Impacts of Feral Pig Rooting Disturbance on Water Quality in Depression Marshes of West Central Florida

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Table of contents

List of Tables

List of Figures

List of Appendices **page**

Abstract: Feral pigs (*Sus scrofa*) can cause widespread impacts in wetland habitats through rooting and soil disturbance when foraging. Other authors have noted accelerated plant decomposition while some have suggested rooting leads to greater turbidity, lower dissolved oxygen, and increased concentrations of various forms of nitrogen and phosphorus. The objective of this study was to determine if feral pig rooting impacted nitrogen and phosphorus fluxes from soil to the overlying water column on undisturbed vs. recently disturbed depression marsh sites. I hypothesized that rooting disturbance would result in higher nutrient flux than that from undisturbed sites and that mounded soil (Mound Treatment) resulting from pig rooting disturbance would have higher nutrient flux due to soil mixing and aeration as compared to nearby ground soils (Ground Treatment) and soils with no visible evidence of recent disturbance (Undisturbed Reference). Using intact soil cores and observing nutrient flux over a period of 7 days, I found that both types of disturbance (Mound and Ground treatments) had greater fluxes of total phosphorus (TP), soluble reactive phosphorus (SRP), total Kjeldahl nitrogen (TKN), ammonium-nitrogen (NH_4-N), and nitrate + nitrite-nitrogen (NO_x-N) than Undisturbed Reference cores. For all nutrient parameters the Mound treatment yielded higher flux rates than the Ground treatment and the Ground treatment fluxes were higher than the Undisturbed Reference. These differences were significant for both Mound and Ground treatments for NH_4-N and Mound treatment only for SRP and TKN as compared to Undisturbed Reference cores. The Mound treatment flux was significantly higher than the Ground treatment flux for TKN only.

Total phosphorus flux rate in the Mound treatment averaged 2.71 $\pm$ 2.24 mg m^{-2} day^{-1} compared to the Undisturbed Reference (0.56 $\pm$ 0.26 mg m^{-2} day^{-1}) resulting in 4.8 times greater flux. Mound treatment had significantly higher average flux for SRP (2.87 $\pm$ 2.57 mg m^{-2} day^{-1}) vs. the Undisturbed Reference which was nearly identical to TP flux (0.57 $\pm$ 0.25 mg m^{-2} day^{-1}) (p=0.04). For TKN the Mound treatment flux (28.19 $\pm$ 3.80 mg m^{-2} day^{-1}) was 2.1 times greater than the Ground treatment (13.45 $\pm$ 3.14 mg m^{-2} day^{-1}) (p=0.034) and 4.3 times greater than the Undisturbed Reference flux (6.51 $\pm$ 1.04 mg m^{-2} day^{-1}) (p = 0.0018). NH_4-N flux showed the greatest response to rooting disturbance and was 26.5 times greater than that of the Undisturbed Reference (20.69 $\pm$ 3.77 mg m^{-2} day^{-1} vs. -0.78 $\pm$ 1.50 mg m^{-2} day^{-1}) (p=0.0001) while the Ground treatment had 13 times greater flux than the Undisturbed Reference (p=0.036). All treatments served as sinks for NO_x-N by the end of the 7-day sampling period. Ground (-1.63 $\pm$ 1.29 mg m^{-2} day^{-1}) and Mound (-0.21 $\pm$ 5.36 mg m^{-2} day^{-1}) treatment fluxes were higher than the Undisturbed Reference (-6.01 $\pm$ 0.27 mg m^{-2} day^{-1}) but were not significant at the p=0.05 level for NO_x-N. Total nitrogen (TN) flux was calculated by adding values for TKN + NO_x-N resulting in the Mound treatment flux being nearly 2.4 times greater than the Ground treatment and nearly 38 times greater than the Undisturbed Reference. Substantial differences in fluxes of phosphorus and nitrogen parameters between pig-rooted

(disturbance) treatments and undisturbed sites could have considerable implications for water quality.

Introduction

Feral pigs are becoming increasingly problematic in numerous countries on every continent with the exception of Antarctica. The southeastern United States is no exception where feral pig activity has implications from agricultural crop damage to environmental and human health concerns. Feral pigs, also known as feral swine or wild boar, are native to Eurasia and North Africa (Pimentel et al. 2005). Spanish explorers are credited with bringing swine to Florida in the 1500s (Giuliano 2010). Feral pigs are found in all 67 Florida counties and are established in 36 states throughout the United States, including Hawaii. The successful expansion of this exotic species is due to a high fecundity rate, translocation by humans, and adaptation to a wide variety of foods and habitats (J. Corn, pers. comm., Fogarty 2007). Ironically, wild boar has been close to extinction in its native range in Eurasia since the beginning of the last century (Wirthner et al. 2012).

Feral pigs are omnivorous and forage for a major part of their diet by grubbing or rooting in soil to obtain plant seeds, roots and bulbs, and vertebrate and invertebrate animals (Ditchkoff and Mayer 2009, Gimenez-Anaya et al. 2008, Seward et al. 2004). There is concern that rooting and wallowing behavior can lead to declining water quality in streams and wetlands through increased turbidity and sedimentation, soil erosion, disruption of nutrient cycling, and alteration of native upland and wetland plant and animal communities (Fogarty 2007, Kotanen 1995, Singer et al. 1984, Dunkell et al. 2011).

Several studies have looked at impacts of feral pig rooting disturbance on nutrient concentrations and nutrient cycling in upland grassland and forested ecosystems. Nutrient turnover rates in disturbed areas may be affected by increased nutrient release due to enhanced decomposition and/or decreased nutrient uptake by plants. Bueno et al. (2013) found that both occurrence and intensity of rooting strongly influenced nitrate-N (NO_3-N) and ammonium-N (NH_4-N) concentrations in alpine grasslands. Nitrogen (N) concentrations were significantly higher in disturbed (rooted) plots as compared to undisturbed plots while phosphorus (P) concentrations were more community-dependent than treatment-dependent. Sims (2005) compared impacts of feral pig rooting on grasslands and woodlands and found that soil NO_3-N and NH_4-N concentrations were significantly greater in rooted vs. unrooted plots in grasslands. Nitrate-N concentrations were also higher in rooted plots in woodlands. She found that there was significantly greater belowground plant biomass in non-rooted vs. rooted plots that could affect uptake rates of nutrients.

Cuevas et al. (2012), working in the Monte Desert of Argentina, found less compaction during the wet and dry season in rooted plots as compared to unrooted plots. Mineral N, consisting of both nitrate + nitrite (NO_x-N) and NH_4-N, was higher in disturbed plots during the dry season due to significantly higher NO_x-N content. Total N and NH_4-N were not significantly different between treatments. Carbon to nitrogen ratios (C:N) were significantly higher in disturbed plots compared to undisturbed plots for both wet and dry seasons. Conversely, C:N ratios were lower in disturbed plots than undisturbed plots in a mixed pine-hardwood forest in Texas (Siemann et al. 2009).

Wirthner et al. (2012) found that rooting in a Swiss hardwood forest led to significant increases in total C and N soil concentrations that did not translate into more plant available N on rooted areas. These authors presented three potential arguments for the lack of plant available N (NH_4-N and NO_3-N) as follows: a) N removal was due to plant uptake-although they did not detect higher levels of plant growth in disturbed plots; b) immobilization of N by microbes that may relate to higher microbial biomass C found in disturbed plots; and c) loss by leaching and erosion due to a reduction in the understory herbaceous layer with a delay in nutrient uptake. Alternatively, Groot Bruinderink and Hazebroek (1996) and Mohr et al. (2005) working in deciduous/coniferous forests of the Netherlands and low mountain oak forests of Germany, respectively, did not find any impacts of pig rooting on soil pH, organic C or N, nor C:N ratios.

At least two studies have looked at impacts of rooting on soil chemical factors in deciduous forests of the Great Smoky Mountains, USA. Singer et al. (1984) noted that rooting accelerated decomposition and loss of nutrients from the forest floor. They found higher NO_3-N and NH_4-N concentrations in soils of rooted stands while P was significantly lower. It was also noted that calcium, magnesium, manganese, zinc, copper and hydrogen and cation exchange capacity (CEC) were significantly lower in rooted stands. Organic matter was not different among treatments. Three years post-rooting, they found an increase in P with no differences for N or NH_4-N on previously rooted areas. Lacki and Lancia (1983) found that organic matter, CEC, and acidity increased on pig-rooted sites while base saturation declined. Increases in acidity did not impact soil pH due to apparent sufficient buffering. They found P values were highest in unrooted controls followed by lightly rooted areas then heavily rooted sites but differences were not statistically significant.

Extent of rooting can be both considerable and quite variable. Felix et al. (2014) measured the spatial extent of feral pig disturbance in over 8,000 rooting polygons in herbaceous seepage slopes, wet flatwoods, and wet prairie over a four-year period on Avon Park Air Force Range in south central Florida. Average rooting polygon area was 25.44 m^2 (n=8,035 polygons) but ranged from 0.00023 m^2 to 4,335 m^2. The majority of polygons measured were less than 5 m^2

with a few cases of polygons reaching over 1,000 m^2. Furthermore, feral pigs often repeatedly root in some plant communities, such as seepage slopes, with long-term implications for biodiversity and community dynamics (Engeman et al. 2007, Brown and Miller 2012). Felix et al. (2014) conducted surveys during the "middle-dry season" (MDS-November through January) and the "late-dry season" (LDS-April through May). They found that the amount of re-rooting between any two MDS seasons averaged 1,719 $\pm$ 940 m^2 with the amount of rooting between any three MDS seasons averaging 373 $\pm$ 193 m^2. Late-dry season surveys showed 2,561 $\pm$ 2,780 m^2 and 361 $\pm$ 263 m^2 for any two seasons or three seasons, respectively.

Some studies have noted relationships between pig rooting disturbance and changes in plant nutrient content. Krull et al. (2013), working in temperate rainforest in New Zealand, found significantly higher plant-available NO_x-N in pig-disturbed areas compared to areas protected from disturbance using ion exchange resin bags. No significant effects of disturbance on ammonium or phosphorus levels were detected in their study but they noted that changes in nutrients such as NO_3-N could lead to changes in plant species composition. They also suggested that pigs may be preferentially disturbing areas with higher soil nitrates. Palacio et al. (2013) studied impacts of wild boar disturbance on geophytes in subalpine grasslands and noted a significant effect between rooting and plant-N concentrations. Disturbance in their study did not result in higher soil nutrient concentrations; however, they concluded that increased plant nutrient value could lead to repeated foraging by wild boar.

Boughton and Boughton (2014) reported on a long-term plant species composition study looking at effects of fire and nutrient addition on a wet prairie community in Florida. Ten years into the study feral pigs breached the fence protecting their site and rooted over half of the study plots; pigs preferentially rooted in N-addition plots and they surmised that N fertilization resulted in a higher percentage of N in plant tissue leading to higher protein content that pigs may seek out. Redroot (*Lachnanthes caroliniana*), a rhizomatous species, had been present in their plots prior to rooting; however, they saw an average increase in redroot cover of 40% resulting in near monocultures on 92% of the area disturbed by pigs. In fact, Boughton and Boughton (2014) suggested a positive feedback mechanism with pig rooting leading to an expansion of redroot and thus perpetuation of an attractive food source.

Other studies conducted in the southern U.S. have discussed impacts of feral pig disturbance on wetland vegetation and plant communities (Arrington et al. 1999, Engeman et al. 2003, Chavarria et al. 2007, Zengel and Conner 2008) but very few studies have been published concerning impacts of rooting on nutrients in wetland systems. Singer et al. (1984) looked at nutrient impacts on aquatic systems in the Great Smoky Mountains National Park, USA. Heavy disturbance by feral pigs in one watershed resulted in significantly higher levels of NO_3-N, Kjeldahl-N and potassium in soil water than a watershed that had no feral pigs present. Similarly they found NO_3-N levels in stream water to be twice as high as those from the undisturbed watershed. Tannourji (2009) reported higher ammonium content in soils of vernal pools impacted by pig disturbance in California.

Doupe et al. (2010) and Doupe et al. (2009) conducted studies in freshwater floodplain lagoons in Australia where they examined feral pig impacts on aquatic macrophytes and water quality. They used open water/bare ground as a proxy for pig rooting disturbance and found significantly higher dissolved oxygen levels in the fenced (protected) treatment as compared to the unfenced (unprotected) treatment. Turbidity was significantly higher in the unfenced treatment and increased over time. Doupe et al. (2009) sampled water quality parameters *in situ* and found large, but not significant, increases in total, dissolved and particulate concentrations of N and P over time in one pair of lagoons. They attributed this to aquatic macrophyte destruction and consumption by pigs and excretion of wastes. Ford and Grace (1998) looked at coastal marshes and noted that removal of aboveground plant biomass by herbivores (e.g. nutria and feral pigs) can lead to increased light penetration thus affecting soil temperatures and ultimately decomposition rates. They felt increased decomposition could have considerable effects on high organic content of wetland soils.

As noted in the brief review of previously published studies above, feral pigs have been shown to affect soil and plant nutrient concentrations and nutrient cycling in upland and wetland systems. However, no studies were found that looked at the effects of feral pig rooting disturbance on nitrogen or phosphorus fluxes in depression marshes. This study investigated the potential impacts that feral pigs have on the flux of nitrogen and phosphorus resulting from soil disturbance in depression marsh systems. Specifically, I wanted to address whether pig rooting disturbance caused an increase in N and P water quality parameters in terms of flux as compared to field control (Undisturbed Reference) sites. I also hypothesized that mounds of soil (Mound treatment) resulting from pig rooting would have higher nutrient flux due to greater soil mixing and aeration as compared to ground locations (Ground treatment) that served as the source of soil material for mounds.

I employed an intact soil core method whereby soil cores were collected in the field and taken to the laboratory for flooding. Core tubes were to remain flooded for a period of 7 days to represent the maximum estimated P release from soil to overlying water as per Dunne et al. (2010) as other studies have indicated that rate of P release is typically greatest for the initial flooding period (Fisher and Reddy 2001, Malecki et al. 2004). For nitrogen, Malecki et al. (2004) found that NH_4-N concentrations under aerobic conditions dropped rapidly between Days 0 and 5 then remained relatively stable. They felt this was due to the immediate nitrification of NH_4-

N to NO_x-N. Thus we felt a 7-day period was adequate to capture peak flux of nutrients examined. Core tube floodwater was sampled at several intervals over the 7-day period. Floodwater samples were collected on Days 1 and 7 to assess flux of total phosphorus (TP) and total Kjeldahl nitrogen (TKN). Floodwater samples were collected on Days 1, 2, 5, and 7 to assess flux of soluble reactive phosphorus (SRP), ammonium-nitrogen (NH_4-N) and nitrate/nitrite-nitrogen (NO_x-N).

Methods

Study Sites

The study was conducted in the Western Valley physiographic province of the Coastal Plain of Florida. The Western Valley is composed of a low, irregular valley resulting from the erosion of soluble sediments with elevations ranging from 75 to 120 feet above sea level (NGVD 29) (Spechler and Kroening 2006). The karst terrain has numerous sinkholes due to dissolution of underlying limestone (Sinclair et al. 1985, Nilsson et al. 2013). Climate is classified as humid subtropical with hot, wet summers and mild, fairly dry winters. Mean annual temperature is 73.0^{o} F (22.8^{o}C) with mean annual rainfall of 52.84 inches (1342 mm). Rainfall is unevenly distributed with about 60% of annual precipitation occurring from June through September. April and November are often the driest months of the year (Spechler and Kroening 2006, Southeast Regional Climate Center 2010).

Three depression marshes, ranging in size from 0.4 to 1.6 ha in area, were selected for study at the Upper Hillsborough Preserve. This public conservation land, managed by the Southwest Florida Water Management District, is located in northwestern Polk and southeastern Pasco counties in west-central Florida (Figure 1). The marshes are classified as Palustrine-Emergent-Persistent-Semipermanently Flooded (Wetlands A4 and A19) or Palustrine-Emergent-Persistent-Seasonally Flooded (Wetland A16) under the U.S. Fish and Wildlife Service's Wetlands and Deepwater Habitats Classification System (Cowardin et al. 1979). All three sites are recognized as Depression Marshes under the Florida Natural Areas Inventory (FNAI) classification system. According to county soil surveys, soil series are as follows: the Pomona Series (Wetland A4 and a portion of Wetland A16) is classified as a sandy, siliceous, hyperthermic Ultic Alaquod with 0-2% slope. The Palmetto series (Wetland A19 and a majority of Wetland A16) is classified as a loamy, siliceous, subactive, hyperthermic Grossarenic Paleaquult with small areas of very poorly drained Zephyr and Sellers soils. Slope is less than 2% (U.S. Dept. of Agriculture-Natural Resources Conservation Service 2014). Depression marshes are embedded in an upland matrix of open mesic pine flatwoods. At the time of sampling, areal extent of rooting ranged from less than 15% in Wetland A19 where disturbance was concentrated along wetland edges to approximately 85% rooting coverage in Wetland A16 (C. Gates, pers. obs.).

Depression marshes can serve as important recharge sites. A 7-year study showed that geographically isolated wetlands in west-central Florida tended to serve as groundwater recharge zones for at least 50% of the time (Nilsson et al. 2013). Depression marshes are fire-dependent communities and upland habitats around each marsh had been prescription burned three to four years prior to the study. Wetland A16 was burned in 2009 (summer) and Wetlands A4 (summer) and A19 (late winter) were burned in 2010. However, for each of the three wetlands, the last fire prior to sampling did not burn into the marshes due to inundation. It is likely that, prior to our study, more than 7 years had elapsed since the marshes have been directly affected by fire (C. Gates, pers. obs.).

At the time of core sampling, the most common and readily identifiable plant species included chalky bluestem (*Andropogon glomeratus* var. *glaucopsis*), red root (*Lachnanthes caroliniana*), maidencane (*Panicum hemitomon*), and ten angle pipewort (*Eriocaulon decangulare*). Undisturbed Reference core sites tended to have a greater diversity of plants or were dominated by chalky bluestem. Mound and Ground disturbance treatment core sites were dominated by bare ground, scattered to occasionally numerous redroot plants, occasional *Eupatorium* sp., and decomposing chalky bluestem (C. Gates, pers. obs.) (Figure 2a).

No quantitative plant surveys were conducted during my study. However, in addition to observations at the time of soil core sampling, a qualitative assessment of soil core sites was made approximately 2.5 months post-sampling on July 5, 2013. At this time wetlands were inundated, plants were actively growing and species were easier to identify. In addition to species observed during the April sampling period other species observed in one or more study wetlands included false fennel (*Eupatorium leptophyllum*), sandweed (*Hypericum fasciculatum*), fascicled beaksedge (*Rhynchospora fascicularis*), yellow hatpins (*Syngonanthus flavidulus*), yellow milkwort (*Polygala rugellii*), Elliott's yellow-eyed grass (*Xyris elliottii*), Baldwin's spikerush (*Eleocharis baldwinii*), thin paspalum (*Paspalum setaceum*), arrowhead (*Sagittaria* sp.), camphorweed (*Pluchea* sp.), meadow beauty (*Rhexia* sp.), flattop goldenrod (*Euthamia caroliniana*), primrose willow (*Ludwigia* sp.), flatsedge (*Cyperus* sp.), marsh mermaidweed (*Proserpinaca palustris*), and witchgrass (*Dichanthelium* sp.) (C.Gates and K. Gruenhagen, pers. obs.). The University of South Florida's Atlas of Florida Vascular Plants (2015) was used for common name conventions.

Field Collection

Selection of depression marshes to sample was based on similarity of hydrologic zone of rooting disturbance (generally within 10-12 m of wetland edge) as well as the severity (moderate rooting disturbance to a depth of 10-20 cm) and estimated time-since-damage (4-6 months) using a categorical ranking system developed by the U.S. Dept. of Agriculture-Wildlife Services Division (2009).

Once depression marshes were selected, polygons within each marsh were demarcated with Geographic Positioning System (GPS) using a Trimble ™ model XT. Soil core sampling sites were selected by randomly choosing a Point of Origin within a disturbance polygon and then identifying a sampling point for the mound treatment and the ground treatment. Mound treatments were identified as areas where soils and litter had been pushed into a mound as a result of rooting activity. Ground treatments were identified as areas near mounds where soil and litter had been removed and translocated to the mound (Figure 2b). Mound sampling sites were identified by randomly selecting a cardinal direction (north, east, south or west) and a random distance, working at 30 cm intervals within 3 meters of the Point of Origin. This point was marked as M1 for that wetland. The ground treatment sampling site was located by randomly selecting a cardinal direction and distance (10 cm increments up to 1 meter) relative to the M1 location. This area was marked as G1. An additional set of soil samples was collected in unrooted areas (Undisturbed Reference sites) within the polygons that were at a similar elevation to that of the disturbed sites. These sites were selected using a random cardinal direction and random distance (one meter increments up to 10 meters) from that replicate's point of origin. In four cases, I was limited in the number of cardinal directions available to stay within the 10-meter distance from the point of origin and to ensure Undisturbed Reference sites were within similar hydrologic zones. Rough approximations of mound height ranged from 5 to 23 cm with an average height of 10 cm. Figures 3, 4, and 5 show disturbance polygons and soil core sampling points for Wetlands A4, A16, and A19, respectively. Appendix A shows the distance from each Undisturbed Reference site to its corresponding Mound and Ground site within each replicate for each wetland.

All wetlands were dry at the time of sampling on April 20, 2013. Samples were collected between 0900 and 1430 hours with temperatures ranging from 20 to 24°C, relative humidity ranging from 53 to 68%, light winds and partly sunny to cloudy skies. Estimated rainfall totaling approximately 1.3 to 1.9 cm had fallen within 72 hours prior to sample collection (Southeast River Forecasting Center 2013).

Intact soil cores were taken to a depth of 20 cm using 7-cm inside diameter polycarbonate tubes. To minimize compaction, a bread knife was used to cut soil around the outside of the core tube. Any litter, standing dead material, and/or live vegetation was left intact in the core tube to simulate field conditions (Figure 6). The top and bottom of each tube was capped and all core tubes were immediately taken to the laboratory following core collection.

<u>Core Flux Experimental Set Up and Water Sample Collection</u>

Harvested rainwater was used to inundate the intact core tubes since this source best represented the type of water that would likely inundate the natural marsh. A collection receptacle was constructed using boards and Visqueen™ plastic (4 mil thickness). A chance rainfall event occurred on March 11, 2013. Once the rain had stopped, collected water was immediately transferred to 5-gallon (18.9 l) plastic buckets. During the rainfall event pollen from nearby pine trees also fell into the rain collection receptacle so rainwater was filtered through clean, undyed knit cloth and stored in the plastic buckets. Periodically, remaining pollen collected at the water's surface near the outer edge of each bucket and was skimmed off to reduce potential for organic material buildup that could affect study results.

Buckets were aerated using an aquarium pump and tubing and placed in a dark room to reduce the likelihood of biofilm development or stagnation until the experiment was initiated. Temperatures during storage ranged from 17 to 23^{o}C. Following field collection of soil cores and just prior to soil core tube flooding, rainwater was transferred to a 30-gallon (113.6 l) clean plastic garbage can for mixing and storage of rainwater for the duration of the experiment.

All 27 field-collected treatment tubes, as well as three Floodwater-Only Control tubes, were randomly placed in a large tub, with top caps removed, and allowed to set for 24 hours prior to flooding with harvested rainwater. Bottom caps were left in place to prevent air and water leakage. On Day 0, floodwater was initially added just to the point of soil saturation. Then soils were flooded with a volume of rainwater to an equivalent overlying floodwater depth of 25 cm (i.e. floodwater was added to a height of 25 cm above each soil core surface). Care was exercised to avoid resuspension of soil or litter material during flooding. Three empty soil core tubes were capped on the bottom and filled with floodwater to the same total height (45 cm) as the soil core tubes to serve as Floodwater-Only Controls. Only samples from Floodwater-Only Controls were taken on Day 0 to be tested for all parameters.

Tap water was added to the tub holding the flooded core tubes to reduce any hydraulic head gradient between the inside and the outside of the core tubes to minimize potential seepage and to moderate temperature changes (Dunne et al. 2010). Core tubes were stored in a dark room to prevent exposure to light to reduce the potential for algae development. The water bath temperature ranged from 23 to 25^{o}C over the course of the 7-day experiment. In order to prevent stratification and maintain a well-mixed water column, air bubbles were slowly released into the water column using air lines attached to a small gauge hypodermic needle (after Pant and Reddy 2003). Each needle was inserted to a depth of approximately 5 cm from the top of the water column of each tube for the duration of the experiment (Figure 7).

Overlying floodwater samples were collected using a 60-ml cartridge syringe with Tygon™ tubing. Tubing attached to the syringe was placed at a depth of ~ 10 cm below the water surface to collect water samples. Each treatment (Floodwater-Only Control, Undisturbed Reference, Ground Treatment and Mound Treatment) had its own cartridge syringe and tubing to prevent the potential for contamination of water samples between treatments. Water samples were analyzed for the following parameters: total phosphorus (TP), soluble reactive phosphorus (SRP), total Kjeldahl nitrogen (TKN), ammonium-nitrogen (NH_4-N), and nitrate+nitrite-nitrogen (NO_x-N). On Days 1 and 7 when all parameters were to be assessed a second draw had to be taken to obtain enough water for analysis. For the Floodwater Control, samples were drawn on Days 0, 1, 2, 5 and 7 for all parameters. Day 0 concentrations for Floodwater Controls were assumed to be the same for all samples within each parameter. Water samples for NH_4-N, NO_x-N, and SRP were drawn from all treatment core tubes on Days 1, 2, 5, and 7. TKN and TP samples were drawn only on Days 1 and 7 to bracket the organic component. After each sampling, an equivalent volume of harvested rainwater was added into core tubes to replace the volume removed for sampling (Pant and Reddy 2003).

As water samples were collected, the appropriate volume was placed into properly marked 20 ml scintillation vials. NH_4-N, NO_x-N, and SRP samples were filtered using 0.45 um disc filters; TKN and TP samples were not filtered. All samples were acidified to pH<2 using concentrated H_2SO_4 as a preservative except for SRP samples. As samples were collected, vials were placed in a refrigerator and kept at a temperature of 4 ± 1^{o}C. At the end of the experimental period, samples were transported on wet ice to the Analytical Research Laboratory at the University of Florida in Gainesville for analysis. Nutrient concentrations were determined colorimetrically using standard U. S. Environmental Protection Agency (USEPA) methods as follows: for TP and SRP-EPA 365.1, for TKN-EPA 351.2, for NH_4-N-EPA 350.1 and for NO_x-N-EPA 353.2 (USEPA 1993).

Based on concentrations derived from laboratory analysis, average and cumulative flux for each analyte were calculated using Excel ™ spreadsheets. Release rates for all parameters were measured following the methods of Pant and Reddy (2003) and Dunne et al. (2010).

Statistical Analyses

A nested, randomized block design was used and blocked for wetland effect. Data were entered into an Excel ™ spreadsheet and nutrient fluxes were estimated by calculating difference in concentration between time 0 and 7 days for each parameter over time as per Dunne et al. (2010). Flux values were adjusted for rainwater contribution (Floodwater) for each parameter. Results were then analyzed using JMP, release 8.0.2 (Statistical Analysis System, Cary, NC) to look at differences in flux rates among Undisturbed Reference, Ground and Mound treatments. Statistically significant differences were determined at the $p < 0.05$ level. One-way analysis of variance (ANOVA) was used to compare treatment means. Where ANOVA detected significant differences the post-hoc the Tukey-Kramer means comparison test was used.

Results

Phosphorus Parameters

Concentration values for TP and SRP are presented in ug L^{-1} while flux values are expressed in mg m^{-2}. The Mound treatment resulted in higher flux than the Undisturbed Reference with the Ground treatment flux levels intermediate for both TP and SRP.

TP concentrations increased substantially for the Ground and Mound treatments between Day 1 and Day 7 (Figure 8). There was very little difference in concentration for the Undisturbed Reference for Days 1 and 7 (29.94 $\pm$ 3.46 ug L^{-1}, and 32.32 $\pm$ 8.30 ug L^{-1}, respectively). Ground concentration doubled (36.57 $\pm$ 0.85 ug L^{-1} and 72.71 $\pm$ 10.85 ug L^{-1}, respectively) and Mound concentration increased 2.7 times between Day 1 and Day 7 (33.74 $\pm$ 5.76 ug L^{-1} and 90.46 $\pm$ 59.76 ug L^{-1}, respectively).

TP was measured only on Days 1 and 7. The flux value for the Undisturbed Reference was much lower for the period Day 2-7 (0.62 $\pm$ 1.36 mg m^{-1}) than on Day 1 (3.30 $\pm$ 0.98 mg m^{-1}) (Figure 9). Ground flux nearly doubled between Day 0-1 and Day 2-7 (5.18 $\pm$ 0.24 and 9.40 $\pm$ 2.85 mg m^{-1}, respectively) while Mound flux was 3.3 times higher on Day 7 (14.59 $\pm$ 14.20 mg m^{-1}) than on Day 1 (4.38 $\pm$ 1.62 mg m^{-1}).

In terms of average TP flux (mg m^{-2} day^{-1}) Mound treatment flux was 4.8 times greater than the Undisturbed Reference flux and the Ground treatment had 3.7 times higher flux than the Undisturbed Reference (Table 1). An outlier occurred for TP from Mound treatment sample A19 M2 resulting in a lack of significance at alpha=0.05. I could not state conclusively that the outlier did not fall within the sample population. The decision was made to retain the outlier to fully represent all data and to retain the ability to block for wetland effect. Thus figures referenced in this section reflect retention of the outlier. However, Appendix B includes a table showing ANOVA and Tukey-Kramer significance levels for all parameters as well as analysis results with and without outliers for TP, SRP, and NO_x-N.

In spite of the high variability associated with the Mound treatment outlier, Tukey-Kramer means comparison resulted in a difference between the Mound vs. Undisturbed Reference treatment at p=0.0666. ANOVA showed a treatment effect at p=0.0718 with wetland effect of p=0.1790. When the Mound outlier was pulled from analysis the Ground treatment was significantly higher for average flux than the Undisturbed Reference (p=0.0471) (Appendix B).

For SRP, peak concentration values were reached at Day 7. Initial concentrations for all treatments were between 3.5 and 8 ug L^{-1} and followed similar curves over the course of the experiment. Peak concentrations on Day 7 averaged 16.93 $\pm$ 8.75 ug L^{-1}, 44.51 $\pm$ 15.05 ug L^{-1}, and 77.31 ± 65.94 ug L^{-1} for the Undisturbed Reference, Ground and Mound treatments, respectively (Figure 10).

All treatments resulted in positive flux of SRP from soil to water column. Normalized SRP flux curves by treatment are shown in Figure 11a. Figure 11b shows best fit curves and R^2 values based on a 2nd order polynomial. Normalized flux values rose steadily through Day 7 for the Undisturbed Reference while both Ground and Mound treatment flux rose steeply between Day 1 and Day 2. The Mound treatment then leveled off while the Ground treatment declined after Day 2. An aberrant value that appears to have been a measurement error for sample A19 UR2 on Day 5 was removed for graphing purposes to provide a more accurate depiction of the Undisturbed Reference curve. This value does not affect the analysis for average flux discussed below.

Outliers occurred from site A19 M2 (the same site that resulted in an outlier for TP analysis) as well as from site A4 UR2. As with the TP analysis, the decision was made to retain the outliers to fully represent all data and to retain the ability to block for wetland effect. Figures referenced in this section reflect retention of the outliers. However, it is interesting to note that the p value (0.08) was higher (i.e. lower significance) with the Mound and Control outliers removed (Appendix B).

Tukey-Kramer means comparison showed that the Mound treatment had significantly higher average flux than the Undisturbed Reference (p=0.04) for SRP. The Mound treatment (mean of 2.87 mg m^{-2} day^{-1} with a range of 0.30 to 5.44 mg m^{-2} day^{-1}) had 5 times greater flux than the Undisturbed Reference (0.57 mg m^{-2} day^{-1} with a range of 0.26 to 0.88 mg m^{-2} day^{-1}). The Ground treatment flux was 2.8 times greater than that of the Undisturbed Reference (Table 1).

It is interesting to note that ANOVA showed treatment effect was significant (p=0.05) with a near significant wetland effect of p=0.0670 for SRP. Indeed a comparison of means by wetland showed that wetland A16 consistently had lower SRP values across treatments compared to wetlands A4 and A19. Qualitative visual and tactile observations of soils at the time of sampling indicated that wetland A16 seemed to have higher soil moisture and more organic matter. If so, this may partially explain the lower flux values for SRP if wetter conditions resulted in more organic matter which would lead to higher retention of P; however, organic matter was not measured directly.

Overall flux values for TP and SRP were very similar for the Undisturbed Reference indicating that SRP contributed nearly 100% to the TP flux for that treatment while contributing ~77% of TP for the Ground treatment. The Mound treatment SRP mean was 6% higher than the TP mean and is likely an artifact of sampling but still indicates that the majority of the TP value was contributed by SRP. In terms of concentration, Day 1 concentration ratios of SRP to TP ranged from 13 to 20%; however, Day 7 concentration ratios showed SRP contribution to TP of 45, 61, and 71 %, respectively, for the Undisturbed Reference, Ground, and Mound treatments.

Day 0 analyses for TP and SRP were only run for Floodwater Controls so it is assumed that all treatments started at the Floodwater average value for Day 0. TP Floodwater Control concentrations dropped approximately 28% between Day 0 and Day 7 while SRP concentrations fluctuated slightly and ended up approximately 20% higher on Day 7 as compared to Day 1 (Appendix C).

Nitrogen Parameters

The Mound treatment yielded higher flux values for all nitrogen parameters as compared to the Ground and Undisturbed Reference treatments. Mound treatment flux was significantly greater ($p<0.05$) than the Undisturbed Reference for all measured parameters except for NO_x-N. The Ground treatment was significantly different from the Undisturbed Reference for NH_4-N.

Mean TKN concentrations were higher on Day 7 than on Day 1 for all flooded core samples. The Undisturbed Reference showed a slight rise in TKN concentration between Days 1 and 7 (0.40 $\pm$0.18 mg L^{-1} and 0.53 $\pm$0.04 mg L^{-1}, respectively). Ground concentrations rose from 0.53 to 0.71 mg L^{-1} and Mound concentrations rose more steeply from 0.61 to 1.10 mg L^{-1} for an increase of 1.8 times between Days 1 and 7 (Figure 12).

TKN flux for the Undisturbed Reference increased nearly 3-fold between Days 1 and 7, Ground treatment flux dropped slightly, and Mound flux increased 1.8 times (Figure 13).

TKN results showed that the Mound treatment average flux was 4.3 times greater than the Undisturbed Reference ($p = 0.0018$) and 2.1 times greater than the Ground treatment ($p = 0.034$). Mean flux for the Mound treatment was 28.19 mg m^{-2} day^{-1} and ranged from 24.39 to 31.99 mg m^{-2} day^{-1}. The Ground treatment mean flux was 13.45 mg m^{-2} day^{-1} with a range of 10.31 to 16.59 mg m^{-2} day^{-1} while the Undisturbed Reference had mean flux of 6.51 mg m^{-2} day^{-1} with a range of 5.47 to 7.55 mg m^{-2} day^{-1} (Table 2).

Generally, NH_4-N concentrations for the Undisturbed Reference stayed relatively flat while the Ground and Mound treatment concentrations increased slowly through Day 5; all treatment concentrations then rose sharply by Day 7. The Mound treatment showed the steepest rise from 0.13 mg L^{-1} on Day 1 to 0.78 mg L^{-1} on Day 7 with a 6-fold increase in concentration. Ground treatment concentrations rose 4-fold over the 7-day period while the Undisturbed Reference concentration increased 2.6 times (Figure 14).

Ammonium-N showed a similar response to that of TKN flux results but with greater magnitude in differences among treatments. The Mound treatment had significantly higher flux than Undisturbed Reference treatment (26.5 times, $p = 0.0001$). The Ground treatment flux was also significantly greater than the Undisturbed Reference (13 times, $p = 0.036$) (Table 2). Looking at NH_4-N flux over the sampling period, normalized flux curves for all treatments show sharp increases in flux for Days 1-2 and Days 5-7 while the curve is flatter between Days 2-5 (Figure 15a). Figure 15b shows flux with best fit lines using a 2nd order polynomial and R^2 values for all treatment curves.

For NO_x-N, all Undisturbed Reference values trended steadily downward while Ground and Mound treatment values fluctuated before ending up with lowest concentrations on Day 7 (Figure 16). Interestingly, all treatments had negative fluxes over the 7-day duration of the experiment; thus all served as sinks for NO_x-N.

Normalized NO_x-N flux curves are shown in Figure 17a. Day by day flux rates showed similar trends to those of concentration with a steady increase in flux for the Undisturbed Reference until Day 5 when the rate dropped to a level slightly above the Day 1 flux. The Ground treatment went steeply from a negative to a positive flux value between Days 1 and 2 ending with a negative flux value of -8.74 mg m^{-2} on Day 7 that fell below its Day 1 value. Mound flux was erratic as shown by the R^2 value of 0.3222 for a second order polynomial (Figure 17b). Mound flux started at a high positive value of 19.13 mg m^{-2} with a standard deviation of $\pm$ 20.33 on Day 1 with a rapid decline to -10.71 mg m^{-2} on Day 2. Flux rebounded somewhat resulting in a positive value on Day 5. On Day 7, all treatment values reflected negative flux between -7.89 and -9.25 mg m^{-2}.

NO_x-N analysis revealed an outlier from site A16 M2. Concentrations for this soil core for days 2, 5 and 7 were at least 3 times greater and mean flux for the sampling period was 7.5 times greater than the average value for other Mound treatment soil cores indicating an anomaly within the core itself (i.e. not a laboratory sampling error). It is possible that this core site was a "hotspot" potentially due to pig or other animal urine or excrement at that location. The outlier was retained for analysis in order to represent all data and to retain the ability to block for wetland effect. In spite of the outlier, Mound treatment average flux (-0.21 $\pm$ 5.36 mg m^{-2} day^{-1}) resulted in a near significant difference ($p=0.0587$) compared to the Undisturbed Reference (-6.01 $\pm$ 0.27 mg m^{-2} day^{-1}) (Table 2). Interestingly, with the outlier removed, both Ground and Mound treatment flux was significantly higher than the Undisturbed Reference flux ($p < 0.0001$ and $p = 0.0019$, respectively) although trendline using a 2nd order polynomial resulted in very poor fit (R^2 of 0.0251) for Mound treatment flux (Appendix B).

TKN is a measure of organic-N plus NH_4-N. For the Ground and Mound treatments, respectively, 77.5% and 73.4% of the TKN flux was attributable to NH_4-N. The Undisturbed

Reference, in contrast, had a negative flux value for NH_4-N and contributed little to the TKN value (-12%). A comparison of Day 1 and Day 7 concentration ratios of NH_4-N:TKN showed Day 1 proportions of 20-22% for all treatments. By Day 7, NH_4-N made up 42, 76, and 69% of TKN for the Undisturbed Reference, Ground and Mound treatments, respectively.

Day 0 analyses for TKN, NH_4-N, and NO_x-N were only run for Floodwater Controls so it is assumed that all treatments started at this average value for Day 0 (Appendix C). Floodwater Control values for NH_4-N were a bit of an anomaly as the Floodwater started out on Day 0 with a concentration of 0.23 mg L^{-1} then dropped to values very similar to the Undisturbed Reference (~0.09 mg L^{-1}) for Days 1, 2, and 5. Between Day 5 and Day 7, all treatments, including the Floodwater Control, increased fairly steeply with Floodwater trending back up to a concentration of 0.26 on Day 7. Conversely, TKN showed an increase in concentration for the Floodwater Control between Day 0 and Day 1 with values returning to Day 0 levels by Day 7. It is possible that a sampling or laboratory analysis error occurred. It is conceivable that some of the pollen that accompanied rainfall into the rainwater collector was not removed and thus contributed some NH_4-N. However, with the contribution of NH_4-N to TKN, TKN concentrations would have been expected to follow a similar trend and drop between Day 0 and Day 1. NO_x-N Floodwater Control values remained even at 0.34 to 0.35 mg L^{-1} throughout the sampling period.

Total nitrogen (TN) is the total of TKN (which includes organic N and NH_4-N) plus NO_x-N. Figure 18 shows a comparison of Day 1 and Day 7 concentrations of TN by treatment. Day 1 and Day 7 TN concentrations for the Undisturbed Reference were very similar at 0.69 $\pm$ 0.24 and 0.70 $\pm$ 0.04 mg L^{-1}, respectively. Ground treatment values were 0.83 $\pm$ 0.09 and 0.99 $\pm$ 0.11 mg L^{-1}, respectively, for Days 1 and 7. The Mound treatment had the highest TN concentrations; Day 1 concentration was 1.00 $\pm$ 0.10 mg L^{-1} and Day 7 concentration was 1.41 $\pm$ 0.18 mg L^{-1}. Thus Mound treatment concentration was twice that of the Undisturbed Reference on Day 7.

In terms of flux, the Undisturbed Reference had similar values for both Day 0-1 and Day 2-7 with high variability (2.49 $\pm$ 66.37 and 2.69 $\pm$ 69.05 mg m^{-2}, respectively). Ground treatment flux values were similar between the two periods while Mound treatment flux was highest among treatments for both periods (89.01 $\pm$ 27.49 and 105.93 $\pm$ 27.90 mg m^{-2} for Day 0-1 and Day 2-7, respectively). Mound treatment flux was 39 times greater than flux for the Undisturbed Reference for Day 2-7 (Figure 20). In terms of average flux (mg m^{-2} day^{-1}), mean Mound treatment flux was nearly 38 times greater than that of the Undisturbed Reference and 2.4 times greater than that of the Ground treatment (Table 2).

Appendix B provides Tukey-Kramer means comparison and ANOVA significance values for all parameters including TP, SRP, and NO_x-N significance values with outliers removed.

Discussion

Reddy and DeLaune (2008) define flux as “the rate of transfer of solutes between soil and overlying water column and from one physical or chemical state to another”. Flux of nutrients in this study is likely attributable to diffusive processes where the flux of solutes diffusing through the medium is proportional to the concentration gradient as per Fick’s first law.

The central focus of my study was to determine if feral pig rooting affected flux of nutrients from depression marsh soils. Clearly disturbance, as defined by Mound and Ground treatments, resulted in higher relative flux for all N and P parameters compared to the Undisturbed Reference. Differences in average flux among nitrogen parameters were more significant statistically than differences for phosphorus parameters.

The Mound treatment was significantly higher (alpha=0.05) than the Undisturbed Reference for three of the five parameters measured (SRP, TKN, and NH_4-N) and nearly significantly for NO_x-N (p=0.0587). In the case of NH_4-N the Ground treatment also had significantly greater flux than the Undisturbed Reference.

In relation to the Undisturbed Reference, the Mound treatment and Ground treatment were 4.8 and 3.7 times greater for TP, and 5.0 and 2.8 times greater for SRP, respectively. TKN flux was 4.3 and 2.1 times greater for the Mound treatment than for the Undisturbed Reference and Ground treatments, respectively. The most dramatic differences among treatment fluxes were for NH_4-N and NO_x-N. The Mound treatment flux was 26.5 and 28.6 times greater than the Undisturbed Reference for NH_4-N (p = 0.0001) and NO_x-N (p = 0.0587), respectively. Ground treatment flux was 13.4 and 3.7 times greater than Undisturbed Reference flux for NH_4-N (p=0.0363) and NO_x-N. However, as previously noted, removal of a single Mound outlier would have resulted in the Ground treatment (p=<0.0001) and Mound treatment (p=0.0019) being significantly higher in flux than the Undisturbed Reference for NO_x-N (Appendix B). Finally, TN flux based on TKN + NO_x-N resulted in the Mound treatment flux being 37.6 and 2.4 times greater than the Undisturbed Reference and Ground treatment, respectively. The Ground treatment was 15.9 times greater than the Undisturbed Reference.

Not only did disturbance treatments result in higher flux than the undisturbed treatment but the Mound treatment consistently had greater flux than the Ground treatment as hypothesized. For TKN the Mound treatment flux was significantly greater than the Ground treatment flux (p = 0.0340). Mound treatment flux was higher than the Ground treatment flux for NH_4-N and approached significance at p = 0.0579.

Several studies have examined N and P flux rates under a variety of conditions. As with this study, values reported by other researchers are not absolute rates as studies were not conducted *in situ*; however, they do provide information on relative flux values in other systems. Malecki et al. (2004) examined dissolved reactive phosphorus (DRP) flux from sediments of the Lower St. Johns River over a 25-day period. DRP flux values averaged 0.13 to 4.77 mg m^{-2} d^{-1} under aerobic and anaerobic conditions, respectively. Under oxic conditions, phosphorus tends to bind with ferric iron and is held in an insoluble complex. However, SRP values would be expected to be higher under anaerobic conditions as anoxic conditions lead to ferric iron being reduced to soluble ferrous iron resulting in release of P to overlying flood waters. Dunne et al. (2010) found P release from wetland and upland soils within grazed, improved pastures ranged from -20 to 77 mg m^{-2} d^{-1}. They found similar P release rates from deep marsh, shallow marsh and upland soils.

SRP concentrations continued to rise steadily through the 7-day experimental period for all treatments in my study (Figure 10). Normalized flux rates for the Mound and Ground treatment rose sharply between Days 1 and 2 (Figure 11a). Mound treatment flux remained high while Ground treatment flux declined. SRP release rates may have been maintained due to increasingly anoxic conditions; however, flux of highly bioavailable SRP is likely also attributable to mineralization of organic P under aerobic conditions during the wetland dry-down period (Aldous et al. 2005, Dunne et al. 2010) and/or increased mineralization due to rooting disturbance (Ground and Mound treatments).

In terms of average nitrogen flux, Malecki et al. (2004) found that under anaerobic water column conditions, NH_4-N release rates were significantly greater as compared to flux under aerobic conditions over a 25-day period. Their average value of 18.03 mg m^{-2} day^{-1} under anaerobic conditions across seasons was similar to my average high value of 20.69 $\pm$ 13.86 mg m^{-2} day^{-1} for the Mound treatment while my Undisturbed Reference showed a negative value of -0.78 $\pm$ 3.25 mg m^{-2} day^{-1}. They found that NH_4-N flux rose quickly under anaerobic conditions during the first 2 days whereas my treatments had slightly negative flux between Days 1 and 2 with the steepest rise between Days 5 and 7. In terms of flux, my study found that NH_4-N flux was 6.4 and 7.7 times higher than NO_x-N flux for the Ground treatment and Undisturbed Reference, respectively, with NH_4-N concentrations increasing over time between Days 2 and 7. Nitrate-N concentrations steadily decreased over time in the Undisturbed Reference while NO_x-N concentrations for the Ground treatment steadily increased until Day 5 when concentrations for both the Ground and Mound treatment dropped considerably between Days 5 and 7.

Several studies have looked at nutrients in intact and soil-disturbed upland and wetland systems. I did not examine soil nutrient concentrations among treatments; however, results from studies on soil nutrients may have implications for this study. For instance SRP flux in the Undisturbed Reference and Mound treatment in my study made up nearly 100% of TP flux with SRP in the Ground treatment making up 77% of TP. In terms of concentration my study found ratios of SRP to TP of 13-20% across treatments on Day 1. By Day 7 these ratios increased from 45% (Undisturbed Reference) to 71% (Mound) SRP as a proportion of TP. All treatments in my study contrasted with Craft and Chiang (2002) working in undisturbed depressional wetlands in southwest Georgia. They found most soil P in their study to consist of the recalcitrant organic form with only 2-4% in labile (including plant-available) form with labile P being 5 to 8 times greater in surface (0-5 cm depth) as compared to subsurface (20-25 cm depth) soils. It is important to note that soils in Craft and Chiang's study had been inundated for approximately 6 months prior to sampling where my depression marsh soils had not been inundated for several months which would affect soil N and P pools as well as fluxes. Dunne et al. (2007) also suggested that most P in emergent marsh wetland soils (58%) was stored in organic forms and was relatively unavailable to overlying waters.

My study showed higher average flux of TKN for Mound and Ground treatments over the Undisturbed Reference; however, inorganic N forms comprised much of the TKN in disturbance treatments. As discussed in the Results section, I found that nearly 75% of the TKN average flux was attributable to NH_4-N for the disturbance treatments whereas NH_4-N contributed little to the Undisturbed Reference TKN average flux (i.e. there was a higher proportion of organic-N in the Undisturbed Reference). An evaluation of TN flux (calculated from measured TKN+NO_x-N values) showed that organic N made up 26% of the Ground treatment flux and 35% of the Mound treatment flux. Craft and Chiang (2002) found that soil organic-N accounted for up to 98% of TN with plant-available NH_4-N and NO_x-N accounting for <1% of total soil N. In fact, the Undisturbed Reference in my study showed organic-N approaching 100% of TN flux (88% of TKN flux), thus approximating organic N contributions found in soil concentrations in the study by Craft and Chiang. In terms of concentration, my study showed a 20-22% contribution on Day 1 and a 42% (Undisturbed Reference) to 76% contribution on Day 7 of NH_4-N to TKN. Rooting disturbance in my study had a considerable impact on the form of nitrogen with a much higher percentage of NH_4-N vs. organic N in the disturbance treatments.

During their study of seasonal wetlands on improved pastures and semi-native range in Florida, Bohlen and Gathumbi (2007) found that net nitrification rates (conversion of NH_4-N to NO_x-N) were highest during the early part of the dry season (December – February), declined through the latter portion of the dry season (March – June), and were lowest following inundation (July – October). They noted that the transition from terrestrial to flooded conditions results in a shift from nitrification as the dominant process to ammonification as the dominant process during flooding. In my study, conducted during the latter portion of the dry season in west-central Florida, NO_x-N mean concentrations made up ~70-78% of the total inorganic N concentration (NO_x-N + NH_4-N) for all treatments, as well as the Floodwater Control, on Day 1. On average, the percentage of inorganic-N concentration attributed to NO_x-N remained high through Day 5 for the Undisturbed Reference and Ground treatments (64 and 70%, respectively) then dropped precipitously for all treatments by Day 7. The steepest decline in percentage of NO_x-N was found in the Mound treatment (26% of total inorganic N) followed by the Ground treatment (35% of total) and Undisturbed Reference (43% of total) as percentage of NH_4-N increased to 74, 65, and 57% of total inorganic nitrogen, respectively, by the end of the sampling period.

Doupe et al. (2009) sampled *in situ* nutrient concentrations in waters of floodplain lagoons from May to October 2008. They found large (though not significant) increases in total, dissolved and particulate concentrations of N and P in an unfenced (unprotected from rooting) lagoon compared to a fenced (protected) lagoon from July through the end of their study. They suggested that significantly lower dissolved oxygen levels in pig-disturbed lagoons may have contributed to the release of phosphorus bound to sediments. Doupe et al. (2010) noted that hydraulic residence time had a more significant effect on total N, total dissolved N, and total P than protection from pig rooting. Additionally, unfenced (rooted) treatments resulted in significantly lower pH compared to undisturbed lagoons (Doupe et al. 2009, Doupe et al. 2010) which could have implications for biogeochemical reactions in terms of nutrients.

Feral pigs have been likened to “rototillers” in that their rooting activity simulates tillage (Nordrum 2014, Arrington et al. 1999). Tillage of intact wetland soils in a large marsh in China resulted in increased soil NH_4-N, NO_3-N, and dissolved organic N (Zhang et al. 2008). Moser et al. (2009) looked at the influence of microtopography on soil nutrients in created vs. reference wetlands. In a comparison of disked vs. non-disked plots in the created wetlands they found that disking disturbance resulted in higher soil NH_4-N concentrations that were seven times greater than the non-disked treatment although total soil N was comparable. They attributed this to more pronounced microtopography although no significant differences were detected in soil NO_x-N concentrations. In my study, NO_x-N flux was higher for Ground and Mound treatments than the Undisturbed Reference but NH_4-N flux values were of much greater magnitude. Interestingly, Moser et al. (2009) found no clear cut effect of disking on soil ortho-phosphate concentrations.

Kotanen (1997) conducted studies in a coastal meadow in California where burial and excavation of vegetation and soil were conducted to simulate pig rooting. He found that buried plots (covered with material excavated to a depth of 9 cm and likely similar in nature to my Mound treatment), consistently had more NH_4-N than controls while NO_3-N varied inconsistently among treatment. Excavated plots, likely more similar to my Ground treatment, had relatively low NH_4-N compared to burial plots which he attributed to removal of organic-rich surface layers. Indeed my study showed progressively lower flux of NH_4-N from Mound >Ground>Undisturbed Reference treatments. In contrast, other studies conducted in California coastal grasslands and oak savanna-grasslands showed no significant impact of feral pig disturbance on NH_4-N or NO_3-N pools or mineralization rates (Cushman et al. 2004, Tierney et al. 2006, Moody and Jones 2000).

Mound treatment cores yielded more variable results in day to day flux as well as cumulative flux than either the Undisturbed Reference or the Ground treatment for TP and SRP. The Mound treatment also had the highest day to day variation in NH_4-N and the greatest variation among treatments for cumulative flux of NOx-N. This could be due to a variation in the mound material itself in terms of mound height, volume, compaction, and amount and decomposition state of vegetation which was not controlled for in the experiment. For instance, if a mound were only 7 cm tall, the 20-cm soil core sample would have contained more soil below the actual soil surface than a mound that was 15 cm tall. Also, the compactness of the mounds would depend on how much and what type of vegetation may have been incorporated during the act of rooting. This could impact soil aeration, soil moisture and soil temperature and have potential impacts on mineralization rates. In a study by Bueno et al. (2013), both occurrence and intensity of pig rooting strongly influenced NH_4-N and NO_3-N concentrations up to a disturbed soil volume of 5 m^3. An inverse relationship with NO_3-N concentrations being higher than NH_4-N on the most intensively disturbed study site led them to postulate that the aeration effect of disturbance affected nitrification of NH_4-N (due to increased oxygen in the soil) thus resulting in higher NO_3-N concentrations. In my study, NOx-N concentrations were higher than NH_4-N concentrations on Days 1 and 2 for all treatments but NO_x-N levels declined as this ion fluxed from the water column back into the soil while NH_4-N concentration increased. Thus flooding will affect which species of N is more common. Crude estimates of mound height prior to sampling averaged ~10 cm but I did not focus on height nor measure volume of mounds. However, neither NH_4-N nor NO_x-N appeared to vary based on estimated mound height.

Management Implications

Few studies have examined the impact of feral pig rooting on soil nutrients or nutrient flux in wetlands. However, several authors have discussed the potential effects of cattle disturbance in marshes. Dunne et al. (2007) examined historically isolated wetlands in the Lake Okeechobee Basin, FL, and noted that long-term P storage could be enhanced with increasing accumulation of soil organic matter in wetland soils. However, they noted that cattle may impact soil organic matter accumulation and nutrient cycling via trampling and grazing in wetlands they examined. Bohlen and Gathumbi (2007) working in seasonal wetlands within improved and semi-native pastures suggested cattle disturbance in flooded soils could alter soil C by affecting productivity and decomposition properties of plants as well as microbial decomposition processes and N cycling with a loss of soil organic matter.

In terms of nutrients and plant communities in seasonal wetlands, Tweel and Bohlen (2008) showed that areas protected from cattle grazing had much greater cover by maidencane and much less bare ground than areas where cattle grazing occurred. They suggested that changes in plant community dynamics could impact wetland P cycling. For instance, a study by Balcer (2006) showed higher P retention in decomposing maidencane as compared to soft rush (*Juncus effusus*), a species that dominates in cattle-grazed wetlands.

Approximately 2.5 months after pulling soil cores, a qualitative visual assessment was conducted in an area approximately 2 m^2 around each of the nine Mound/Ground pair core sites in my study. Redroot was the single most dominant plant in six cases and was secondarily dominant in the other three cases. In contrast, Undisturbed Reference sites were dominated by chalky bluestem, maidencane, and a variety of other herbaceous species (C. Gates and K. Gruenhagen, pers. obs.). Boughton and Boughton (2014) noted that feral pig rooting resulted in a shift from a bunchgrass dominated wet prairie system to a near monoculture of redroot. Further study should be considered to examine long-term plant community recovery and effects on nutrient cycling in areas affected by feral pig rooting.

Feral pigs do not root in inundated wetlands thus disturbance is primarily limited to dry down periods when soils are moist (Arrington et al. 1999). Repeated rooting of dry to moist wetland soils by feral pigs could have implications as organic matter is more quickly decomposed and converted to inorganic forms. Pig rooting activity in non-flooded soils likely results in less compaction (Cuevas et al. 2012) as opposed to trampling by cattle in flooded wetlands.

My soil core study showed that pig rooting disturbance resulted in greater nutrient flux for all parameters as compared to undisturbed sites. Relative magnitude of fluxes and the potential for wetland water quality impacts can be put into perspective. In the study by Felix et al.

(2014), rooting polygon area ranged from 0.00023 m^2 to 4,335 m^2. The majority of polygons measured were less than 5 m^2 but some polygons covered over 1,000 m^2. In my study feral pig rooting disturbance in the Mound treatment resulted in the highest cumulative flux rates followed by the Ground treatment. To illustrate the potential impact of rooting, the following example takes a simple mean and range of cumulative flux over 7 days for Mound and Ground treatments (assuming half of the disturbance results in mounded soil displaced from adjacent ground sites). Using nutrient flux data from this study and extrapolating to a 1,000 m^2 dry wetland upon inundation could result in a contribution of 16,800 mg TP (range 6,400 to 27,100 mg), 15,650 mg SRP (range 3,040 to 28,250 mg), 139,330 mg TN (range 66,790 to 211,880 mg), and 108,920 mg NH_4-N (range 65,870 to 151,980 mg) fluxing from soil to water column. Assuming primarily a negative flux for NO_x-N and extrapolating for data with Mound outlier removed, NO_x-N flux could average 15,925 mg (range 5,450 to 26,400 mg) fluxing from water column to soil over a 7-day period. Scattered areas of rooting may have relatively benign impacts on nutrient flux. However, feral pigs are capable of intensive rooting over extensive portions of wetlands and this could have major implications for water quality and thus aquatic plant and animal life.

Conclusions

My hypothesis, that feral pig rooting would result in higher nutrient fluxes for Mound and Ground disturbance treatments compared to undisturbed sites, was borne out. Tukey-Kramer means comparison tests detected significant differences ($p = 0.05$) for SRP, TKN and NH_4-N. In terms of phosphorus, the Mound treatment flux was significantly greater than the Undisturbed Reference for SRP and approached statistical significance for TP ($p = 0.067$). NO_x-N flux differences between the Mound treatment and the Undisturbed Reference were nearly statistically significant at $p = 0.059$. I also hypothesized that the Mound treatment would have greater flux than the Ground treatment due to a potentially greater degree of soil mixing and aeration. Mound and Ground treatments were not significantly different from each other for either phosphorus parameter or for NO_x-N. However, Mound treatment flux was significantly higher than both the Ground treatment and Undisturbed Reference for the TKN parameter. Both the Mound and Ground treatment had significantly higher flux than the Undisturbed Reference for NH_4-N but were not significantly different from each other.

For all analyses, feral pig rooting disturbance impacts had higher significance for nitrogen parameters as compared to phosphorus parameters. Concentration and flux values may not be interpreted as being particularly high; however, the nuances of feral pig disturbance have implications for biogeochemical processes in depression marshes that could impact long-term system dynamics.

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Table 1. Average flux in phosphorus parameters for Undisturbed Reference, Ground and Mound treatments. Lower case letters below average values represent statistical comparison between treatments for the same parameter. Different letters represent statistically different means (p=0.05) within the same row.

	Undisturbed Reference **$mg\ m^{-2}\ d^{-1}$**	**Ground** **$mg\ m^{-2}\ d^{-1}$**	**Mound** **$mg\ m^{-2}\ d^{-1}$**
TP	0.56 $\pm$ 0.32	2.08 $\pm$ 0.40	2.71 $\pm$ 2.24
SRP	0.57 $\pm$ 0.31 a	1.60 $\pm$ 0.57 ab	2.87 $\pm$ 2.57 b

Table 2. Average flux in nitrogen parameters for Undisturbed Reference, Ground and Mound treatments. Lower case letters below average values represent statistical comparison between treatments for the same parameter. Different letters represent statistically different means (p=0.05).

	Undisturbed Reference **$mg\ m^{-2}\ d^{-1}$**	**Ground** **$mg\ m^{-2}\ d^{-1}$**	**Mound** **$mg\ m^{-2}\ d^{-1}$**
TKN	6.51 $\pm$ 1.04 a	13.45 $\pm$ 3.14 a	28.19 $\pm$ 3.80 b
NH_4-N	-0.78 $\pm$ 1.50 a	10.43 $\pm$ 1.13 b	20.69 $\pm$ 3.77 b
NO_x-N	-6.01 $\pm$ 0.27	-1.63 $\pm$ 1.29	-0.21 $\pm$ 5.36
TN*	0.74 $\pm$ 0.79 a	11.79 $\pm$ 4.33 ab	27.85 $\pm$ 7.06 b

*no blocking

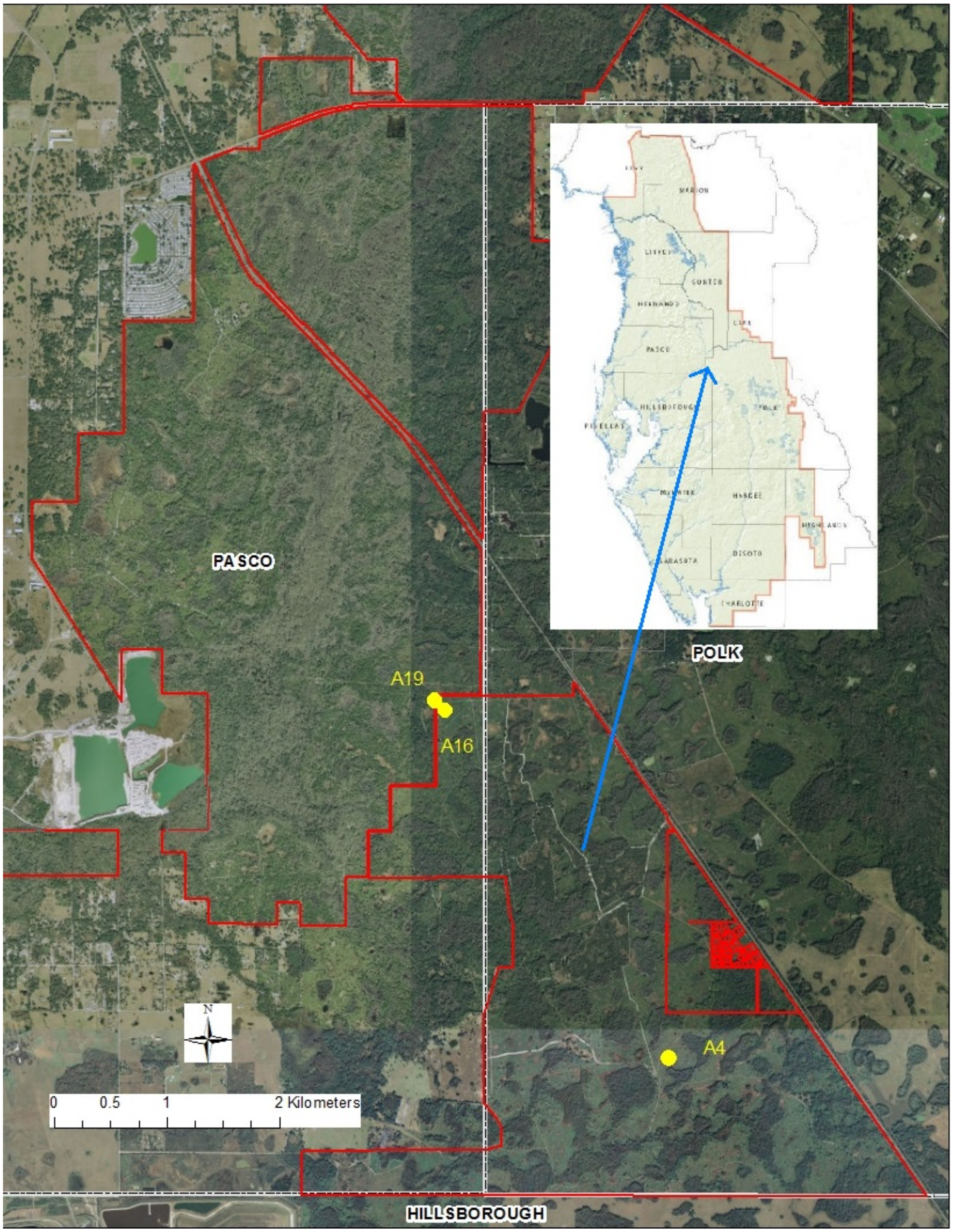


Figure 1. Soil core study location at the Upper Hillsborough Preserve in Pasco and Polk counties, Florida. Yellow dots show locations of study wetlands. Inset shows relative location of the study area within the Southwest Florida Water Management District.

Figure 2a. Mound core site on left in foreground showing decomposing bluestem and scattered redroot. Undisturbed Reference core site is in background.

Figure 2b. Example of Mound (left flag with pushed up soil) and Ground (right flag) pair

Figure 3. Wetland A4 showing disturbance polygon (green outline) and soil core and origin points (in yellow) with inset showing wetland overview and relative location of polygon within wetland.

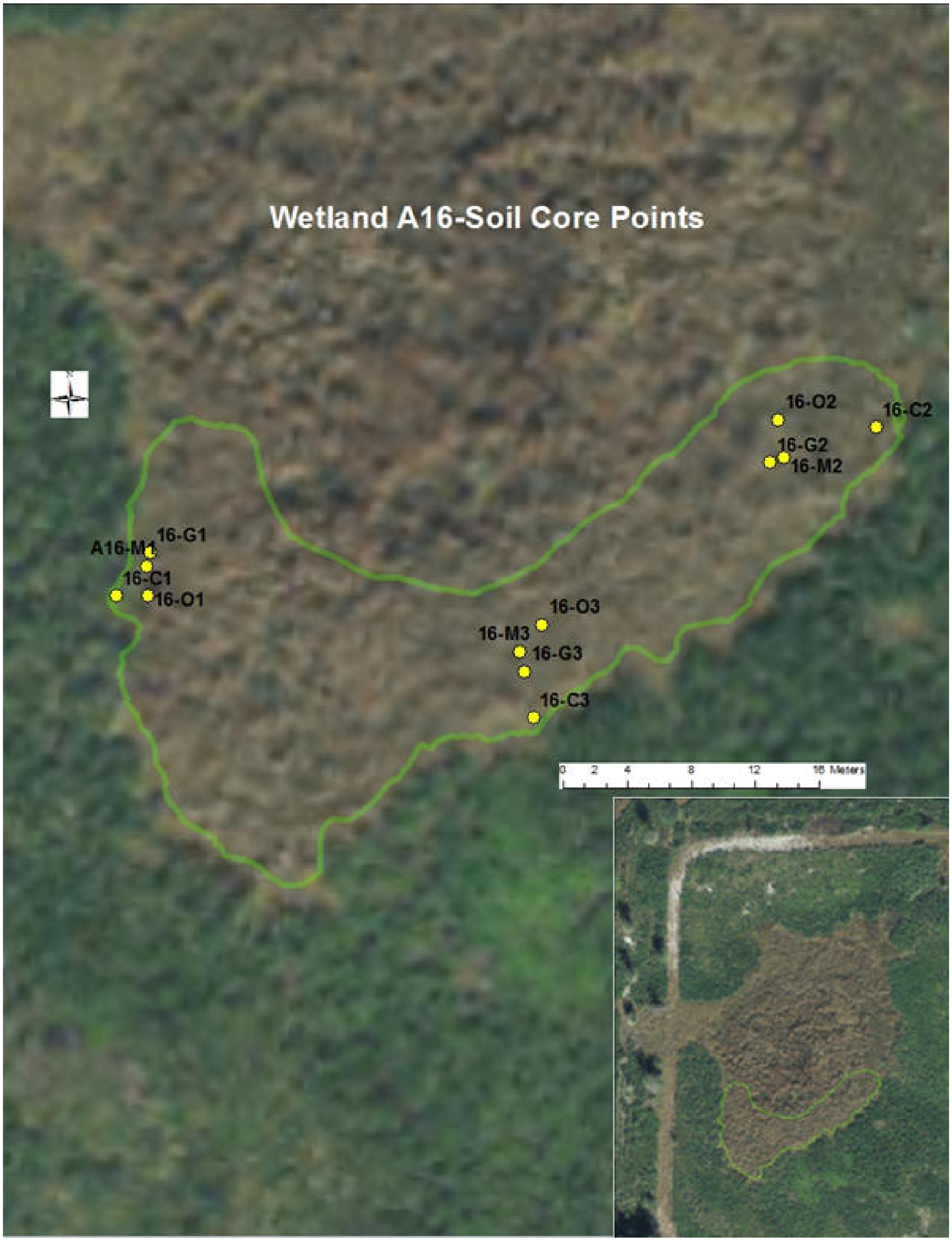


Figure 4. Wetland A16 showing disturbance polygon (green outline) and soil core and origin points (in yellow) with inset showing wetland overview and relative location of polygon within wetland.

Figure 5. Wetland A19 showing disturbance polygon (green outline) and soil core and origin points (in yellow) with inset showing wetland overview and relative location of polygon within wetland.

Figure 6. Field collected soil core from Undisturbed Reference site

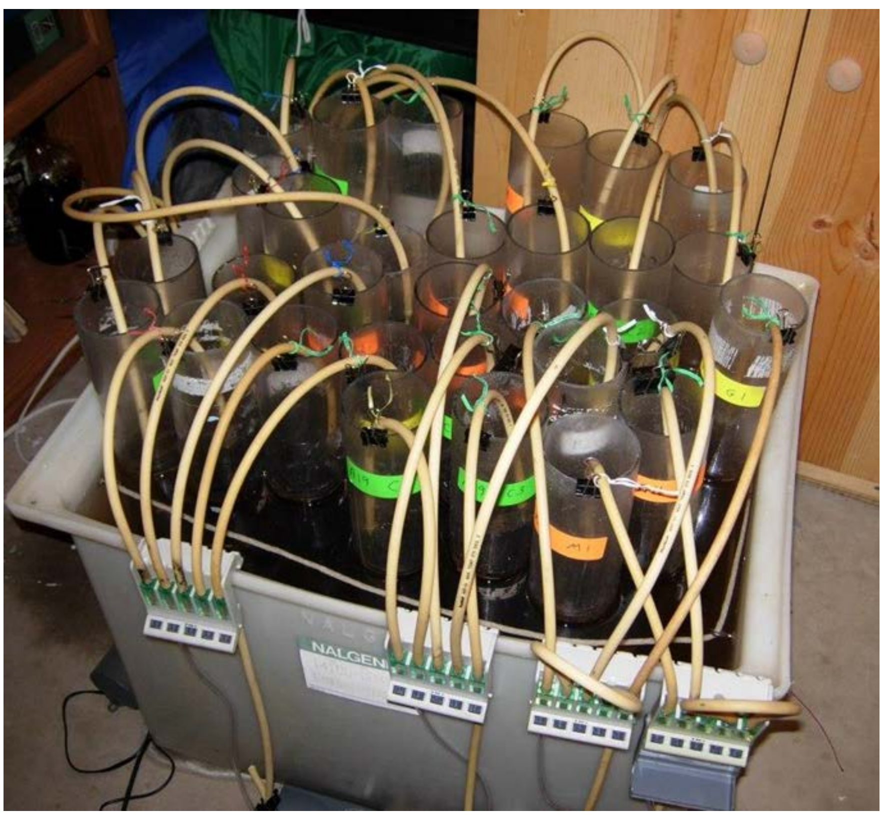


Figure 7. Flooded soil core laboratory set-up showing randomly placed core tubes and mixing apparatus

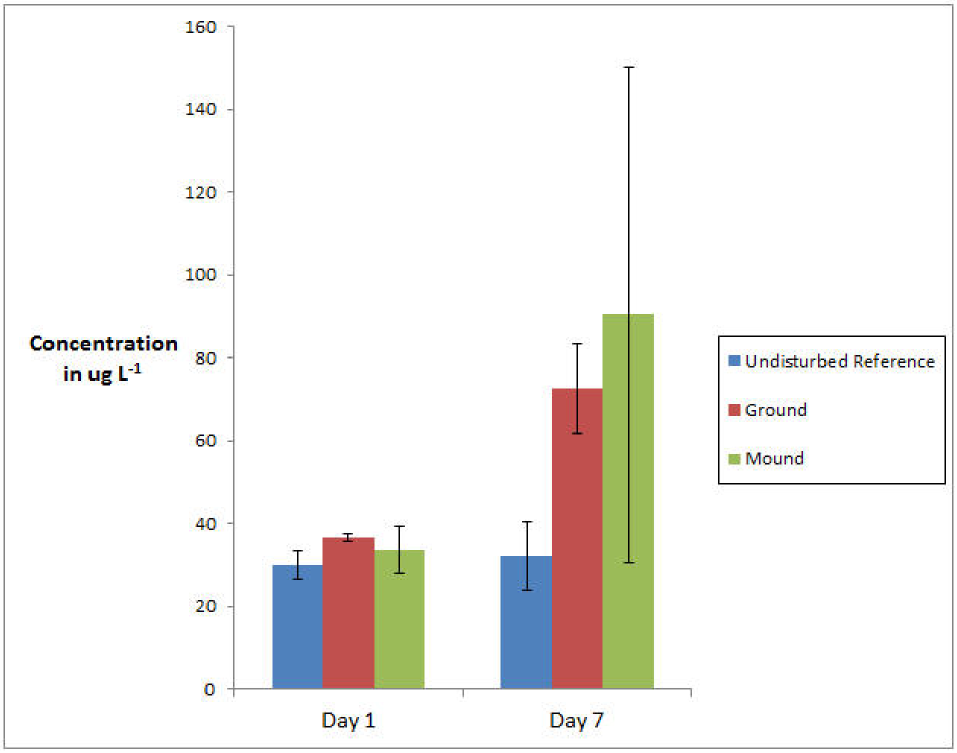


Figure 8. Total phosphorus concentration in ug L^{-1} by treatment for Days 1 and 7. Values are shown as means $\pm$ 1 SD.

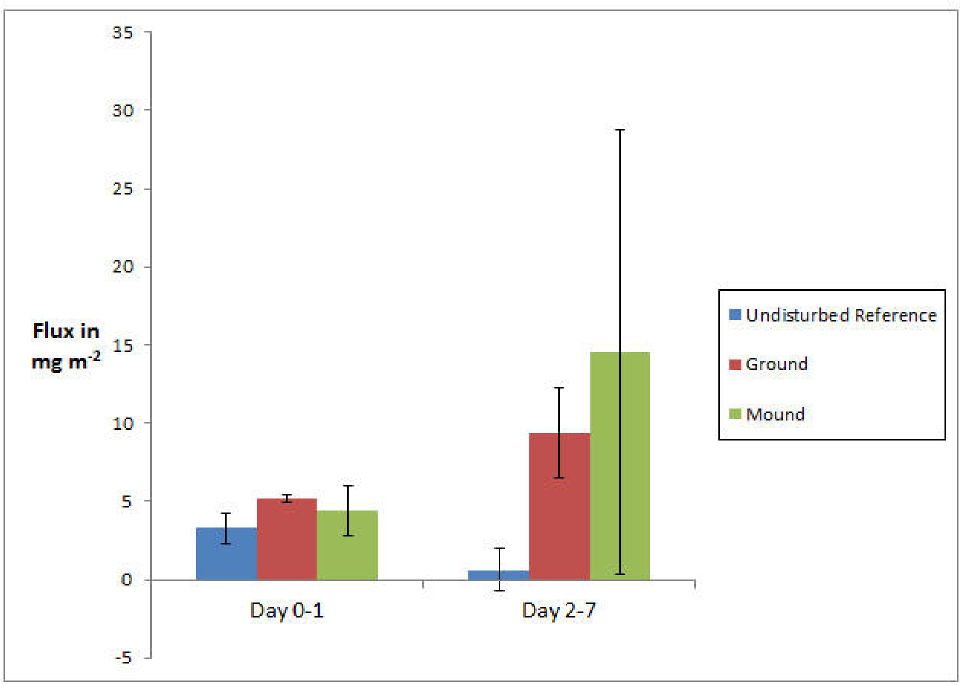


Figure 9. Total phosphorus flux by treatment for Days 0-1 and 2-7. Values are shown as means $\pm$ 1 SD.

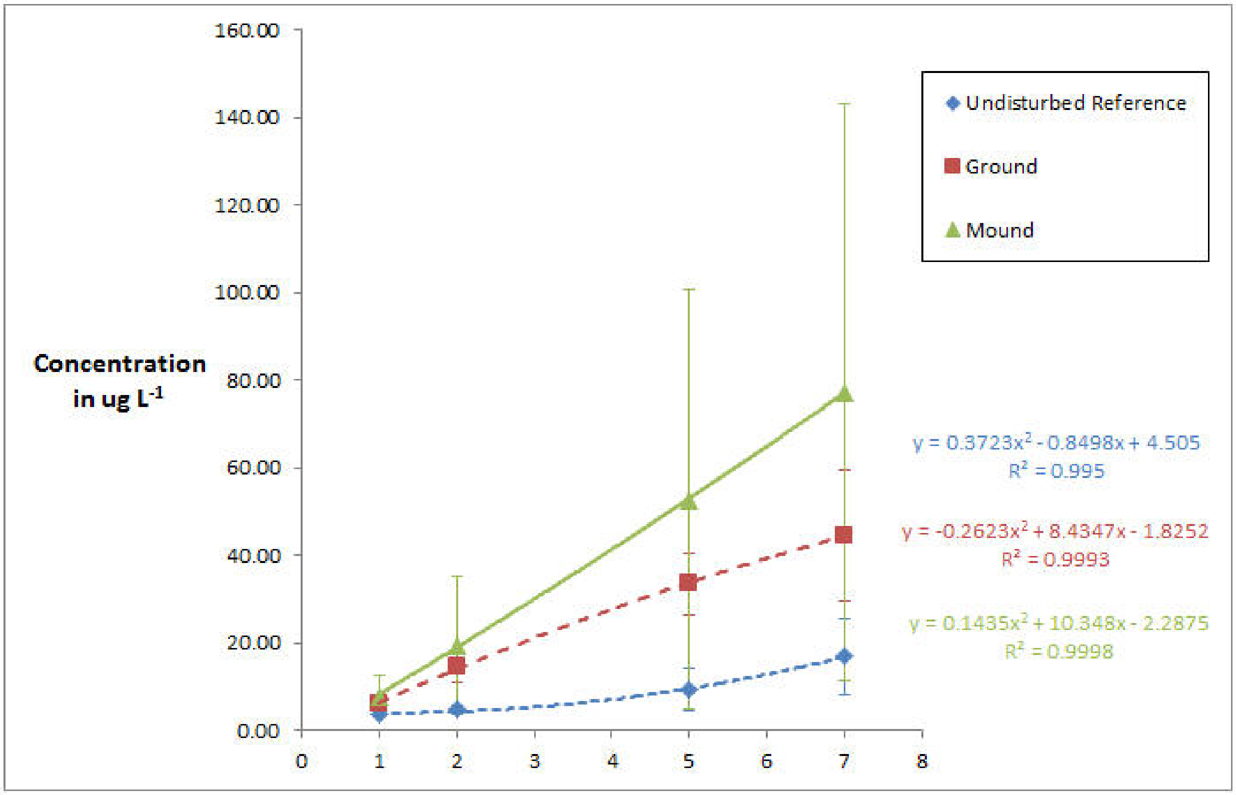


Figure 10. Soluble reactive phosphorus concentration curves by treatment for Days 1, 2, 5, and 7 based on 2nd order polynomial. Values are shown as means $\pm$ 1 SD.

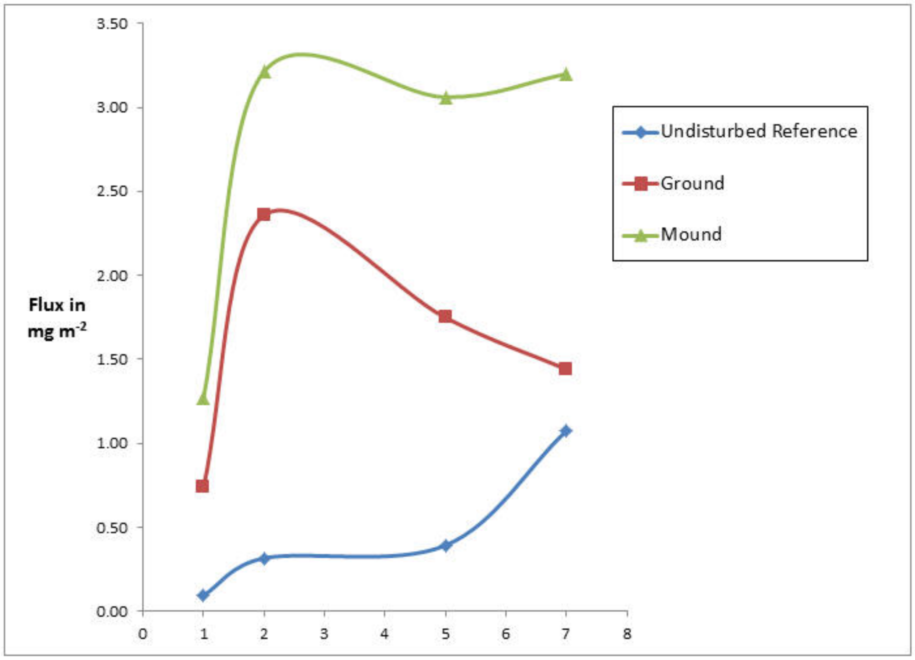


Figure 11a. Soluble reactive phosphorus normalized flux by treatment for Days 1, 2, 5 and 7.

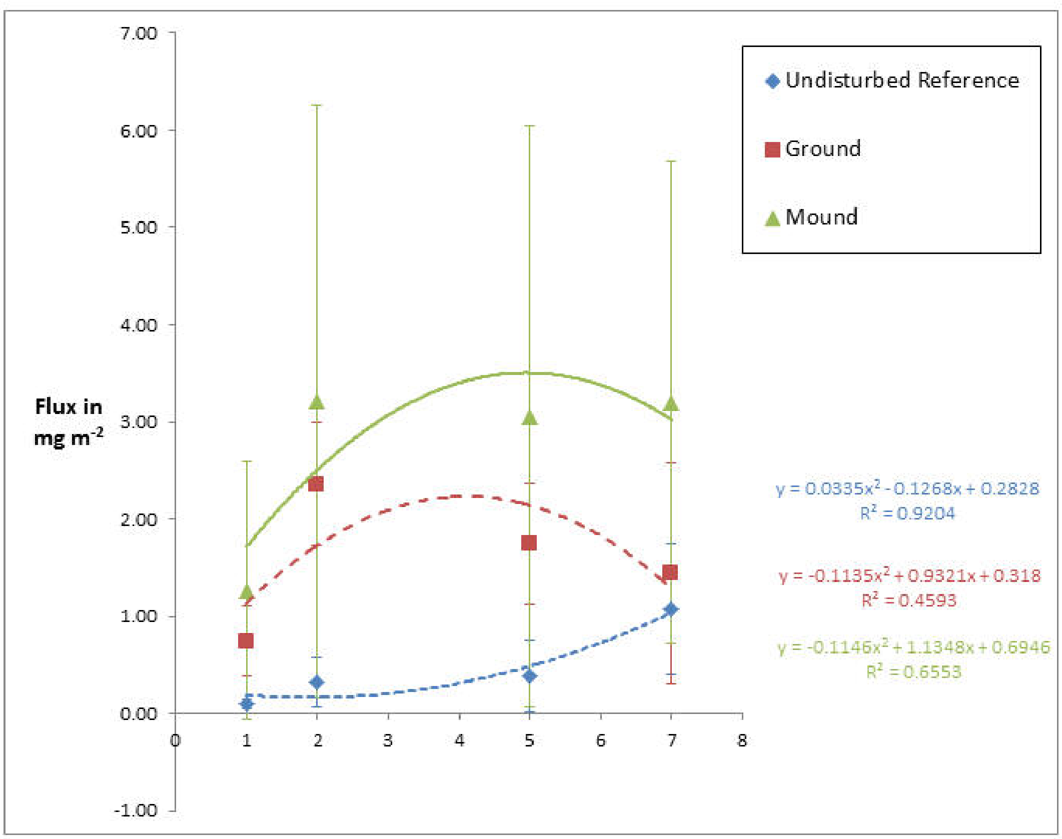


Figure 11b. Soluble reactive phosphorus normalized flux curves based on 2[nd] order polynomial. Values are shown as means ± 1 SD.

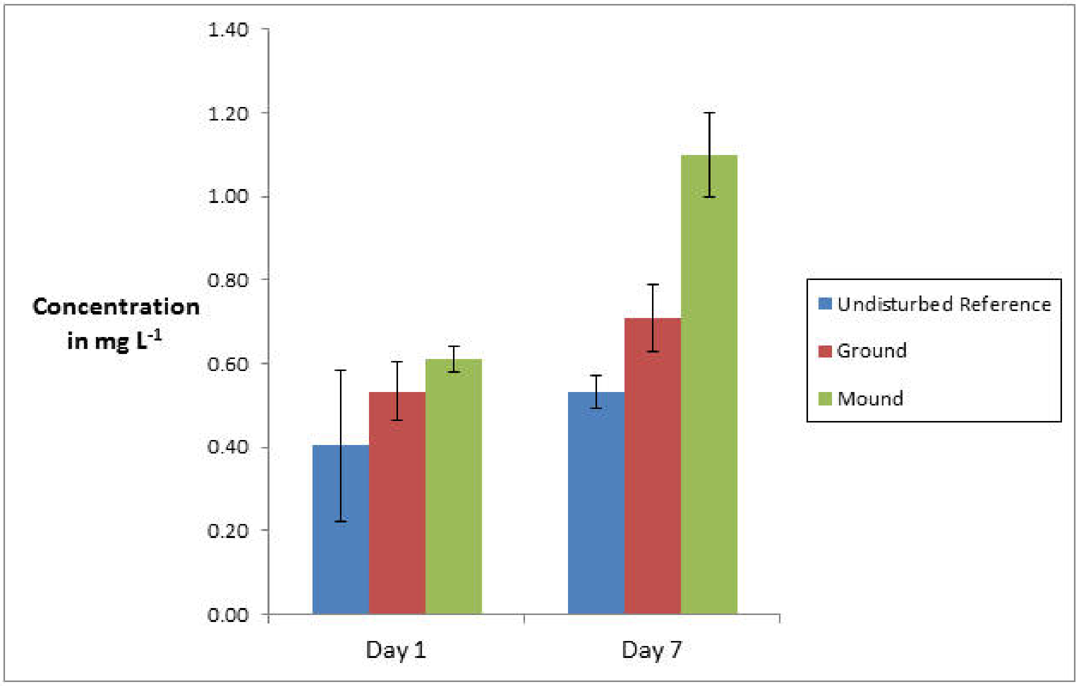


Figure 12. Total Kjeldahl nitrogen concentration by treatment for Days 1 and 7. Values are shown as means ± 1 SD.

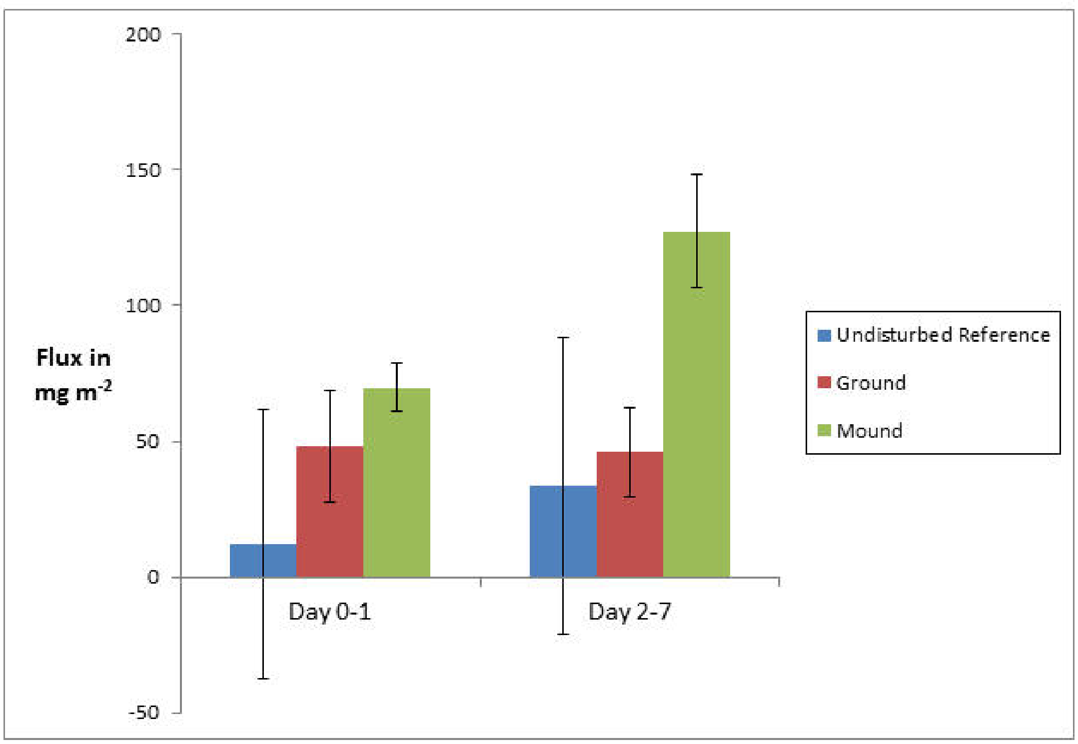


Figure 13. Total Kjeldahl nitrogen flux by treatment for Days 0-1 and 2-7. Values are shown as means ± 1 SD.

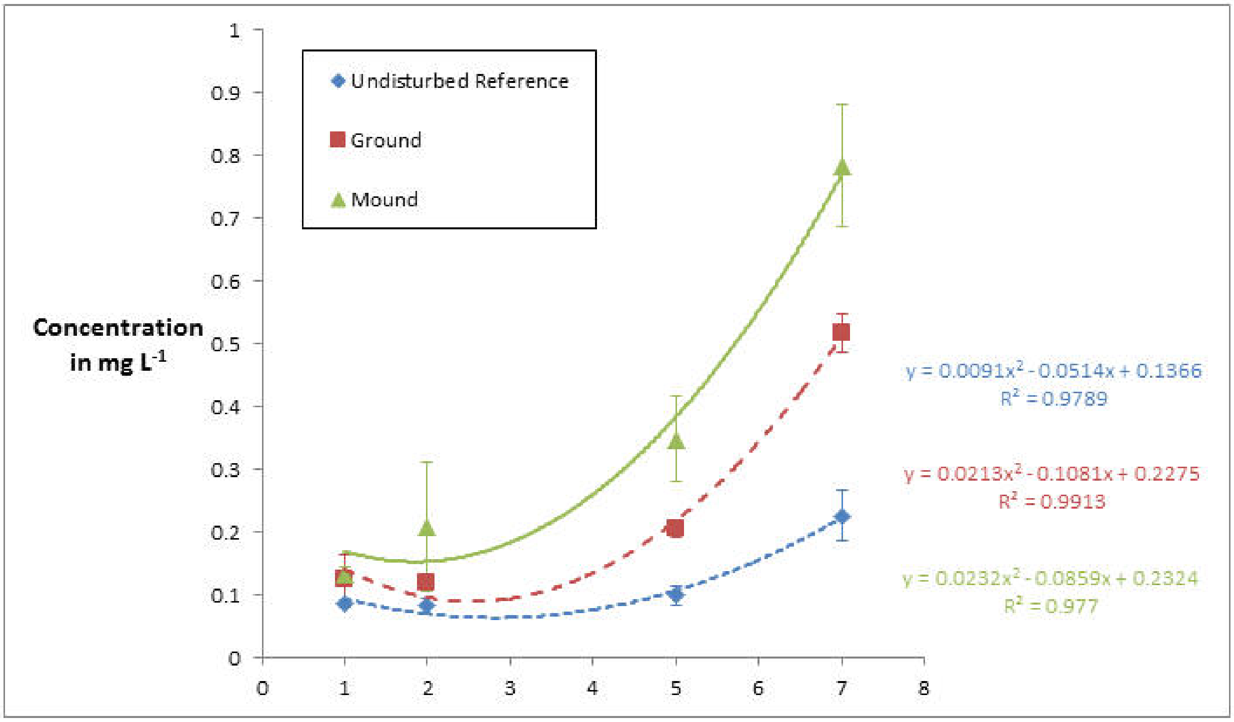


Figure 14. Ammonium-N concentration by treatment for Days 1, 2, 5, and 7 based on 2nd order polynomial. Values are shown as means ± 1 SD.

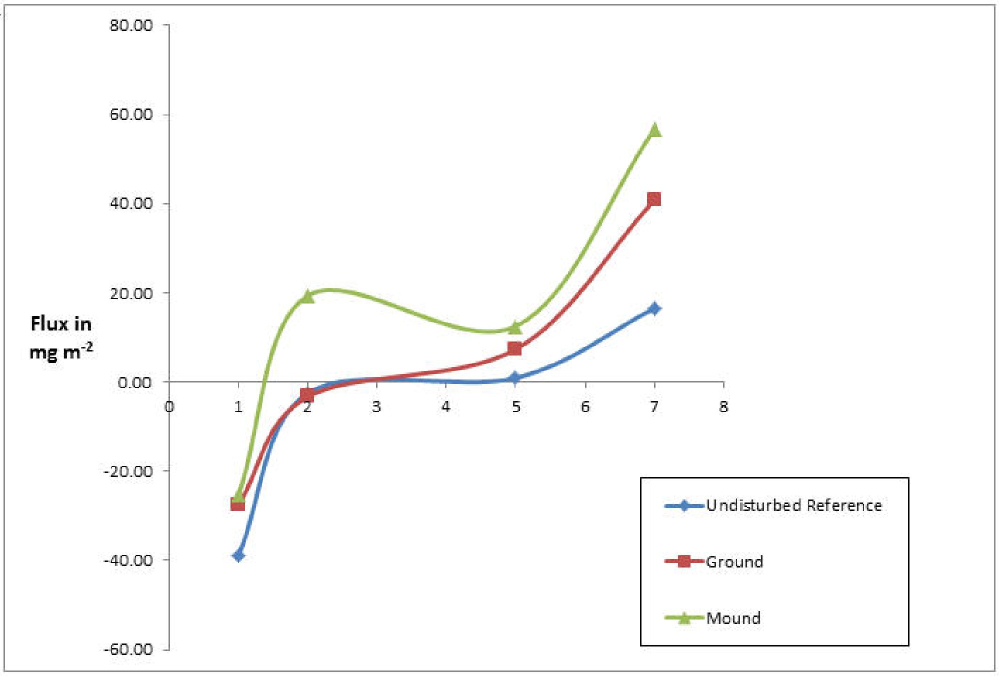


Figure 15a. Ammonium-N normalized flux by treatment for Days 1, 2, 5 and 7.

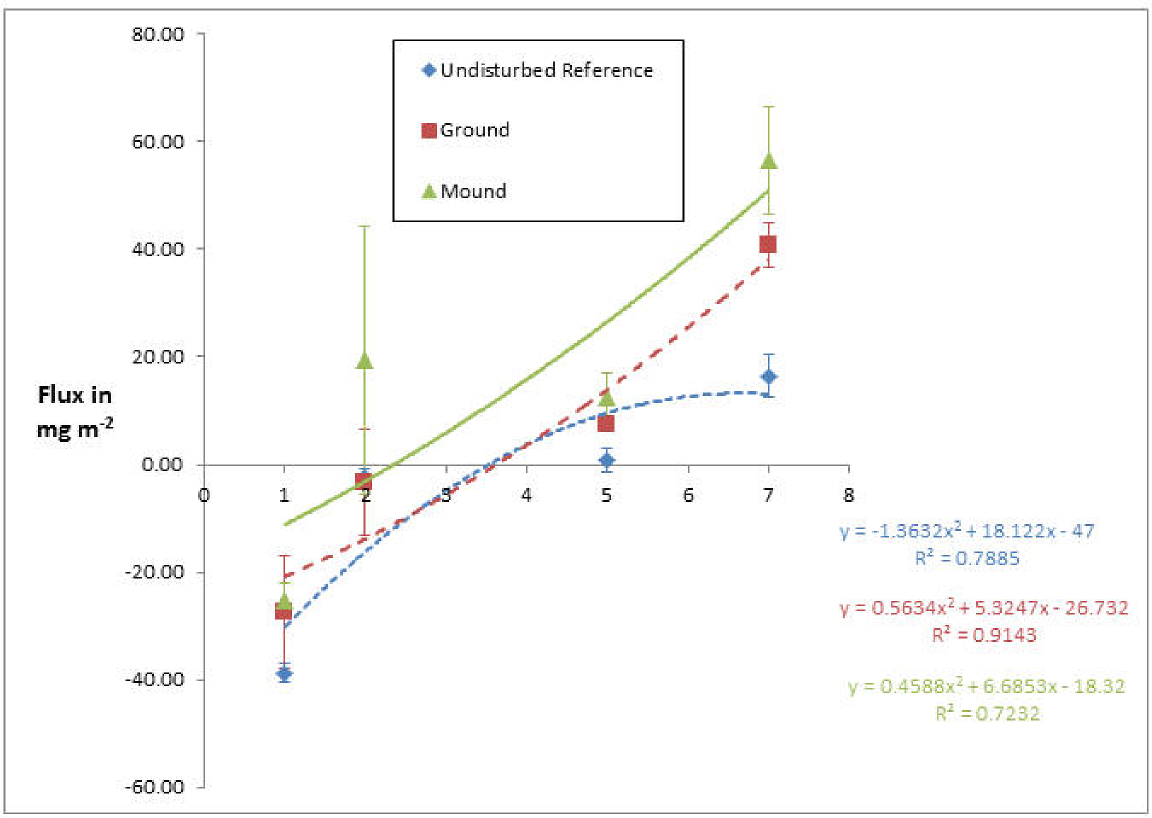


Figure 15b. Ammonium-N normalized flux curves by treatment for Days 1, 2, 5, and 7 based on 2nd order polynomial. Values are shown as means + 1 SD.

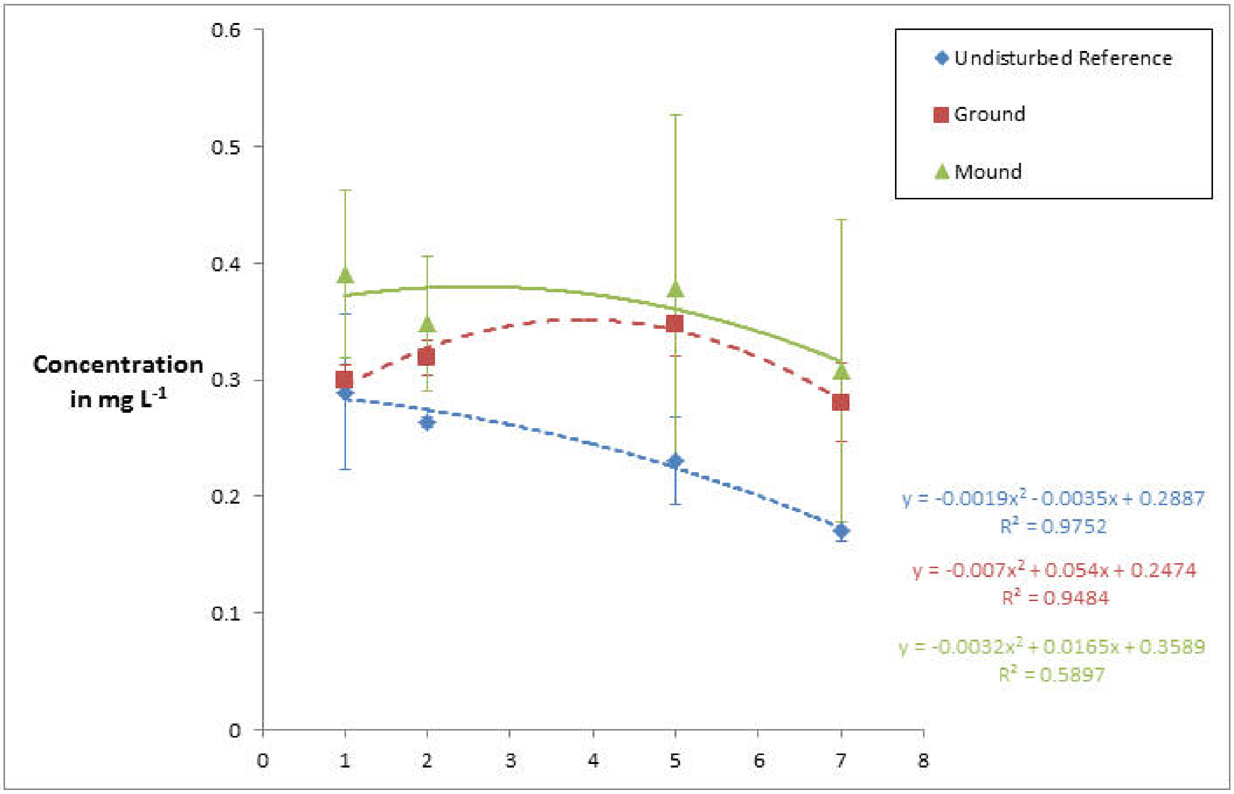


Figure 16. Nitrate+nitrite-N concentration by treatment for Days 1, 2, 5, and 7 based on 2nd order polynomial. Values are shown as means ± 1 SD.

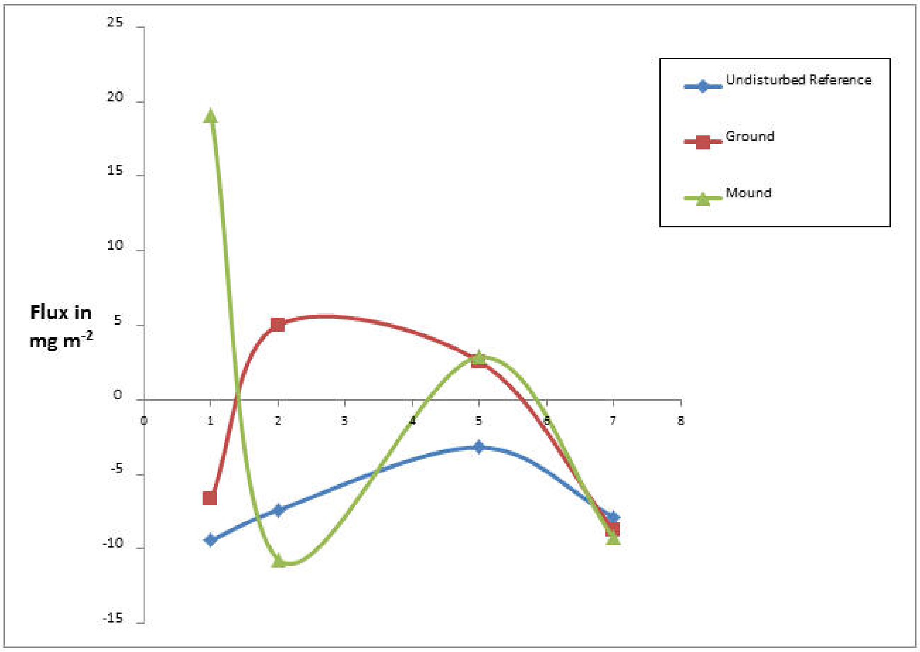


Figure 17a. Nitrate+nitrite-N normalized flux by treatment for Days 1, 2, 5 and 7.

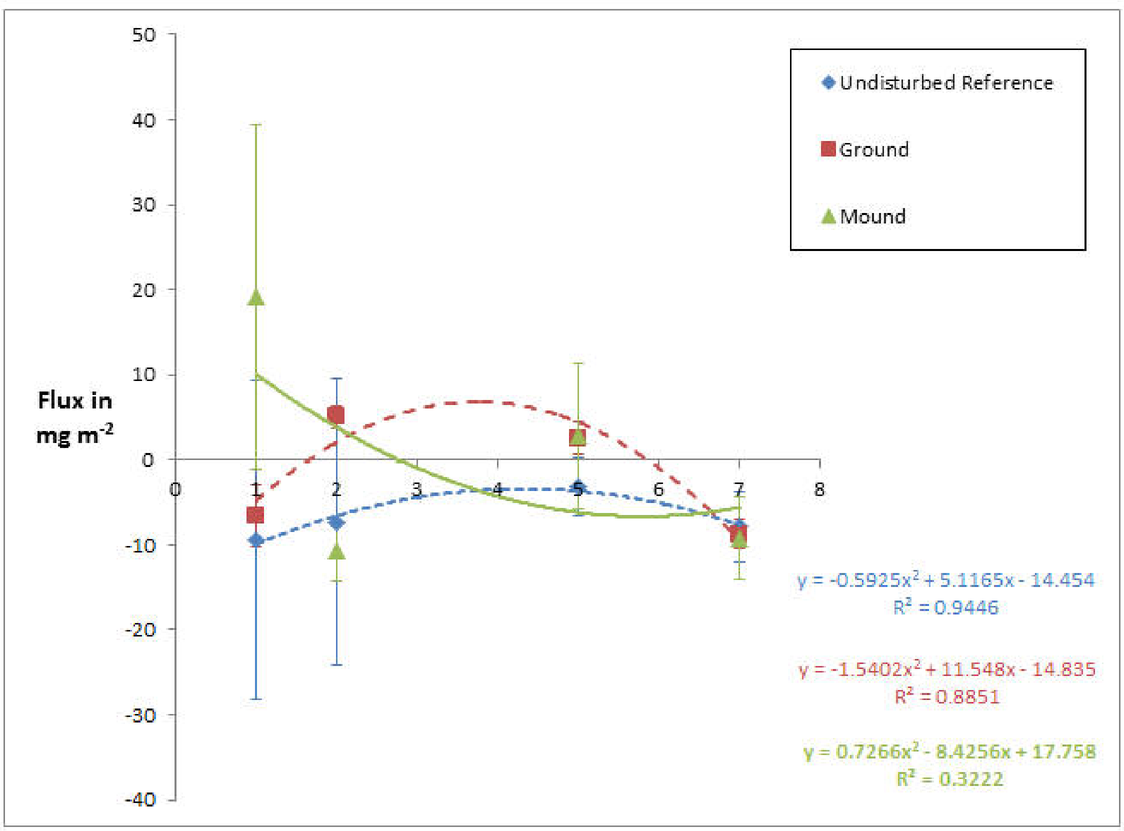


Figure 17b. Nitrate+nitrite-N normalized flux curves by treatment for Days 1, 2, 5, and 7 based on 2nd order polynomial. Values are shown as means ± 1 SD.

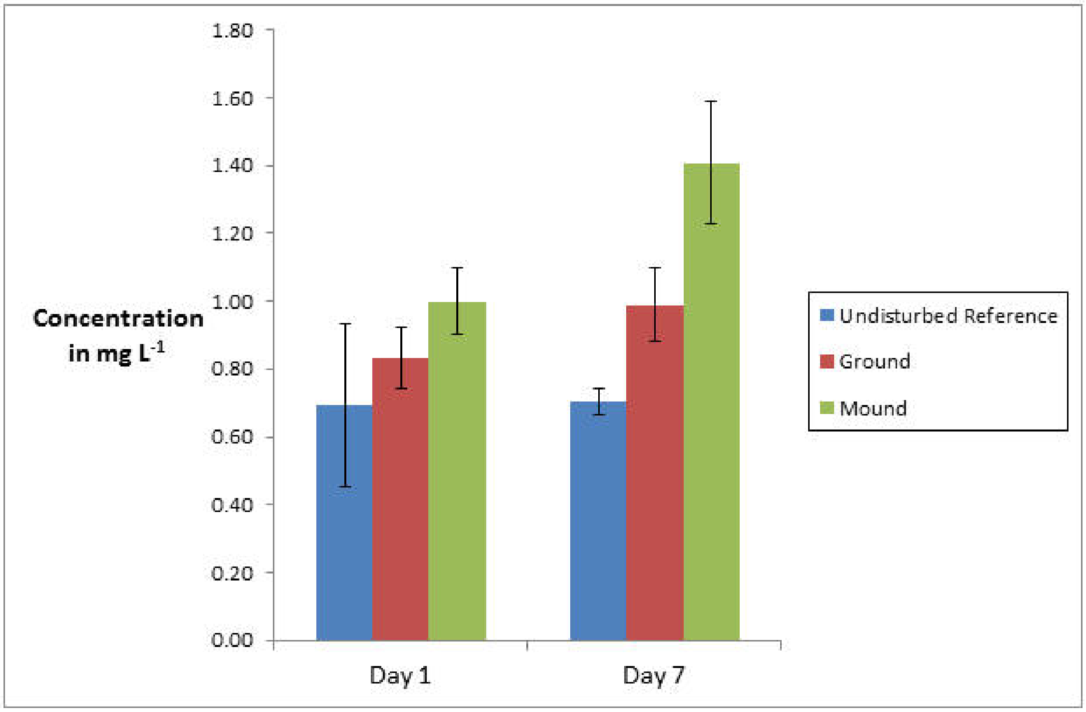


Figure 18. Total nitrogen concentration by treatment for Days 1 and 7. Values are shown as means $\pm$ 1 SD.

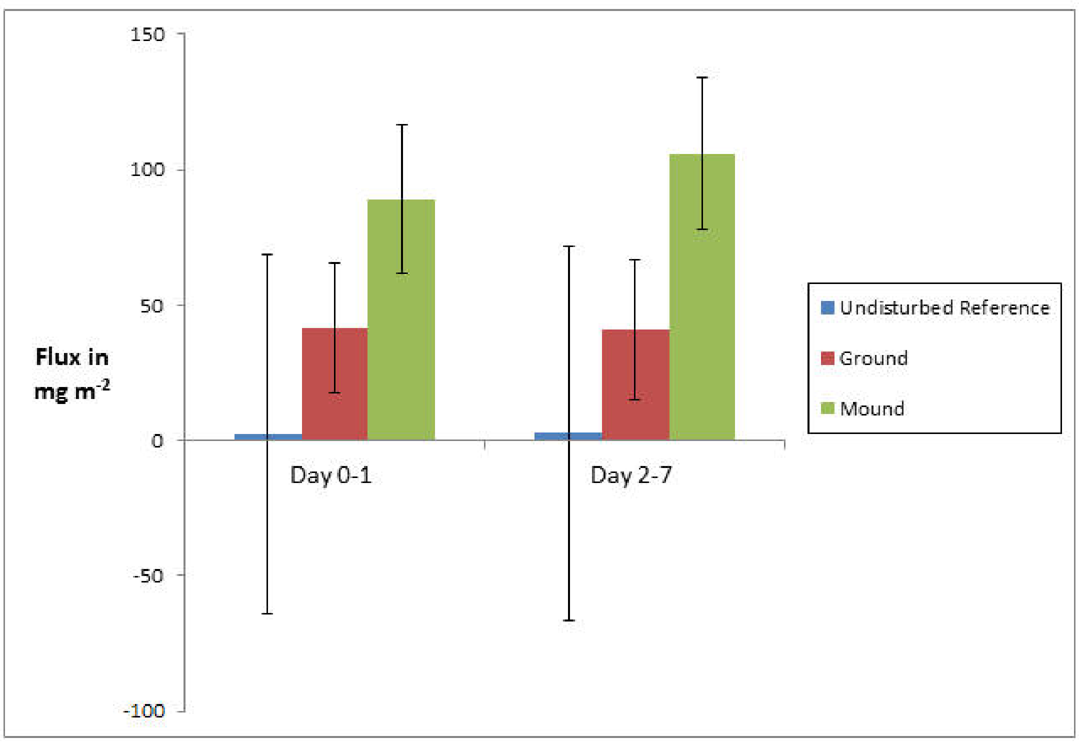


Figure 19. Total nitrogen flux by treatment for Days 0-1 and 2-7. Values are shown as means $\pm$ 1 SD.

APPENDIX A.

Table A1. Distance (in meters) between Soil Core Sites, by Replicate, for All Parameters (TP, SRP, TKN, NO_x-N, and NH_4-N)

UR=Undisturbed Reference
G=Ground
M=Mound

Soil Core Site	Distance UR-G	Distance UR-M	Distance G-M
A4-1	8.0	8.9	0.9
A4-2	6.3	6.5	0.8
A4-3	7.9	8.6	0.7
A16-1	3.4	2.7	0.8
A16-2	6.9	6.1	0.9
A16-3	3.0	4.3	1.3
A19-1	2.6	2.3	0.6
A19-2	4.7	4.6	0.3
A19-3	2.6	3.0	0.7

APPENDIX B.

Table B1. Significance levels (p) by nutrient parameter for Tukey-Kramer means comparison tests and analysis of variance. Includes all values for all parameters-no outliers removed.

	Tukey Kramer Means	**ANOVA**	
Nutrient Parameter		TMT	Wetland Effect
TP	0.0666 M-UR	0.0718	0.1790
SRP	0.0399 M-UR	0.0500	0.0670
TKN	0.0018M-UR/0.0340 M-G	0.0022	0.9193
NH_4-N	0.0001 M-UR/0.0363 G-UR	0.0002	0.7073
NO_x-N	0.0587 M-UR	0.0594	0.3459

Table B2. Significance levels (p) by nutrient parameter (TP, SRP, NO_x-N) for Tukey-Kramer means comparison tests and analysis of variance. Outliers removed from analysis.

	Tukey Kramer Means*	**ANOVA***
Nutrient Parameter		TMT
TP	0.0471 G-UR	0.0429
SRP	0.0821 M-UR	0.0619
NO_x-N	<0.0001 G-UR/0.0019 M-UR	<0.0001

*Outliers removed

Table B3. Average flux by treatment with outlier removed from analysis. Values are shown as means ± 1 SD. Lower case letters below average values represent statistical comparison between treatments for the same parameter. Different letters represent statistically different means (p=0.05) within the same row.

	Undisturbed Reference **$mg\ m^{-2}\ d^{-1}$**	**Ground** **$mg\ m^{-2}\ d^{-1}$**	**Mound** **$mg\ m^{-2}\ d^{-1}$**
TP	0.56 ± 0.61 b	2.08 ± 1.41 a	1.81 ± 1.41 ab
SRP	0.39 ± 0.13	1.60 ± 1.07	1.94 ± 1.84
NO_x-N	-6.01 ± 1.93 b	-1.63 ± 1.46 a	-2.91 ± 1.23 a

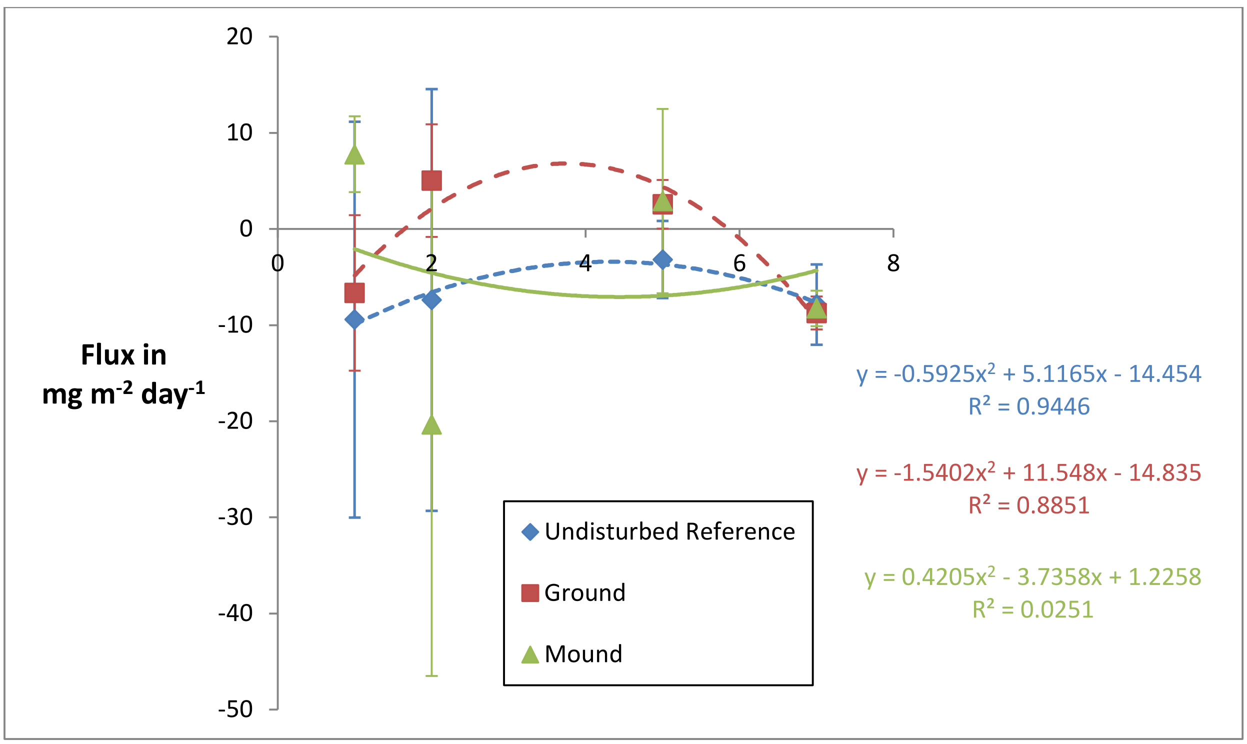
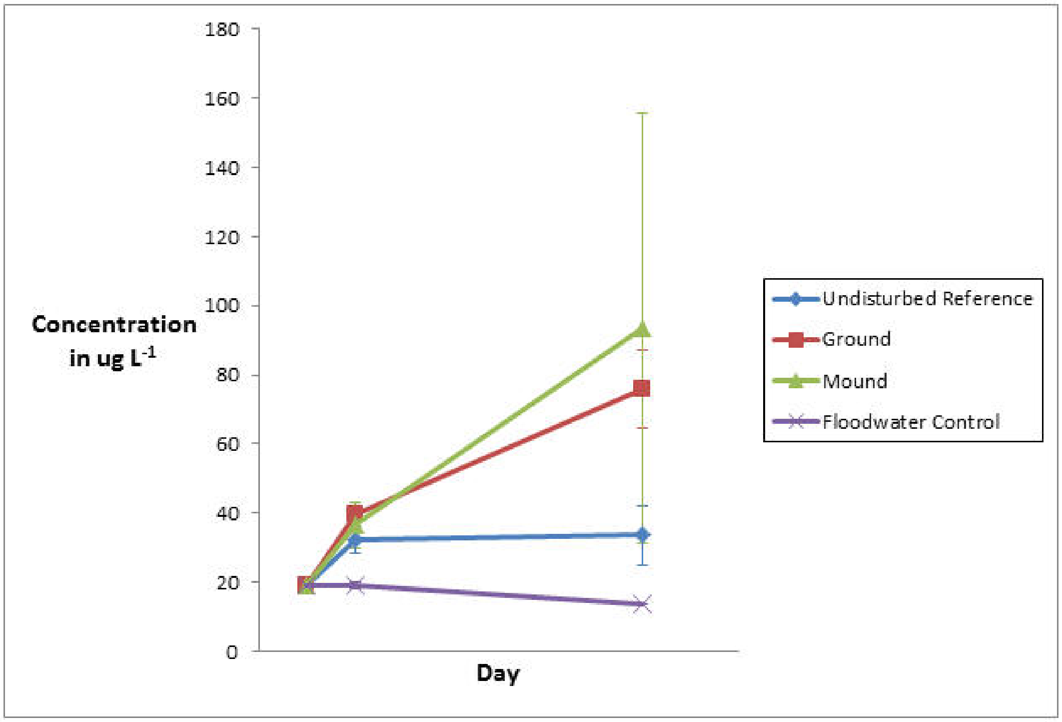
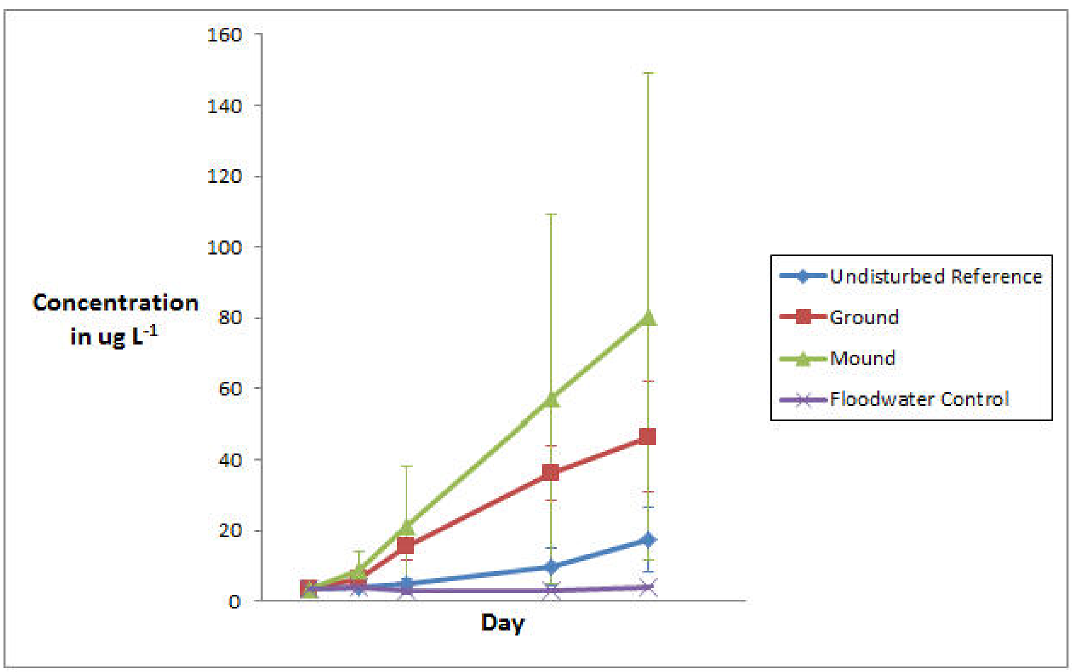


Figure B1. Nitrate+nitrite-N average flux by treatment for Days 1, 2, 5, and 7 based on 2nd order polynomial. Values are shown as means ± 1 SD. Outlier removed (A16 M2).

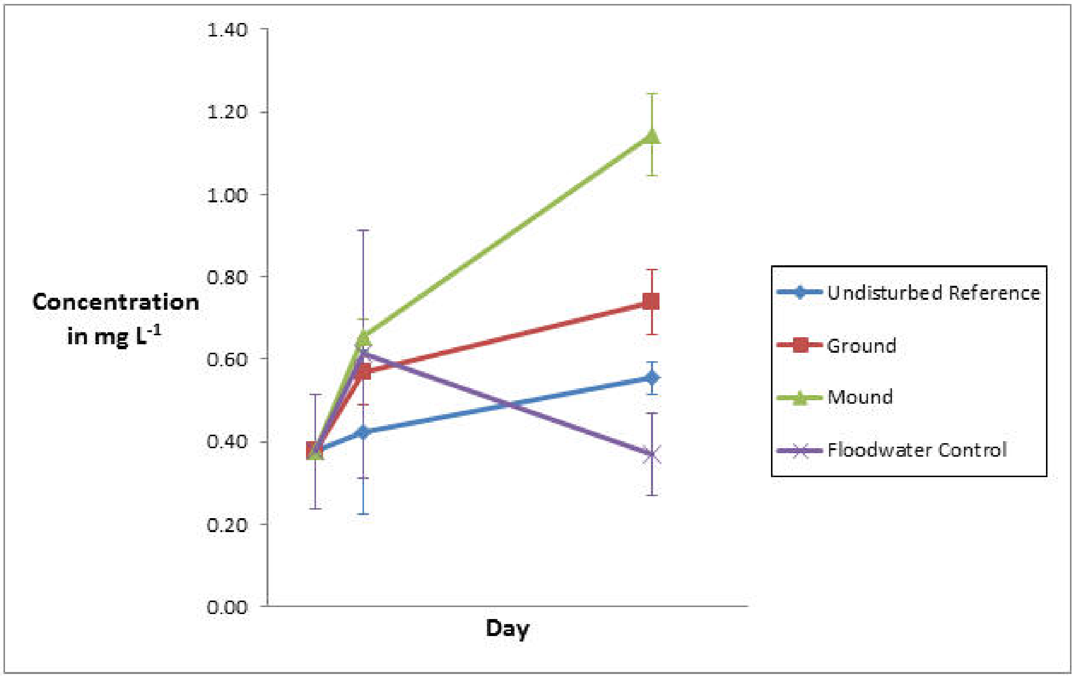
APPENDIX C. Figures showing mean concentrations of phosphorus and nitrogen parameters and relationship to Floodwater Control concentrations.



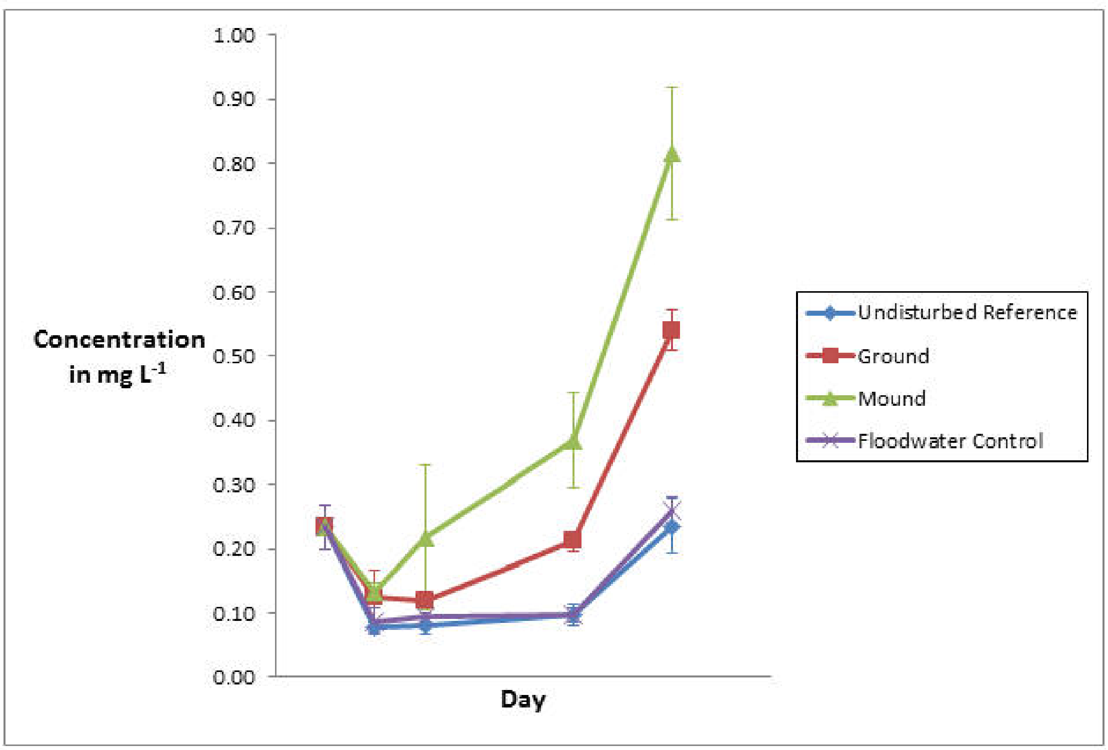
C1. Total phosphorus concentration for all treatments and Floodwater Control for Days 0, 1 and 7.



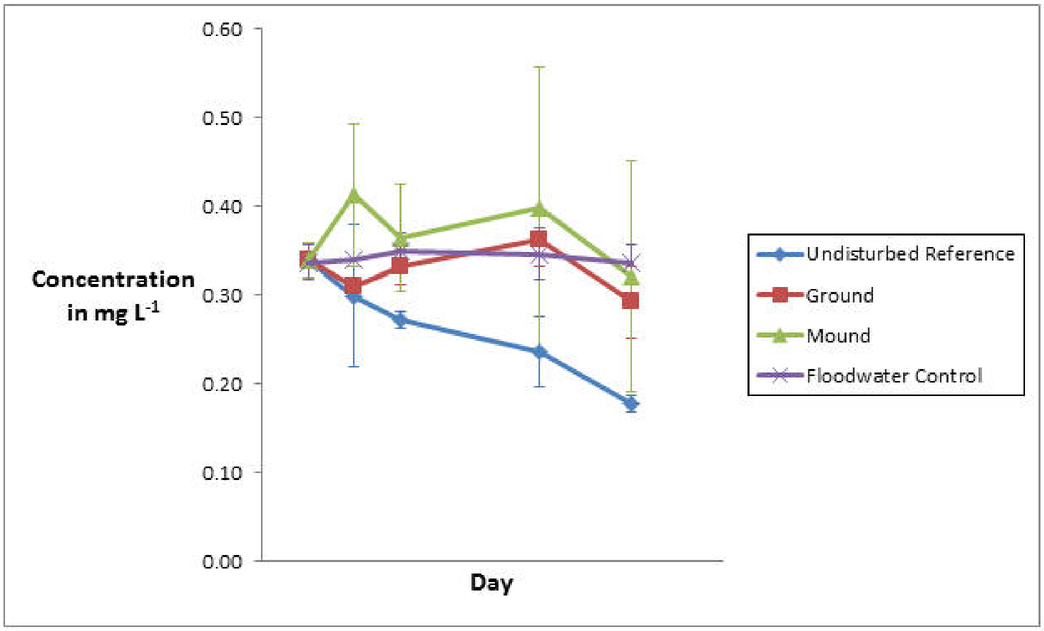
C2. Soluble reactive phosphorus concentration for all treatments and Floodwater Control for Days 1, 2, 5 and 7.



C3. Total Kjeldahl nitrogen concentration for all treatments and Floodwater Control for Days 0, 1 and 7.



C4. Ammonium-N concentration for all treatments and Floodwater Control for Days 1, 2, 5 and 7.



C5. Nitrate+nitrite-N concentration for all treatments and Floodwater Control for Days 1, 2, 5 and 7.