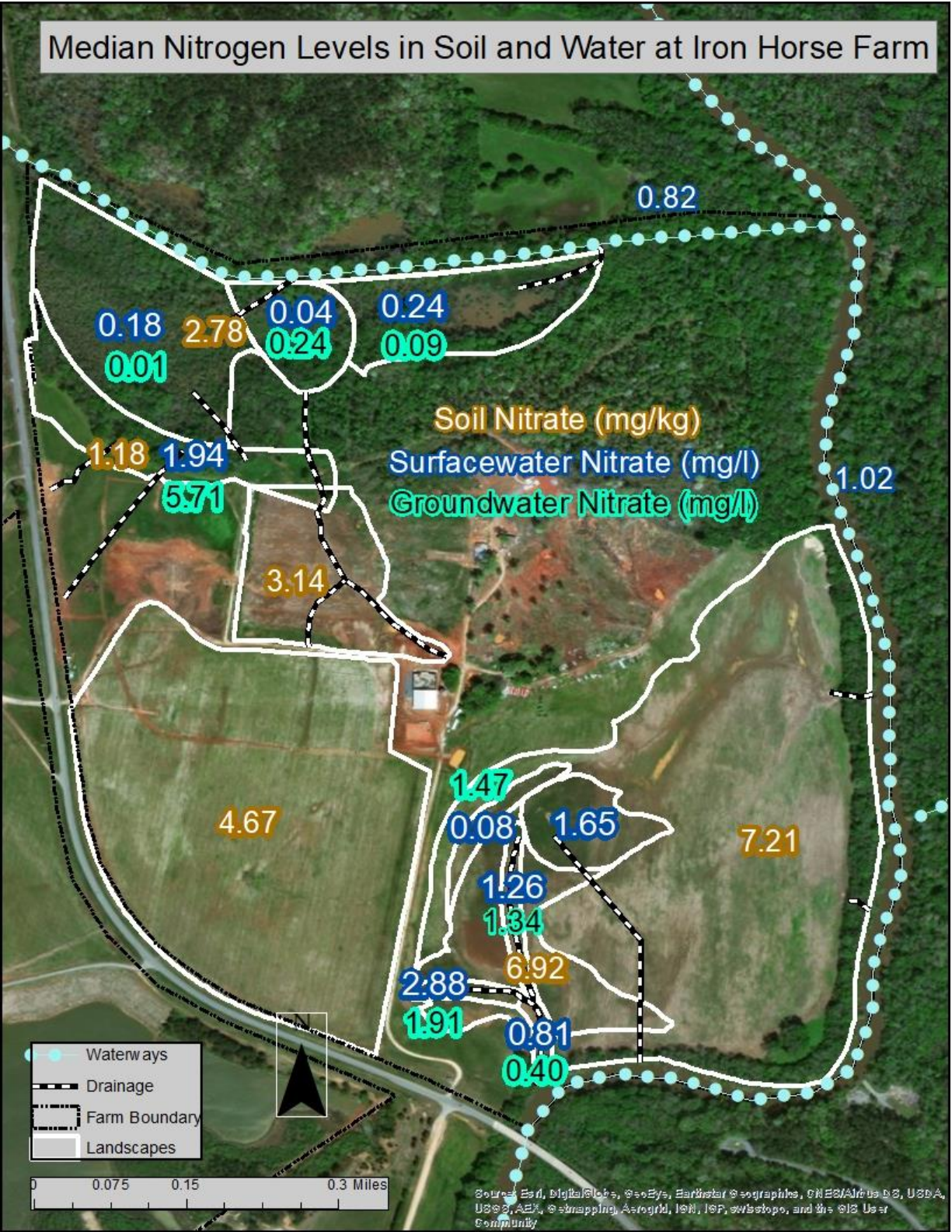


Figure 3.6: Map of nitrate median values in each of the studies landscape assessment areas. White lines indicate landscape portions compared using ANOVA/Kruskal-Wallis.

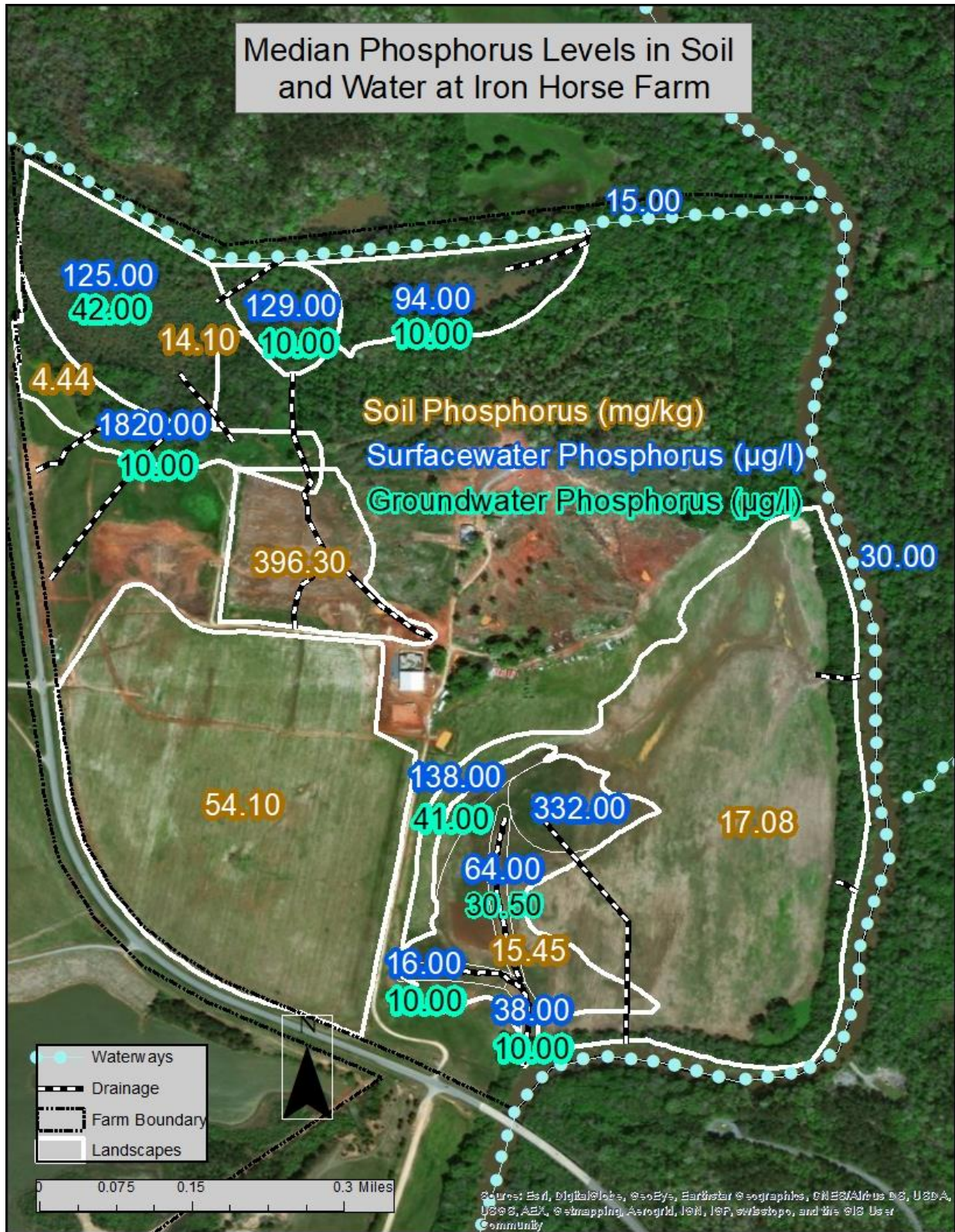


Figure 3.7: Map of phosphorus median values in each of the studies landscape assessment areas. White lines indicate landscape portions compared using ANOVA/Kruskal-Wallis.

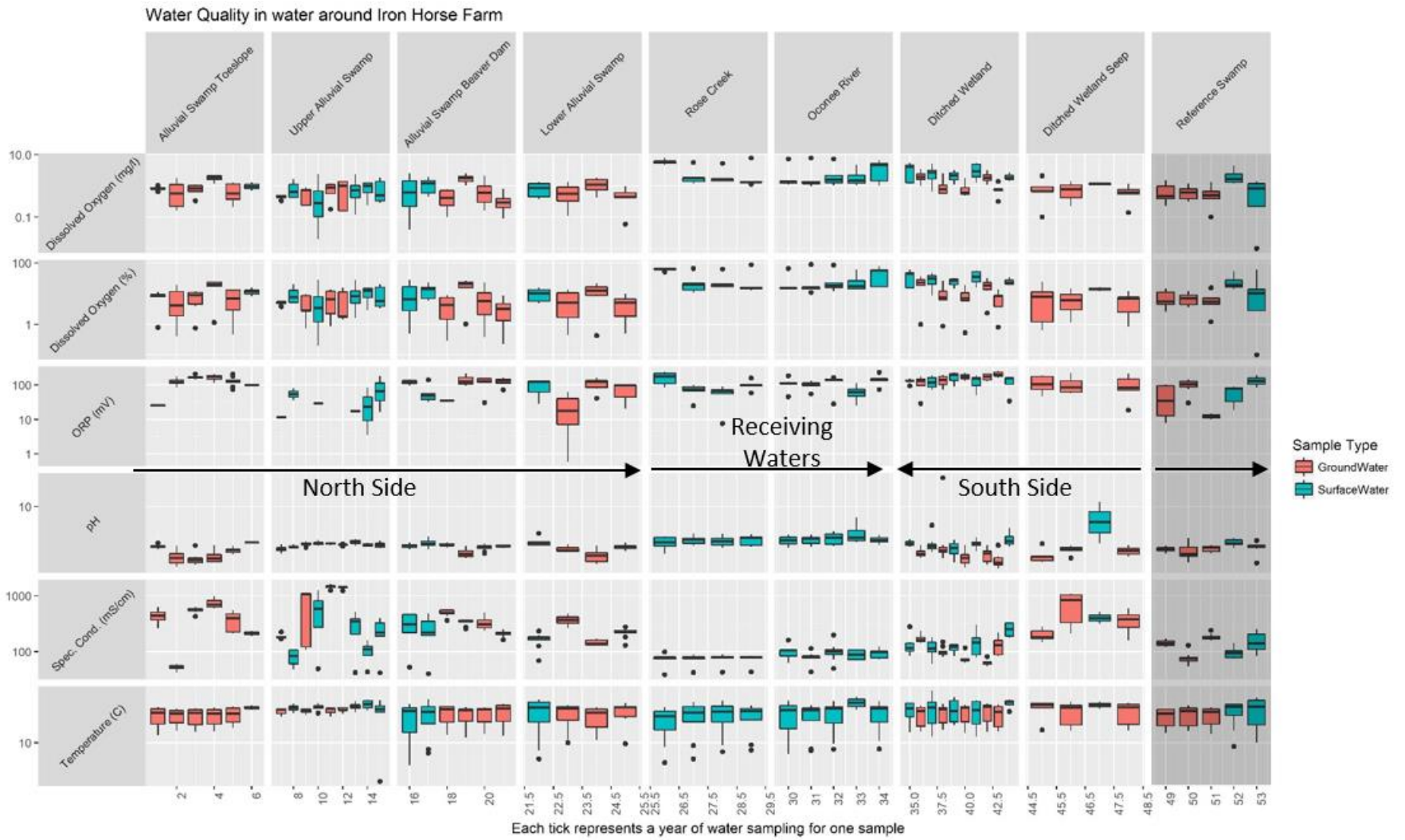


Figure 3.8: Water quality in surface water and groundwater, arrows point from upland landscapes to receiving waters. Distribution reflects monthly samples collected over a year.



Figure 3.9: Correlation matrix of water quality and nutrients, no strong correlations exist but specific conductivity and ORP are moderate positively related to nitrate + nitrite.

Table 3.1: Mean daily precipitation on days of purging wells (Purging) and bimonthly water sampling at Iron Horse farm (Sampling).

<b>Activity</b>	<b>Date</b>	<b>Precipitation (in)</b>
Sampling	10/3/2016	0
Purging	12/12/2016	0.02
Sampling	12/13/2016	0.26
Purging	2/9/2017	0.26
Sampling	2/10/2017	0
Purging	3/28/2017	0
Sampling	3/29/2017	0
Sampling	4/3/2017	0.64
Sampling	4/5/2017	2.57
Purging	5/23/2017	1.2
Sampling	5/24/2017	0.32
Purging	6/19/2017	2.11
Sampling	6/20/2017	1.63
Purging	7/17/2017	0
Sampling	7/18/2017	0
Purging	8/14/2017	0.17
Sampling	8/15/2017	0
Purging	9/4/2017	0
Sampling	9/5/2017	0.63

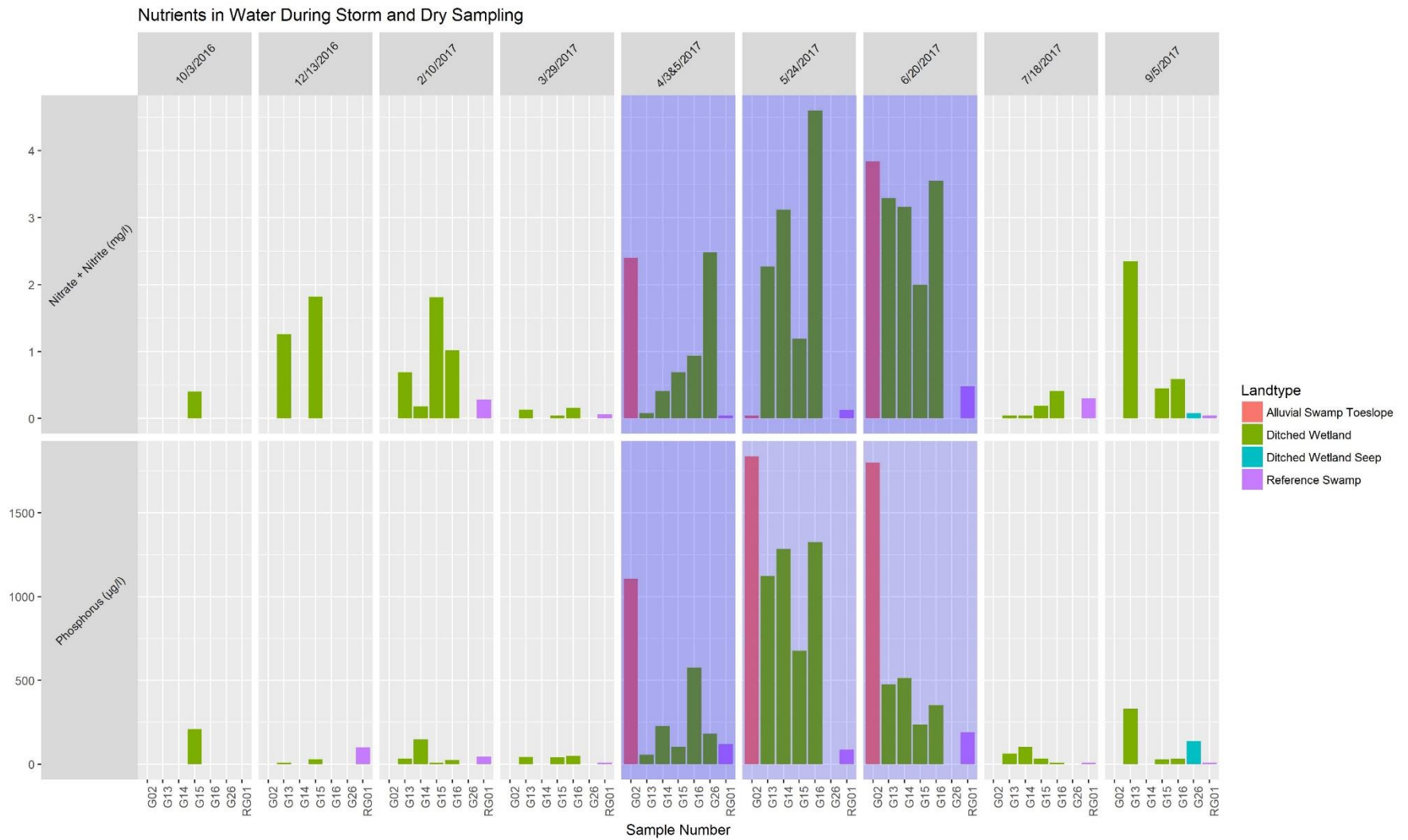


Figure 3.10: Nutrients in water by sampling event emphasizing storm events (blue shading), nutrients are elevated in sampling events taking place during storms. Sample sites selected by those collected during April storm sampling.

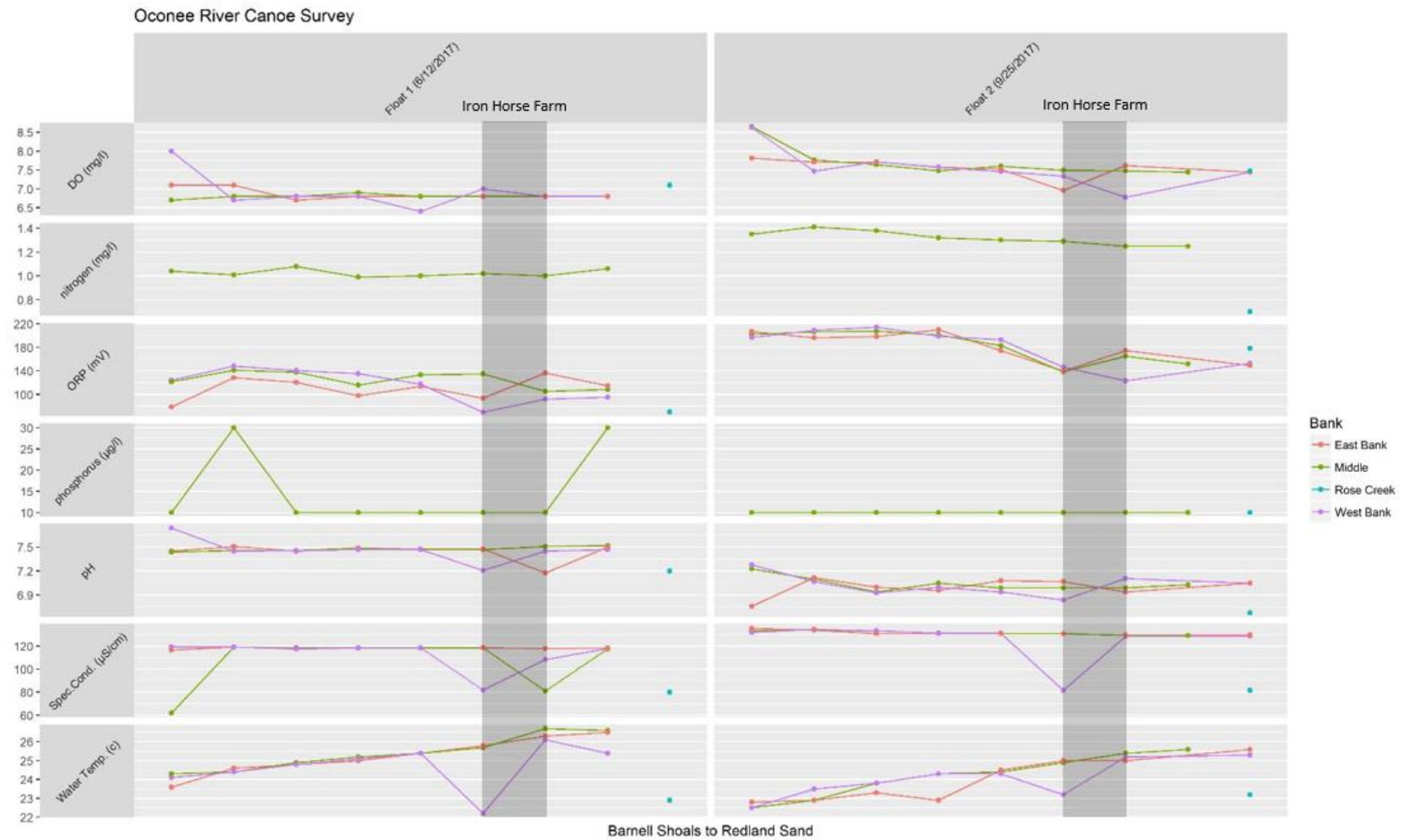


Figure 3.11: Longitudinal water quality variation in the Oconee River determined by sampling from a canoe with measurements taken every 4-5 km near the dam, 1-2 km in the middle of the reach, and 0.5 km near Iron Horse Farm. The study site did not influence water quality in the Oconee River. The limit of quantification (LOQ) for phosphorus is 10.00 µg/l, this value was reached in most samples down the Oconee River.

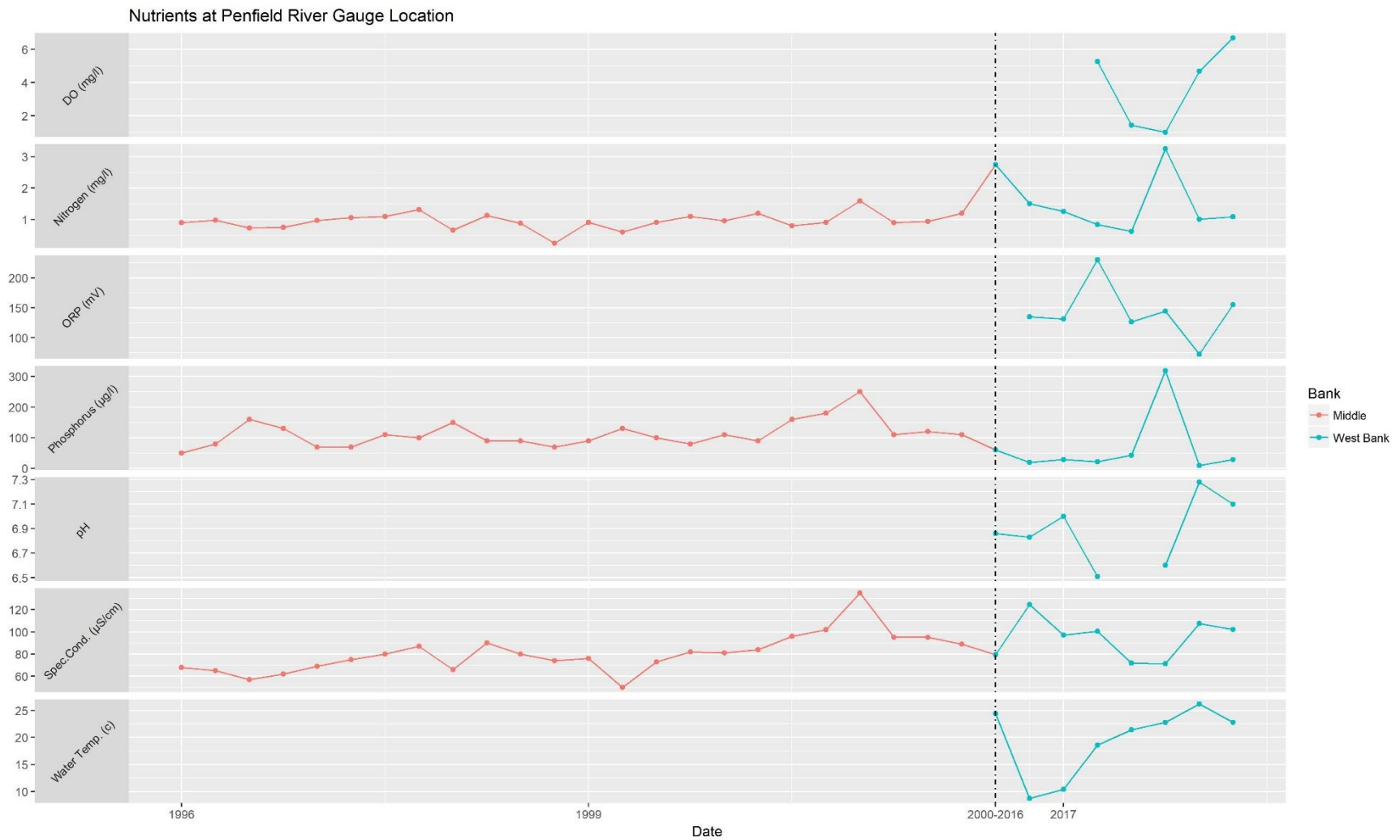


Figure 3.12: Oconee River data at the USGS Penfield River Gauge (02218300), the orange line is USGS data, while the blue line is data collected during this study.

Appendix 3.1

Iron Horse Farm Soil Nutrients

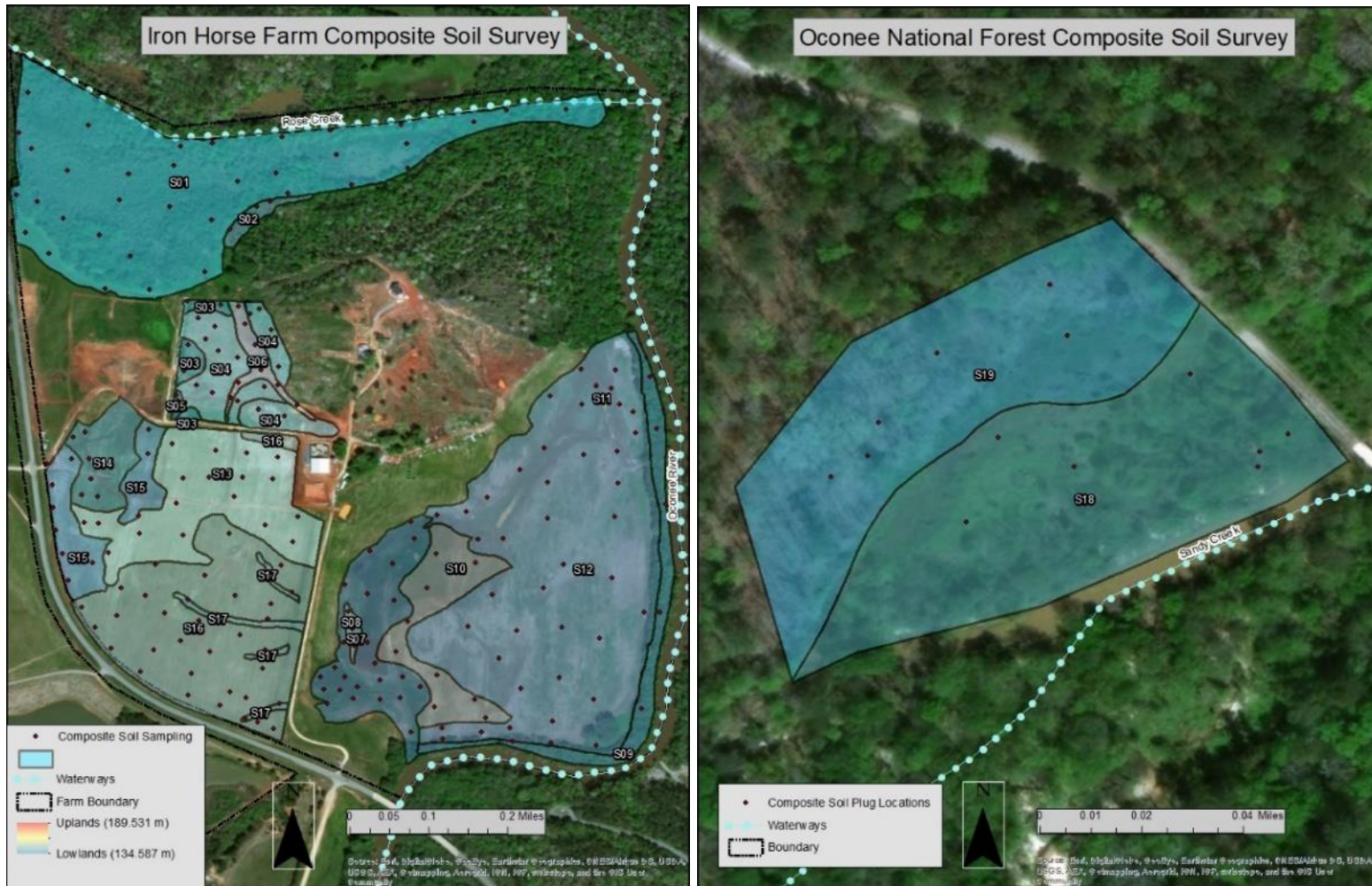


Figure 3.1.1: Composite soil sampling at Iron Horse farm and Oconee National Forest.

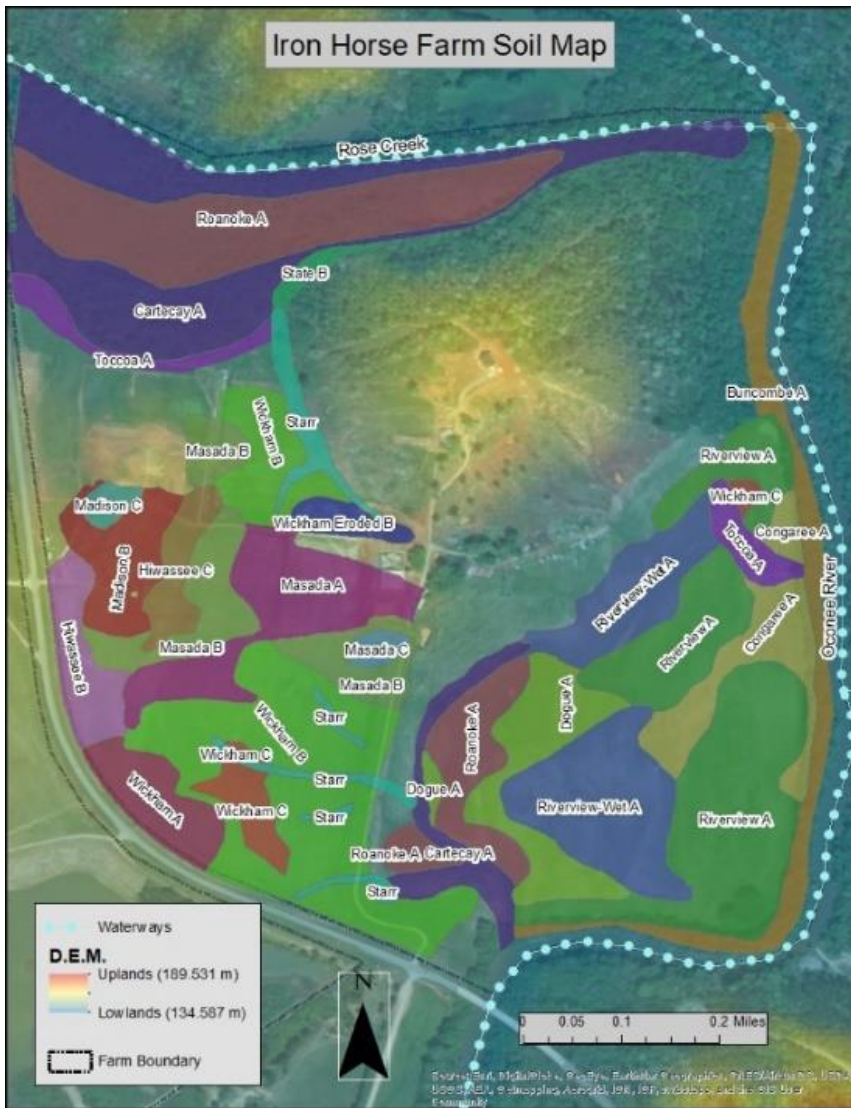


Figure 3.1.2: Soil map and digital elevation model of Iron Horse Farm assessed landscapes.

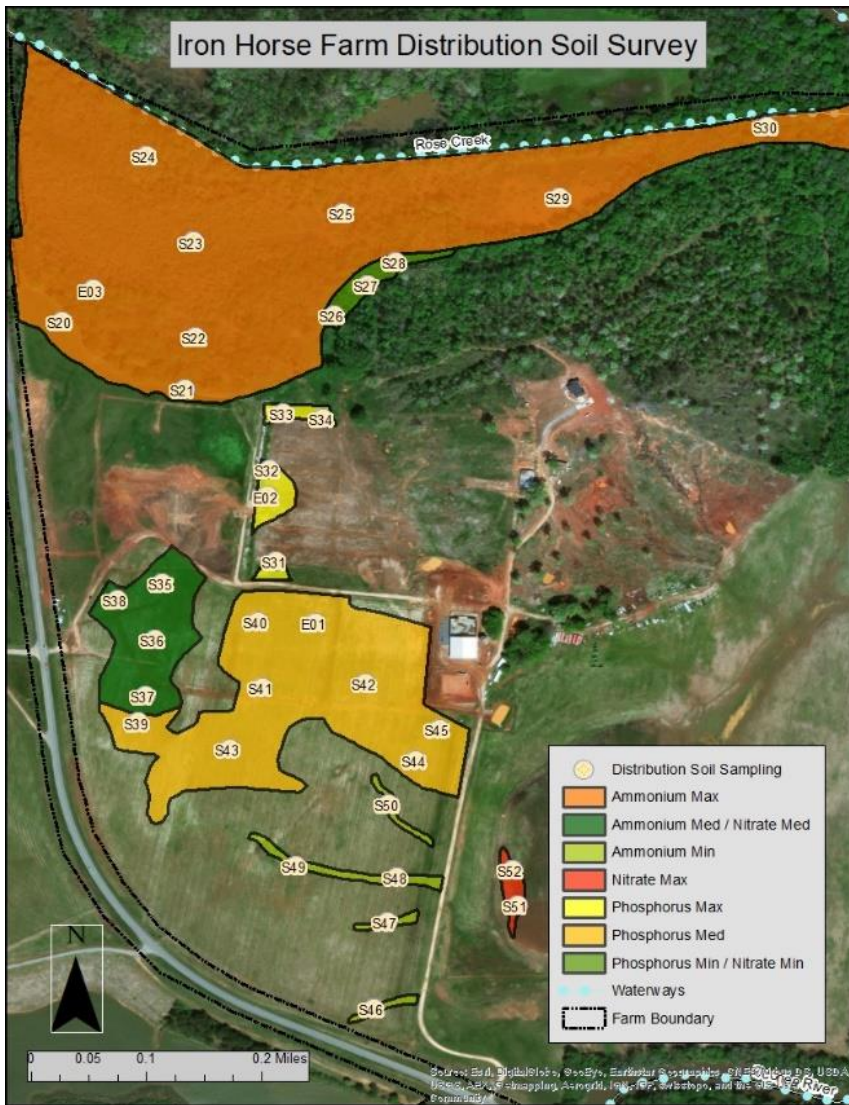


Figure 3.1.3: Distribution soil sampling at Iron Horse Farm and Oconee National Forest.

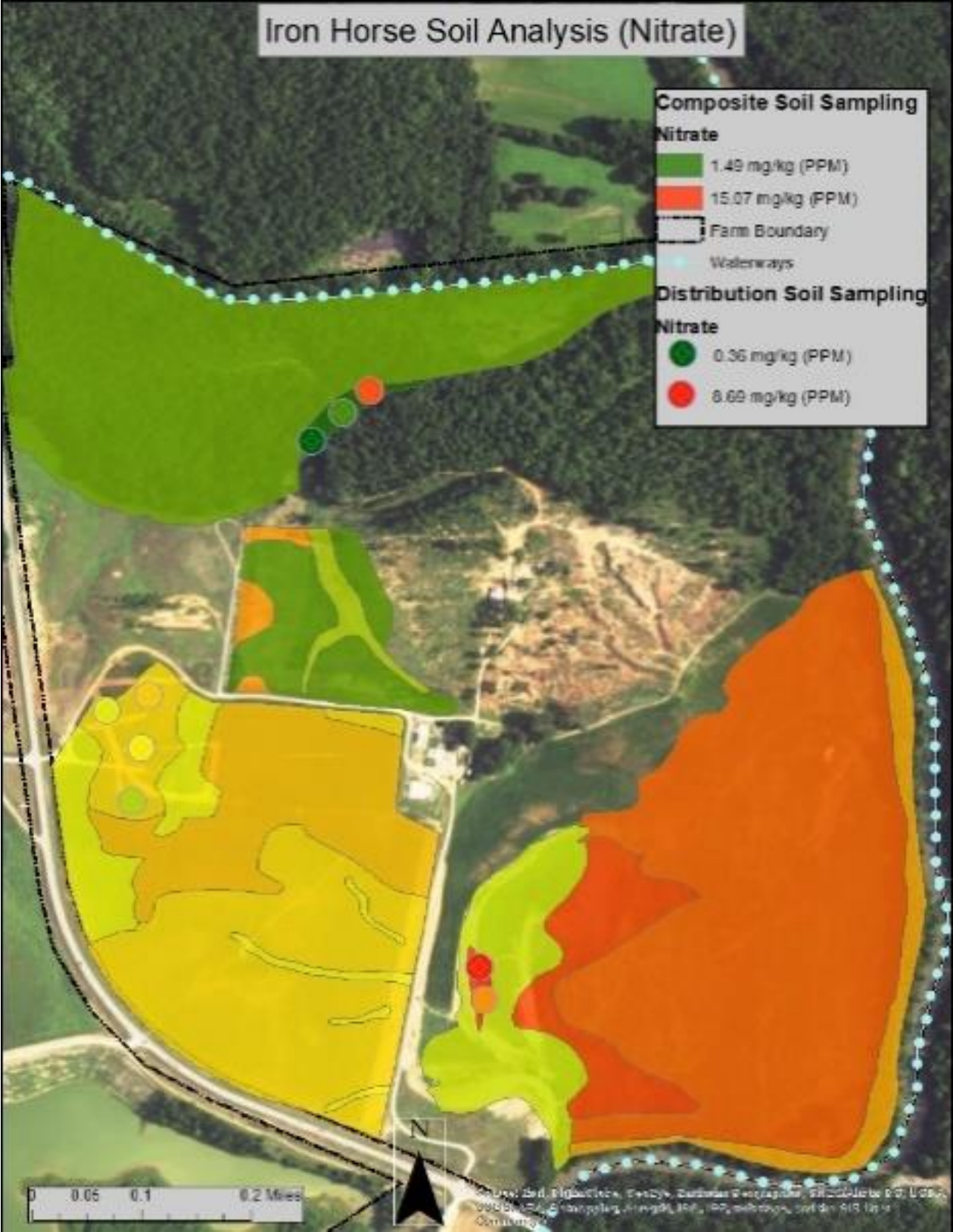


Figure 3.1.4: Nitrate, ammonium, and phosphorus in Iron Horse Farm soils.

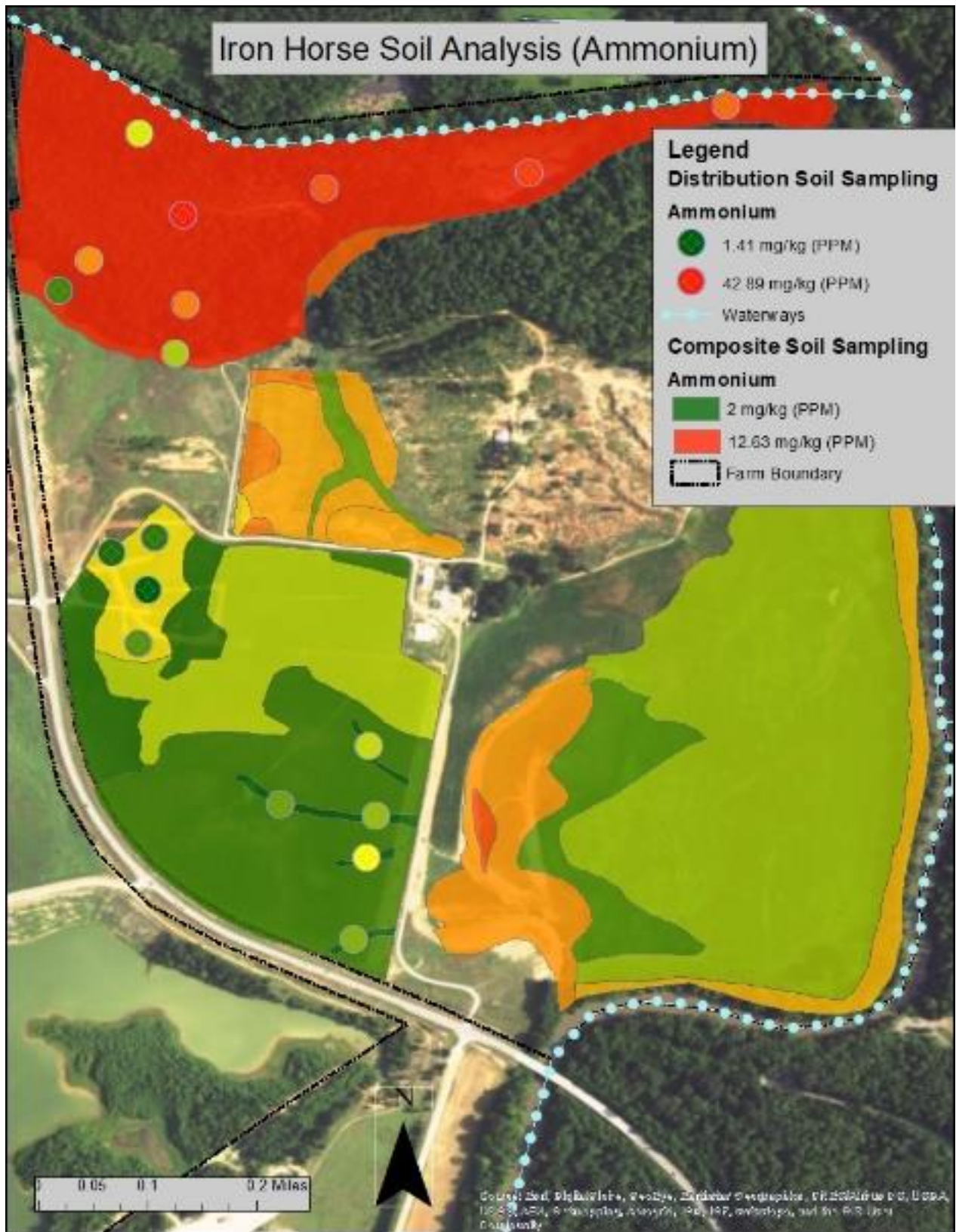


Figure 3.1.4 cont.: Nitrate, ammonium, and phosphorus in Iron Horse Farm soils.



Figure 3.1.4 cont.: Nitrate, ammonium, and phosphorus in Iron Horse Farm soils.

Appendix 3.2

Iron Horse Farm Water Nutrients

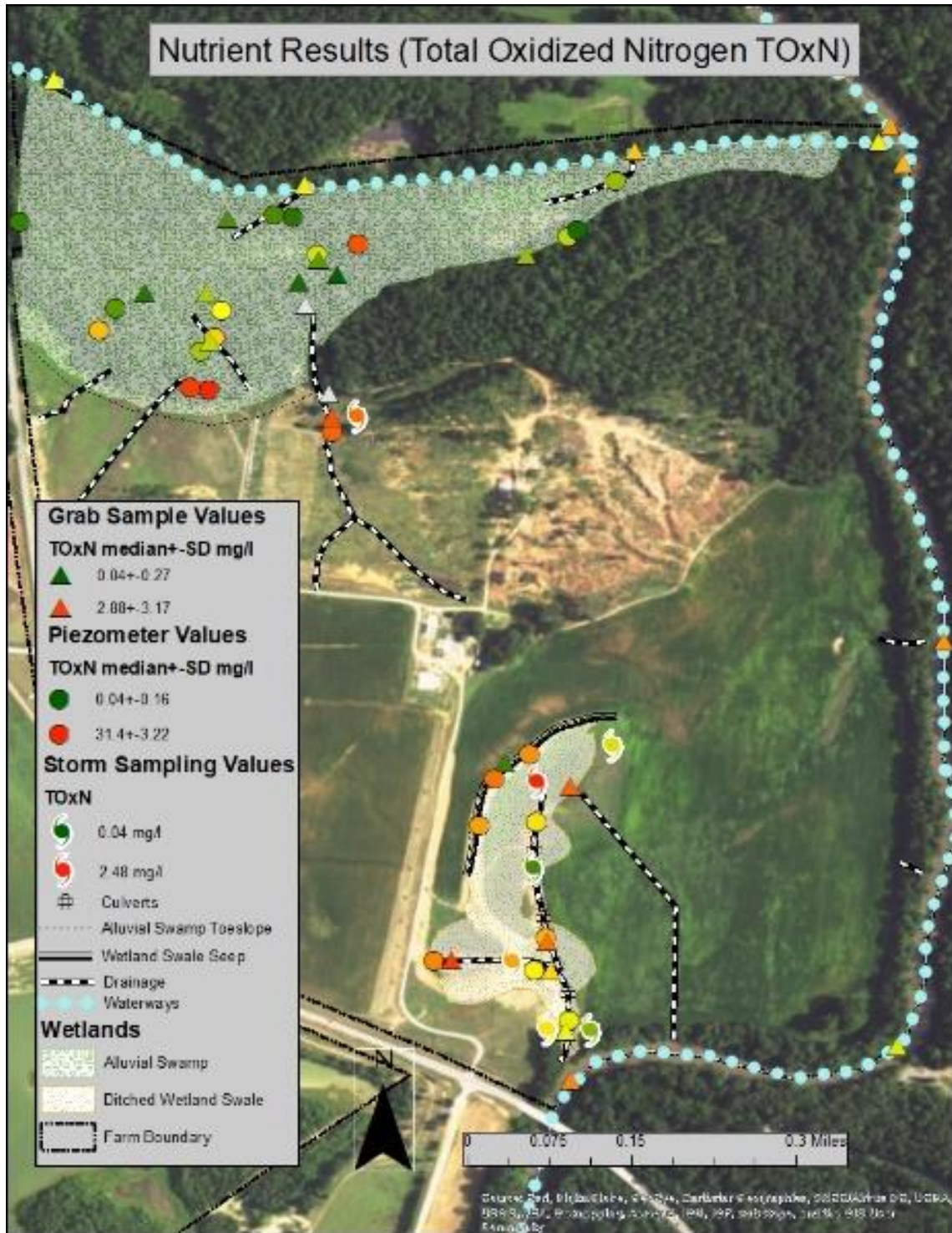


Figure 3.2.1: Nitrate, ammonium, and phosphorus levels in surface water and groundwater.

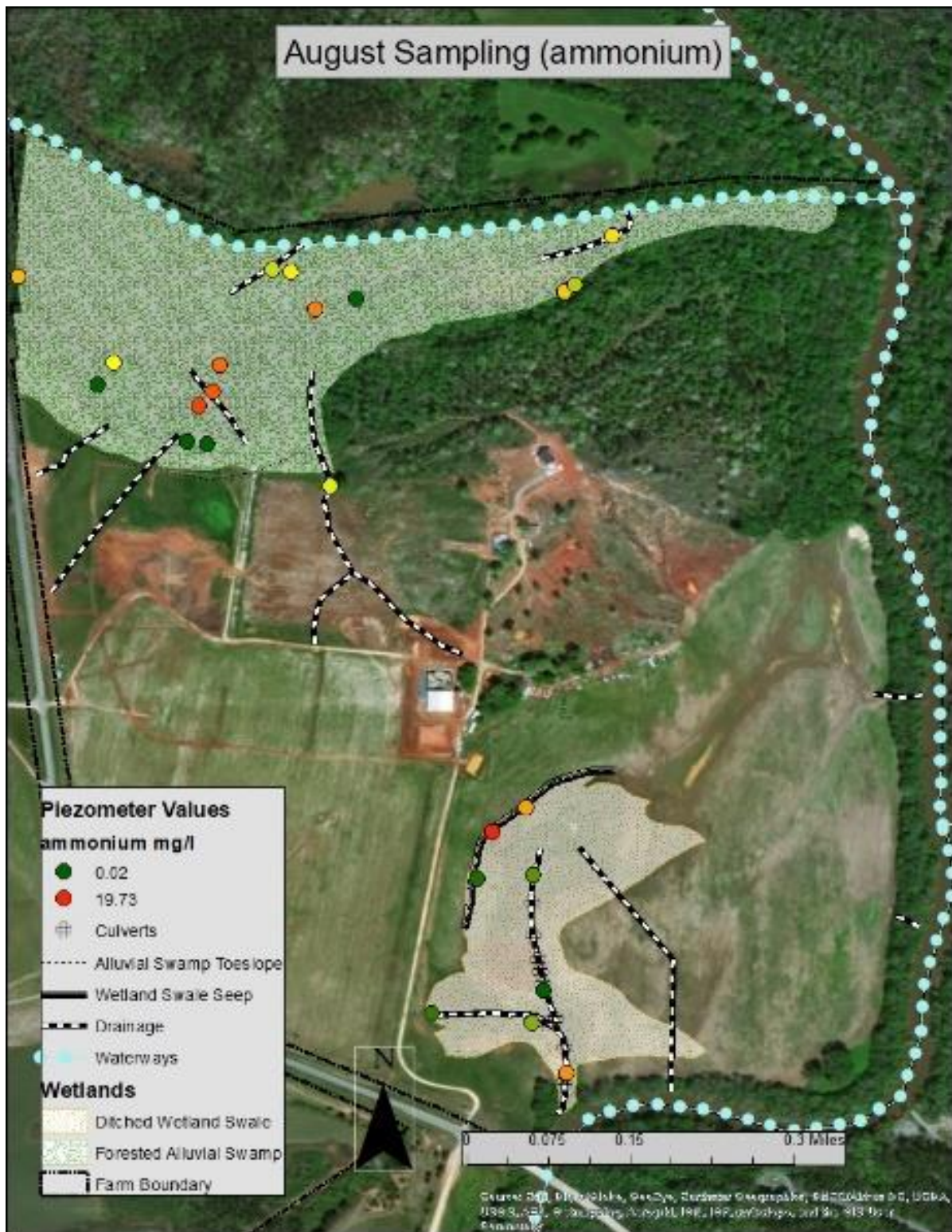


Figure 3.2.1 Cont.: Nitrate, ammonium, and phosphorus levels in surface water and groundwater.

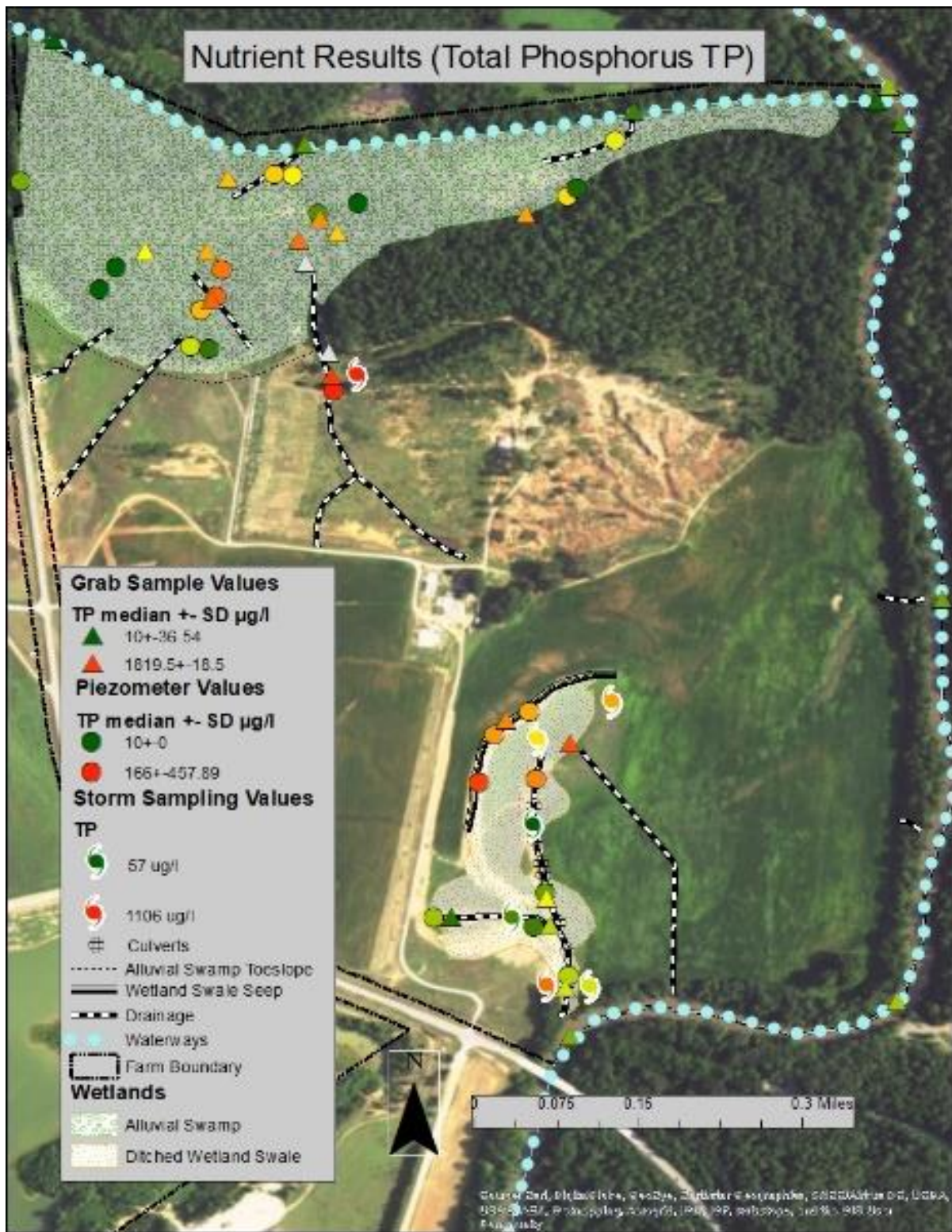


Figure 3.2.1 cont.: Nitrate, ammonium, and phosphorus levels in surface water and groundwater.



Appendix 3.3

Oconee Survey-Nutrients

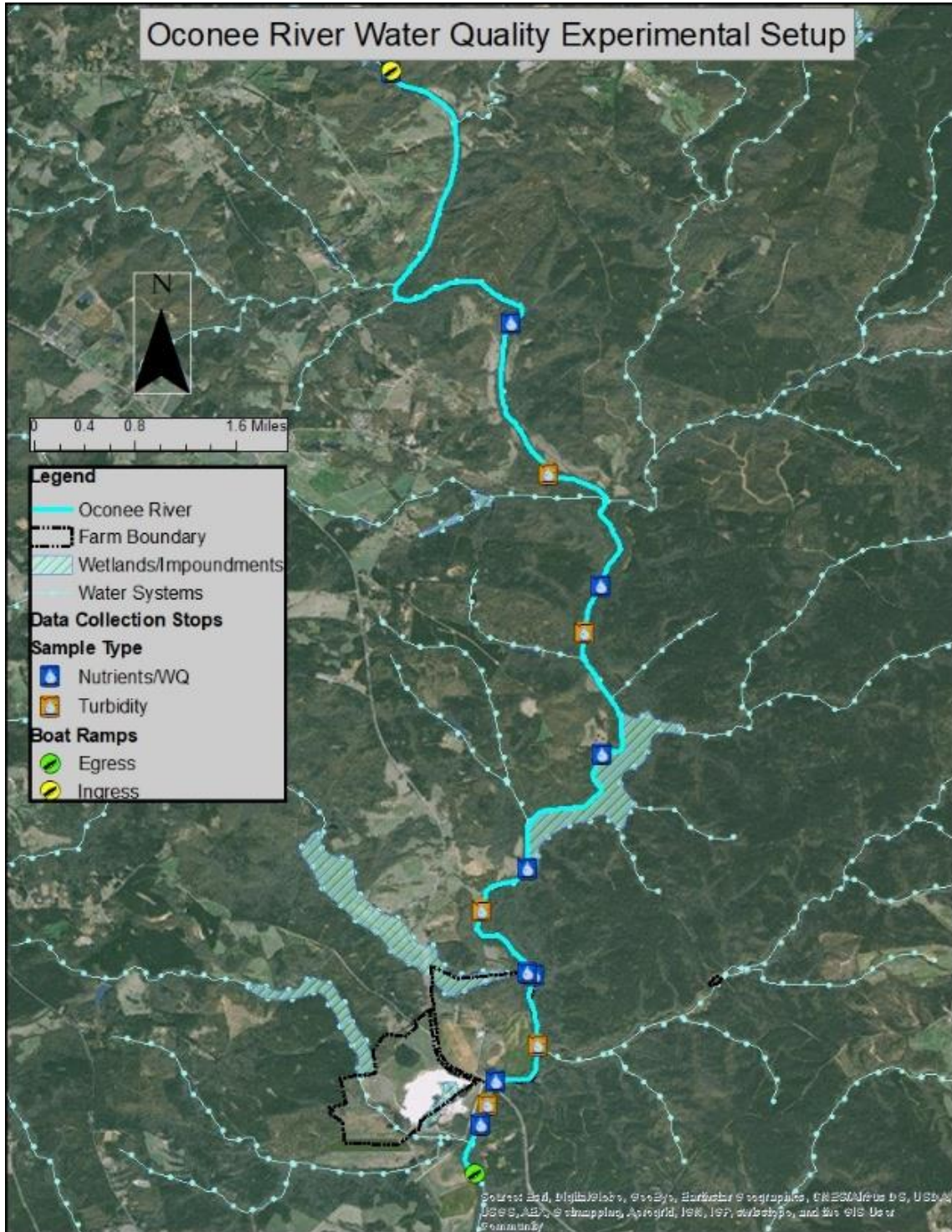


Figure 3.3.1: Oconee River survey setup.

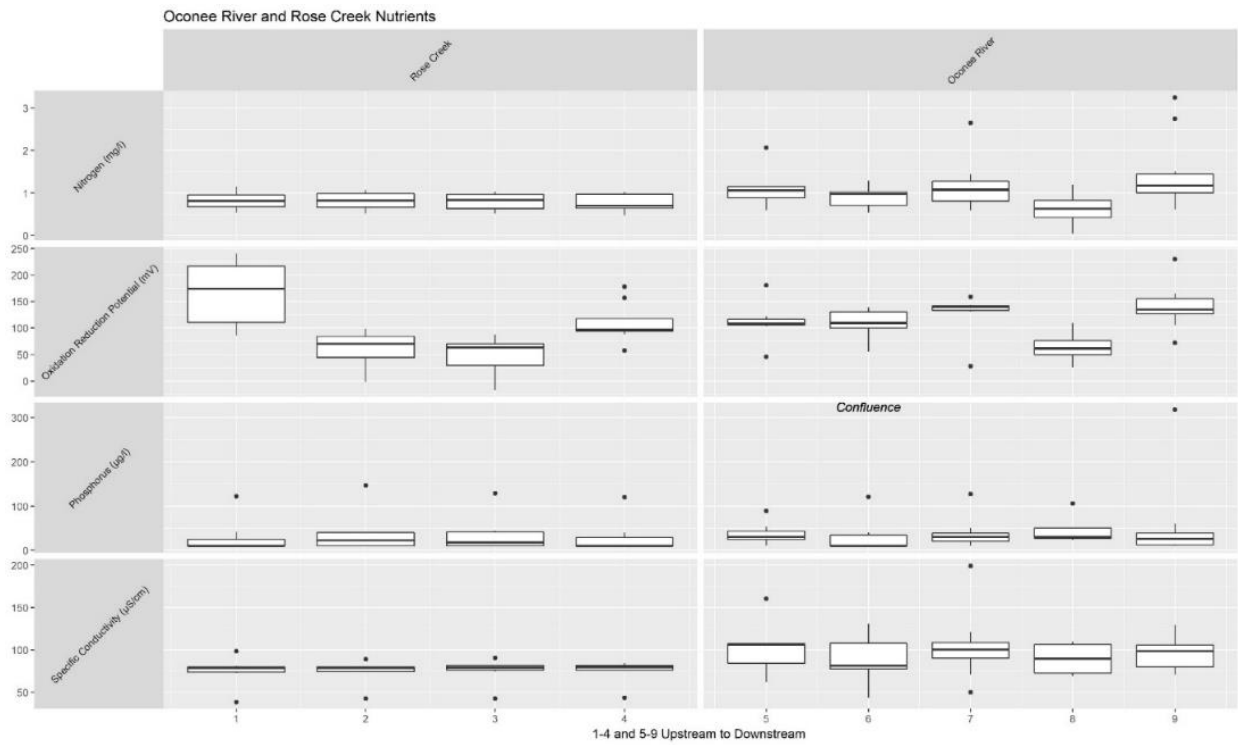


Figure 3.3.2: Nutrients in Rose Creek and Oconee River adjacent to Iron Horse Farm.

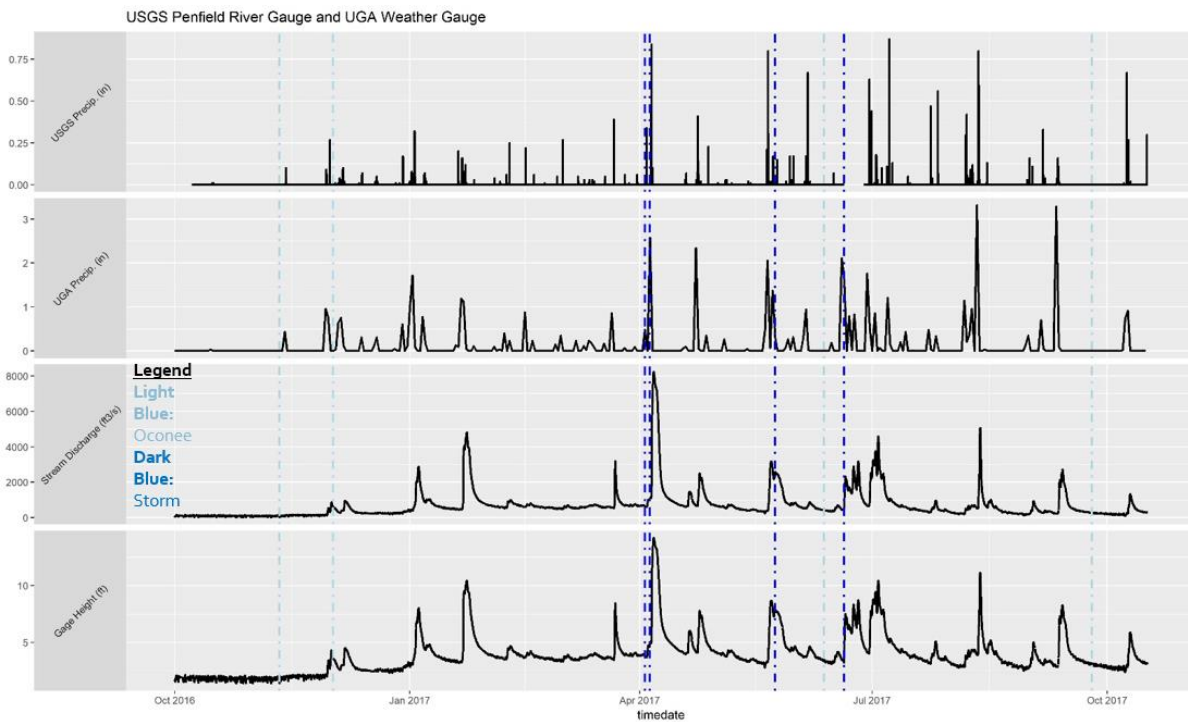


Figure 3.3.3: USGS Penfield River Gauge and UGA Oconee and weather Data during the sampling period.

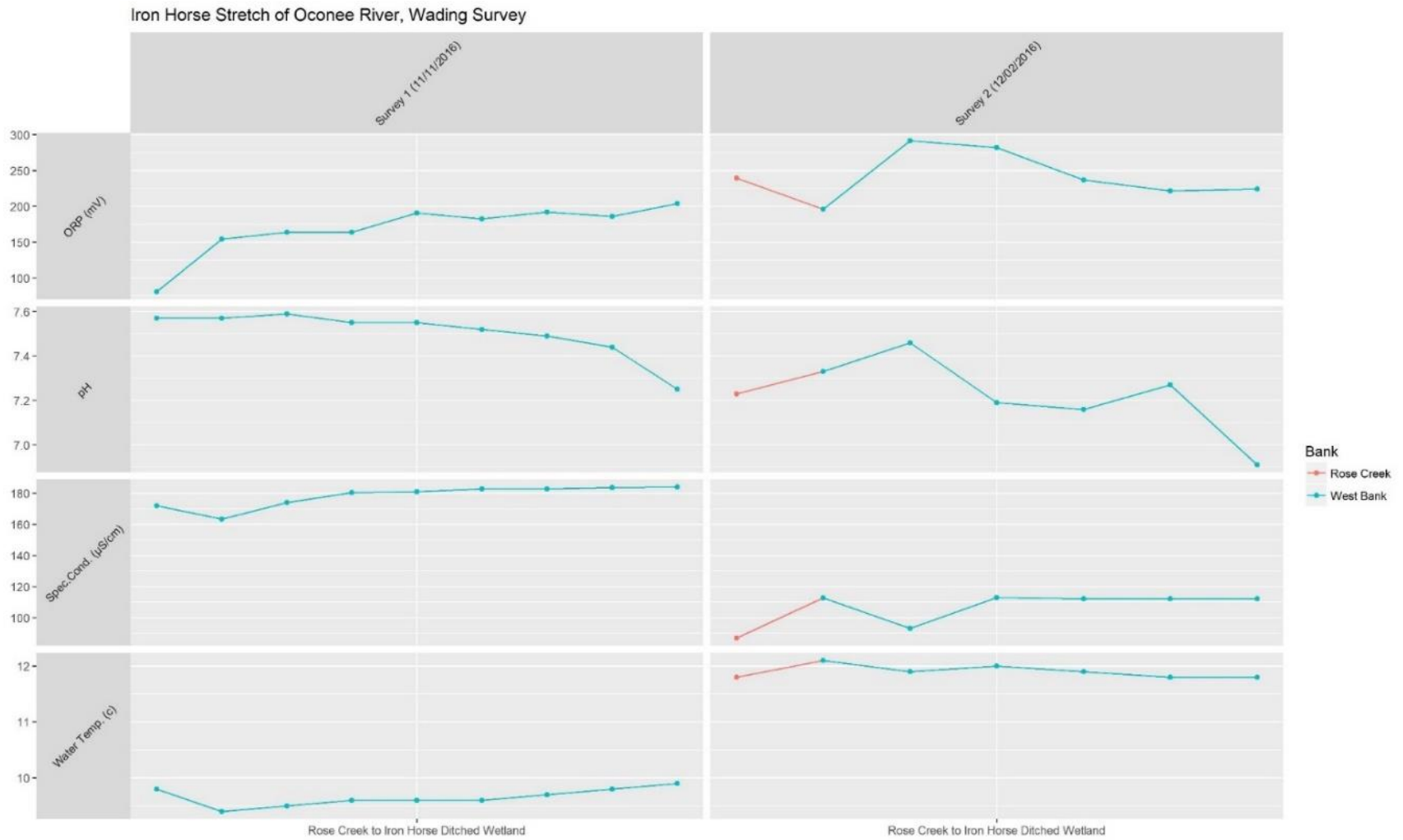


Figure 3.3.4: Survey of Oconee River by Iron Horse Farm.

## CHAPTER 4

### MICROBIOLOGICAL INDICATORS FOR BIOASSESSMENT IN AGRICULTURAL WETLANDS

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<sup>1</sup>Matteson, C. T., S.B. Wilde, C.R. Jackson, D.B. Batzer, J.L. Shelton, J.B. Jeffers, To be submitted to *Wetland Ecology and Management*.

## Abstract

This study utilizes microbial assemblages (esp. Cyanophyta, Bacillariophyta) as biotic indicators of water quality in agricultural wetlands. During 2016 and 2017, water samples collected from surface water of a forested alluvial swamp and ditched wetland swale were assessed by microscopy for algal, protozoan, and microzooplankton species richness and taxon specific biovolume. Nitrate + nitrite, phosphorus, and sunlight intensity influenced microbiotic communities in agricultural wetlands. The highest overall species diversity was found in the shaded, lower nutrient forested alluvial swamp bordering the northern side of the study farm. This forested wetland receives organic loading from the upslope retired feedlot, and the algal species assessment included high richness in mixotrophic algal species adapted to taking up dissolved organic compounds under low light conditions (Euglenophyta, Cryptophyta). There was higher biovolume and lower species richness in a ditched wetland swale used in crop production that is exposed to full sunlight and higher relative nutrients in the surface water. This open ditched wetland supported elevated biovolume of photosynthetic, Charophyte *Spirogyra*, a species that thrives in high nutrient conditions. Cyanobacteria species richness is greater in the wetland swale but also present in both agricultural wetlands. The forested alluvial swamp supports a more diverse community of microorganisms including species adapted to low light conditions and turbid water. This diverse community may help reduce potential for the formation of dense monospecific blooms of toxic cyanobacteria.

INDEX WORDS: wetlands, agriculture, cyanobacteria, bioassessment, nitrate, phosphorus.

## Introduction

Microbiota diversity and abundance provide integrated assessment of temporal and spatial integrity and variability in aquatic sites (Lavoie et al. 2004; Bellinger and Sigeo 2010b). Algal assemblage shifts have been shown to be an effective, visible predictor of changes in watershed inputs and lake, stream, or wetland trophic status (Smucker 2014). Many species can help remove impurities and certain species thrive in high/low nutrients, and drought/floods (Biggs and Kilroy 2000; Liang et al. 2003). Microbiota can be used as bioindicators of impaired water quality and will likely increase in use as non-point source pollution indicators (Bellinger and Sigeo 2010a; Smucker 2014) and general water quality (Lavoie et al. 2004; Dodson 2005). Important features of indicator species include a narrow ecological range, rapid response to changes, easy to see taxonomy, reliable identification, and a large geographic range (Bellinger and Sigeo 2010). With their rapid rates of reproduction and short life cycles, they are valuable indicators of short-term or pulsed impacts. Tracking hydrologic conditions, chemical and physical water quality parameters, and microbial bioassessments can provide rapid, effective detection of farm management practices resulting in erosion and degradation of the agricultural landscape and potential pollutant inputs to downstream waters.

Interactions between light regimes and nutrient levels can affect the taxa and volume of organisms (Murkin et al. 1991). Generally, decreasing intensity of light on surface water will result in lower phototrophic biomass and increased mixotrophic or heterotrophic organisms (Hillebrand et al. 1999). Algae which proliferate in high light and nutrient regimes typically respond to the nutrients in the water; nutrient rich wetlands generally result in greater biomass

than those with low nutrients (Murkin et al. 1991). Phosphorus is considered a limiting nutrient in freshwater (Falconer and Humpage; Dodson 2005; Schindler 2010) so biovolume increases when it is introduced (Schindler 2010).

Cyanobacteria, Bacillariophyta, and turbidity/organic indicator species were tracked over the study site. Cyanobacteria has been indicated in the poisonings of humans and livestock all over the world (Falconer and Humpage), as well as waterfowl (Wilde et al. 2005). A majority of these poisoning events take place on small farm ponds and lakes but some have even been noted on rivers and large reservoirs (Falconer and Humpage). The need for additional research on cyanobacteria is accelerating due to future conditions which will likely be more conducive to even more prevalent cyanobacterial blooms (Falconer and Humpage). Elevated temperatures, excess nutrients, and stagnant flow conditions can result in increased abundance of potentially toxic species within the Cyanophyta, or blue-green algae. Dense blooms of cyanobacteria called cyanoHABS (harmful algal blooms) not only indicate degraded water quality conditions, but they produce toxins that may be lethal to aquatic consumers and have deleterious allelopathic effects on beneficial algal species (Paerl and Otten 2013; Ibelings et al. 2016).

Bacillariophyta and turbidity/organic indicators are useful at sites with high potential for nutrient pollution. Diatoms provide excellent insight into the status of a waterbody (Lavoie et al. 2004) due to their distinct water quality requirements (Spaulding et al.). There are as many as 5,000-12,000 species of diatoms, and specific taxa proliferate under various water quality conditions (Vymazal 1995). In addition, relative increases in percentage of motile diatom taxa

indicate more turbid water resulting from resuspension of sandy sediments or active erosion within the watershed (Bellinger and Sigg 2010a; Stevenson 2014). Vertical changes in algal assemblages within the water column or across horizontal landscapes can often be attributed to gradients in environmental parameters including light, dissolved oxygen (DO), temperature, and pH (Murkin et al. 1991, Rader and Richardson 1992, Burt et al. 2013).

This study was conducted at a southeastern Piedmont farm with a forested alluvial swamp, ditched wetland swale, shaded stream, and surrounding river. We quantified algal, protozoan, and microzooplankton species in agricultural wetland systems with contrasting light and nutrient regimes. Algal data collected in the wetland bioassessment included taxa common in more impaired aquatic sites, primarily Cyanophyta, Bacillariophyta, Cryptophyta, and Euglenophyta. This study specifically investigated wetland environmental conditions supporting increases in potentially toxic cyanobacterial species. Additionally, the relative abundance of motile diatoms and taxa favored under high turbidity/organic conditions were tested to evaluate temporal correlation to hydrologic events resulting in sediment and organic nutrient pollution. No previous studies of microbiological species diversity, richness, and biovolume have been conducted in the wetlands and waterways surrounding the farm. Iron Horse Farm offers a unique ecological opportunity to observe microbial changes in agricultural wetlands, a creek, and a river, at one site.

## Methods

### *Site Description*

The University of Georgia recently acquired the 300 ha Iron Horse Farm for active agricultural research, the farm consists of an upland row-crop field, forested area, and farm operations yard sloping down into a retired feedlot, wetlands, and additional lowland (floodplain) row-crop field (Figure 4.1). On average, piedmont Georgia receives 1240 mm of rain per year, and the mean annual temperature is 16.5°C (Cowell 1998). Iron Horse Farm received 1344 mm of rain and a mean annual temperature of 18°C during the study period.

The 17 ha forested alluvial swamp is comprised of two large beaver dams holding water in two flooded pools, each pool has its own discharge point into Rose Creek, which forms the northern border of the farm. Both pools have unique light regimes; the littoral edges are shaded by Willow, Ash, Elm, Maple, and Chinese Privet, and shade free interior pools support Arrow Arum, grasses, Duckweed and smaller Maples and Willows. A 4 ha ditched wetland swale is drained by a ditch complex extending to the Oconee River; ditches extend to the west, north, and east, and have been established since at least 1875 (Coulter 1964). This wetland swale and ditch complex is termed the ditched wetland swale and has no tree cover and a fluctuating water table. The Oconee National Forest served as a reference site of water quality supporting different microbiological communities. This 0.80 ha reference swamp is located on Sandy Creek ~2.4 km from the Oconee River (Figure 4.1).

### *Surface Water Collection*

Surface water grab samples were collected for nutrient and microbial assessments at Iron Horse Farm, the surrounding water ways, and the Oconee national Forest over the course of a year, 25 at the farm and 2 in the reference swamp. Surface water samples were chosen after visual inspection of the field site, based on low spots on the landscape and in the proximity of installed piezometers utilized in a hydrologic analysis of the study site. Samples were 125 ml unless the sample site was running dry in which case as much water was collected as possible. Visible filamentous green algae at a grab site was collected when observed and surface water sample depth was measured. All sample bottles were rinsed in the sample water three times before a sample is taken. After sample collection they were placed on ice for the remainder of the sampling event. Water quality parameters were taken using a YSI Professional Plus Quatro Cable (YSI), which includes sensors for oxidation reduction potential (ORP) (mV), dissolved oxygen (DO) (% and mg/l), potential of hydrogen (pH), temperature (°C), and specific conductivity ( $\mu\text{s}/\text{cm}$ ).

### *Microscopy Identification*

Microscopic screening of live samples was conducted to facilitate species identification in the individual water samples. Within 24 hours of collections, Lugols Iodine solution (10% potassium iodine and 5% iodine) was added to water samples for preservation/staining for subsequent species presence data, taxon specific counts, and biovolume estimates. Water samples were sub-sampled using a pipette and ~5 ml of each sample were placed in a 12-well

microplate. All observed species were recorded using a Zeiss inverted microscope under a 100X magnification.

#### *Algal Taxa Density and Biovolume Estimation*

We conducted genus/species counts and biovolume estimates on a subset of seasonal water samples from contrasting wetland types. Samples from March 2017, July 2017, and September 2017; from the forested alluvial swamp, ditched wetland swale, and reference swamp. Within the selected months, six samples were selected for complete algal density estimation including three from sunlit water locations, and three from shaded water locations. Selected samples were sub-sampled using a pipette and placed on a 0.1 ml Palmer Nanoplankton Counting Cell.

Species population biovolume were calculated using geometric equations from published literature and data obtained from species counts (Hillebrand et al. 1999). For each commonly observed species, 10 individuals (Hillebrand et al. 1999) were measured using AmScope Software which functions with an AmScope Camera and compound microscope at a 100X magnification. Algal length, width, diameter, and area were calculated by tracing the outline of the cell or filament on the computer screen (Figure 4.2). Width was used as a proxy for depth of the cells since this varies with vertical position and is assumed to be proportional (Verity et al. 1992; Hillebrand et al. 1999). Medians of dimensions were fed into geometric equations in Microsoft Excel to determine mean unit biovolume for selected species (Hillebrand et al. 1999). Mean unit biovolume of each species was multiplied by cell density estimates to calculate total species biovolume.

### *Oconee River Analysis*

Sampling began 6 km upstream of the farm at Barnett Shoals Dam outside of Watkinsville, GA in Oconee County and ended at the Redland Sand mining site 1.6 km downstream from Iron Horse Farm in Green County, GA (Appendix 4.1, Figure 4.1.1). Eight samples were taken to analyze microbiota in the water combined with YSI and nutrients.

### *Light Measurements*

Photosynthetically active radiation (PAR) was measured at the study site to test for taxon specific biovolume responses to light level. Ambient and surface water light measurements were collected using a LI-193 Spherical Quantum Sensor and recorded in Photosynthetic Photon Flux Fluence Rate (PPFFR) (LI-COR 2015). Light measurements were taken in three spots, one in the forested alluvial swamp, one in the confluence of the ditched wetland swale, and a final one in the reference swamp. Measurements were repeated in August 2017 to represent leaf-on, and December 2017 for leaf-off.

### *Storm Effects on Microbiota Species Richness*

Monthly sampling in May and June 2017 were conducted during storm conditions (Table 4.1). Microbial data was evaluated to determine if microbial taxa commonly occurring in a swamp were flushed into the adjacent channels.

### *Statistics*

Data was first assessed using parametric statistics to determine the most observed phyla. Normality of variances using a Bartlett test was run, followed by a Shapiro Test on the normality of residuals on selected phyla richness. Microbiological organisms in samples from

around the farm were analyzed by One-way ANOVA blocked for season and a post hoc Tukey Test was run on the ANOVA to assess specific differences in species richness by landscape features (Dowdy et al. 2003; Dalgaard 2008). If data did not meet assumptions of ANOVA, a non-parametric Kruskal-Wallis test was run followed by a Dunn Test in place of Tukey. Individual surface water samples at Iron Horse Farm were grouped into landscapes for assessment and sampling months were grouped into seasons. Water samples were assigned light and nutrient bins, within those groups; high, medium, and low values of nitrate + nitrite and phosphorus were selected, and the phyla biovolume compared by wetland type and light intensity. Analysis focused on indicators groups, Cyanobacteria, Bacillariophyta, organic indicator species, and turbidity indicator species. A correlation analysis was conducted to test for relationships in parameters cited to affect microbiological biovolume and cell counts. Correlations between species and tested variables was assessed and correlation bins included strong linear relationships between  $+1$  and  $+0.5$ , medium between  $+0.49$  and  $+0.3$ , and weak between  $+0.29$  to  $+0.1$ . A Principle component analysis was run on microbiota counts and biovolume to further assess correlation between species and tested variables. Shannon diversity index (H) and species richness (S) were compared over landscapes and sampling periods.

## Results

The two most cited indications of a healthy system or impaired one are the presence of Diatoms in a healthy system and Cyanobacteria as degraded and potentially toxic (Lavoie et al. 2004; Wilde et al. 2005; Bellinger and Sigeo 2010a; Burt et al. 2013). Cyanobacteria found at

Iron Horse Farm included *Oscillatoria*, *Anabaena*, *Microcystis*, *Anacystis*, *Nostoc*, *Phormidium*, and *Leptolyngbya*. Organic pollution indicators included Euglenophyta (*Trachelomonas*, *Euglena*, and *Phacus*), Cryptophyta (*Cryptomonas*) and Ochrophyta (*Ochromonas*). *Euglenoids* are ideal taxa for indicating excess organics in a system (Abboud-Abi Saab and Hassoun 2017), and can be toxic in some cases (NOAA 2009). Organic waste in receiving water can indicate sewage (Abboud-Abi Saab and Hassoun 2017), but in the case of a farm a likely source is animal waste. Species indicating turbidity included motile diatoms (*Nitzschia*, *Navicula*, *Synedra*, *Pinnularia*), Chlorophytes (*Ankistrodesmus*) and protozoa.

#### *Spatial and Temporal Changes in Microbiological Species Richness and Biovolume*

Cell counts of cyanobacteria have a strong positive relationship with phosphorus (P), and a strong negative relationship with light. A moderate negative relationship exists with cyanobacteria count data and nitrate + nitrite (N) as well. Cyanobacteria biovolume is strongly negative correlated with light, and positively with phosphorus and its own cell counts. Bacillariophyta biovolume is strongly positive correlated with nitrate + nitrite (N) and Bacillariophyta cell counts, and moderately with phosphorus (Figure 4.3).

Cyanobacteria cell counts eigenvector is in the same direction as phosphorus, while the biovolume eigenvector does not match up as easily. Bacillariophyta cell count and biovolume point in similar directions; coupled with nitrate + nitrite, and close to phosphorus (Figure 4.4). Microbiota identified came from 158 surface water samples and yielded 70 species in 15 different phyla. The largest proportion of species sampled were Bacillariophyta at 44% of the

total species. Remaining phyla found in water samples can be found on Table 4.2, and Figures 4.5 and 4.6.

Cyanobacteria had the highest species richness in the ditched wetland swale. Summer samples had the most species with 24, species richness of cyanobacteria was significantly different between seasons ( $\chi^2(3) = 14.20, P = 0.002$ ). Bacillariophyta species richness was greatest in the forested alluvial swamp with 81 species and a significant difference in the number of Bacillariophyta species was detected by season ( $\chi^2(3) = 13.76, P = 0.003$ ) between spring and summer. Turbidity indicator richness was greatest in the forested alluvial swamp with 59 different species, and in the summer (79 species). Organic indicator richness was greatest in the forested alluvial swamp with 47 different species, significant differences in the number of turbidity indicators was documented between the seasons ( $\chi^2(3) = 22.13, P < 0.001$ ).

Microbiological species were assessed for biovolume using published equations (Table 4.3). Green algal species had the highest biovolume, *Spirogyra* made up 56% of the total biovolume followed by *Closterium* at 16%. The diatom *Aulacoseira* represented 5% of the total biovolume. Species population biovolume was highest in the ditched wetland swale, and in March (Figure 4.7). Differences between biovolumes of individual phyla sampled are significant ( $\chi^2(6) = 35.03, P < 0.001$ ). Cyanobacteria biovolume was highest in the ditched wetland swale at  $73,703.57 \mu\text{m}^3/0.1\text{ml}$  and during July at  $73,922.28 \mu\text{m}^3/0.1\text{ml}$ . Bacillariophyta were most successful in the ditched wetland swale with  $1,611,855.4 \mu\text{m}^3/0.1\text{ml}$  and in September  $1,211,305.7 \mu\text{m}^3/0.1\text{ml}$ .

Species counts averaged 61 cells in the forested alluvial swamp, 35 in the reference swamp, and 55 in the ditched wetland swale. Counts conducted using March samples averaged 67 cells per species, 68 in July, and 28 in September. Charophyta exhibited the greatest number of cells and cell counts varied significantly between phyla ( $\chi^2(8) = 22.39, P = 0.004$ ). Shannon's Diversity Index (H) yielded highest values in the forested alluvial swamp (H: 1.99) during September 2017, sampling (Figure 4.11).

Light measured during leaf-on conditions yielded PAR values of 785 nm in the ditched wetland swale, 303.1 nm in the forested alluvial swamp, and 2683 nm at the reference swamp. During leaf-off conditions, PAR increased at all sites except for the reference swamp that was recorded at 1754 nm. The ditched wetland swale increased to 1233 nm, and the forested alluvial swamp to 470.1 nm. Nitrate + nitrite and phosphorus levels at selected sample sites were split into high, medium and low bins (Table 4.4, Table 4.5). The largest biovolume was documented in the ditched wetland swale during leaf-on conditions, when nitrate + nitrite and phosphorus were in the high category (Figure 4.8). The phyla dominant in the ditched wetland swale was Charophyta; light intensity in the alluvial swamp was significant in determining biovolume of phyla present ( $\chi^2(1) = 9.69, P = 0.002$ ).

Species were categorized into taxonomic and functional groups (autotrophs, plankton, and sediment feeders) (Appendix 4.1, Figure 4.1.2). Microbiota categories in the Oconee River include 74 autotrophic species, 8 plankton, and 3 sediment feeders. This shift in species richness was significant ( $\chi^2(1) = 4.35, P = 0.03$ ) between the Oconee River and Rose Creek but does not vary by season (Appendix 4.1, Figure 4.1.3).

Two major storm events occurred during the sampling periods (Figure 4.9). There was no significant difference between the species richness observed overall between months of dry sampling and those taking place during heavy precipitation events (Figure 4.10). Species richness did shift during these storm events, but not statistically, toward lower (35 species) in the swamp and higher in the creek (29) during the storm. Species richness was highest (46) in the swamp and lowest (11) in the creek during dry weather. During the storm, the wetland reflected diatom assemblages typical in Rose Creek samples rather than species richness of turbidity indicators.

### Discussion

A possibility for the decrease in PAR at the reference swamp during leaf-off could be the grasses around the surface water sample site; they hung down during the growing season but stood more upright when yellowing as the seasons changed to leaf-off. Time of day causing lower light intensity could also have played a role in the reduced PAR value.

Overall biovolume did seem to respond to light changes over the sampling period; although different wetlands showed different types of responses. When a wetland is cleared and drained, less species will be able to survive, but those that do will have all competitors taken away, and will thrive. The ditched wetland swale had the largest biovolume of the sampling period, but it only consisted of Charophyta *Spirogyra*. Nutrients and light regimes are clearly both important factors in microbiological richness and growth. Major *Spirogyra* blooms took place during leaf-on while nitrate + nitrite and phosphorus were high. In the same wetland in samples with low nitrate + nitrite, and with low phosphorus, biovolume was low. In

the forested alluvial swamp, water samples were taken from an open pool with tree cover. High phosphorus and low nitrate + nitrite samples had low biovolume during leaf-on, but high biovolume during leaf-off. The majority of this biovolume was *Chlorophyta* and *Charophyta*. The composition of species in the shaded samples changed in samples with high nutrients, supporting filamentous green algae which thrive in sunlight, but also respond to elevated levels of phosphorus (Figure 4.8). The reference site biovolume was similar between leaf-off and leaf-on and nutrients were low in assessed samples.

Cyanobacteria species richness varied within individual agricultural wetlands, likely due to sunlight intensity and nutrients. Cyanobacteria displayed more species richness and greater biovolume in the ditched wetland swale than the forested alluvial swamp or reference swamp. Cyanobacteria tend to outcompete eukaryotic algae under drought, excessive nutrients, and lower light intensity seem to be more attractive for Cyanobacterial species. Managers should keep an eye on cleared, eutrophic wetlands, especially during warm months; Cyanobacteria is likely to respond with increased cells and biovolume to higher phosphorus. Based on Figure 4.3, cell count will decrease with increased light, and nitrate + nitrite. Increased light appears to reduce the number of cells and biovolume, which could be a factor of Cyanobacteria not being as competitive in intense light as green algae. The biovolume of Cyanobacteria is strongly affected by number of cells found at a site.

More Bacillariophyta species were found in the forested alluvial swamp, but there was greater biovolume of Bacillariophyta in the ditched wetland, this is the same dynamic that was seen in other species in the study. More Chlorophyta were observed in the reference swamp

during leaf-on, the decrease during leaf-off may be due to the larger biovolume of Bacillariophyta occurring in the low nutrient samples. Another possible reason Bacillariophyta did well is they are able to grow during colder months when other species do not produce biovolume. Bacillariophyta biovolume increases with elevated nitrate + nitrite and phosphorus, but more so nitrate + nitrite. Since Cyanobacteria are primarily related to phosphorus, Bacillariophyta or Cyanobacteria presence can be used as an indicator of certain elevated nutrients. Bacillariophyta are typically small cells, so the number of cells in a sample only moderately affects accumulation of biovolume.

Turbidity, and therefore turbidity indicators are common in the Southeast United States due to past farming practices (Jackson et al. 2005). Turbidity indicators in Rose Creek show that much of its water likely stems from agricultural wetlands. There is lower species richness of turbidity indicators in the reference swamp and Oconee River, indicating lower turbidity from the forested hillslope, and most of the turbidity in the Oconee may be at the bottom of the water column. Most samples taken from the Oconee River were clear, even though the water in the river looks very turbid. Organic indicators are more diverse in the forested alluvial swamp due to the retired feedlot just upslope of the forested alluvial swamp.

Storm events do not seem to dictate entirely which species are in a wetland, but probably affect them based on the size of the storm. Bacillariophyta exemplified this difference between Rose Creek and the alluvial swamp during dry weather and was more similar during storms. If there are more Bacillariophyta than normal in a waterbody, there is likely a recent flooding event adding pollutants to a creek or river. Organic indicator species were found in

Rose Creek during a storm, indicating that upstream agricultural wetlands likely only contribute surface water containing organic pollutants to Rose Creek during storms.

Between the two floats down the Oconee River, effects of more intense sunlight during warmer months is visible. More species of Autotrophs, proliferated in June, and in September while the Autotrophs decline, Plankton, and Sediment Feeders can increase in species richness. A shift in the species richness between Rose Creek and the Oconee River can also be indicative of the water quality of each waterbody. Since there is greater species richness in Rose Creek, the assumption of less polluted water quality may be made. The two systems are both exposed to different pollutants, from agriculture around Rose Creek, and a mixture of urban and agriculture in the Oconee watershed, which is also much larger. Since no Cyanobacteria was found during the canoe float of the Oconee River, a fair assumption that Cyanobacteria in the Oconee could be coming from Rose Creek as its water is heavily supplied by agricultural wetlands. Large rivers often have many inputs and light regimes along their flow path which can alter the microbial communities in the water.

### Conclusions

Microbiological organisms can tell us a great deal about water quality. Here we found that organic indicator species were predominantly found in the forested alluvial swamp, which also supported the largest overall species diversity (H). The diversity of species, number of cells, and their biovolume is largely due to the intensity of the sunlight that hits the sample sites, and the nutrient levels. The alluvial swamp supported non-photosynthetic algae with low nutrients, but photosynthetic algae took over when shading lifted. In the ditched wetland

where sunlight is constant, the nutrient regime is the only limiting factor controlling algal blooms. The increased species of cyanobacteria in the ditched wetland indicates that when nutrients aren't mitigated, and the water is exposed to sunlight all year, potentially toxic algae can proliferate in agricultural settings. Agricultural wetlands which are shaded and able to process nutrients will support healthier communities of microbiota, and communities observed in wetlands can be used to assess long term inputs and light regimes. Species in the forested alluvial swamp did match up more with species observed in Rose Creek during storm events. This can serve as an early indication that excess nutrients or other pollutants have discharged into an adjacent creek.

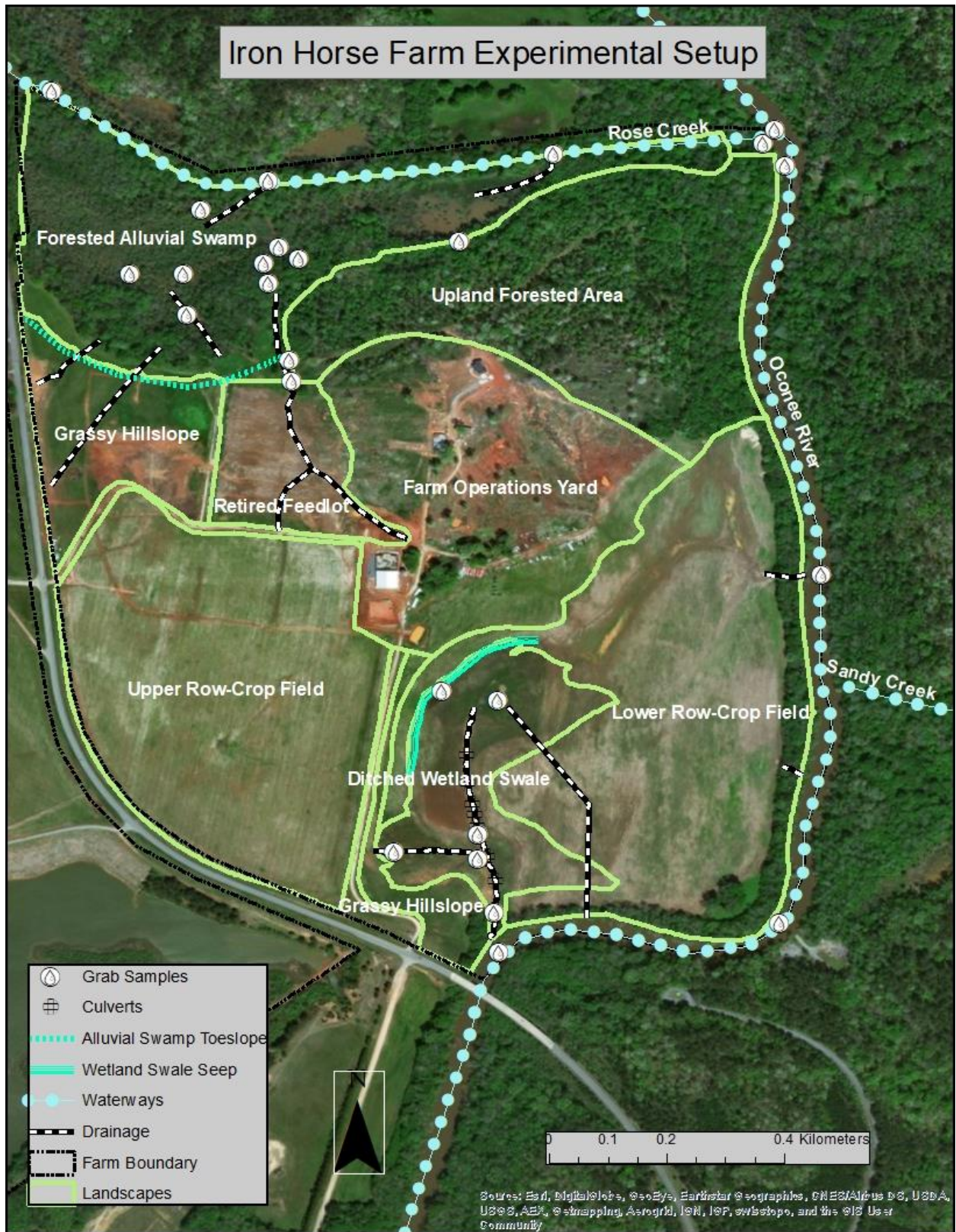


Figure 4.1: Experimental setup of Iron Horse Farm microbiota collection.

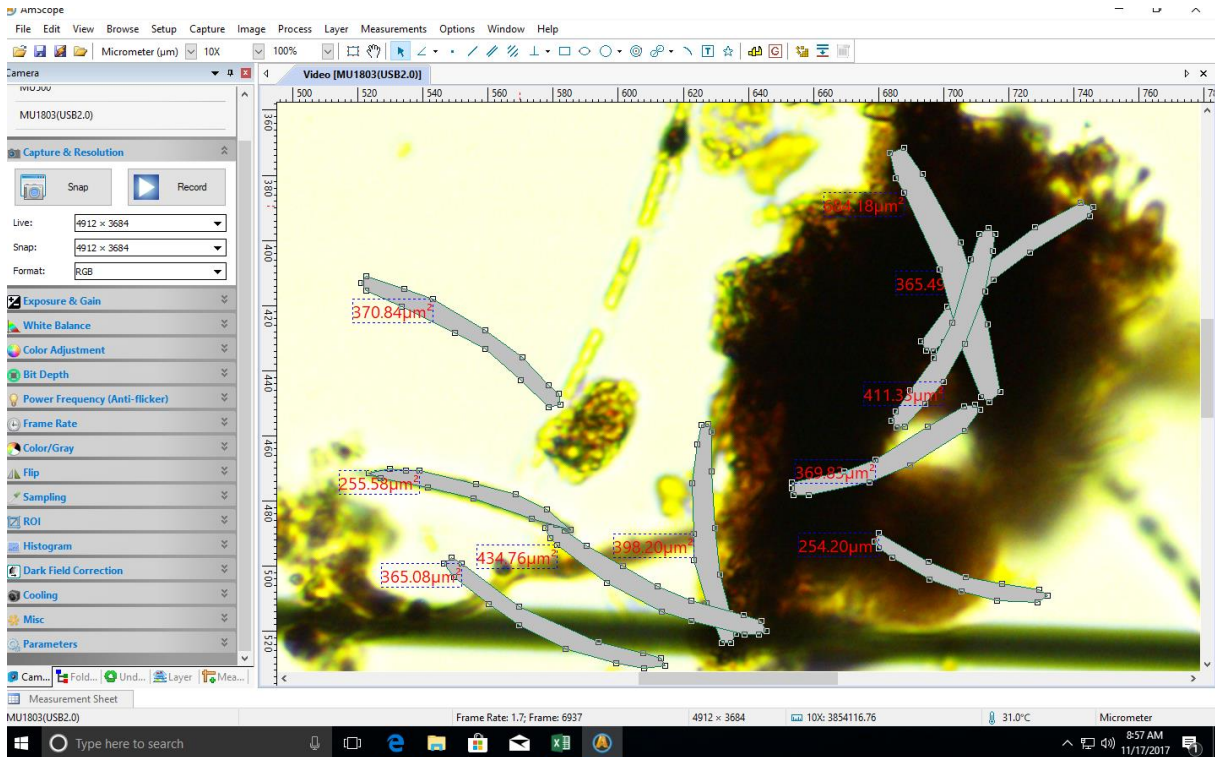


Figure 4.2: AmScope software used to measure dimensions of microbiota following species cell counts.

Table 4.1: Mean daily precipitation on days of bimonthly water sampling at Iron Horse farm (Sampling).

Activity	Date	Precipitation (in)
Sampling	10/3/2016	0
Sampling	12/13/2016	0.26
Sampling	2/10/2017	0
Sampling	3/29/2017	0
Sampling	4/3/2017	0.64
Sampling	4/5/2017	2.57
Sampling	5/24/2017	0.32
Sampling	6/20/2017	1.63
Sampling	7/18/2017	0
Sampling	8/15/2017	0
Sampling	9/5/2017	0.63

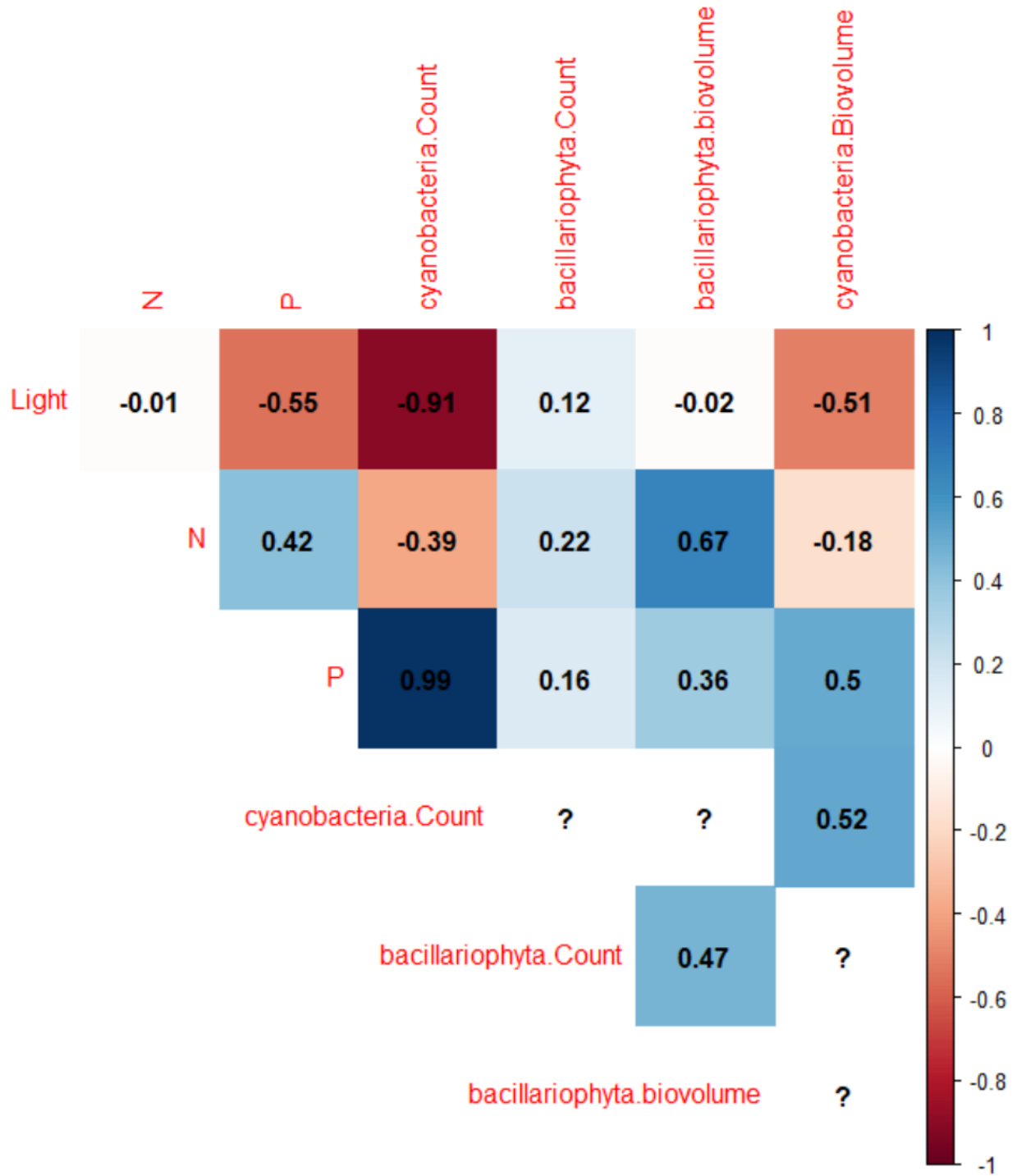


Figure 4.3: Correlation matrix of cyanobacteria and bacillariophyta cell count, biovolume, and water quality.

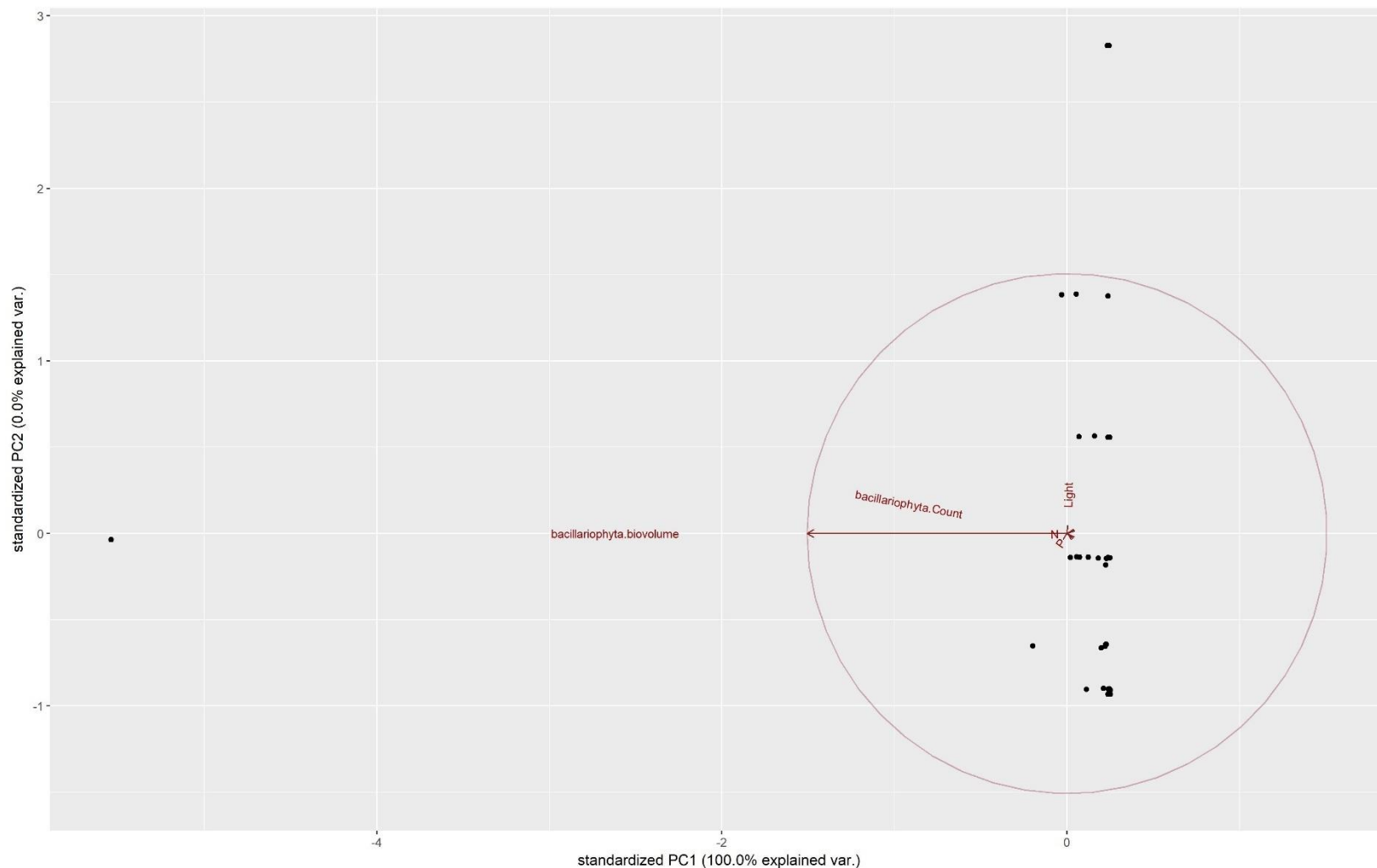


Figure 4.4: Principle component analysis of cyanobacteria biovolume and cell count (bottom), and bacillariophyta biovolume and cell count (top).

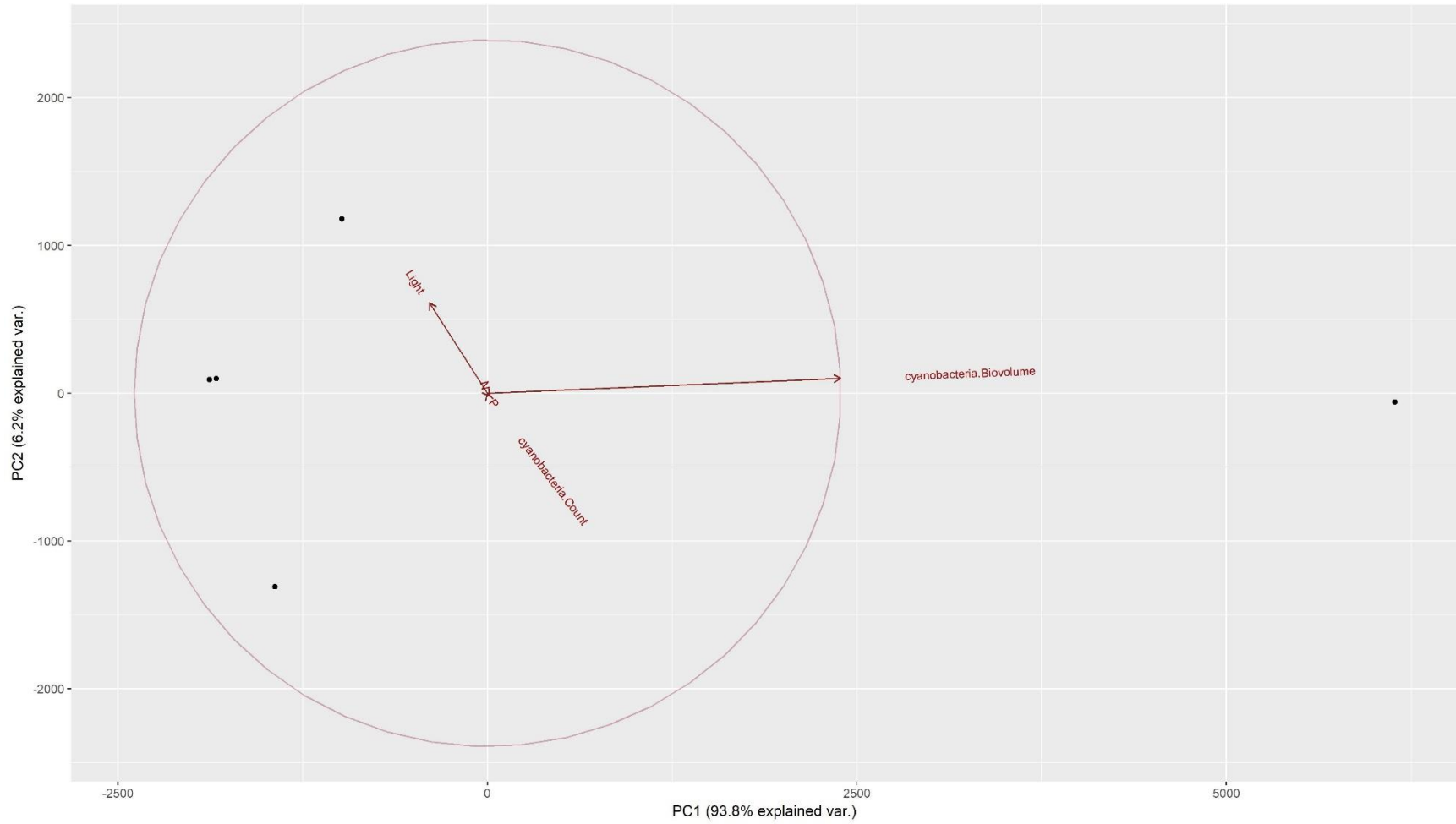


Figure 4.4 cont.: Principle component analysis of cyanobacteria biovolume and cell count (bottom), and bacillariophyta biovolume and cell count (top).

Microbiota Species Richness

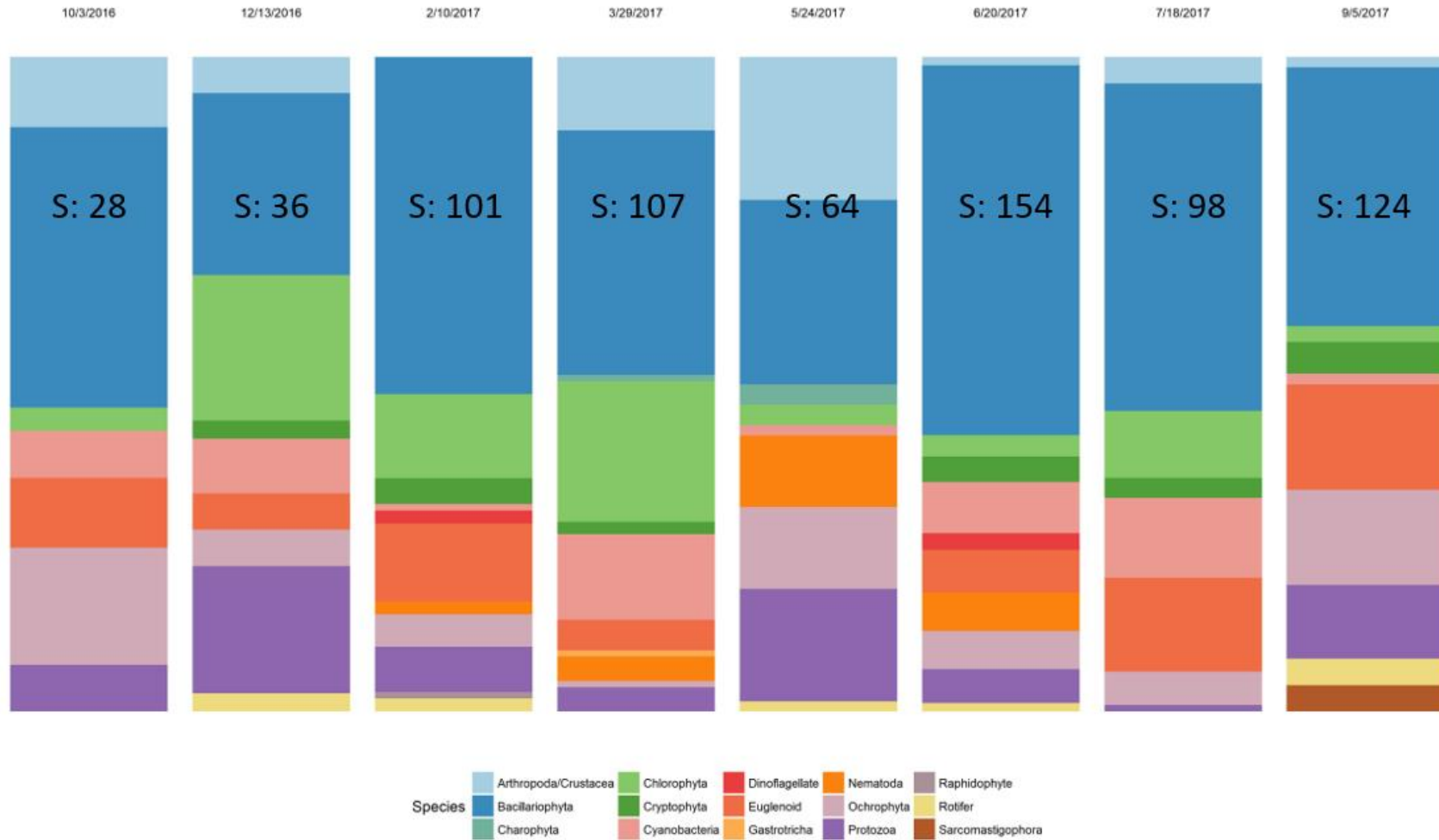


Figure 4.5: Microbiota species richness by month.

Microbiota Species Richness

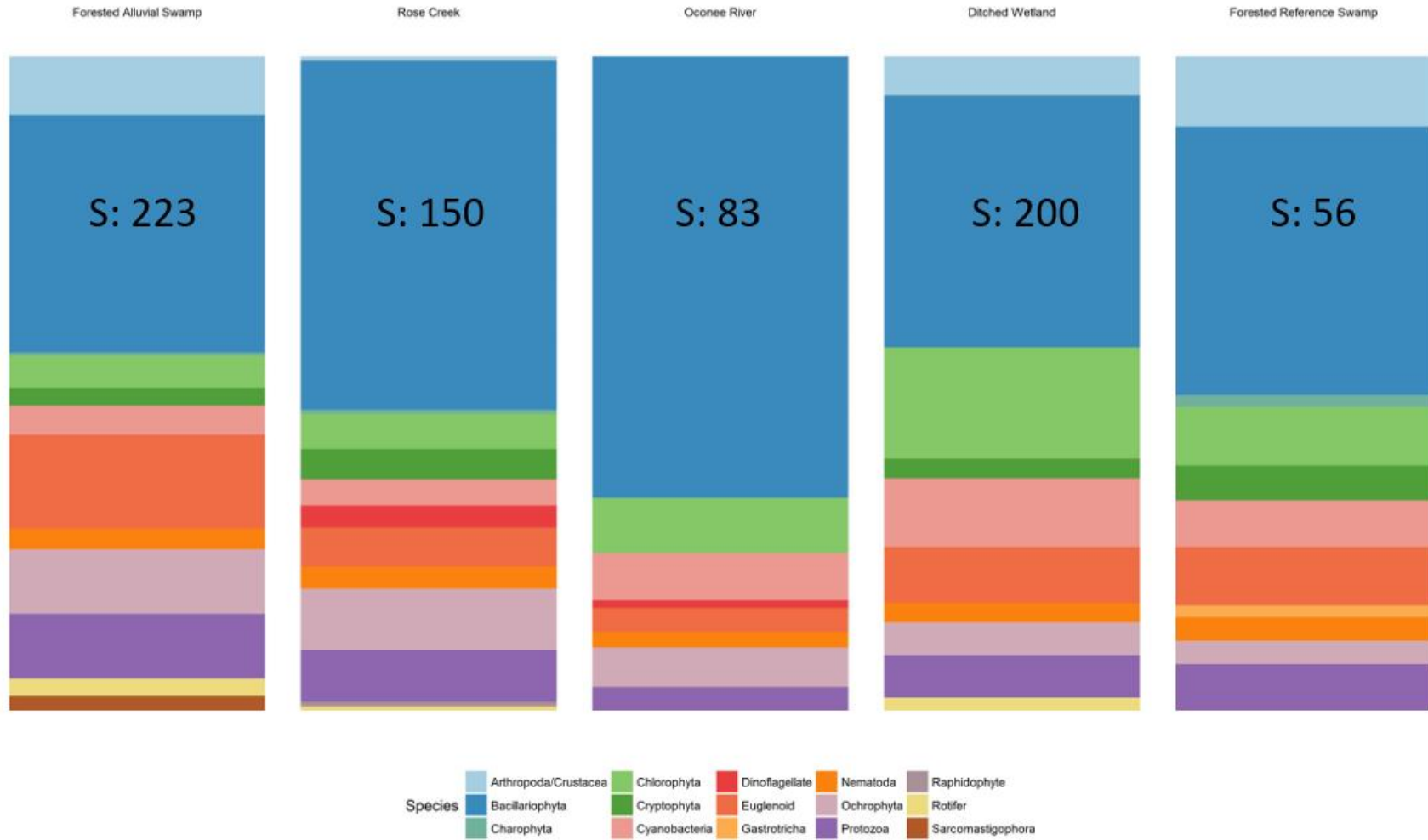


Figure 4.6: Microbiota species richness by sampling landscapes.

Table 4.2: Microbiota Species Identified at Iron Horse Farm.

	Common Name	phylum	Species
Autotrophs	Cyanobacteria	Cyanobacteria	<i>Oscillatoria, Anabaena, Microcystis, Anacystis, Nostoc, Phormidium, Leptolyngbya</i>
	Euglena	Euglenozoa	<i>Trachelomonas, Euglena, Phacus</i>
	Ochromonas	Ochrophyta	<i>Ochromonas, Synura, Gonyostomum</i>
	Cryptomonas	Cryptophyta	<i>Cryptomonas</i>
	Dinoflagellate	Miozoa	<i>Ceratium, Peridinium, Gymnodinium</i>
	Green Algae	Chlorophyta	<i>Chlamydomonas, Pandorina, Ulothrix, Scenedesmus, Spirogyra, Oedogonium, Pithophora, Volvox, Ankistrodesmus, Dunaliella</i>
	Green Algae	Charophyta	<i>Zygnema, Closterium, Mougeotia, Cosmarium, Staurastrum, Desmidiium</i>
	Diatom	Bacillariophyta	<i>Nitzschi, Navicula, Surirella, Melosira, Synedra, Fragilaria, Gyrosigma, Asterionella, Pinnularia, Gomphonema, Cymbella, Cyclotella, Achnanthes, Cymbella, Coscinodiscus, Tabellaria, pleurosigma</i>
	Plankton	Protozoa	Protozoa
Rotifer		Rotifer	<i>Bdelloid, Caratella</i>
Crustacean		Arthropoda/Crustacea	<i>Bosmina, Chydorus, Calanoid Copepod, Cyclopoid Copepod, Chironomid Larvae, Daphnia, Ostracod, Nauplii</i>
Gastrotrich		Gastrotricha	<i>Gastrotrich</i>
Sediment Feeders	Nematode	Nematoda	<i>Nematode Cyst</i>
	Amoeba	Sarcomastigophora	<i>Arcella, Giardia</i>

Table 4.3: Species, shapes, and equations used in biovolume calculations.

Common Name	phylum	Species	Shape in (Hillebrand et al 1999)	Shape Used in Current Study	Equation Used in Current Study	
Green Algae	Charophyta	<i>Spirogyra</i>	cylinder	cylinder	$V = \left(\frac{\pi}{4}\right) * Di^2 * H$	
Cyanobacteria	Cyanobacteria	<i>Leptolyngbya</i>	cylinder	cylinder		
Green Algae	Chlorophyta	<i>Oedogonium</i>	Not in (Hillebrand et al 1999)	cylinder		
Green Algae	Chlorophyta	<i>Pithophora</i>	Not in (Hillebrand et al 1999)	cylinder		
Green Algae	Chlorophyta	<i>Ulothrix</i>	cylinder	cylinder		
Green Algae	Charophyta	<i>Zygnema</i>	cylinder	cylinder		
Diatom	Bacillariophyta	<i>Melosira</i>	cylinder	cylinder		
Green Algae	Charophyta	<i>Mougeotia</i>	cylinder	cylinder		
Green Algae	Chlorophyta	<i>Microspora</i>	Not in (Hillebrand et al 1999)	cylinder		
Cyanobacteria	Cyanobacteria	<i>Oscillatoria</i>	cylinder	cylinder		
Cyanobacteria	Cyanobacteria	<i>Nostoc</i>	cylinder	cylinder		
Diatom	Bacillariophyta	<i>Aulacoseira</i>	cylinder	cylinder		
Green Algae	Charophyta	<i>Closterium</i>	2 cones	half elliptic prism		$V = \left(\frac{\pi}{4}\right) * L * W * D$
Euglena	Euglenazoa	<i>Euglena</i>	half ellipsoid + cone on elliptic base	half-elliptic prism+prism on elliptic base		$V = \left(\frac{\pi}{4}\right) * L * W * D$ $V = \left(\frac{\pi}{4}\right) * L * W * D$
Diatom	Bacillariophyta	<i>Achnanthes</i>	elliptic prism	prism on elliptic base	$V = \left(\frac{\pi}{4}\right) * L * W * D$	
Diatom	Bacillariophyta	<i>Navicula</i>	elliptic prism	prism on elliptic base		
Diatom	Bacillariophyta	<i>Surirella</i>	elliptic prism	prism on elliptic base		
Diatom	Bacillariophyta	<i>Pleurosigma</i>	prism on parallelogram	prism on elliptic base		
Phacus	Euglenazoa	<i>Phacus</i>	elliptic prism	prism on elliptic base		
Cryptomonas	Cryptophyta	<i>Cryptomonas</i>	prolate spheroid	prolate spheroid	$V = \left(\frac{\pi}{6}\right) * Di^2 * H$	
Trachelomonas	Euglenazoa	<i>Trachelomonas</i>	prolate spheroid	prolate spheroid		
Green Algae	Chlorophyta	<i>Scenedesmus</i>	prolate spheroid	prolate spheroid		
Diatom	Bacillariophyta	<i>Synedra</i>	box	rectangular box	$V = L * W * D$	
Diatom	Bacillariophyta	<i>Pinnularia</i>	box	rectangular box		
Golden-Brown Algae	Ochrophyta	<i>Ochromonas</i>	cone+half sphere	sphere	$V = \left(\frac{\pi}{6}\right) * Di^3$	
Diatom	Bacillariophyta	<i>Coscinodiscus</i>	cylinder	sphere		
L = Length		D = Depth (Same as Width)				
W = Width		Di = Diameter				

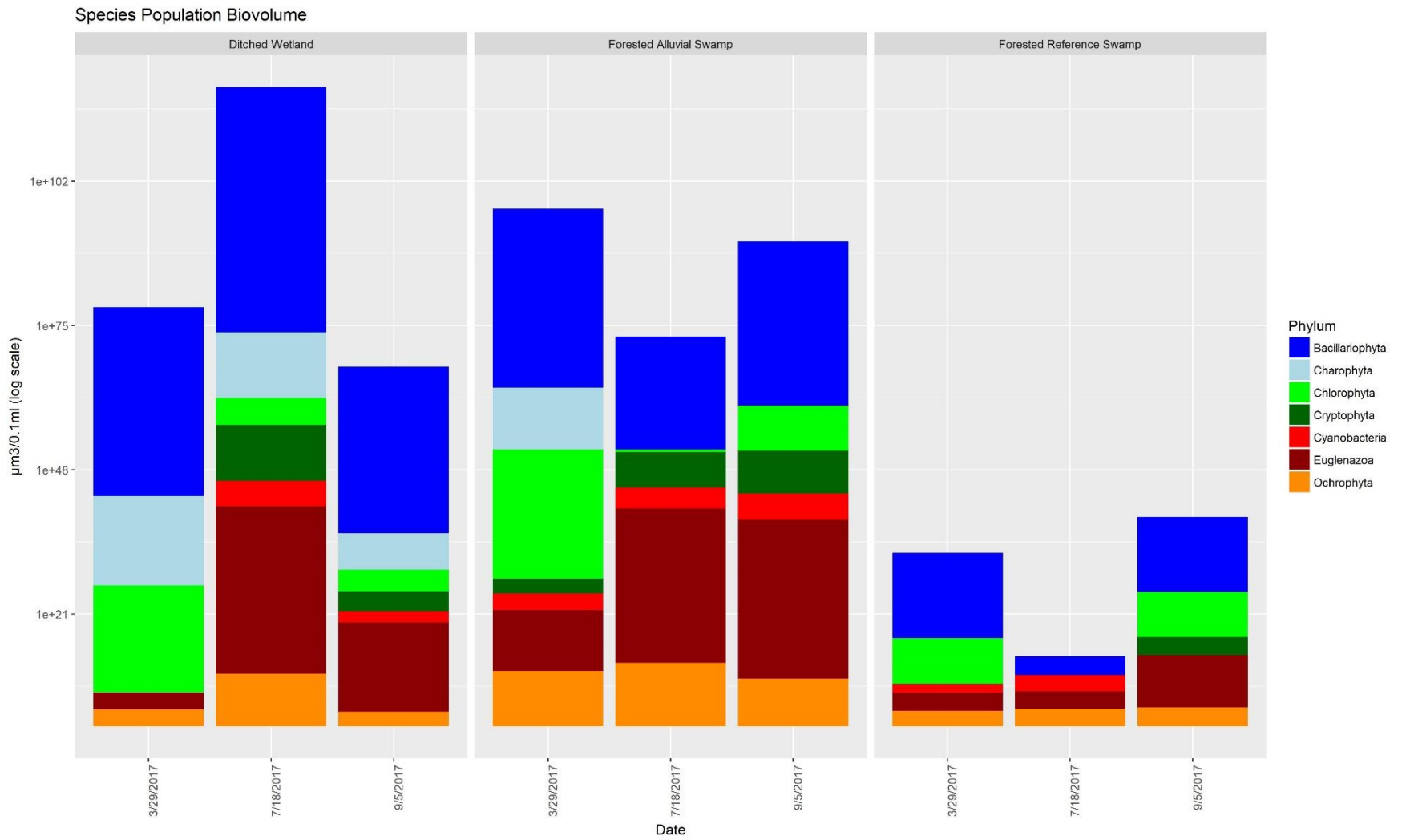


Figure 4.7: Biovolume of microbiota by sampling date and landscape.

Table 4.4: Nutrient bins for light and nutrient analysis.

	Nitrate + Nitrite (mg/l)	phosphorus (µg/l)
High	2.35-1.58	330-223
Medium	1.57-0.81	222-106
Low	0.80-0.04	105-10

Table 4.5: Biovolumes by light intensity, wetland type, and nutrient levels.

Nutrient Bin	Species Population Biovolume µm <sup>3</sup> /0.1ml	Wetland	Light	Leaf
High NO <sub>3</sub>	65,479,900,000,000.00	Ditched Wetland Swale	785	Leaf on
high P	65,479,900,000,000.00	Ditched Wetland Swale	785	Leaf on
	2,650.29	Forested Alluvial Swamp	785	Leaf on
	432,746.20	Forested Alluvial Swamp	470.1	Leaf off
Medium NO <sub>3</sub>	None available for assessment			
Medium P	19,198.76	Forested Alluvial Swamp	303.1	Leaf on
	5,135.80	Forested Alluvial Swamp	470.1	Leaf off
Low NO <sub>3</sub>	923,697,600,000.00	Ditched Wetland Swale	785	Leaf on
	8,661.56	Forested Alluvial Swamp	785	Leaf on
	6,528.84	Reference Swamp	785	Leaf on
	119,057.10	Ditched Wetland Swale	1233	Leaf off
	210,240.10	Forested Alluvial Swamp	1233	Leaf off
	11,569.30	Reference Swamp	1233	Leaf off
Low P	923,697,600,000.00	Ditched Wetland Swale	785	Leaf on
	5,378.21	Forested Alluvial Swamp	785	Leaf on
	6,528.84	Reference Swamp	785	Leaf on
	119,057.07	Ditched Wetland Swale	1233	Leaf off
	2,495.89	Forested Alluvial Swamp	1233	Leaf off
	11,569.30	Reference Swamp	1233	Leaf off

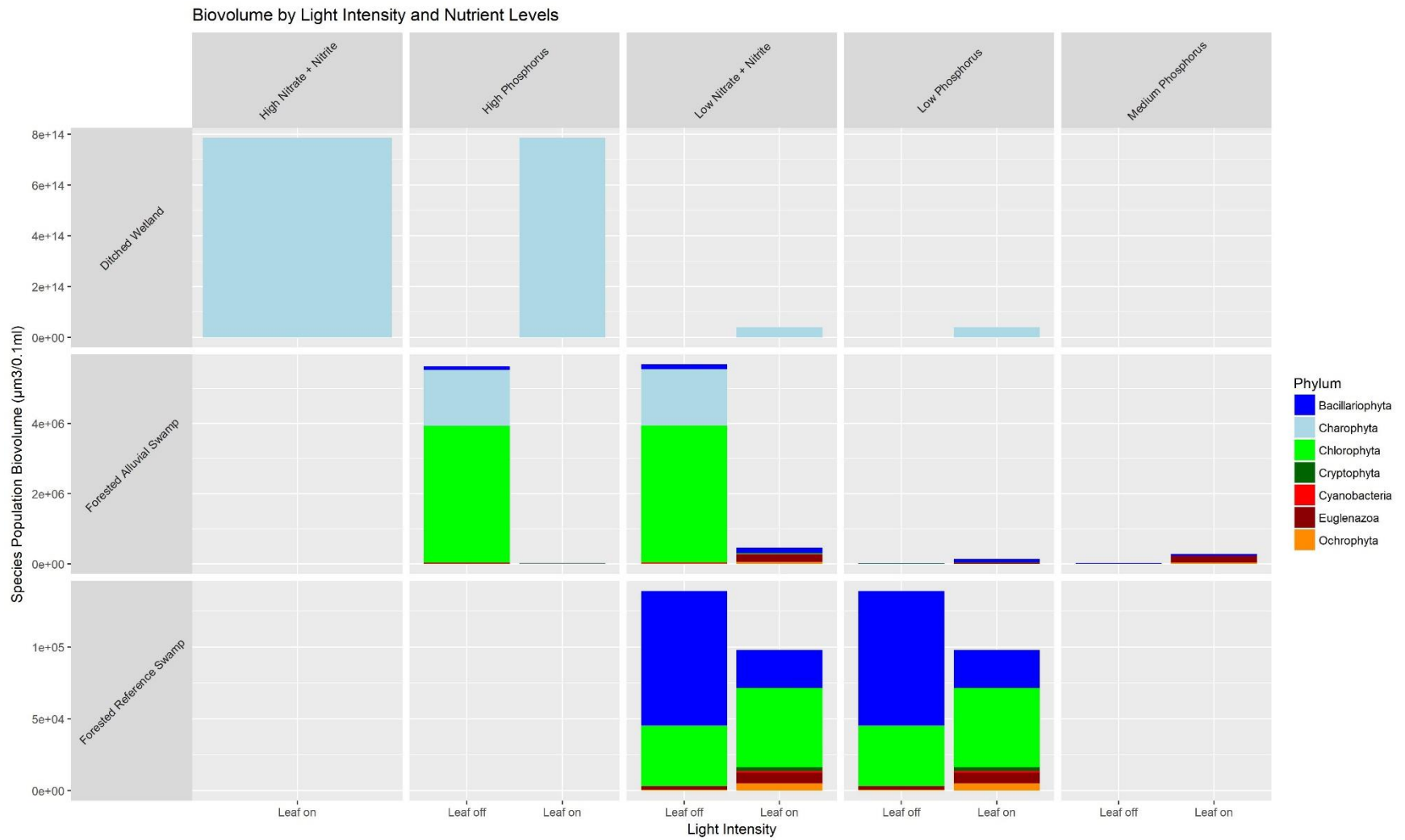


Figure 4.8: Biovolume of microbiota by light intensity, wetland type, and nutrient levels.

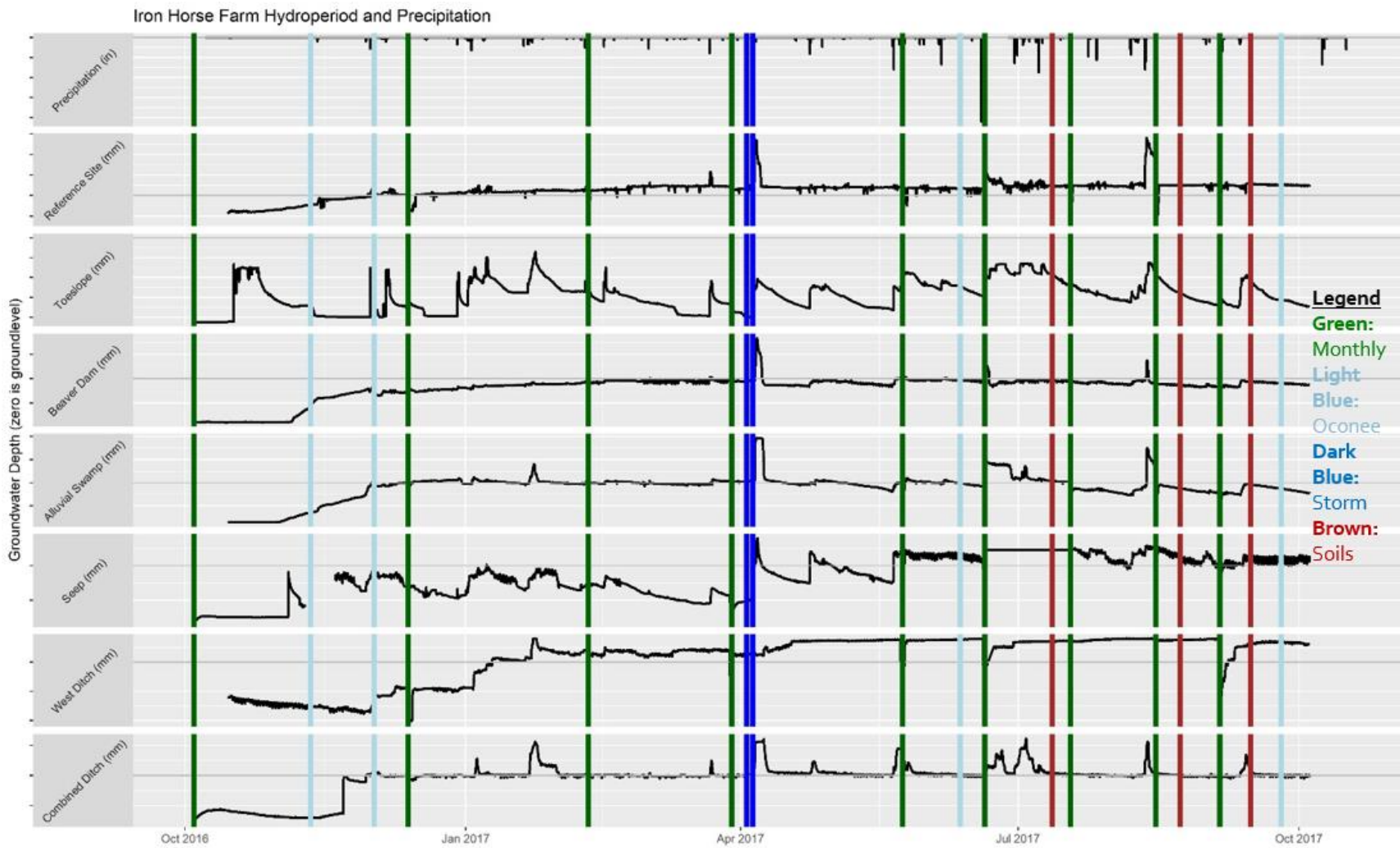


Figure 4.9: Iron Horse Farm hydroperiod and precipitation over the sampling period. Lines correspond to sampling events.

Microbiota Precipitation Relationship

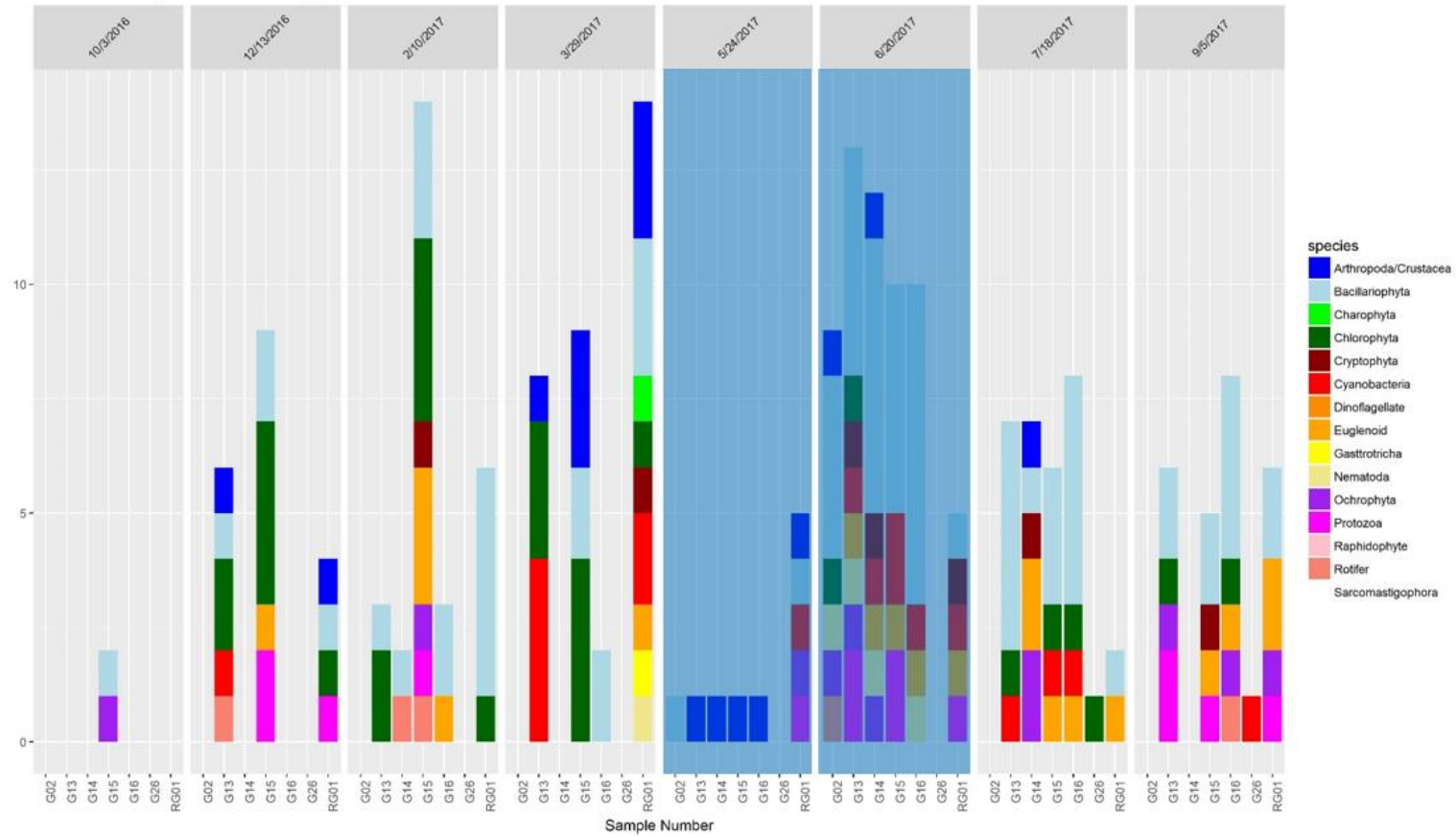


Figure 4.10: Microbiota in water by sampling event emphasizing storm events (blue shading), more species show up in June sampling event, but not May sampling event. Sample sites selected by those collected during April storm sampling.

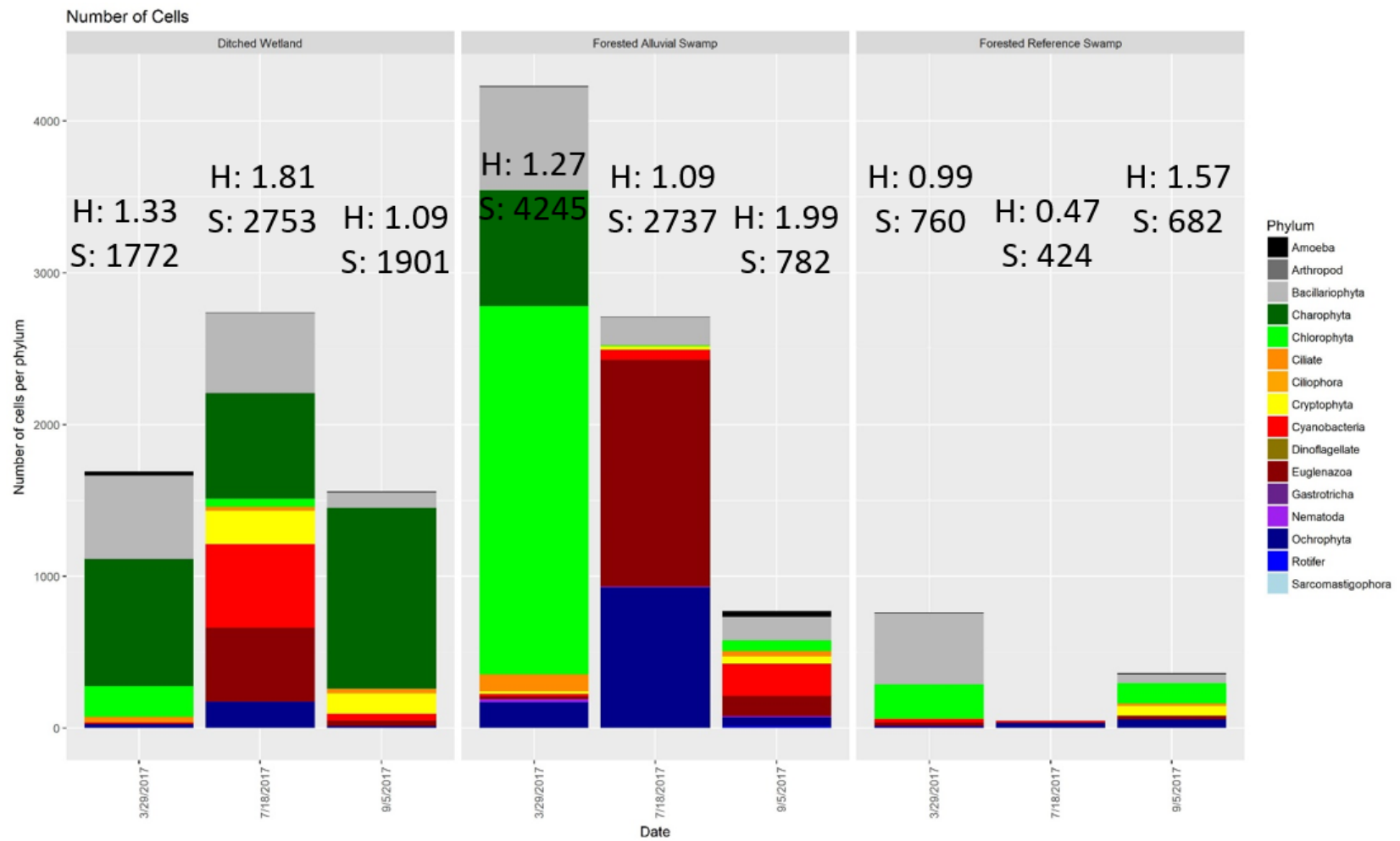


Figure 4.11: Cell counts of microbiota by date and landscape.

Appendix 4.1

Oconee Survey-Microbiota

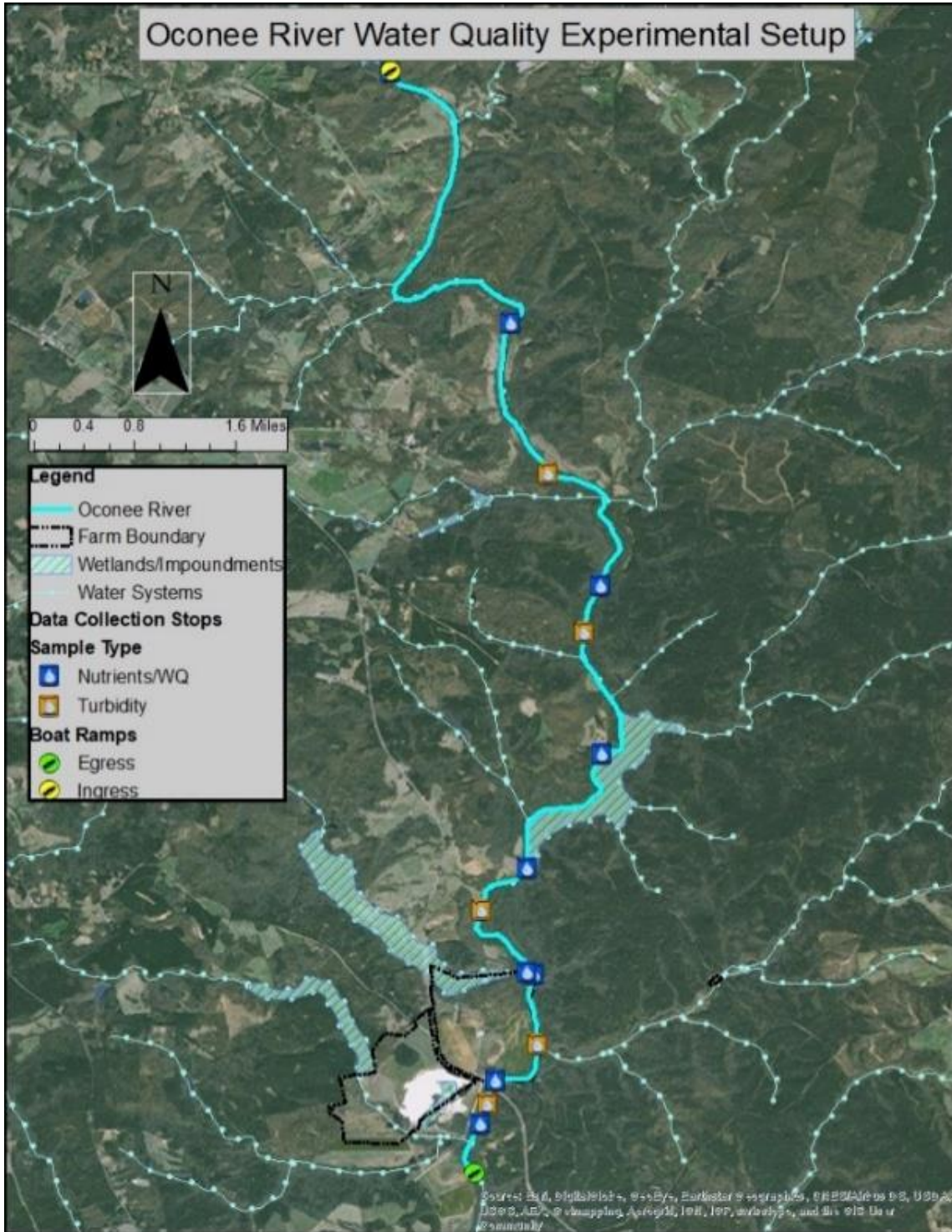


Figure 4.1.1: Oconee River survey setup.

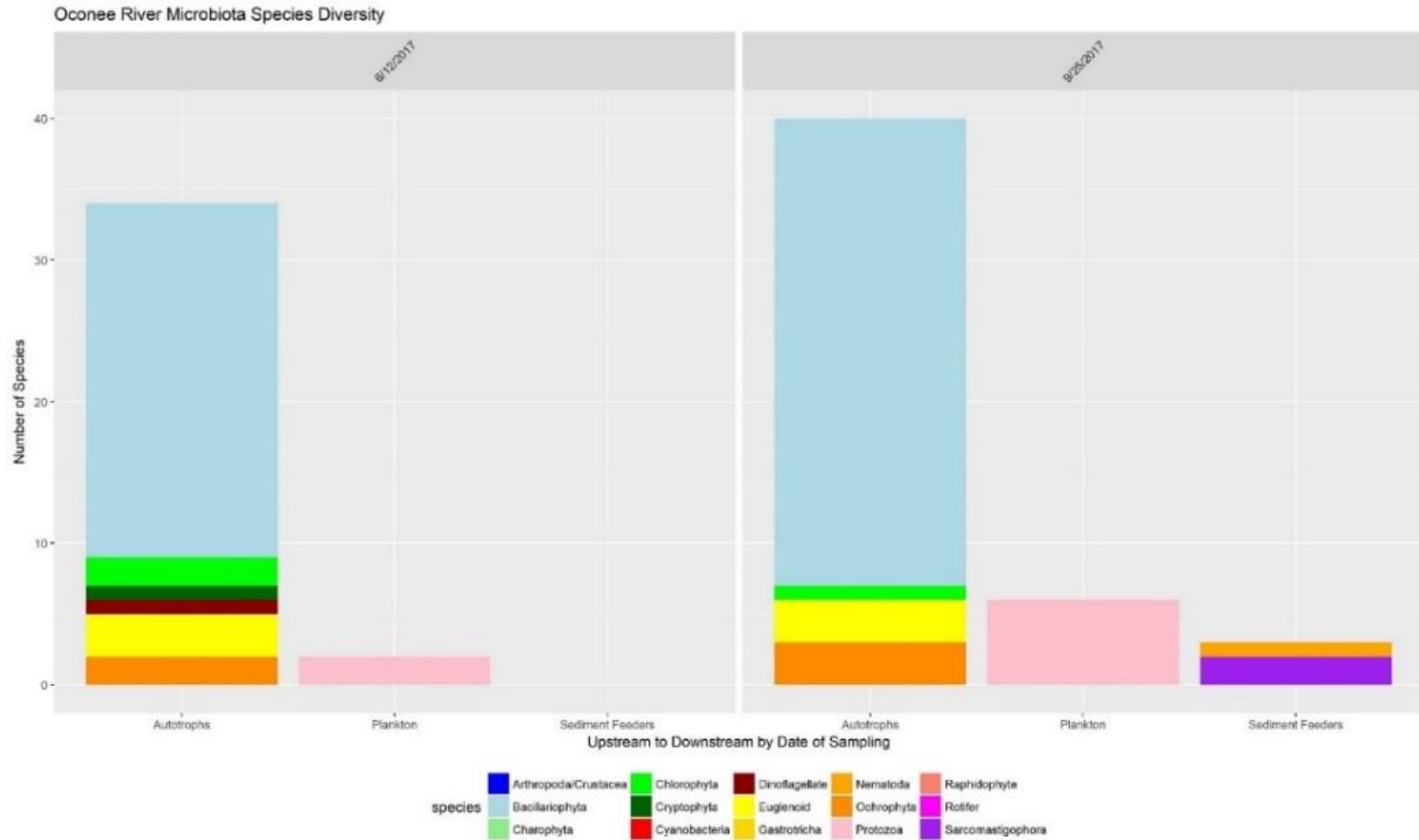


Figure 4.1.2: Species Richness in the Oconee River from the Barnett Shoals Dam to the Redland Sand mining site.

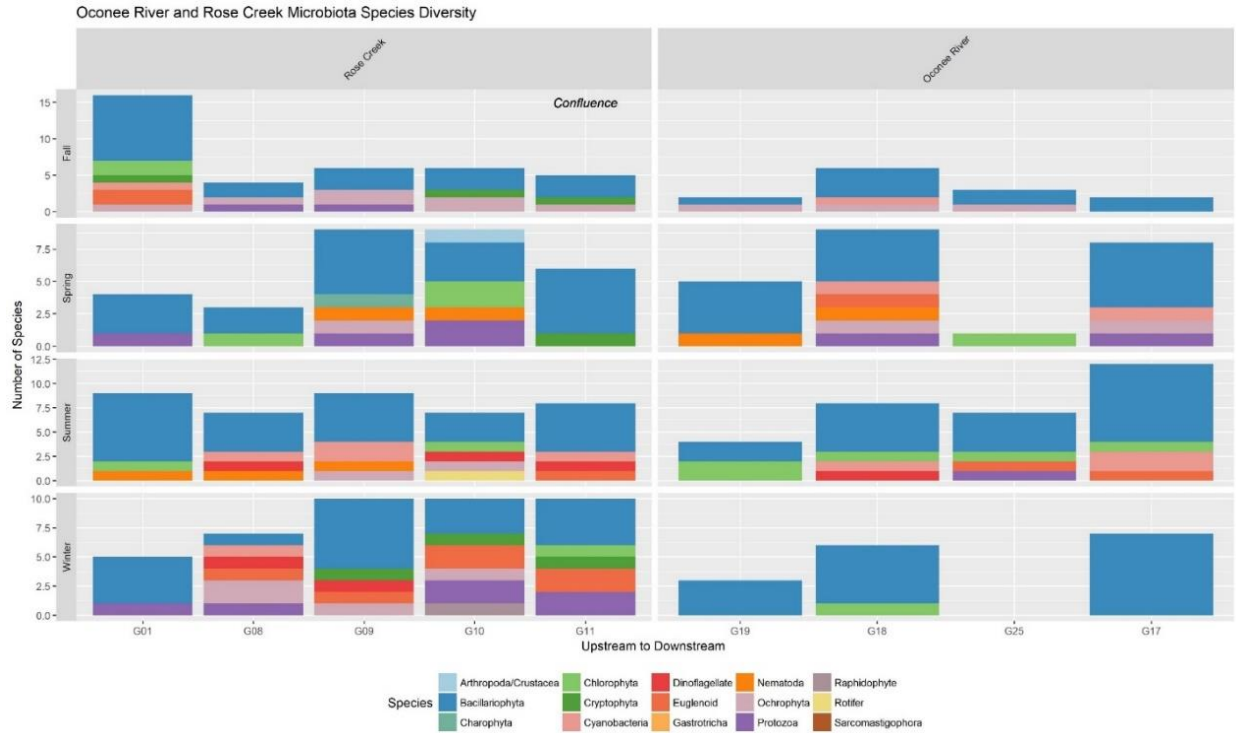


Figure 4.1.3: Species Richness in the Oconee River and Rose Creek around Iron Horse Farm.

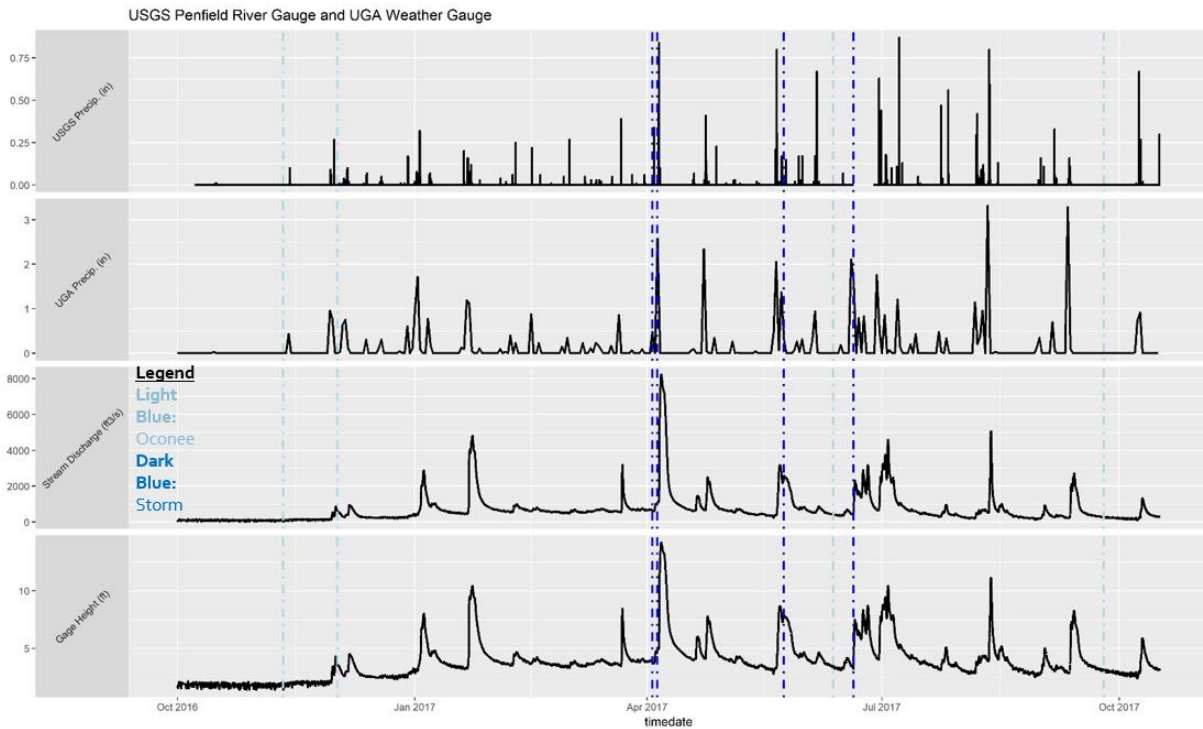


Figure 4.1.4: USGS Penfield River Gauge and UGA Oconee and weather Data during the sampling period.

## CHAPTER 5

### NUTRIENTS AND MICROBIOTA RESULTS AND CONCLUSIONS

#### Results

Nutrient regimes were significantly different between the forested alluvial swamp and the ditched wetland swale ( $\chi^2(4) = 60.23, P < 0.001$ ). Nitrate + nitrite and phosphorus both statistically decline as they move through the forested alluvial swamp ( $\chi^2(3) = 39.44, P < 0.001$ ) and ( $\chi^2(3) = 17.75, P = 0.0005$ ), but do not through the ditched wetland swale. Nitrate + nitrite and phosphorus both increase in samples taken during storms.

More species of microbiota were found in the alluvial swamp (Figure 4.6), but the biovolume of all these species was less than that found in the sunlit ditched wetland (Figure 4.7). Cyanobacteria is more likely to colonize the ditched wetland swale while more organic indicators were found in the forested alluvial swamp. Bacillariophyta are prevalent in all waters but more species prefer low nutrients where they do not have to compete with photosynthetic algae. Species shifts occur in wetlands and rivers after storm events.

#### Discussion

##### *Water quality and microbiological organisms in a forested alluvial swamp*

In the forested alluvial swamp at Iron Horse Farm, elevated nitrate levels in specific piezometers may indicate hotspots of denitrification (Starr and Gillham; Cey et al. 1999) in fluctuating areas of the water table (Cheschair et al. 1991). An inverse relationship between

nitrate and ammonium (Appendix 3.2, Figure 3.2.4) at each sample site exists because anoxic conditions allow ammonium to be converted to nitrate (Vymazal 1995; Simon 2002; Boon et al. 2014; Funk and Wagnalls 2017). Ammonium is nitrified to nitrate when oxic conditions allow it (Reddy and Patrick 1975; Boon et al. 2014) by microbes (Vymazal 1995; Funk and Wagnalls 2017). Elevated nitrate discharging into the alluvial swamp appears to have no effect on Rose Creek, but this probably changes during storms (Appendix 3.2 and Figure 3.10).

Some phosphorus is sequestered in the soils of the alluvial swamp but levels in the soil were low. There are plants in and around the swamp that could be substantial in mitigating phosphorus in the soils and water. The alluvial swamp does not seem to have an impact on phosphorus in Rose Creek but likely does during storms (Appendix 3.2 and Figure 3.10).

Cyanobacteria, potentially toxic algae detrimental to farms with livestock and to downstream habitats, thrive in areas of high nutrients, particularly nitrogen as they are nitrogen fixers (Power et al. 1988; Liess and Kahlert 2009). A positive correlation between cyanobacteria and phosphorus was found in this study (Figure 4.3, 4.4, and 4.5). Biovolume of photosynthetic algae in the alluvial swamp may be kept in check by trees shading the water, allowing increased diversity of other species. Bacillariophyta species diversity and richness is greatest in the alluvial swamp, but due to their small size do not make up large portions of the biovolume. High nutrients favor many of the filamentous algal species, but shade inhibits them, so more bacillariophyta can colonize. Higher richness of turbidity indicators are in the agricultural wetlands and Rose Creek than in the reference wetland or Oconee River. This indicates that Rose Creek water is heavily supplied by upstream agricultural wetlands, there is

less sediment accumulating in the forested reference hillslope, and the Oconee River probably has more sediment at the bottom of the water column. Most samples taken from the Oconee River were very clear when compared to how turbid the water looks in the river. Indicators of organic pollution display more species richness in the forested alluvial swamp, which is probably from organic runoff into the alluvial swamp from the upslope retired feedlot and row-crop field.

Shaded samples allow more species to proliferate, thereby keeping the filamentous green algae which operate by photosynthesis in check. Once this sunlight restriction is lifted, photosynthetic organisms become very abundant. Not only did this occur in the ditched wetland swale, but also in the forested alluvial swamp during leaf-on compared to leaf-off light conditions (Figure 4.8). In both agricultural wetlands, filamentous green algae needed high phosphorus levels to increase their biovolume as well.

Bacillariophyta can be used as an indirect indicator that wetlands and creeks have mixed (Figure 4.10). If more cells are observed in a creek than normal, it is probably due to the inclusion of an alluvial wetland in the water flow. Organic indicators were found in Rose Creek during a storm event but were not observed during dry weather. Turbidity indicator species richness in the alluvial swamp and Rose Creek matched up during storm events.

#### *Water quality and microbiological organisms in a ditched wetland swale*

Ammonium in the ditched wetland is converted to nitrate due to oxic conditions created by anthropogenic drainage (Kadlec 1962; Reddy and Patrick 1975; Scholz et al. 2002; Boon et al.

2014). Since nitrate levels do not change throughout the wetland and ditch it is probably stored in the water and released during storm events.

The ditches are not sequestering phosphorus, only the west ditch is significantly lower than the groundwater seep and this ditch does not receive water from the seep. Groundwater intrusion as indicated by an Odyssey probe in the ditches piezometer, is responsible for dilution of phosphorus levels in the west ditch (Figure 3.3, *West Ditch*). High levels of phosphorus in the ditched complex runoff into the Oconee River during minor flooding and storm events (Appendix 3.3, Figure 3.3.3). Phosphorus levels have improved in the Oconee River since 1996, this change is likely from sample location or upstream improvements of nutrient catchments (Figure 3.12).

The ditched wetland swale is ideal habitat for species of microbiota which increase their growth output with increased light. During biovolume assessment in the ditched wetland, only Charophyta was found, but it achieved the highest biovolume of the sampling period (Figure 4.8). This large Charophyta bloom occurred during leaf on, indicating light intensity is not a limiting factor in this wetland. This bloom did not occur in samples which had low phosphorus, and low nitrate + nitrite values. Cyanobacteria species were seen in both agricultural wetlands and the reference wetland, but the most species observed occurred in the ditched wetland swale (Figure 4.6). Wetlands with high nutrients which are exposed to intense sunlight are more likely to be ideal habitat for these potentially toxic algae. There were more species of bacillariophyta (Figure 4.6) in the alluvial swamp but the biovolume was greater in the wetland swale (Figure 4.7). Bacillariophyta biovolume did best where phosphorus was low, and nitrate +

nitrite was low. The likely relationship is that sunlight allows photosynthetic algae to grow, but nitrate + nitrite and phosphorus dictate how much biovolume is produced. Lower nutrients allow other microorganisms to make up the species richness observed. A similar relationship is seen in the reference swamp, green algae grow but are not able to reach the biovolume achieved in the ditched wetland swale.

### Conclusions

The forested alluvial swamp which receives runoff from Iron Horse Farm is more efficient in processing nitrate + nitrite and phosphorus than a ditched wetland swale on the same site. Both lowland areas received similar nutrient inputs, but the alluvial swamp also received additions from a retired feedlot. The wetland swale would likely be an efficient landscape feature for nutrient catchment and mitigation if its previous hydrology and vegetation were restored.

Since increased processing of nutrients in the alluvial swamp lowers nutrient levels, the biovolume of filamentous green algae and species diversity and richness of cyanobacteria are lower. Along with nutrients, shading from larger plants allows for many non-photosynthetic algal species to thrive. Also preventing potentially toxic blooms of cyanobacteria on a farm landscape. Light regimes and nutrients play a key role on the biovolume, richness, and species diversity of microbiological species.

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