

Phosphorus Retention and Storage by Wetlands in the Okeechobee Drainage Basin

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Executive Summary

Wetlands are known to accrete nutrients and other contaminants and are sometimes managed to improve their overall performance to maintain water quality. The extent of management required depends upon the nutrient/contaminant retention capacity of wetlands, contaminant load to wetlands, and the desired effluent quality. Management scenarios can vary, depending on type of wetland and hydraulic loading rate. For example, small-scale wetlands can be managed efficiently by altering the hydraulic loading or integrating them with a conventional treatment system while large-scale systems can be managed by controlling nutrient/contaminant loads.

Accretion of organic matter has been reported as a major mechanistic sink for P in wetlands. Wetland soils tend to accumulate organic matter due to the production of detrital material from biota and the suppressed rates of decomposition. Restoring the hydrology of these presently ditched and drained wetlands to store increased amounts of water and P could help mitigate P loss from surrounding agricultural pasture within the Okeechobee Basin. However, the surrounding cow-calf grazed, pasture- and subsequently wetland soils have been P loaded for many years. The ability of the restored wetland soils to store more P may be confounded by the legacy of phosphorus already in the drainage basin. Therefore, it is important to determine the P characteristics of wetland soils in these basins and estimate wetland soil potential to sorb P and determine if these soils have any additional P storage capacity.

Overall hypothesis of the study is the hydrologic restoration will enhance the retention of P in isolated wetlands. Results presented in this report is a continuation of prior efforts for the purpose of gaining the appropriate level of information to effectively integrate wetlands P storage concepts into water quality best management practices (BMPs). To accomplish this objective the following tasks have been formulated for the project:

1. Determine the effect of hydrological restoration on water storage and flow paths.
2. Determine, (if any) the change in P storage in wetland and surrounding upland soils and vegetation, as a result of restoring hydrology and grazing.
3. Determine the composition and stability of soil P in the LOB.
4. Validate hydrologic and P models for adaptation to the LOB and use these models to simulate P retention capacity.

Experiments and work, which form components of the various tasks within the project are in different stages of completion at this time. During 2011 we initiated a new experimental variable (grazed vs. non-grazed treatments) and the response to this new variable was significant, complex and not necessarily what we expected and will require additional growing and hydrologic cycles to confirm initial findings. This report presents initial findings and results to date.

Only initial effects of hydrologic restoration and grazing exclosures can be evaluated at the current time because long-term monitoring is required to confirm the effect of these treatments on soil nutrient storage and vegetation dynamics. This may be more than 3 years and could be up to 10 years. Murkin et al. (2000) undertook similar work in isolated wetlands (prairie potholes) in northern portions of the US. They found that there was a time lag between the time of hydrological restoration, where wetland water levels were permanently increased, and when wetland components (vegetation) responded to these new water level regimes. Vegetation is the source material for plant litter, which in turn is the source material for soil organic matter, which in turn is considered critical for long-term phosphorus storage. Because of the significant

influence cattle grazing has on vegetative biomass, understanding how grazing might affect long-term P storage in wetlands was investigated.

These tasks were accomplished using two approaches: (1) Review the available data and determine long-term trends in P storage and release in the drainage basin, and (2) Conduct additional monitoring of soil phosphorus in two isolated wetlands located on Larson Dixie Ranch of the Okeechobee Basin. We used a pair-wise approach with hydrologic restoration of one wetland and allowed the other to be used as a control. In March 2009, a dam was installed on the east wetland which completely blocked ditch flow into or out of the wetland. Each wetland was instrumented with pressure transducers to monitor wetland stage and groundwater level to compare the influence of restoration. Also the center of each wetland was equipped with an automated water sampler in order to determine relationships with wetland processes and total phosphorus concentrations. In February 2011 cattle exclosures were established within the two wetland sites to determine long-term trends on P storage under grazed and ungrazed conditions.

First objective was to determine the effect of hydrological restoration on water storage and flow paths.

Based on the pre-restoration water budgets computed for the study wetlands during the project period, outflow from the drainage ditch accounted for approximately 50% of the water loss from the wetland. Evapotranspiration accounted for more than 30% of the water loss and drainage to groundwater was the remainder. Water budget modeling results found that hydrologic restoration through weir construction in the drainage ditch would increase the hydroperiod and result in a reduction in the ditch export fraction.

An unexpected finding was that inflow from the drainage ditch accounts for at least 10% of the water coming into the wetland. Thus, while installation of the weir in the study wetland resulted in additional retention of water during drainage periods, it also restricted the inflow of water from downstream. This could be the result of culverts later installed downstream of the hydrologically restored wetland resulting in restricted flow and backwatering upstream during certain storm events.

Effective and efficient hydrologic restoration of ditched wetlands must consider the morphology of the wetland- ditch system. We found that the ditches in some of the study wetlands intersected the wetland closer to the lowest elevation of the wetland bottoms, while other wetlands were intersected by ditches that connected at points well above the lowest elevation. Ditches that connected closer to the wetland bottoms were more effective at draining the wetlands resulting in large fractions of the wetland water exported through the ditches. In wetlands where ditches connected near the wetland highest elevations, very little water drained from the wetland. Finally, in wetlands drained by small ditches that connect directly to much larger regional ditches, backwater effects in the larger ditches may result in backflow from the ditch into the wetland. In such instances, weirs will retain water in the wetland but will also hold water from entering the wetland. The net effect of these processes on wetland hydroperiod should be considered when designing wetland hydrologic restoration.

Second objective was to determine, (if any) the change in P storage in wetland and surrounding upland soils and vegetation, as a result of grazing and hydrology.

Findings for this objective indicate that grazing within wetlands and adjacent uplands can significantly reduce the amount of aboveground biomass and associated phosphorus that is contributed directly to litter and possibly to long-term soil storage. Depending on assumptions regarding which aboveground standing stock biomass best represents the contribution of grazed areas, the effect of grazing can be a factor as high as 110 times less organic matter and P input to litter than in non-grazed areas, with the greatest reductions occurring in wetland centers where organic matter accretion rates are expected to be the highest.

This apparent impact of grazing on P storage potential however needs to be tempered by the uncertainty associated with the fate of manure deposited by cattle, and the effects of grazing on soil P storage measured during the first year of this study (objective 3). Looking at grazing effects on aboveground biomass alone would indicate that reducing grazing in wetland areas will result in a significant increases in potential P storage associated with organic matter contributed from aboveground biomass as long as hydrologic conditions are conducive to additional organic matter accumulation. However, results from changes in soil P storage during this same period indicate an as yet unknown interaction between cattle grazing and soil P storage where grazed areas had lower soil P release than areas that were not grazed. It is likely that some of the soil P loss was translocated and stored in aboveground biomass, however that only explained less than 20 % of the change in soil P storage.

Due to the annual cycle of biomass production, senescence and litter production in non-grazed areas relative to an almost continuous cycling of aboveground biomass in grazed areas, the potential benefit of additional organic matter input to the wetlands as a result of non-grazing can only be estimated at this time. The long-term net effect of the observed changes in soil P storage between grazed and non-grazed treatments, relative to the predicted increase in soil P accretion from increased aboveground plant litter inputs will require further monitoring of the exclosure experiment.

Third objective the study was to determine the composition and stability of soil P along the hydrologic gradient (wetland to upland).

This study applied standard biogeochemical analysis and solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy to track the microbial mediated transformation in surface soils of phosphorus (P) inputs (cow-manure, Bahia grass leaf-litter, and inorganic P) characteristic of the agricultural landscape. Bench top mesocosms containing either upland or wetland soils augmented with organic matter or an inorganic P standard were held at field capacity or under simulated flooded conditions for up to 150 days. The aim was to establish how altered redox conditions and associated biogeochemical conditions impact both native and introduced soil P forms.

Organic matter inputs to the landscape contain significant quantities (up to 60% of total P in cow manure) of inorganic P forms that are readily labile in soils, especially under flooded conditions. There is only limited impact of redox conditions upon organic P forms found in soils and studied organic matter inputs. There is a significant impact of redox conditions upon inorganic P forms; including, labile phosphate, acid extractable 'inorganic P' and orthophosphate identified by ^{31}P NMR. Effective P sequestration in soils is dependent upon the accumulation of organic P forms which appear to be relatively insensitive to the influence of altered redox conditions.

Phosphorus and N sequestration in isolated wetlands is strongly linked organic matter accumulation. Results of this study indicate that soils with longer hydroperiod (wetland center) tend accumulate P, N, and C as compared to sites with shorter hydroperiod (edge and upland sites). Approximately 80 to 85% of total P was present in reactive pools with most of it present as organic P. Increasing the hydroperiod can significantly slow down the mineralization of organic P, suggesting hydrologic restoration can potentially increase the P retention. Results also suggest that grazing may not have any negative impact on P retention in wetlands.

Fourth objective of study was to validate hydrologic and P models for adaptation to the LOB and use these models to simulate P retention capacity.

Hydrologic and P measurements were used to validate water- and P-budget models. Both advective and diffusive processes were found to be important components of the total P fluxes from wetlands that are affected by flooding and drying cycles. Based on correlation of the stage measured at the study site and a nearby site with a longer-term record, for the five-year period 2004-2008 the study site wetlands have been dry for 63% (± 19) of the time, over the last five years; and have undergone at least 23 drawdown events. This could have a negative effect on nutrient loading to the wetland, because alternating flooding and drying conditions induce redox-related P release from the soils generating higher P fluxes than perpetually flooded conditions.

The internal loading of P to the wetland via advection and diffusion accounted for nearly 18% of the total P entering the wetland. The development of future best management strategies for cattle pastures should equally address reducing P from internal as well as external sources. By far the greatest export of P from the wetland was via ditch flow, accounting for 49% of the total P loads out of the wetland. The advective transport of P from the water column into the ground via infiltration accounted for 14% P loss from the wetland.

The water flows and the P loads in the LOB are highly unequal in time. That is, the vast majority (as much as 80%) of the total flow in the rivers and the total P load to the Lake occurs during only a few days of the year (as little as 10%, or 36 days). River flows vary over several orders of magnitude, but P concentrations are by comparison relatively stable. Thus, the large variability in P loads is controlled almost entirely by the variability in flows. Thus, the most effective interdiction measures are those that are capable of handling the largest flows, and thus the largest loads.

Recommendations

Management of vegetation and hydrology are key to long-term accretion of P, N, and C in isolated wetlands. Stability of soil P as linked to the stability of soil organic matter needs more detailed evaluation. In addition, management strategies that will increase the proportion non-reactive pools of P needs to be explored. At this we are uncertain if plant uptake of P from soil and potential for litter mineralization and leaching is offset by increased P accretion in OM deposition when compared to grazing effects on biomass and P lability. We believe future studies should be conducted using two approaches: (1) Establish role of wetlands in macro-nutrients (C, N, and P) at watershed scale, and (2) Determine the macro-nutrient storage and associated cycling of C and N on P reactivity and mobility. These studies should be conducted over a long-term under grazed and ungrazed conditions and capture spatial and temporal hydrologic patterns related sequestration and stability of macronutrients in the drainage basin.

Introduction

Wetlands are known to accrete nutrients and other contaminants and are sometimes managed to improve their overall performance and to maintain expected water quality. The extent of management required depends upon the nutrient/contaminant retention capacity of wetlands, contaminant load to wetlands, and the desired effluent quality. Management scenarios can vary, depending on type of wetland and hydraulic loading rate. For example, small-scale wetlands can be managed efficiently by altering the hydraulic loading or integrating them with a conventional treatment system while large-scale systems can be managed by controlling nutrient/contaminant loads.

Phosphorus (P) retention by wetland soils includes surface adsorption on minerals, precipitation, microbial immobilization, and plant uptake, and these processes may be combined into two distinct P retention pathways: sorption and burial. Phosphorus sorption in soils is defined as the removal of phosphate from the soil solution to the solid phase, and it includes both adsorption and precipitation reactions. Phosphorus immobilization through microbial activity and plant uptake are also significant pathways for P removal. However, when plants and microbes die off, the P contained in cellular tissue may either recycle within the wetland, or it may be buried with refractory organic compounds.

Accretion of organic matter has been reported as a major mechanistic sink for P in wetlands. Wetland soils tend to accumulate organic matter due to the production of detrital material from biota and the suppressed rates of decomposition. Soil accretion rates for constructed wetlands are on the order of millimeters per year, although accretion rates in productive natural systems such as the Everglades have been reported as high as one centimeter or more per year. The genesis of this new soil is a relatively slow process, which may affect the P sorption characteristics of the wetland. With time, productive wetland systems will accumulate organic matter (which ultimately forms peat) that has different physical and biological characteristics than the underlying soil. Eventually, this new material settles and compacts to form new soil with perhaps different P sorption characteristics than the original soil. As the wetland ages, steady accumulation of organic matter can potentially decrease the efficiency of the wetland to assimilate additional P and alter the hydraulic flow paths, as organic accretion is seldom uniform throughout space. These conditions can result in elevated effluent P concentrations. However, management of newly accreted material by consolidation or removal can improve the overall P retention capacity of wetland.

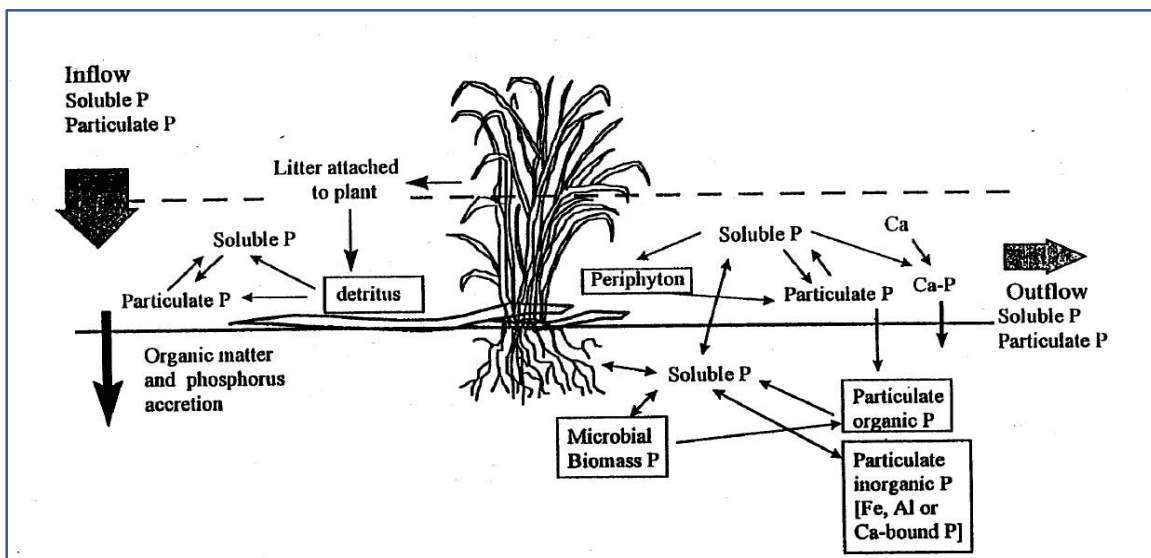


Figure 1. Schematic showing phosphorus stores and fluxes in wetlands

Small historically isolated wetlands, which are a common feature throughout the Lake Okeechobee Basin (LOB) cover about 12,000 ha of the four priority sub-basins. These systems (about 50%) are presently ditched and drained. Hydrologic restoration of these wetlands may help to provide water storage and long-term P retention within LOB's four priority basins—the S-191, S-1154, and Pools D and E in the Lower Kissimmee River as defined by the Northern Everglades and Estuaries Protection Program (Chapter 373.4595, Florida Statutes). There is interest in using these systems to store water and nutrients in the landscape, as the P load to Lake Okeechobee still needs to be reduced to achieve its target Total Maximum Daily Load TMDL of 140 metric tons of P by 2015.

Experiments and work, which form components of the different tasks within the project are in different stages of completion at this time. The proposed work is part of on-going project to address the objectives presented above. Only initial effects of restoration can be evaluated at the current time because long-term monitoring is required to determine the effect of restoration on soil nutrient storage and vegetation dynamics. This may be more than 3 years and could be up to 10 years. Murkin et al. (2000) undertook similar work in isolated wetlands (prairie potholes) in northern portions of the US. They found that there was a time lag between the time of hydrological restoration, where wetland water levels were permanently increased, and when wetland components (vegetation) responded to these new water level regimes. Vegetation is the source material for plant litter, which in turn is the source material for soil organic matter, which in turn is considered critical for long-term phosphorus storage.

Restoring the hydrology of these presently ditched and drained wetlands store increased amounts of water and P, could help mitigate P loss from surrounding agricultural pasture within the Okeechobee Basin. However, the surrounding cow-calf grazed, pasture- and subsequently wetland soils have been P loaded for many years. The ability of the restored wetland soils to store more P may be confounded by the legacy of phosphorus already in soil, akin to the previously mentioned lake sediment example. Therefore, it is important to determine the P characteristics of wetland soils in these basins and estimate wetland soil potential to sorb P and determine if these soils have any additional P storage capacity.

Project Objectives

Overall hypothesis of the study is the hydrologic restoration will enhance the retention of P in isolated wetlands. This research project is proposed as a continuation of prior efforts for the purpose of gaining the appropriate level of information to effectively integrate wetlands P storage concepts into water quality best management practices (BMPs). To accomplish this objective the following tasks have been formulated for the project:

- Task 1. Determine the effect of hydrological restoration on water storage and flow paths.
- Task 2. Determine, (if any) the change in P storage in wetland and surrounding upland soils and vegetation, as a result of restoring hydrology.
- Task 3. Determine the composition and stability of soil P in the Lake Okeechobee Basin.
- Task 4. Validate hydrologic and P models for adaptation to the Lake Okeechobee Basin and use these models to simulate P retention capacity.

These tasks were accomplished by using two approaches: (1) Review the available data and determine long-term trends in P storage and release in the drainage basin, and (2) Conduct additional monitoring of soil phosphorus in two isolated wetlands located on Larson Dixie Ranch of the Okeechobee Basin. The two wetlands on Larson Dixie cow-calf ranch were again selected to for this study (Figure 2). We used a pair-wise approach with hydrologic restoration of one wetland and allowing the other to be used as a control. In March 2009, a dam was installed on the east wetland which completely blocked ditch flow into or out of the wetland. Each wetland was instrumented with

pressure transducers to monitor wetland stage and groundwater level to compare the influence of restoration. Also the center of each wetland was equipped with an automated water sampler in order to determine relationships with wetland processes and total phosphorus concentrations. Three transects were added in order to determine baseline vegetation and soils information for exclosures. In February 2011 exclosures were established at two wetland sites to determine long-term trends on P storage under grazed and ungrazed conditions. All six transects were sampled in the center, edge, and upland with respect to above and below ground productivity and nutrient storage and soil nutrient characteristics 0-20 cm January 2011, August 2011, January 2012. It should be noted that the results presented in this represent short-term effects of grazing over a period of less than one year.

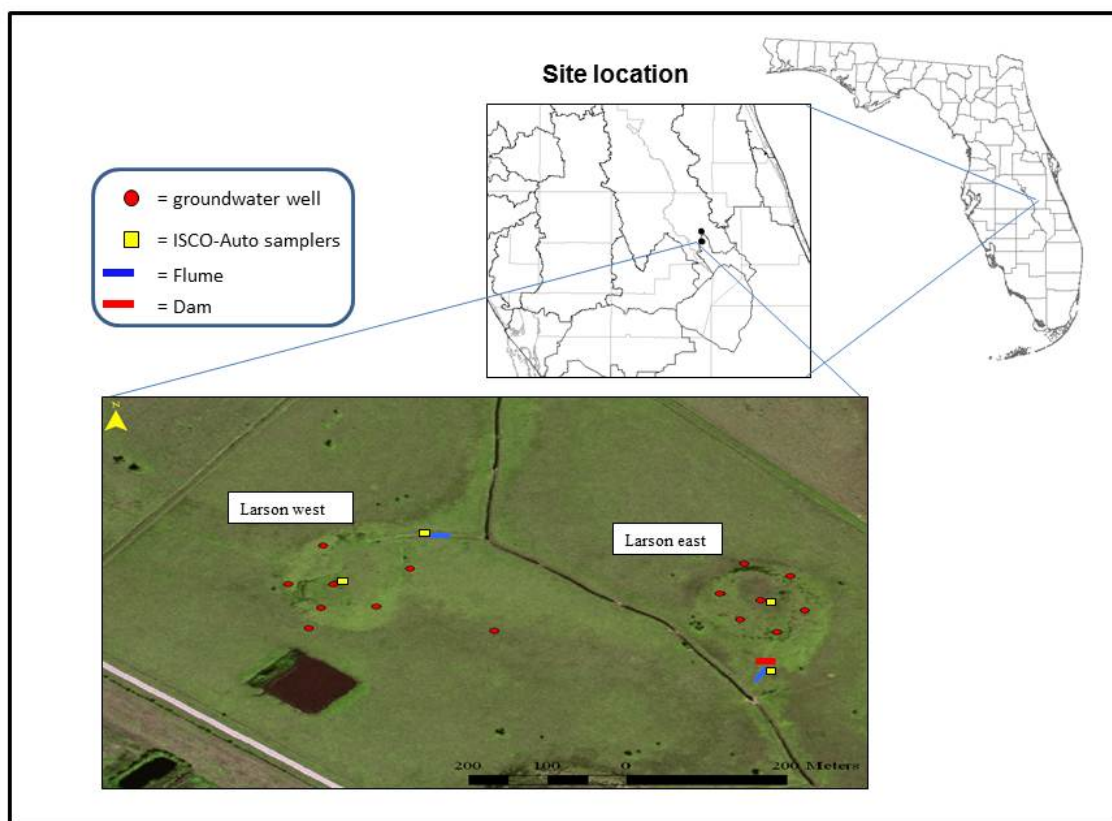


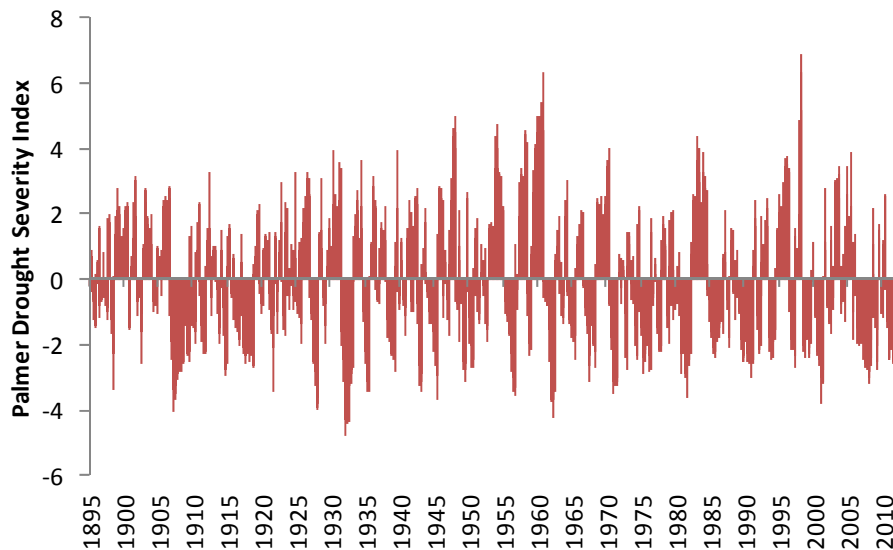
Figure 2. Paired isolated wetlands located in Larson Ranch used in the study

Task 1

Effect of Hydrological Restoration on Water Storage and Flow Paths

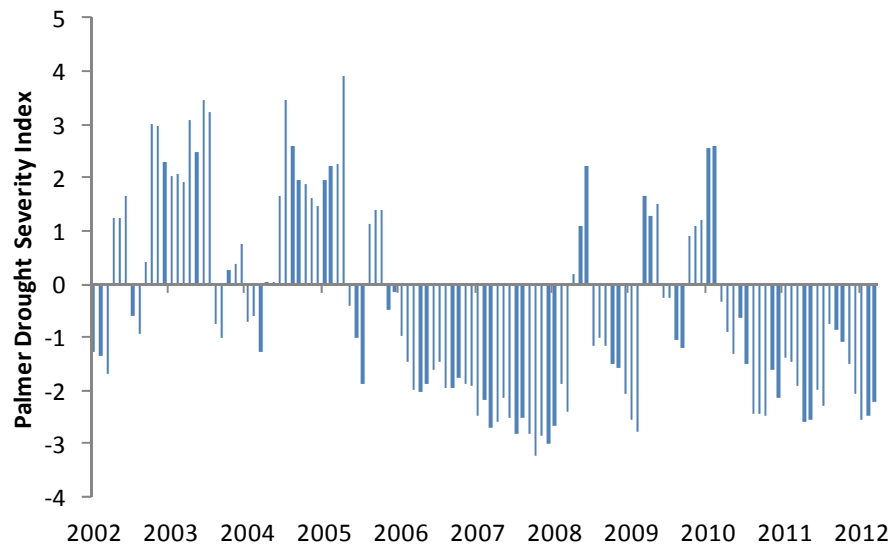
1.0 Introduction

Long-term drought conditions in the Okeechobee basin continued during this phase of the study. The Palmer Drought Severity Index (PDSI) is a quantitative measure of drought, and data are available from the National Climatic Data Center, a division of NOAA. The first figure below shows the PDSI for Florida Climatic Division 4 (the central portion of the state which encompasses Okeechobee County) from 1895 through May 2012. Values greater than zero indicate wet conditions, while increasingly negative values indicate increasingly severe drought. These data reveal cyclic wet-dry periods on a sub-decadal scale. The second figure shows only the data for the past 10 years. Again a cyclic pattern is evident. The period since mid-2010 is classified as drought, and much of the total study period (since 2002) is also shown to be drought conditions.



Data source: NCDC, <http://www.ncdc.noaa.gov/>, Florida climatic division 4.

Because of these drought conditions, the study wetlands were dry during the recent study period. Long-term hydrologic monitoring was able to capture periods when the wetlands were inundated. Water quality sampling of surface waters during these inundation periods was coupled with the hydrologic data to develop models of both the water and nutrient budgets. Included within those budgets was the role of fluxes across the sediment-water interface. The text below describes these models.



1.1 Background and Motivation

Isolated wetlands within the Lake Okeechobee basin (LOB), Florida, have been used to store water, and shown to reduce phosphorus (P) loss within the landscape continuum between the upland and the lake (Hiscock et al. 2003; Leibowitz and Nadeau 2003). However, wetlands located on fertilized agricultural or manure-impacted lands such as those used for beef operations can also potentially release some of the P stored in soils back into the water column (Fisher and Reddy 2001; Pant and Reddy 2003; Dunne et al. 2007). Such internal loading of P from impacted sediments and soils has been identified as a critical component of the nutrient budget that can control the trophic conditions of aquatic systems such as lakes and wetlands (Berner 1980; Håkanson 2004). The internal release of P from shallow aquatic systems can occur via two different mechanisms: (i) release at or below the sediment-water interface during hypoxic or anoxic conditions, and the subsequent *diffusion* of dissolved P to the overlying water column driven by concentration gradients (Moore et al. 1998; Fisher and Reddy 2001; Pant and Reddy 2003); and (ii) *advection* as a result of a fluctuating water table (Corstanje and Reddy 2004), bioturbation (Biswas et al. 2009) or sediment re-suspension (Sondergaard et al. 2001). Phosphorus fluxes from the soil to the overlying water column depend on factors such as, physico-chemical characteristics of the soil, P concentration of the floodwaters, and changes in soil redox conditions. These factors determine whether wetland soils behave as a sink of P, or as an internal source of P, which may equal or even exceed the external load entering the system (Pant and Reddy 2003).

The implementation of phosphorus control programs within the LOB resulted in a decline in P loading from the early 1980s to 1990, however P loads to Lake Okeechobee still remain over the legally mandated target primarily because of discharge from tributary basins such as Nubbin Slough and Taylor Creek (NSTC) that have active beef cattle and dairy operations within its watershed (Hiscock et al. 2003). External loading of P from agricultural and cattle areas has been addressed through the implementation of Best Management Practices (Bottcher et al. 1999), and interception strategies that involve the restoration of isolated wetlands in the state of Florida (Rice et al. 2002). However, internal loading of P through

impacted soils within the NSTC still exceeds the desired levels, and continues to prevent the rapid improvement of water quality in the basin (Flaig and Reddy 1995; Pant and Reddy 2003).

Fluxes of P from wetland soils have been routinely estimated using a general procedure that involves exposing a soil core to a layer of P-free water (Olila et al. 1997; Fisher and Reddy 2001; Pant and Reddy 2003; Dunne et al. 2006), and measuring the P concentration of the water column over time. However, these are typically abiotic experiments with static water levels. Hence, such experiments only capture the diffusive flux, placing a limit on how much labile P can be released from the soil to the water column (Kadlec and Wallace 2008). Corstanje and Reddy (2004) demonstrated that fluxes of dissolved reactive P were significantly greater under microbially active anaerobic floodwaters using incubation core experiments, and that subsurface reflooding also resulted in significant increase in fluxes from the soils.

Internal load from advective fluxes are potentially important in wetlands that receive groundwater inflow. Depressional wetlands in the LOB are seasonally inundated and may experience multiple wetting/drying cycles as the regional water table rises/falls. In a two-year study of four isolated wetlands in the LOB, Min et al. (2010a) found mean annual hydroperiods of 71 ± 10 % with groundwater inflow occurring 15% of the time the wetlands were inundated. The inflowing shallow groundwater (pore water) may act as a potential internal advective source of P.

In addition, wetlands that are unsaturated during drawdown events, soil oxidation is increased and re-flooding may subsequently mobilize P (Bostic and White 2006). Episodic drops in water levels can increase organic matter decomposition, potentially increasing P loading to the water column on re-flooding (Watts 2000; DeBusk and Reddy 2003). Also, the P retention capacity of soils that have undergone repeated drawdown have been shown to diminish on re-flooding compared to perpetually flooded soils (Klotz and Linn 2001; Bhadha et al. 2010).

A simple one-dimensional (depth) diagenetic model such as Fick's first law of diffusion is often used to estimate the vertical flux based on close-interval estimation of pore water concentration gradients (Berner 1980; Harper et al. 1997). Such models assume microbially-mediated processes are laterally uniform (Kana et al. 1998), and nutrient gradients can be measured using pore water equilibrators (Hesslein 1976; Webster et al. 1998) or multisamplers (Martin et al. 2003). Quantifying nutrient fluxes via advective modes is complicated by transient processes such as wind effects, wave action, and fluctuating water table. Some of the techniques that have been used to quantify advective fluxes include chemical tracers (Rama and Moore 1996), ground water modeling (Pandit and El-Khazen 1990), and seepage meters (Micheal et al. 2003).

In this paper we report on a field study to estimate P fluxes in and out of the water column from two impacted isolated wetlands within the Lake Okeechobee drainage basin (Figure 1-1). Wetland 1 was our main site from which water samples were periodically collected and analyzed. Both diffusive and advective fluxes were estimated from Wetland 1, in addition to a P budget assessment. Wetland 2 was used only to estimate diffusive fluxes using pore water equilibrators for comparison with Wetland 1. It was hypothesized that under fluctuating water table conditions the advective internal load of P within such wetlands may be significant compared to diffusion. The objectives of the study were to: (i) calculate diffusive and advective fluxes of total P (TP) based on *in-situ* measured pore water concentrations, and (ii) develop a P budget to evaluate the importance of diffusive and advective internal load in wetlands affected by transient hydrologic conditions involving flooding and drying cycles. Quantifying diffusive and advective P fluxes from these wetlands can be useful for ecosystem managers in determining the importance of internal versus external P loading.

1.2 Site Description

The majority of the P load to Lake Okeechobee has been identified from four sub-basins, which are functionally defined as the “priority basins” (Flaig and Reddy 1995). The two wetlands selected for this study were located within the S-154 priority basin, and are historically drained emergent marshes located on an active beef farming facility (north 27° 20' 56", west 80° 56' 28"). Neither wetland has any ditch inflows, and each has only one drainage ditch outflow that connects to a larger ditch network transporting water to Lake Okeechobee (Figure 1).

The wetlands are surrounded by cow-calf grazing pastures that are dominated by *Bahia* grass species. The wetland soils at the site have been classified as Ultisols because they consist of stratified soil horizons more than a meter deep. In contrast, the upland soils exhibit pedologically formed soil horizons and are classified as Alfisols because of the presence of an argillic clay horizon within the profile (Bhadha and Jawitz 2010). Wetland soil characteristics from the surface to 10-cm depth are summarized as follows: total-P $236 \pm 53 \text{ mg kg}^{-1}$, oxalate extractable-P $130 \pm 153 \text{ mg kg}^{-1}$, water extractable-P $8.9 \pm 4.8 \text{ mg kg}^{-1}$, P-sorption capacity $288 \pm 38 \text{ mg kg}^{-1}$, organic matter $38 \pm 14\%$, and pH 6.5. The surficial water table fluctuates from as much as 60 cm above the soil surface during the wet season to more than 100 cm below ground surface during the hot-dry periods (Min et al. 2010a). Prior to 1992, the site was used for dairy operations, and since 1992 the land surrounding the wetland has been used for a cow-calf operation with about 140 cattle enclosed within the pasture (yearly average), at a stocking rate of 1.2-2.0 head ha^{-1} .

2.0 Materials and Methods

2.1 Field Sampling and Analyses

2.1.1 Hydrologic monitoring and water budget analysis

The wetland stage (H_{wet}), upland groundwater level (H_{up}), and rainfall for Wetlands 1 and 2 were monitored for a two-year period between March 2004 and March 2006 (Min et al. 2010a). This study is based on the eleven-month period between May 2005 and March 2006 during which intensive water quality data were also collected (described below). During this period, hydrologic pathways of the study wetlands were measured or estimated independently (Min et al. 2010a and b). The methods used to determine each volume-based water budget component are summarized in Table 1-1, and the average daily fluxes ($\text{m}^3 \text{d}^{-1}$) including water budget error are illustrated in Figure 1-2.

For wetland-groundwater exchange, the hydraulic conductivity of the soil surrounding the wetlands was calculated at the wetland scale using the modified Dupuit equation under a constrained water budget framework ($GW = -\Delta H_{wet} - ET$). Intermittent natural drawdown events were observed at the study wetlands and the temporal mean hydraulic conductivities (Wetland 1 = 5.5 m d^{-1} and Wetland 2 = 21.3 m d^{-1}) were used to estimate the continuous daily volumetric groundwater inflow (Q_{IN} , $\text{m}^3 \text{d}^{-1}$) and outflow (Q_{OUT} , $\text{m}^3 \text{d}^{-1}$) during the period (Min et al. 2010a). For detailed information regarding the overall hydrologic monitoring effort, including well installation, bathymetry survey, and quantification of water budget components, see Min et al. (2010a and b).

2.1.2 Surface and pore water sampling

Surface and pore water samples were collected six times from Wetland 1 during the monitoring period. On each of these occasions surface water samples were collected by simple dip and grab method in 100 ml polypropylene bottles. Pore water samples were collected using multilevel piezometers (“multisamplers”, Martin et al. 2003) specifically designed for this project. The multisamplers consist of ten ports screened with 250- μm mesh built into a 5-cm diameter, 120-cm long schedule 80 PVC pipe. The

ports were glued to PVC tubing that extended up the interior of the PVC pipe to the surface. The pipe was pushed into the soil, and the tubing led to the surface and hooked to a peristaltic pump to extract water. The sampling ports were located at 10 cm depth increments up to 110 cm below the soil-water interface. Approximately 100 ml of pore water was purged out prior to sampling. Water samples were, acidified, and transported to the University of Florida where they were analyzed for TP (soluble reactive P + dissolved organic P) by EPA (1979) standard method 365.4 (Technicon AAI autoanalyzer). Analytical precision was based on duplicates every tenth sample, with relative percent difference for each sampling event of < 5%.

In addition to the multisamplers, in August 2008 three pore water equilibrators (PWEs) were deployed in Wetland 1 and 2 to capture vertical changes in solute concentrations at every 1 cm depth interval near the soil-water interface, up to a depth of 25 cm. Both PWEs (from Wetlands 1 and 2) and the multisamplers (from Wetland 1) were deployed strategically, one near the connection to the drainage ditch, the second in the center of the wetland, and the third at the boundary of the wetland farthest from the ditch. Using PWEs helps identify the precise depth (± 1 cm) below the soil-water interface where P concentration in groundwater is highest. In contrast to the multisampler, PWEs can provide a much finer resolution in concentration gradients. Pore water samples were analyzed for soluble reactive P (SRP) using this technique. .

The PWEs used in this study were similar in design to the device described by Hesslein (1976), consisting of $2 \times 10 \times 60$ -cm laths of acrylic into which are milled 8-cm³ cells vertically spaced every 1 cm. The cells were filled with deionized water, covered by a sheet of 0.45- μ m polyethersulfone membrane and a sheet of 300- μ m silk netting placed over it. An acrylic cover was tightly screwed on to the lath, and the entire apparatus was placed in a container, sealed, and purged of O₂ using a N₂ gas line for 48 hours prior to field deployment. On site, the PWEs were pushed into the ground so that 10 cells (10 cm) were in the water column, and the remaining cells buried below ground. Equilibrium studies have shown that between 2-3 weeks is sufficient time for pore water P to equilibrate with the solution inside the cells (Carignan et al. 1985). The equilibration period for this study was 19 days, and during this time tropical storm Fay passed directly over the wetlands (NOAA 2008). After the equilibration period, the PWEs were retrieved from the soils, and the water samples drawn out of individual cells using a syringe, acidified, and stored at 4°C until analysis.

2.2 Flux Calculations

Vertical diffusive flux, J_{Diff} [M T⁻¹], was calculated using Fick's first law (Berner 1980):

$$J_{Diff}(t) = -\frac{\phi D}{\theta^2} \times \frac{\partial(C_{sw}(t) - C_{pw}(t))}{\partial z} \times A_{wet}(t) \quad (1)$$

where ϕ is porosity [L³ L⁻³], D is the bulk diffusion coefficient [L² T⁻¹], θ is soil tortuosity [L³ L⁻³] estimated from Berner (1980) and Sweerts et al. (1991):

$$\theta = \sqrt{0.47\phi + 1.91} \quad (2)$$

$C_{sw}(t)$ and $C_{pw}(t)$ are TP concentration in wetland water column and pore water [M L⁻³], z is effective diffusive depth [L], and $A_{wet}(t)$ is wetland flooded area. Porosity of 0.5 was used based on the depth average measured from intact cores up to 10 cm (Bhadha and Jawitz 2010). Li and Gregory (1974) reported the bulk diffusion coefficients of HPO₄²⁻ and H₂PO₄⁻ as 7.34×10^{-6} and 8.46×10^{-6} cm² s⁻¹, respectively. An average of the two values (7.9×10^{-6} cm² s⁻¹) was used as the pH of the soils was near neutral at 6.5.

While diffusion can occur at all times when the wetland is flooded, advection from groundwater to the wetland water column is generally limited only to times when the water table rises across the soil-water interface, and rarely occurs when the water table is constant. The advective flux was positive when pore

water was transported into the water column (J_{GW_IN}) [$M\ T^{-1}$], and negative when the water table dropped below the soil-water interface carrying TP with it (J_{GW_OUT}) [$M\ T^{-1}$]. Fluxes were calculated as the product of daily Q_{IN} or Q_{OUT} [$L^3\ T^{-1}$] and either C_{pw} of TP sampled from 10 cm depth using multisamplers for fluxes into the wetland, or C_{sw} of TP for fluxes out of the wetland:

$$J_{GW_IN} = Q_{IN} C_{pw} \quad (3)$$

$$J_{GW_OUT} = Q_{OUT} C_{sw} \quad (4)$$

Both diffusive and advective fluxes calculated as part of this study represent the monthly average for all days of the month (including wet and dry days), and reported as $mg\ m^{-2}\ d^{-1}$ for the individual months.

2.3 Phosphorus Budget

The monthly P budget was determined from the summed P mass inputs and outputs over the eleven month monitoring duration:

$$\Delta S_{sw} = J_P + J_O + J_{D_IN} + J_{Diff} + J_{GW_IN} - J_{GW_OUT} - J_{D_OUT} \pm J_R \quad (5)$$

where the subscripts refer to the change of P stored in the wetland water column (S_{sw}) resulting from input flux parameters such as atmospheric loading (P), surface runoff (O), backflow via ditch (D_IN), and ground water loading into the wetland associated to diffusive ($Diff$) and advective fluxes (GW_IN), and output parameters such as ground water load out of the wetland related to the advective flux (GW_OUT), and load associated with the flux exiting the wetland via ditch flow (D_OUT). The J_R term closes the monthly P budget and is considered in this study as a lumped retention process (sedimentation, uptake by biota and sorption) or release process (resuspension, plant decay, and desorption).

Ahn and James (2001) showed that a substantial source of P to the LOB was from atmospheric deposition. They estimated P deposition rates from the atmosphere to vary between 6.8 to 32.6 $\mu g\ L^{-1}$ depending on wet and dry seasons respectively, with a bulk annual average of 95.4 $\mu g\ P\ m^{-2}\ d^{-1}$. The bulk atmospheric deposition rate was multiplied to the daily based wetland flooded area that was calculated from the stage-area relationship (Min et al. 2010a) to estimate atmospheric loading to the wetland. Concentration of P from pasture runoff was not measured as part of this study; however, runoff TP concentration of 0.63 $mg\ L^{-1}$ (Capece et al. 2007) was used in this analysis as a mean concentration of wide ranges (0.15 to 1.20 $mg\ L^{-1}$) reported from Bottcher et al. (1999) and Capece et al. (2007). The input of P from the drainage ditch was calculated from the estimated backflow volume and monthly averaged wetland water column TP concentration, assuming ditch inlet TP concentration is close to the wetland water column level. Phosphorus load exiting the wetland via the ditch was calculated using product of the volumetric loss of water via the ditch and the monthly averaged concentration of surface water in the wetland for periods when the wetland stage was above the bottom of the ditch (H_{ditch} ; 0.3 m).

3.0 Results and Discussion

3.1 Wetland Hydrography and Hydroperiod

Based on the stage data, Wetland 1 remained flooded for 240 of the 314 days of monitoring period, in three cycles of wetting and drying corresponding to 104, 91, and 34 days (Figure 1-3). The remaining 74 days the water table remained below the ground surface. One of the key controls on ground water nutrient cycling processes in seasonally flooded wetlands is the alternation between flooded and dry conditions. The maximum water table fluctuations were between 56 cm above ground and 88 cm below ground surface. The average yearly rainfall in Okeechobee County is between 1.3 and 1.6 m (Lewis et al. 2001). During the monitoring period precipitation was just below the annual average at 1.1 m. Drought conditions render these wetlands dry for prolonged periods, with only 205 inundated days between April

2006 and July 2008, when these wetlands were flooded once again and the water level in the wetland was 120 cm above ground following tropical storm Fay (19 August 2008).

The longer-term hydroperiod of the wetland was estimated by correlation with a five-year record of stage from Cypress slough (USGS station 02272676, <http://waterdata.usgs.gov/nwis/rt>), near Basinger, FL, approximately 30 km from the study site. The Cypress slough region located in Basinger comprised similar limestone bedrock geology, and similar soil series (Basinger fine sand, Bhadha and Jawitz, 2010) as the field site. Polynomial regression ($r^2 = 0.59$; $p < 0.001$) between Cypress slough and Wetland 1 stage during the 314 day monitoring period of our study is shown in Figure 1-4a. This relation indicates that inundation of the study wetland corresponds to approximately 9.6 m at Cypress slough. The regression equation was used to estimate the average yearly hydroperiod of Wetland 1 from 2004 through 2008 (Figure 1-4b). During this period, the estimated annual hydroperiod was $37.2 \pm 19.5\%$, with 4 ± 1.6 inundation events.

Such drastic fluctuations in soil saturation can induce long-term effects in soil chemistry (mineralogy) due to changes in redox conditions. While P stored in newly accreted soils is typically stable and refractory under continued inundation, ecosystem dryout has a negative effect on P storage, increasing internal P loading to the system (Corstanje and Reddy 2004; Bostic and White 2006). In addition, wetland vegetation may rapidly release stored P during senescence (up to 75% of the total plant associated P) as a result of prolonged dryouts, potentially increasing shallow groundwater and water column P concentrations (White et al. 2006).

3.2 Porewater Phosphorus

Porewater P was evaluated based on the pore water samples collected using multisamplers (during 2005-2006 monitoring period), and porewater equilibrators (in August 2008). During the 2005-2006 monitoring period, Wetland 1 porewater concentrations of P showed both spatial and temporal variability (Figure 1-5). Concentration of TP (reported here as mean $\pm$ SD) was highest at 10 cm depth for all six sampling periods: 3.9 mg L^{-1} (± 2.1) at MS1, 3.4 mg L^{-1} (± 1.4) at MS2, and 2.2 mg L^{-1} (± 0.4) at MS3. It is not unusual for porewater P concentrations to be high in the surface horizons due to inputs from plant-associated P during senescence, enhanced microbial decomposition, and manure inputs from cattle activity. The concentration of P decreased to less than 0.02 mg L^{-1} below 80 cm depth at MS2 and MS3, however, P concentrations increased beyond 70 cm at MS1. This could be a result of P desorbing from the deeper soil horizons associated with the clay horizons (Bhadha and Jawitz 2010). At all three sampling locations the lowest concentrations at the surface (10 cm depth) were observed during the February (winter) sampling event, while the highest concentrations were observed between July-October (hurricane season) sampling events.

The importance of sorption kinetics on P transport through the wetland surface soils was evaluated based on the Damköhler number (ω), which is the ratio of residence time to reaction time: $\omega = kL/v$, where k is the first order rate constant [T^{-1}] used to evaluate the sorption reaction between the soil and pore water as the front moves upwards within the soil profile; L is the soil length; and v is the pore water velocity [L T^{-1}]. Chen et al. (1996) estimated SRP desorption rate constants for a two-site reactive transport model from columns of sandy spodic soils (Myakka) ranging between 1.2×10^{-3} to $4.4 \times 10^{-3} \text{ s}^{-1}$. Velocity of the wetting front was determined from a study of the wetland-groundwater interaction at this site (Min et al. 2010a), and varied between 0.02 to 0.081 cm d^{-1} . The soil length of interest was chosen to be the top 10 cm, because pore water-surface water concentrations gradients were highest at that depth. Different scenarios of high and low velocities resulted in $4.7 \times 10^3 < \omega < 1.2 \times 10^5$. Because these values were all large ($\gg 10$), it was concluded that TP in pore water and the soil were in equilibrium, and therefore appropriate to be used to calculate J_{GW_IN} .

Pore water SRP concentrations were also measured at three locations within Wetlands 1 and 2 using PWEs in August 2008 (Figure 1-6). In Wetland 1 pore water concentration of SRP at P1 was highest at 11 cm below the surface (12.5 mg L^{-1}). At P2 and P3 SRP concentrations were highest at 9 cm (6.7 mg L^{-1}) and 8 cm (6.2 mg L^{-1}), respectively. The highest concentration depths measured using PWEs were consistent with those measured using the multisamplers in 2005, supporting $z = 10 \text{ cm}$ to calculate diffusive fluxes. In Wetland 2, pore water concentration of SRP at P4 was highest at 17 cm (7.2 mg L^{-1}) below the surface. At P5 and P6 SRP concentrations were highest at 8 cm (4.6 mg L^{-1}) and 7 cm (7.0 mg L^{-1}), respectively. The concentration of pore water SRP from the PWEs nearest to the ditch were relatively higher than those from either the center of the wetland or its boundary. Regardless of the sampling locations SRP pore water concentrations measured using the PWEs in August 2008 were significantly ($p < 0.01$) greater than TP measured using multisamplers during the 2005-2006 sampling events, even though SRP comprises of 92% of TP in these wetland systems. This increase in pore water concentrations may be directly related to an event-based phenomenon associated with tropical storm Fay, due to rapid flooding of the oxidized soils. Olila et al. (1997) showed that SRP from drained soils that were rapidly reflooded were 4-times higher compared to controlled, continuously flooded soils. Higher external P loading to the wetland in 2008 than in 2005-2006 is also possible, although there is no evidence of changes to surrounding land-use practices or livestock activity in this period to support this theory.

3.3 Fluxes Across the Soil-Water Interface

3.3.1 Diffusive Flux of P Based on Fick's First Law

Based on the concentration gradients between the pore water and the surface water, the direction of the diffusive fluxes at the soil-water interface was always from the ground to the water column (positive). The upward flux of TP from the soil into Wetland 1 was estimated for all six sampling events, with the highest rates at MS1 (Figure 1-7). The pore water TP concentration at 10 cm depth was $3.2 \pm 1.5 \text{ mg L}^{-1}$ ($n = 18$ for all six sampling events at all three multisampler locations), and the mean TP concentration of the surface water in Wetland 1 was $0.53 \pm 0.17 \text{ mg L}^{-1}$ ($n = 18$). The highest monthly TP flux to the water column was $0.97 \text{ mg m}^{-2} \text{ d}^{-1}$ recorded during the September 2005 sampling period, while the lowest flux was observed February 2006 from all three sites (MS1, MS2, and MS3). The diffusive flux measured using all three multisamplers and for all six sampling events was $0.32 \pm 0.14 \text{ mg m}^{-2} \text{ d}^{-1}$, where the variability includes both the spatial distribution of the multisamplers, and also temporal variations, because diffusive fluxes are highly redox sensitive. Malecki et al. (2004) and Sen et al. (2007) showed that the flux of soluble P from sediments under anaerobic conditions was significantly greater than under aerobic conditions. During the study, the wetlands were only seasonally flooded, hence groundwater concentrations (and therefore the fluxes) are highly influenced by changing redox conditions. Prolonged anaerobic conditions within the soils may have led to the higher pore water concentrations, and associated diffusive fluxes, during September and October 2005. Nearly 78% (± 18) flux reduction was observed between September (high-flux) and February (low-flux) months from sites MS1, MS2, and MS3.

Diffusive flux of SRP was also calculated using the surface water-pore water concentration gradients collected from all six PWEs that were deployed in Wetlands 1 and 2 in 2008 (Figure 1-8). The SRP flux calculated using PWEs from both Wetlands 1 and 2 was $0.84 \pm 0.31 \text{ mg m}^{-2} \text{ d}^{-1}$. These were nearly double the annual mean TP flux determined using multisamplers during 2005-2006. However, the multisampler-based fluxes showed variability based on inundation and exposure duration between flooding events. The mean multisampler-based flux from the sampling events with the highest pore water TP concentrations (September and October 2005: $0.61 \pm 0.27 \text{ mg m}^{-2} \text{ d}^{-1}$) was only 26% less than the 2008 PWE-based mean flux. These comparisons highlight the importance of considering temporal variability in estimating diffusive fluxes.

The diffusive fluxes calculated from this study were compared to P fluxes that have been previously reported in a variety of aquatic systems using intact soil incubation (Table 1-2). The fluxes reported here are up to two orders of magnitude lower than those estimated from the peat soils within the Everglades Water Conservation Area (WCA) 2A (Fisher and Reddy, 2001), but are consistent with those from within the sandy zones from within Lake Okeechobee (Moore et al. 1998). The concentration gradient between pore water and surface water is greater for P-rich peat soils as compared to sandy sediments. It is evident that while the accretion of P-rich material at the soil-water interface could increase the diffusive fluxes from the soil to the water column, an increase in surface water concentrations from runoff inputs can reduce diffusive fluxes from shallow soils to the water column, as the P concentration gradient between the two also reduces (Dunne et al. 2006).

3.3.2 Advective Flux of P Based on Groundwater Fluctuations

On three occasions during the eleven-month monitoring period the water level in the study wetlands dropped below the soil surface, rendering the wetland surface completely dry exposing the soils, and then rose back up filling the wetlands once again (June 2005, October 2005, and February 2006). Based on the daily estimation of groundwater-surface water exchange, groundwater moving into the wetland water column occurred for 57 days, generating a positive advective flux of $1.31 \pm 4.03 \text{ mg m}^{-2} \text{ d}^{-1}$ (Figure 1-7). While during the same period water from the wetland water column drained into the ground (groundwater out) almost every month totaling to 181 days, generating a negative advective flux of $2.42 \pm 4.70 \text{ mg m}^{-2} \text{ d}^{-1}$ associated with seepage/infiltration.

The advective fluxes of P to the wetland via groundwater were greater than the diffusive fluxes for all sampling events. Positive advective fluxes were greatest during October 2005 at $1.1 \text{ mg m}^{-2} \text{ d}^{-1}$, driven by the high volumetric loading rate of $6.9 \text{ m}^3 \text{ d}^{-1}$. Advective fluxes of TP out of the wetland water column and into the ground were greatest during January 2006 at $4.7 \text{ mg m}^{-2} \text{ d}^{-1}$. During the monitoring period, the average advective TP fluxes out of the wetland water column were 26% greater than those entering it via ground water fluctuation. In comparison, diffusion temporally dominated the wetland's hydrologic regime (240 out of 314 days), even though it accounted for smaller fluxes than advection via groundwater movement. This implies that advective modes of P transport across the soil-water interface cannot be ignored while calculating P loads in and out of wetlands particularly affected by fluctuating water tables.

3.4 Phosphorus Budget Estimation from May 2005 - March 2006

Based on diffusion alone the cumulative P internal load to the wetland water column was 0.7 kg accounting for 7% of the total P load entering the wetland. In comparison, the cumulative P load associated with advection into the wetland water column was 1.2 kg accounting for 11% of the total load. The average daily P loading rates into the wetland for diffusion and advection were 2.3 and 4.0 g d^{-1} estimated over the entire monitoring period (314 days). The cumulative P loads entering the wetland water column via runoff, ditch inflow, and groundwater (via diffusion and advection) were estimated at 5.5, 1.0, and 2.2 kg, accounting for 51, 9, and 20% of the total load to the wetland, respectively (Figure 1-9). During the monitoring period the cumulative bulk atmospheric load of P to the wetland water column was 0.2 kg, accounting for only 2% of the total P loads entering the wetland, and by far the smallest input. The cumulative load of P exiting the wetland water column via infiltration was 1.6 kg, representing 14% of the total load leaving the wetland. The cumulative P load exiting the wetland via the ditch flow was estimated at 5.6 kg during the monitoring period, accounting for 49% of the P load out of the wetland. Based on the P budget described in equation 5, P stored in the water column (S_{sw}) accounted for 4.2 kg or 37% of the overall P budget. The P retention/release pattern from groundwater was highly related to the wetland stage fluctuation. In particular, the release pattern during the months when the wetland water table rose above the soil surface (June 2005, November 2005, and February/March 2006) was consistent with biogeochemical transformations during and shortly after dry-out events negatively affecting P

storage, and increasing internal P loading to the system. It is important to note that wetland vegetation is a vital component of the P budget that can sequester dissolved P by incorporating it into non-labile above and below ground biomass (Dunne et al. 2007; Kadlec and Wallace 2008). Bostic and White (2006) showed that only in P-enriched soils ($TP > 618 \text{ mg kg}^{-1}$), soluble P was mobilized by the plants on reflooding, and that no significant effect of vegetation on release of soluble P was observed in P-unenriched soils ($TP < 499 \text{ mg kg}^{-1}$). This component to the wetland P budget was not evaluated as part of this study since TP concentrations of our soils were well within the P-unenriched range.

4.0 Conclusions

This study demonstrates that both advective and diffusive processes are important components of the total P fluxes from wetlands that are affected by flooding and drying cycles. Between May 2005-March 2006, advective fluxes to the water column of the study wetlands associated with water table fluctuations were 26% greater than diffusive fluxes, based on concentration gradients between surface and pore water. During this period, diffusive fluxes were calculated when the wetland was inundated, representing 76% of the year, and generated a maximum flux of $0.97 \text{ mg m}^{-2} \text{ d}^{-1}$. During the same period advection occurred for only 57 days, representing only 18% of the year, yet generated a maximum flux of $2.85 \text{ mg m}^{-2} \text{ d}^{-1}$.

Based on correlation of the stage measured at the study site and a nearby site with a longer-term record, for the five-year period 2004-2008 the study site wetlands have been dry for 63% (± 19) of the time, over the last five years; and have undergone at least 23 drawdown events. This could have a negative effect on nutrient loading to the wetland, because alternating flooding and drying conditions induce redox-related P release from the soils generating higher P fluxes than perpetually flooded conditions.

The internal loading of P to the wetland via advection and diffusion accounted for nearly 18% of the total P entering the wetland. The development of future best management strategies for cattle pastures should equally address reducing P from internal as well as external sources. Because there is often little that can be done about internal loads, knowledge of the magnitude of the internal load is important to help target further reductions from external loads. By far the greatest export of P from the wetland was via ditch flow, accounting for 49% of the total P loads out of the wetland. The advective transport of P from the water column into the ground via infiltration accounted for 14% P loss from the wetland. Managers planning wetland restoration should also consider how effects of increased hydroperiod will affect both advective and diffusive internal load.

1 **Table 1-1.** Methods for determination of wetland water budget components.

Components		Methods
IN	Rainfall (P)	Measured onsite using data-logging tipping buckets or at nearby South Florida Water Management District (SFWMD) weather stations (SFWMD 2010)
	Total runoff (O)	Estimated as a sum of direct (O_D) plus indirect runoff (O_I) (see Min et al. 2010b) O_D is overland flow driven by high-intensity P exceeding the threshold of the NRCS runoff curve number method. Variable source areas are determined by equating the volume of infiltrated water in the area contributing runoff to the soil water storage capacity of this area. O_I is defined as low intensity P-induced surface or subsurface flow toward the wetland when P falls on the wetland-upland border area where the water storage capacity of the soil is very limited due to capillary rise.
	Ditch inflow (D_{IN})	Estimated using Manning's equation when $H_{wet} > H_{ditch}$ and lower than the downstream ditch stage (Manning's roughness coefficient = $0.07 \text{ s/m}^{1/3}$).
	Groundwater inflow (GW_{IN})	Estimated using the modified Dupuit equation ($H_{wet} < H_{up}$) (see Min et al. 2010a).
OUT	Evapotranspiration (ET)	Estimated using the FAO Penman-Monteith equation (Allen et al. 1998) with monthly averaged crop coefficients (0.53 to 0.85) reported by Mao et al. (2002).
	Ditch outflow (D_{OUT})	Estimated using Manning's equation when $H_{wet} > H_{ditch}$ and higher than the downstream ditch stage (Manning's roughness coefficient = $0.07 \text{ s/m}^{1/3}$).
	Groundwater outflow (GW_{OUT})	Estimated using the modified Dupuit equation ($H_{wet} > H_{up}$).
	Change in storage (ΔV_{WET})	Calculated from the measured H_{wet} and stage-area relationship.
Budget residual (ϵ)		Defined as $\sum IN - \sum OUT - \Delta H_{WET}$ and expressed as the percentage of $\sum IN$.

2 **Table 1-2.** Diffusive flux of SRP from sediments to overlying water in selected aquatic systems.

Aquatic System	Diffusive Flux (mg P m⁻² d⁻¹)	Reference
<i>Wetland 1 (multisamplers)</i>	<i>0.06-0.97</i>	<i>This study (2005-2006)</i>
<i>Wetland 1 (PWEs)</i>	<i>0.61-1.1</i>	<i>This study (2008)</i>
<i>Wetland 2 (PWEs)</i>	<i>0.53-1.2</i>	<i>This study (2008)</i>
Lake Apopka, Florida	1.0-5.3	Reddy et al., 1995
Lake Apopka, Florida	0.3-0.7	Olila et al., 1997
Lake Okeechobee, Florida		Moore et al., 1998
Mud zone	0.1-1.9	"
Peat zone	0.2-2.2	"
Sand zone	0.1-0.5	"
Littoral zone	0.6-1.5	"
Indian River Lagoon, Florida	1.6	Reddy et al., 1999
Lower St. Johns River Estuary, Florida	0.13-4.55	Malecki et al., 2004
Everglades (Water Conservation Area-2A), Florida	1.5-6.5	Fisher and Reddy, 2001
Beaver Reservoir, Arkansas	0.01-1.77	Sen et al., 2007
Lake Eucha, Oklahoma	1.03-4.4	Haggard et al., 2005

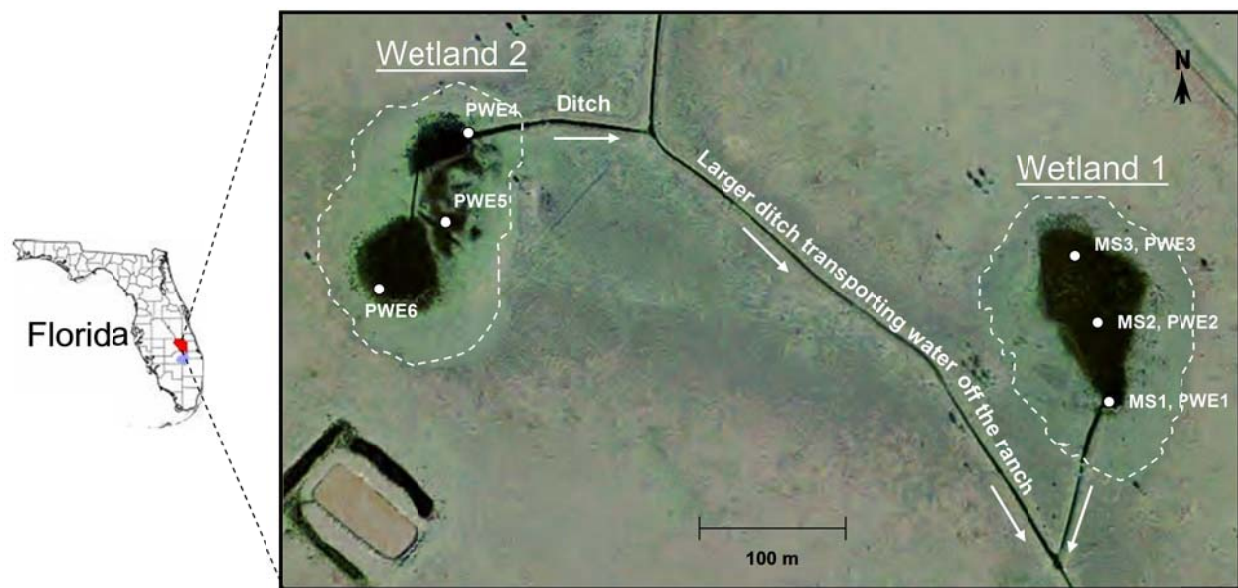


Figure 1-1. Map of ground water sampling locations. Three multisamplers (MSs) and three pore water equilibrators (PWEs) in Wetland 1 (Larson West), and three PWEs in Wetland 2 (Larson East). The larger ditch transports water off the ranch and ultimately drains into Lake Okeechobee.

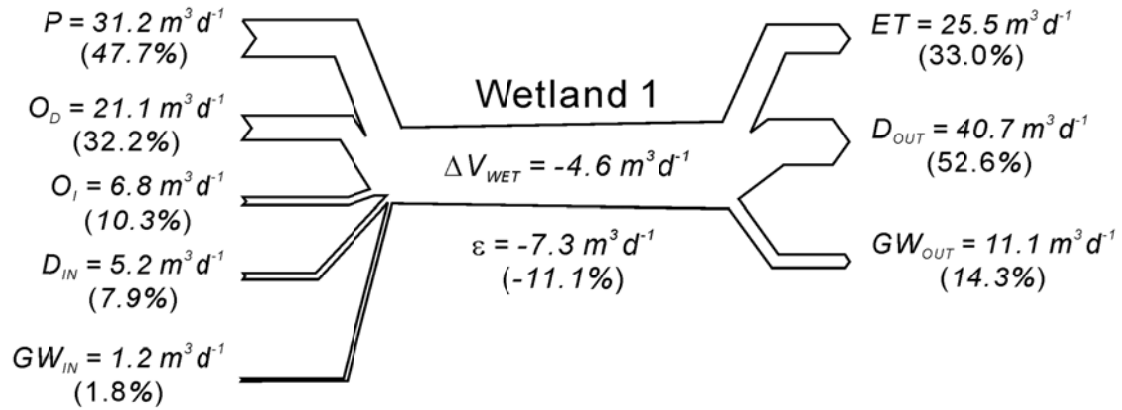


Figure 1-2. Wetland 1 (Larson East) water budget during the study period (May 2005 to March 2006). All input, output, and storage parameters described in Table 1.

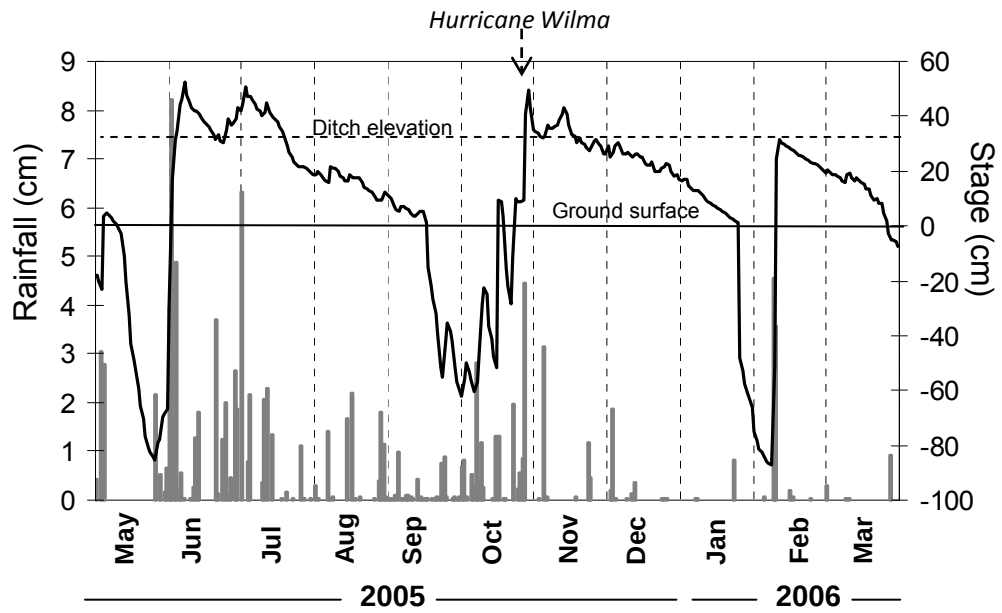


Figure 1-3. Rainfall measured onsite and water table (stage) fluctuation in Wetland 1 during the 314-day monitoring period from May 2005 to March 2006.

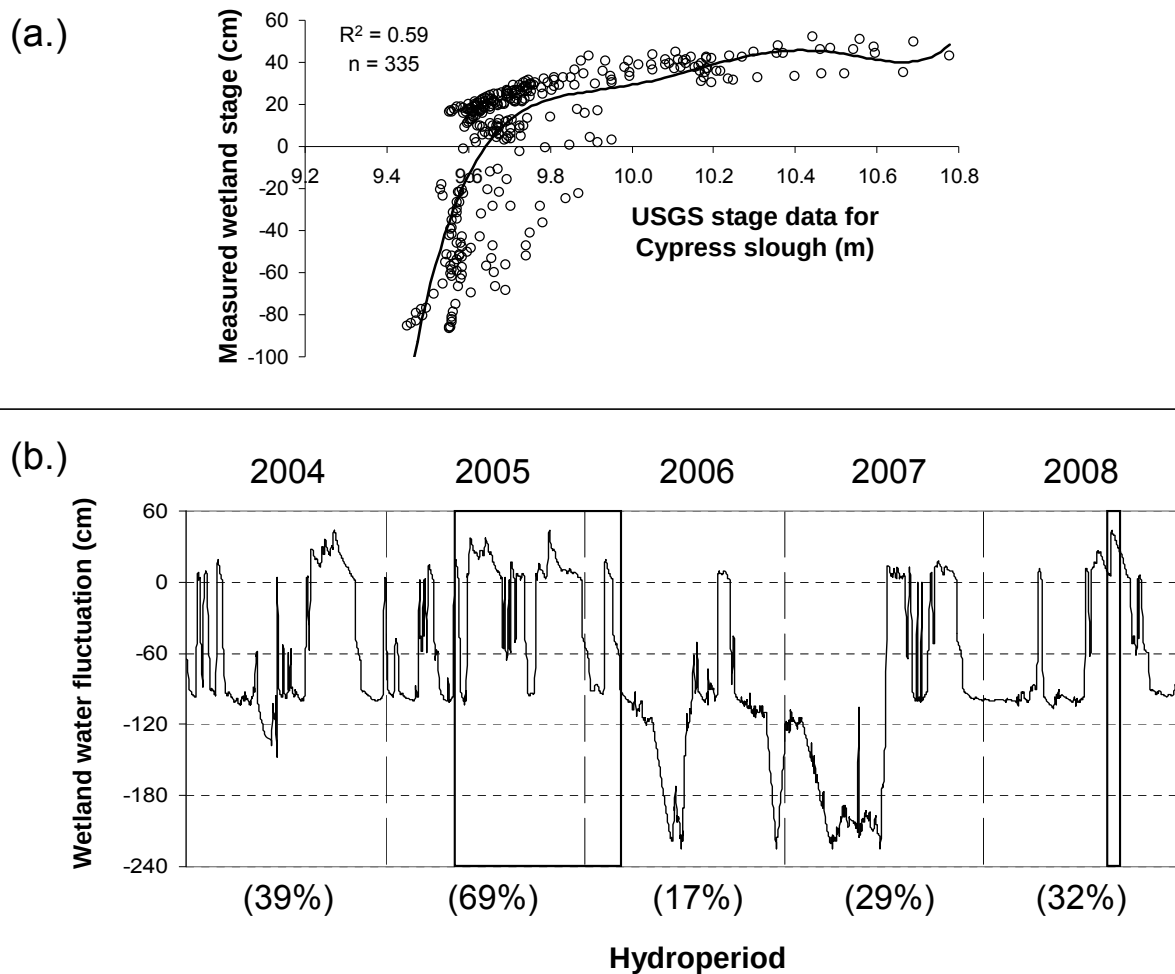


Figure 1-4. (a) Relationship between USGS stage data (Cypress slough, near Basinger, FL) and measured stage in Wetland 1 for 314 days. Trendline represents a 6th-order polynomial regression. (b) Wetland 1 (Larson East) hydroperiod (%) estimated for five years from 2004 to 2008. The two boxed areas represent the sampling period used for this study.

1

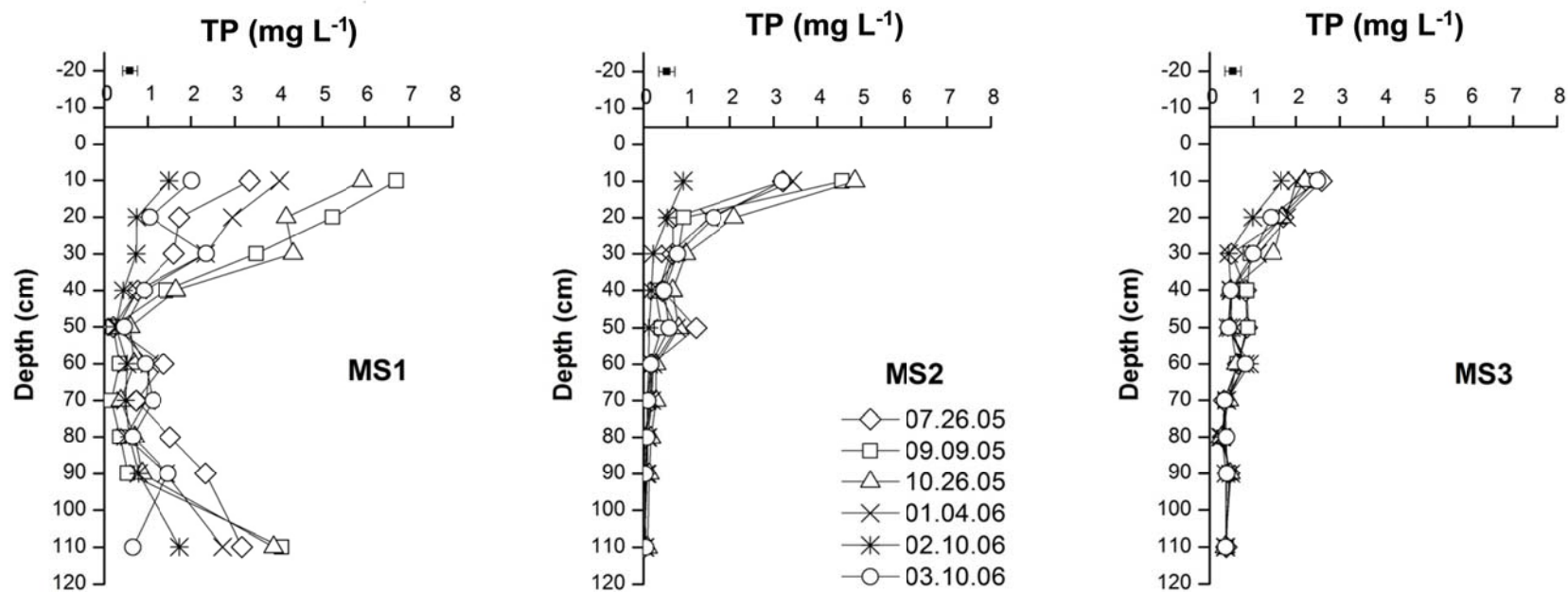


Figure 1-5. Pore water concentration of total phosphorus (TP), measured with depth at three multisampler locations in Wetland 1 six times throughout the monitoring period. Solid black squares represent surface water concentration ($0.53 \pm 0.17 \text{ mg L}^{-1}$, $n = 18$).

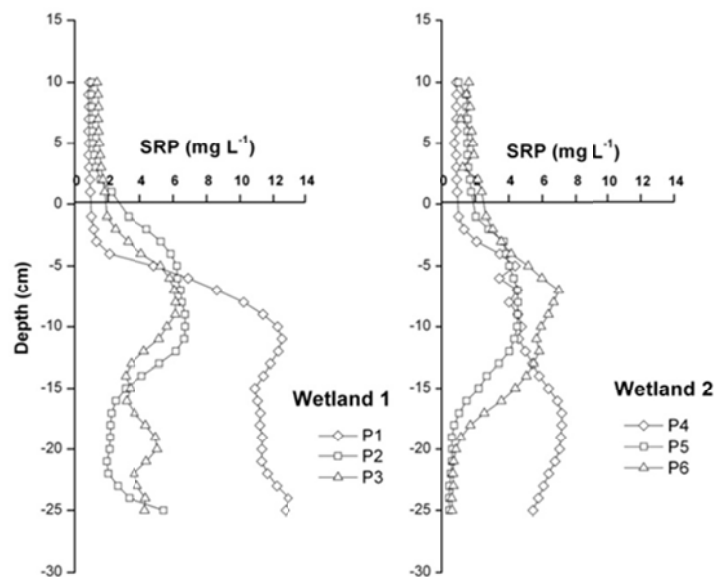


Figure 1-6. Pore water concentrations of SRP with depth measured using pore water equilibrators (PWEs) in Wetlands 1 and 2 in August 2008.

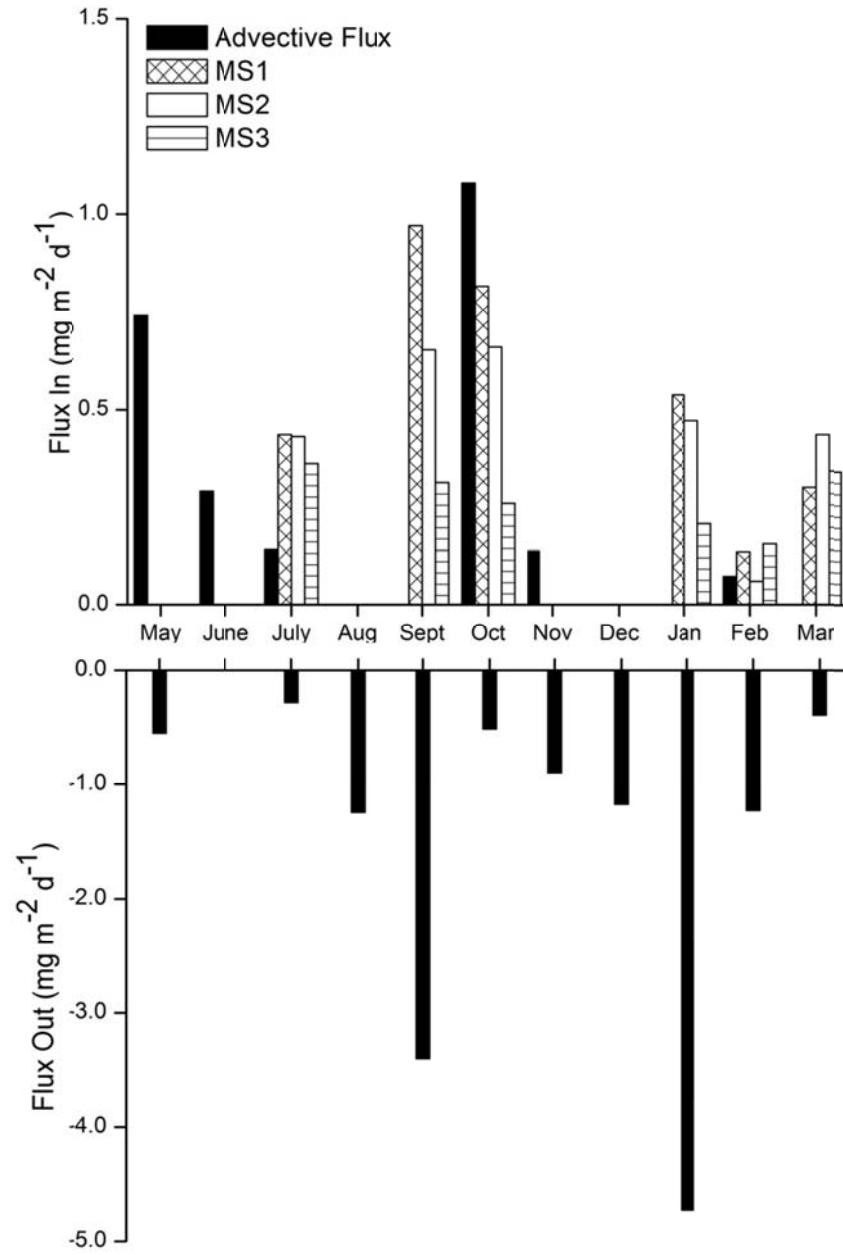


Figure 1-7. Fluxes (mg m⁻² d⁻¹) of TP in and out of the wetland water column May 2005 to March 2006.

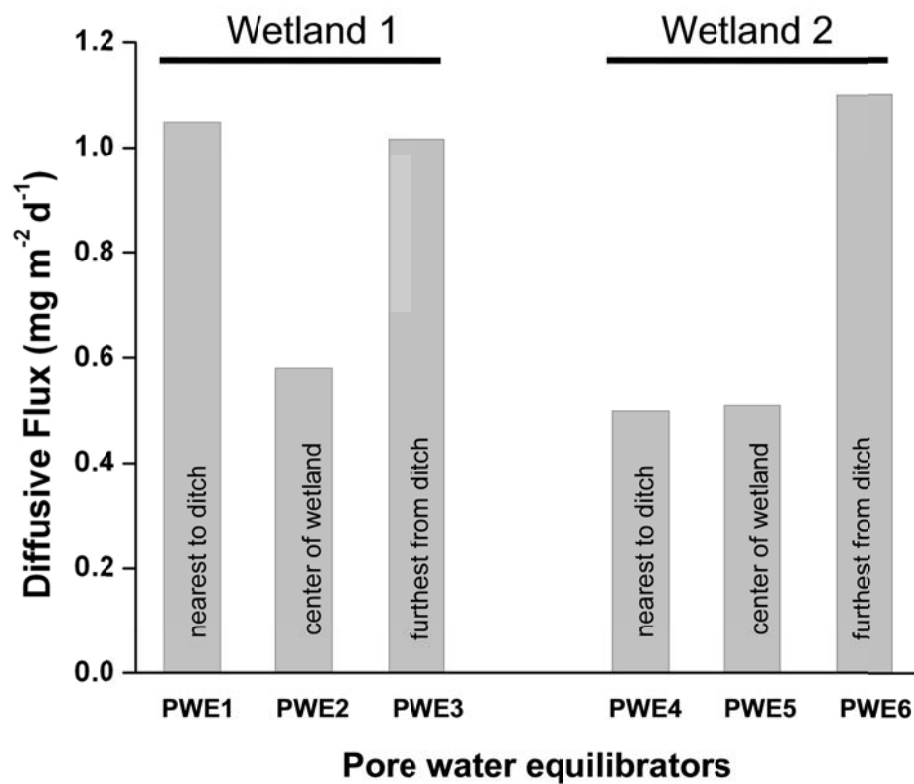


Figure 1-8. Diffusive flux ($\text{mg m}^{-2} \text{d}^{-1}$) of SRP calculated using PWEs from Wetland 1 and 2 during August 2008.

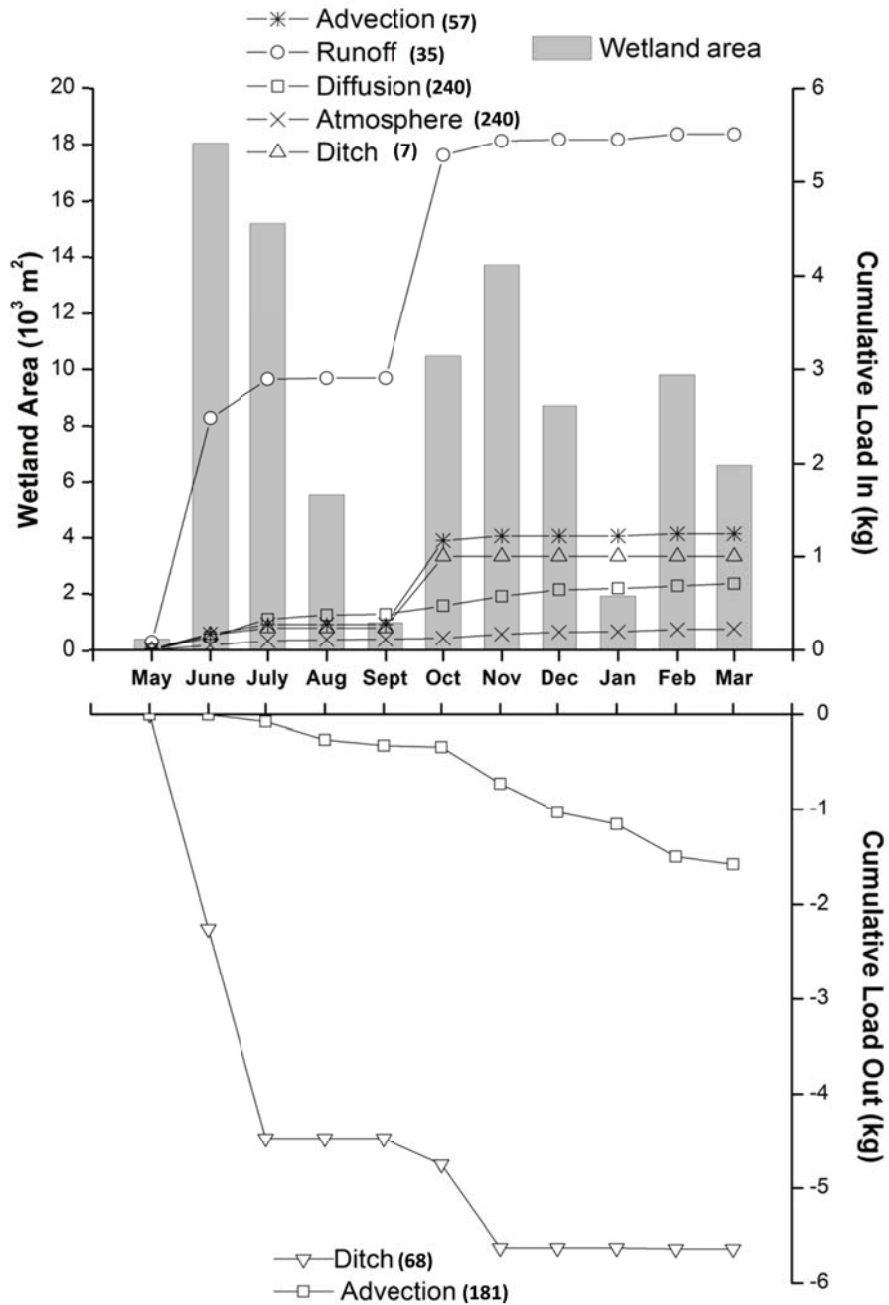


Figure 1-9. Cumulative load (kg) of P entering and exiting Wetland 1. Atmospheric deposition, surface runoff, advection and diffusion contributed P load into the wetland, while ditch flow and advection (infiltration) contributed load out of the wetland. Number of days the processes occurred during the 314-day monitoring period is in brackets.

Additional results from this work published during this fiscal year.

- Bhadha, J.H., Harris, W.G., and Jawitz, J.W., 2010. Soil phosphorus release and storage capacity from an impacted subtropical wetland, *Soil Science Society of America Journal*, 74(5): 1816-1825, doi:10.2136/sssaj2010.0063.

Abstract: Cattle-based agriculture on sandy soils has generated concern over phosphorus (P) leaching within the basin of Lake Okeechobee, FL. Elevated P concentrations in surface and groundwater relate to P retention capacity of soils. To evaluate P release and storage capacity of impacted soils with fluctuating water tables, four intact soil cores were analyzed (one upland and three wetland) to determine their maximum sorption capacity (S_{max}), equilibrium P concentration (EPC0), and soil P storage capacity (SPSC) up to 160 cm depth. Sorption isotherms were measured for samples at 10-cm depth increments, and data fitted to a Langmuir model ($r^2 \geq 0.88$). Both EPC0 and water soluble P (WSP) were higher at the surface and decreased with depth. Oxalate-extractable Fe+Al correlated with (i) oxalate-extractable P ($r^2 = 0.83$), and (ii) S_{max} ($r^2 = 0.71$). In situ pore water concentrations collected using multisamplers were consistently higher than EPC0 values. This discrepancy was much greater for near-surface horizons for which the difference may partially be due to redox-induced release of P previously scavenged by Fe under anaerobic (flooded) conditions. However, SPSC values were positive even when discounting Fe (i.e., assuming in-situ chemical reduction) for samples from deeper horizons, suggesting that the small discrepancies for these horizons are not attributable to Fe oxidation. All cores had negative SPSC at the surface and high EPC0, suggesting that neither wetland nor upland soils safely sequester additional P. High pore water P values in the surface horizons are consistent with the inference of the surface soils potentially being a source for P.

- Min, J-H., Perkins, D.B., and Jawitz, J.W., 2010. Wetland-groundwater interactions in subtropical depressional wetlands. *Wetlands*, 30: 997–1006, doi:10.1007/s13157-010-0043-9.

Abstract: Restoration of ditched and drained wetlands in the Lake Okeechobee basin, Florida, USA is currently under study for possible amelioration of anthropogenic phosphorus enrichment of the lake. Here we focus on the dynamic hydrology of these systems, with emphasis on understanding the interaction between wetland surface water and adjacent upland groundwater. Based on natural drawdown events observed over two years at four depressional wetlands, hydraulic conductivities (K) of the soils surrounding the wetlands were calculated at the wetland scale (approximately 2 ha) using the modified Dupuit equation under a constrained water budget framework. The drawdown-based average $K = 6.6$ m/d (range 0.9 to 21.3 m/d) was about three times greater than slug test-based values (1.9 ± 1.5 m/d), which is consistent with scale-dependent expectations. Net groundwater recharge rate at each depressional wetland, calculated based on the mean K, corresponded to approximately 40% of rainfall in the same period (10.0 m³/d). The average net groundwater recharge decreased by approximately 15% if ET was increased by 30%. Variability in estimated K and groundwater flow between the study wetlands was likely due to the relative difference of ditch bottom elevation controlling the surface outflow, as well as the spatial heterogeneity of the soils.

Task 2

Phosphorus Storage and Release in Wetland and Surrounding Uplands: Influence of Grazing and Hydrology

Introduction

It is well understood that the increased depth, duration and frequency of flooding in wetlands, relative to uplands, can influence vegetative species composition as well as soil biogeochemical processes. Changes in vegetative community structure along a hydrologic gradient are the result of varying degrees of species tolerances to reduced oxygen availability in the soil profile. This same lack of oxygen can reduce microbial decomposition rates of plant litter and result in accumulations of various elements including phosphorus. In previous investigations we have shown a significant relationship between soil phosphorus and soil organic matter leading to the assumption that increased organic matter accretion in wetlands should increase the overall phosphorus storage capacity. However, when wetlands occur in areas of active cattle grazing, such as in much of the landscape north of Lake Okeechobee, consumption of plant biomass by grazers may influence the amount and composition of organic matter entering the wetlands and may add additional stresses to vegetation as a result of regular grazing (Figures 2-1, 2-2).

As part of our continuing investigation of phosphorus dynamics in isolated wetlands in ranchlands, we carried out several experiments to investigate the effects of grazers on vegetation and phosphorus dynamics at the Larson Dixie Ranch where two exclosures were established in two wetlands (Figure 2). This experimental design provided a side by side comparison between grazed and ungrazed wetlands and adjacent upland areas allowing us to begin to address the following question.

- 1) What effect(s) does grazing have on primary production and productivity and how could that influence phosphorus retention in wetlands?

Although findings from this investigation have provided some insight into this question, results are limited to a single growing season and therefore some caution is warranted when inferring the broader application of these results.

Materials and Methods

As previously indicated, the principal experimental treatment was implemented by constructing an exclosure around three long-term transects within two wetlands at the Larson Dixie Ranch (Figure 2-1). At the same time three new transects were established equidistant between the three long-term transects. This resulted in three transects within the exclosure (no grazing treatment) and three outside of the exclosure (grazing treatment) in each wetland. Each ray of the exclosure is 10 meters wide and between 100 and 150 meters in length (Figure 2-3).

Each transect was further divided into three zones based on dominant vegetation present at the beginning of the experiment (February 2011). The “upland” zone was delineated as those areas upland of the point at which *Paspalum notatum* (Bahiagrass) was less than 50% of the vegetative cover. This edge was often very easily defined and occurred as more of a line than a gradient of *P. notatum* dominance. The wetland “edge” zone was delineated by the area upland of the inner edge of *Juncus effusus* (Soft rush) and waterward of the *P. notatum* line. The wetland “center” was delineated as the area waterward *J. effusus*.

Prior to each sampling event a random sampling distance along each transect within each zone was identified. To locate the sampling point a tape measure was laid out from a permanent post at the center

of the wetland and connected to a permanent post at the end of the transect. Once the sampling point was located along the tape measure a 1 m² PVC quadrat was placed on the clockwise side of the tape and off-set 2 meters to avoid any possible disturbance that may have occurred when laying out the tape measure. Vegetation was then harvested from within the 1 m² quadrat using electric grass clippers or hand clippers depending on water depth. Vegetation was clipped to approximately 0.5 cm of the soil surface.

Biomass production within non-grazed areas was determined by taking the average difference in biomass within each zone between sampling periods. When determining biomass production in grazed areas, a temporary exclosure was used to prevent grazing of biomass between sampling periods. After aboveground biomass was harvested along grazed transects, a 1.5 m diameter exclosure made of bull fencing was placed over the harvested plot (Figure 2-4). Two small survey flags were placed at opposite corners of the sampling quadrat so that future placement of the quadrat could be situated exactly over the top of the previously harvested area. During the next sampling period, the 1 m² plot was harvested again and the harvested biomass was used to provide an estimate of production within the grazed area.

Aboveground biomass was harvested eight times during the experimental period (Table 2-1). Two sampling events were in conjunction with long-term semi-annual sampling when belowground, litter and soils were also collected and 6 events were conducted when only harvested aboveground biomass and production biomass were collected.

Harvested aboveground biomass was first sorted into live and dead fractions and then oven dried at 70C for at least 72 hrs. Biomass was then weighed and used to determine various biomass production parameters. Tissue samples from the 8/17/2011 sampling event were analyzed for tissue P, N and C concentration and used to determine overall effects of grazing on biomass phosphorus, nitrogen and carbon content among the three hydrologic zones.

Two main metrics of aboveground biomass were calculated to determine the influence of grazing on biomass and production. The first metric relates to the overall standing stock of aboveground biomass which represents the potential biomass that could become litter and ultimately soil organic matter. The next parameter is the difference between the standing stock of biomass in grazed areas and the productivity measured within the short-term exclosures. The difference between these two values provides an estimate of the biomass consumed by grazers during the time interval.

Results and Discussion

Shortly after installation of the exclosure in February 2011 significant differences in aboveground biomass were visually apparent in the center and edge zones with less significant changes in the upland hydrologic zone (Figure 2-3 to 2-5). No significant rainfall was measured at the site before mid-June which likely influenced seed bank germination in the wetland as well as growth rates early in the growing season.

Aboveground Biomass Production

Aboveground live biomass standing stock showed very different patterns between grazed and non-grazed areas (Figure 2-6 and Table 2-2). Standing stock in non-grazed areas peaked in the center zone during the 8/17/2011 sampling event and in the edge and upland zones during the 10/23/2011 event. Grazed areas generally peaked later in the year during the 12/18/2011 sampling event in the center and edge zones and during the 10/23/2011 sampling event in upland areas. The difference in standing stock peak periods between grazed and non-grazed areas is likely the result of natural cycles in productivity and senescence

as reflected in the non-grazed area and reduced grazing pressure in the later part of the year as reflected in the grazed areas.

Actual aboveground biomass production within grazed areas was much greater than that expressed in the standing stock biomass (Figure 2-7) with the difference in cumulative production being the amount of biomass that cattle consumed while grazing (Figure 2-8). Cumulative biomass grazed within the three hydrologic zones was similar with the exception that grazing rates decreased in the center and edge zones later in the year. Although some fraction of this grazed biomass is likely accreted as soil organic matter, for this investigation it is assumed that no grazed biomass is available for soil organic matter accretion and only standing stock biomass will contribute to litter and eventual soil organic matter. The main reason for not including the manure fraction is that its' rate of decomposition or phosphorus loss has not yet been quantified.

Biomass nutrient composition

Biomass harvested during the 8/17/2011 semi-annual sampling was analyzed for tissue TP, TN and TC. Biomass was partitioned into aboveground live, aboveground dead, belowground and litter. Results indicate some significant effects of grazing on tissue nitrogen and phosphorus concentration. Whether this effect is the result of changes in species composition, age of plant tissue or some other factor cannot be determined at this time

In the case of tissue phosphorus concentrations, grazing had a statistically significant effect on all tissue types in the center hydrologic zone and also on aboveground live tissue in edge zones (Table 2-3). There was also a significant difference in upland zone litter P concentration due to grazing. In all cases where grazing had a significant effect, with the exception of upland zone litter P concentration, grazed areas had higher P concentrations than non-grazed areas. In some instances the difference was as much as 1.7 times higher P in grazed areas vs. non-grazed areas (aboveground live tissue in center zones). There were also some significant differences among hydrologic zones within the same grazing treatment. In the case of belowground biomass and litter, grazed tissue in the center zone had significantly higher tissue P concentrations than uplands. No significant differences in tissue P concentration were identified among hydrologic zones in the non-grazed treatment.

Tissue nitrogen concentration showed similar significant differences between grazed and non-grazed treatments where aboveground live and dead tissue nitrogen was significantly higher in center and edge hydrologic zones (Table 2-4). Litter nitrogen concentration was higher in grazed edge zones and belowground biomass was higher in non-grazed upland zones. There was also a significantly higher nitrogen concentration in all grazed tissue harvested from the center hydrologic zone when compared to the upland hydrologic zone. No significant differences in hydrologic zone tissue nitrogen concentration were found within non-grazed treatments.

Tissue carbon concentration did not show the same significant differences between grazed and non-grazed treatments, but did show a significantly lower tissue carbon concentration in the grazed treatment within the center hydrologic zone when compared to litter in either the grazed edge or upland zones (Table 2-5).

Effect of Grazing on Organic Matter and Phosphorus Accretion Potential

Using aboveground live biomass as an estimate of the potential organic matter that could be contributed to litter (Table 2-2), the mass of phosphorus likely contributed by biomass was calculated using the aboveground dead tissue concentration from the 8/17/2011 sampling event. One reason that aboveground dead tissue P concentration as used, not live tissue P concentration, was that there is a considerable

decrease in tissue P concentration between live and dead aboveground biomass (Table 2-3). This may be due to resorption by the plant as the biomass senesces or the result of P leaching from the senesced tissue. On average the difference between aboveground live tissue P concentration and aboveground dead P tissue concentration was a factor of 2.5 ± 0.33 .

The potential P mass contribution from aboveground biomass standing crop ranged from $108 \pm 53.7 \text{ mg m}^{-2}$ in upland grazed areas to $604 \pm 311 \text{ mg m}^{-2}$ in non-grazed center areas (Table 2-6). In all instances the maximum potential aboveground P mass that could be contributed to soils was greater in non-grazed areas than grazed areas. Near the end of the year standing stocks of aboveground biomass in some grazed hydrologic zones was actually higher than in non-grazed zones. However, this potential contribute to litter could be deceiving. It is assumed that a reduction of grazing pressure in these pastures during the latter part of the year is why standing stock of grazed areas increase and was an attempt to accumulate forage for grazing later in the winter and early spring. As a result, although the standing stock of aboveground biomass is relatively high in the last sampling period, 100% of this biomass is not likely to be added to the litter fraction as would be the case for non-grazed areas. Therefore, the difference in potential P contribution to litter between grazed and non-grazed areas will be provided as a range based on the maximum standing stock found in grazed areas (likely an overestimate of grazed treatment contribution) and the aboveground standing stock value harvested on 4/4/2011, which likely represents the remaining standing stock after grazing has consumed much of the accumulated forage from the previous growing season.

Using the above assumptions, potential P contributions to litter from non-grazed areas are estimated to be 604 ± 311 , 368 ± 251 and $257 \pm 122 \text{ mg m}^{-2}$ for center, edge and upland zones respectively. Grazed areas are estimated to contribute between 5.49 ± 3.42 and $524 \pm 326 \text{ mg m}^{-2}$ in upland zones, between 13.7 ± 17.3 and $233 \pm 127 \text{ mg m}^{-2}$ in edge zones and between 28.7 ± 16.8 and $108 \pm 53.7 \text{ mg m}^{-2}$ in upland zones. This would suggest that there is a reduction of potential P contributions to litter as a result of grazing that range by a factor of 1.2-110 times in center zones, 1.57-26.9 times in edge zones and 2.38-8.95 times in upland zones.

The above findings relate to the effect of grazing on the potential contribution of aboveground biomass to litter. By applying a decomposition rate to this biomass input, the amount of organic matter and P that might remain after one year can be estimated. For this purpose aboveground biomass decomposition rates were calculated from a litterbag study conducted at the ranch in center, edge, transitional and upland hydrologic zones (Figure 2-9). Rates of organic matter loss after one year for a range of common species were determined to be 55.8%, 63.3% and 55.1% for center, edge and upland zones respectively

The estimated effect of grazing on aboveground contributed organic matter and phosphorus after one year of decomposition is summarized in Tables 2-7 and 2-8. Based on estimated decomposition rates and initial aboveground biomass contributions from grazed and non-grazed areas, organic matter remaining after one year ranged from $1.73 \pm 1.08 \text{ g m}^{-2}$ (likely estimate for grazed center zone) to $322 \pm 166 \text{ g m}^{-2}$ (likely contribution from non-grazed center zone). In all cases grazed areas contributed significant less organic matter to soils after one year than non-grazed areas.

The same results are true for aboveground biomass P contributed to soils after one year of decomposition with values ranging from $2.41 \pm 1.50 \text{ mg m}^{-2}$ to $266 \pm 137 \text{ mg m}^{-2}$.

Conclusions

Findings suggest that grazing within wetlands and adjacent uplands can significantly reduce the amount of aboveground biomass organic matter and P that is contributed directly to litter and ultimately long-term soil storage. Depending on assumptions regarding which aboveground standing stock biomass best

represents the contribution of grazed areas, the effect of grazing can be a factor as high as 110 times less organic matter and P input to soils than in non-grazed areas, with the greatest reductions occurring in wetland centers where accretion rates are expected to be the highest.

This apparent impact of grazing however needs to be tempered by the uncertainty associated with the fate of manure deposited by cattle, the significant higher tissue phosphorus concentrations found in live vs. dead aboveground biomass and the significant differences in tissue N and P concentration found between grazed and non-grazed aboveground biomass. If results are taken at face value then reducing grazing pressure in wetland areas will result in a significant increases in potential P storage associated with organic matter contributed from aboveground biomass. However, if cattle are efficient in retaining higher levels of P found in aboveground live biomass from grazed areas then the net difference between grazed and non-grazed areas would be significantly less. Alternatively if organic matter or P labiality is greater in manure relative to aboveground dead biomass or litter then the effect of grazing on P retention in wetlands would again be significant and potentially as high as that found in this study.

Evaluating the fate of carbon and P in forage when vectored through cattle and what the decomposition rates of manure are along the hydrologic gradient from wetland center to adjacent upland will be necessary to clarify these uncertainties.

Table 2-1. Sampling dates and other pertinent events that occurred during the experimental period.

- 1/12/2011 Semi-annual sampling (aboveground, belowground, litter and soils)
- 2/1-2/15/2011 Exclosure fencing installed
- 4/4/2011 Aboveground biomass and productivity Sampling
- 5/11/2011 Aboveground biomass and productivity Sampling
- 6/16/2011 Aboveground biomass and productivity Sampling
- Mid June First appreciable rainfall since March
- 7/20/2011 Aboveground biomass and productivity Sampling
- 8/17/2011 Semi-annual sampling (aboveground, belowground, litter and soils)
- 10/23/2011 Aboveground biomass and productivity Sampling
- 12/18/2011 Aboveground biomass and productivity Sampling

Table 2-2. Mean and standard deviation of aboveground live biomass in grazed and non-grazed areas along three hydrologic zones during each sampling event. Bold values represent maximum biomass levels for each zone and treatment.

Zone	Treatment	1/12/2011	4/4/2011	5/11/2011	6/16/2011	7/20/2011	8/17/2011	10/23/2011	12/18/2011
		----- g m ⁻² -----							
Center	not grazed	68.1 ± 90.8	121 ± 48.9	343 ± 113	334 ± 193	674 ± 197	731 ± 376	618 ± 529	285 ± 179
	grazed	16.3 ± 9.6	3.93 ± 2.45	18.1 ± 25.0	7.37 ± 8.23	27.5 ± 21.6	142 ± 227	154 ± 116	375 ± 234
Edge	not grazed	39.1 ± 28.9	32.3 ± 31.1	95.0 ± 91.4	83.3 ± 53.2	149 ± 138	394 ± 324	430 ± 293	368 ± 176
	grazed	31.9 ± 41.4	12.8 ± 16.1	7.56 ± 4.85	9.11 ± 8.86	26.6 ± 18.5	24.4 ± 15.5	86.0 ± 60.6	217 ± 119
Upland	not grazed	84.1 ± 81.7	81.4 ± 42.0	304 ± 196	64.3 ± 48.3	136 ± 99.1	246 ± 70.8	338 ± 161	268 ± 83.6
	grazed	83.4 ± 75.0	40.0 ± 23.3	39.2 ± 13.7	31.4 ± 16.2	68.5 ± 40.0	106 ± 31.2	151 ± 74.7	144 ± 24.9

Table 2-3. Mean and standard deviation of vegetation and litter tissue phosphours concentrations collected on 8/17/2011 duirng semi-annual sampling event. Letters to right of values represent statistical comparison among hydrologic zones within the same grazing treatment. Capitol letters campare among grazed areas and lower case letters compare among non-grazed areas. Different letters represent statistically different values ($\alpha = 0.05$). Aestrix to the right of values represent statistical comparision between grazing treatments within the same hydrologic zone. Astrix(s) to right of values represent degree of statistical difference as follows * = P of 0.1-0.051, ** = P of 0.05-0.011, *** = P of < 0.01.

Zone	Treatment	Aboveground	Aboveground				Belowground		Litter	
		Live	Dead		mg kg ⁻¹					
Center	grazed	3033 ± 1072	A,***	1395 ±	484	A,**	1160 ± 157	A,*	1522 ± 354	A,**
	not grazed	1754 ± 604	a	826 ±	78	a	945 ± 260	a	1136 ± 125	a
Edge	grazed	2899 ± 986	A,***	1071 ±	311	A	1064 ± 178	A	861 ± 309	B
	not grazed	1977 ± 590	a	858 ±	266	a	987 ± 113	a	1083 ± 217	a
Upland	grazed	1987 ± 670	A	718 ±	198	A	782 ± 101	B	662 ± 105	B,*
	not grazed	2266 ± 480	a	758 ±	151	a	842 ± 140	a	837 ± 199	a

Table 2-4. Mean and standard deviation of vegetation and litter tissue nitrogen concentrations collected on 8/17/2011 duirng semi-annual sampling event. Letters to right of values represent statistical comparison among hydrologic zones within the same grazing treatment. Capitol letters campare among grazed areas and lower case letters compare among non-grazed areas. Different letters represent statistically different values ($\alpha = 0.05$). Aestrix to the right of values represent statistical comparision between grazing treatments within the same hydrologic zone. Astrix(s) to right of values represent degree of statistical difference as follows * = P of 0.1-0.051, ** = P of 0.05-0.011, *** = P of < 0.01.

Zone	Treatment	Aboveground	Aboveground				Belowground	Litter	
		Live	Dead		mg kg ⁻¹				
Center	grazed	26.4 ± 9.03	A***	15.2 ± 2.29	A,**	15.7 ± 2.98	A	17.7 ± 3.00	A
	not grazed	12.5 ± 4.53	a	11.8 ± 1.53	a	14.3 ± 4.38	a	16.4 ± 0.91	a
Edge	grazed	22.6 ± 6.82	A***	12.9 ± 2.06	AB,*	16.1 ± 3.42	A	11.4 ± 2.71	B,**
	not grazed	13.5 ± 3.49	a	10.3 ± 2.37	a	16.3 ± 1.64	a	16.4 ± 3.48	a
Upland	grazed	13.9 ± 2.11	B	9.49 ± 0.737	B	10.7 ± 1.59	B,*	11.9 ± 2.39	B
	not grazed	13.2 ± 0.795	a	9.21 ± 1.27	a	12.6 ± 1.51	a	13.7 ± 1.86	a

Table 2-5. Mean and standard deviation of vegetation and litter tissue carbon concentrations collected on 8/17/2011 during semi-annual sampling event. Letters to right of values represent statistical comparison among hydrologic zones within the same grazing treatment. Capital letters compare among grazed areas and lower case letters compare among non-grazed areas. Different letters represent statistically different values ($\alpha = 0.05$). Asterix to the right of values represent statistical comparison between grazing treatments within the same hydrologic zone. Asterix(s) to right of values represent degree of statistical difference as follows * = P of 0.1-0.051, ** = P of 0.05-0.011, *** = P of < 0.01 .

		Aboveground		Aboveground		Belowground		Litter	
Zone	Treatment	Live		Dead					
		----- g kg ⁻¹ -----							
Center	grazed	403 ± 35.2	A	405 ± 17.3	A	413 ± 9.05	A	393 ± 19.9	A
	not grazed	430 ± 19.3	a	422 ± 21.7	a	408 ± 10.7	a	392 ± 32.0	a
Edge	grazed	417 ± 17.5	A	395 ± 38.0	A	414 ± 17.2	A	427 ± 13.2	B
	not grazed	414 ± 22.1	a	426 ± 4.57	a	420 ± 6.75	a	418 ± 10.2	a
Upland	grazed	432 ± 16.3	A	430 ± 10.6	A	423 ± 8.55	A	424 ± 8.51	B
	not grazed	414 ± 26.0	a	432 ± 7.18	a	414 ± 11.2	a	417 ± 17.5	a

standard deviation of estimated aboveground biomass phosphorus likely to be contributed to litter in grazed and non-grazed
ones. Values are calculated based on aboveground live biomass harvested during each sampling event (Table 2-2) and
phosphorus concentrations collected on 8/17/2011 (Table 2-3). Bold values represent maximum biomass levels for each

	1/12/2011	4/4/2011	5/11/2011	6/16/2011	7/20/2011	8/17/2011	10/23/2011	12/18/2011
	-----mg m-2 -----							
	56.2 ± 75.0	100 ± 40.4	283 ± 92.9	276 ± 159	557 ± 162	604 ± 311	510 ± 437	236 ± 148
	22.8 ± 13.4	5.49 ± 3.42	25.2 ± 34.8	10.3 ± 11.5	38.4 ± 30.2	198 ± 316	215 ± 162	524 ± 326
	33.5 ± 24.7	27.6 ± 26.6	81.3 ± 78.2	71.3 ± 45.5	127 ± 118	337 ± 277	368 ± 251	315 ± 151
	34.1 ± 44.3	13.7 ± 17.3	8.10 ± 5.19	9.8 ± 9.49	28.5 ± 19.8	26.2 ± 16.6	92.1 ± 64.9	233 ± 127
	63.8 ± 61.9	61.7 ± 31.9	231 ± 148	48.7 ± 36.6	103 ± 75.1	187 ± 54	257 ± 122	203 ± 63.4
	59.9 ± 53.9	28.7 ± 16.8	28.1 ± 9.8	22.6 ± 11.6	49.2 ± 28.7	76.1 ± 22.4	108 ± 53.7	104 ± 17.9

Table 2-7. Mean and standard deviation of potential organic matter deposition one year after input of aboveground biomass in grazed and non-grazed areas along a hydrologic gradient. Estimated remaining biomass after one year is based on litter bag studies that indicated an average organic matter loss of 55.8% for center zones, 63.3% for edge zones and 55.1% for upland zones. Values under the minimum column heading represent likely contributions from grazed areas. Values in the column titled maximum represent likely contributions from non-grazed areas, and an upper end maximum for grazed areas.

		Potential OM deposition	
		minimum	maximum
Zone	Treatment	----- g m ⁻² -----	
Center	not grazed		322 ± 166
	grazed	1.73 ± 1.08	165 ± 103
Edge	not grazed		164 ± 111
	grazed	4.85 ± 6.14	82.6 ± 45.2
Upland	not grazed		152 ± 72.2
	grazed	18.0 ± 10.5	67.7 ± 33.6

Table 2-8. Mean and standard deviation of potential phosphorus deposition one year after input of aboveground biomass in grazed and non-grazed areas along a hydrologic gradient. Estimated remaining phosphorus after one year is based on litter bag studies that indicated an average mass loss of 55.8% for center zones, 63.3% for edge zones and 55.1% for upland zones. Values under the minimum column heading represent likely contributions from grazed areas. Values in the column titled maximum represent likely contributions from non-grazed areas, and an upper end maximum for grazed areas.

		Potential P deposition	
		minimum	maximum
Zone	Treatment	-----mg m ⁻² -----	
Center	not grazed		266 ± 137
	grazed	2.41 ± 1.50	230 ± 144
Edge	not grazed		140 ± 95.2
	grazed	5.20 ± 6.57	88.5 ± 48.4
Upland	not grazed		115 ± 54.8
	grazed	12.9 ± 7.53	48.6 ± 24.1

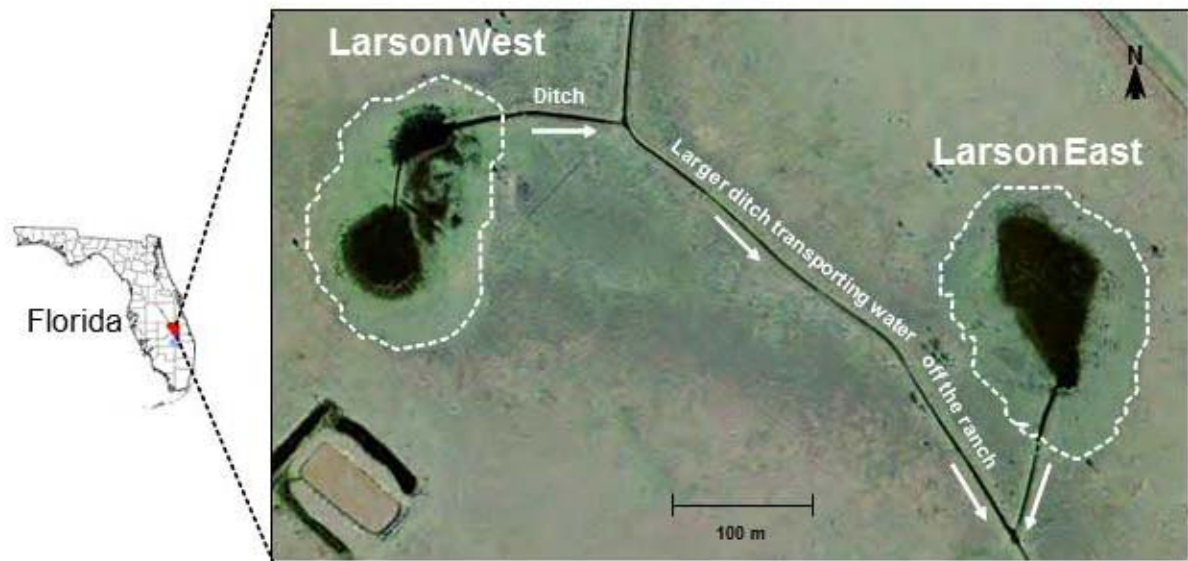


Figure 2-1. Map showing study sites in the Okeechobee Basin, Florida



Figure 2-2. Example of grazing effect on vegetation within a pasture wetland. Left side of fence is grazed and right side of fence has not been grazed for 4 months during the dry season.

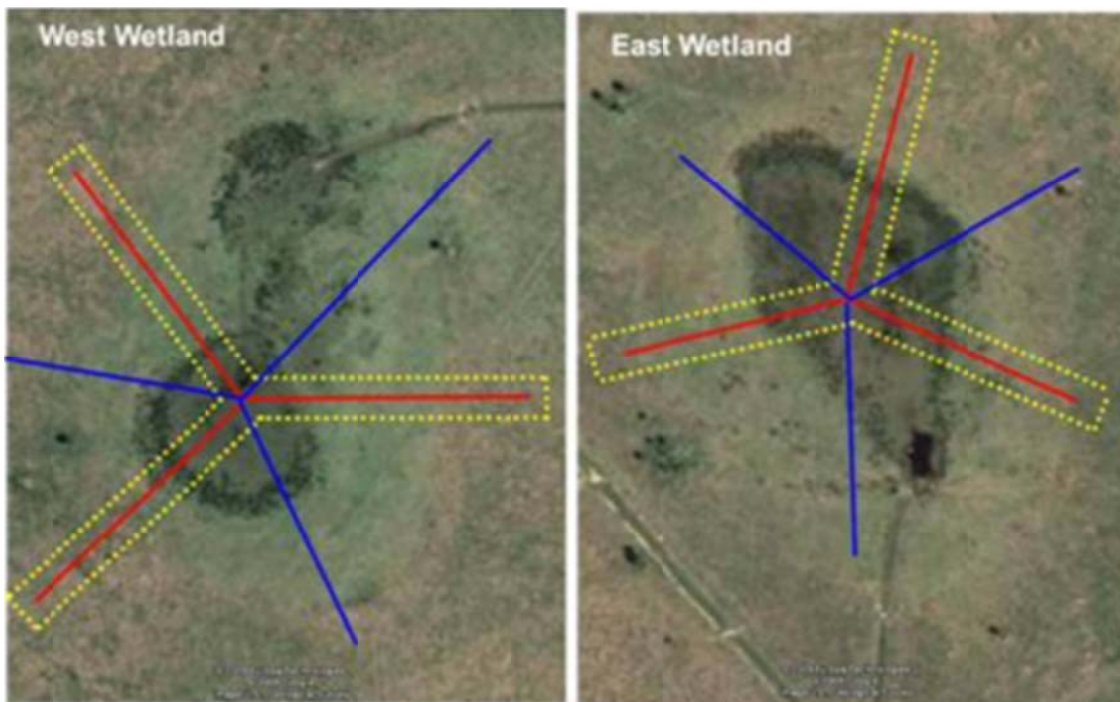


Figure 2-3. Aerial image of two experimental wetlands at the Larson Dixie Ranch. Blue and red colored lines originating at the center and radiating out to the adjacent upland are sampling transects. Blue transects indicate “grazed” treatments and the red lines indicate “non-grazed” treatments. The yellow dotted line indicates the approximate location of the fence enclosure



Figure 2-4. Images of temporary bull fencing exclosures used to determine biomass production in grazed areas. Image at left shows clipped plot within exclosure at beginning of time period. Image at right shows non-grazed biomass within exclosure, and grazed biomass outside of exclosure.



(a)



(b)



(c)

Figure 2-5. Photographs taken along the fence line enclosure in the upland (a), edge (b) and center (c) hydrologic zones 4 months after enclosure establishment.

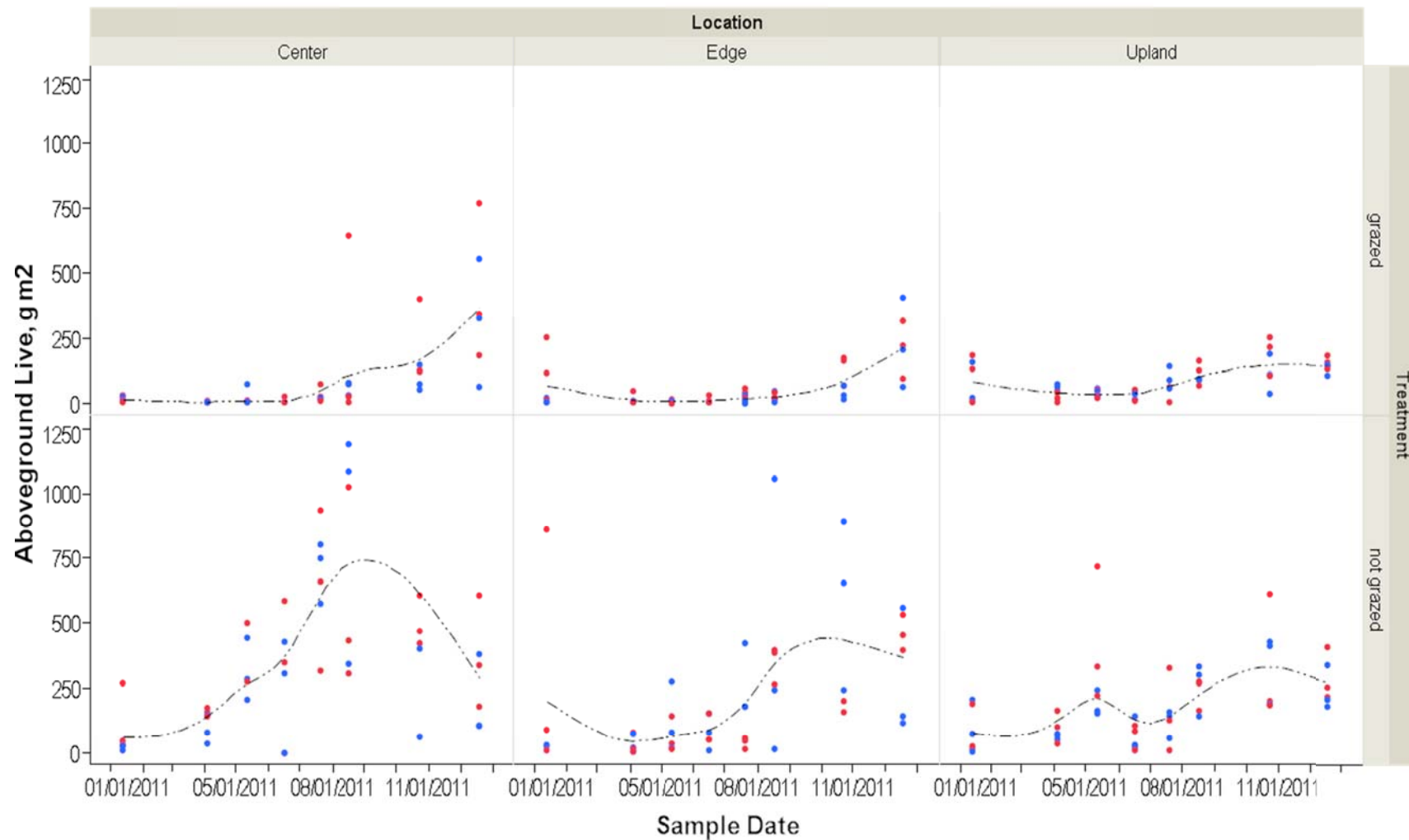


Figure 2-6. Plot of aboveground live biomass by sampling date, hydrologic zone and grazing treatment. Red points represent biomass from the Larson East wetland and blue point represent values from the Larson West wetland. Dashed line through points represent least squares fit of data.

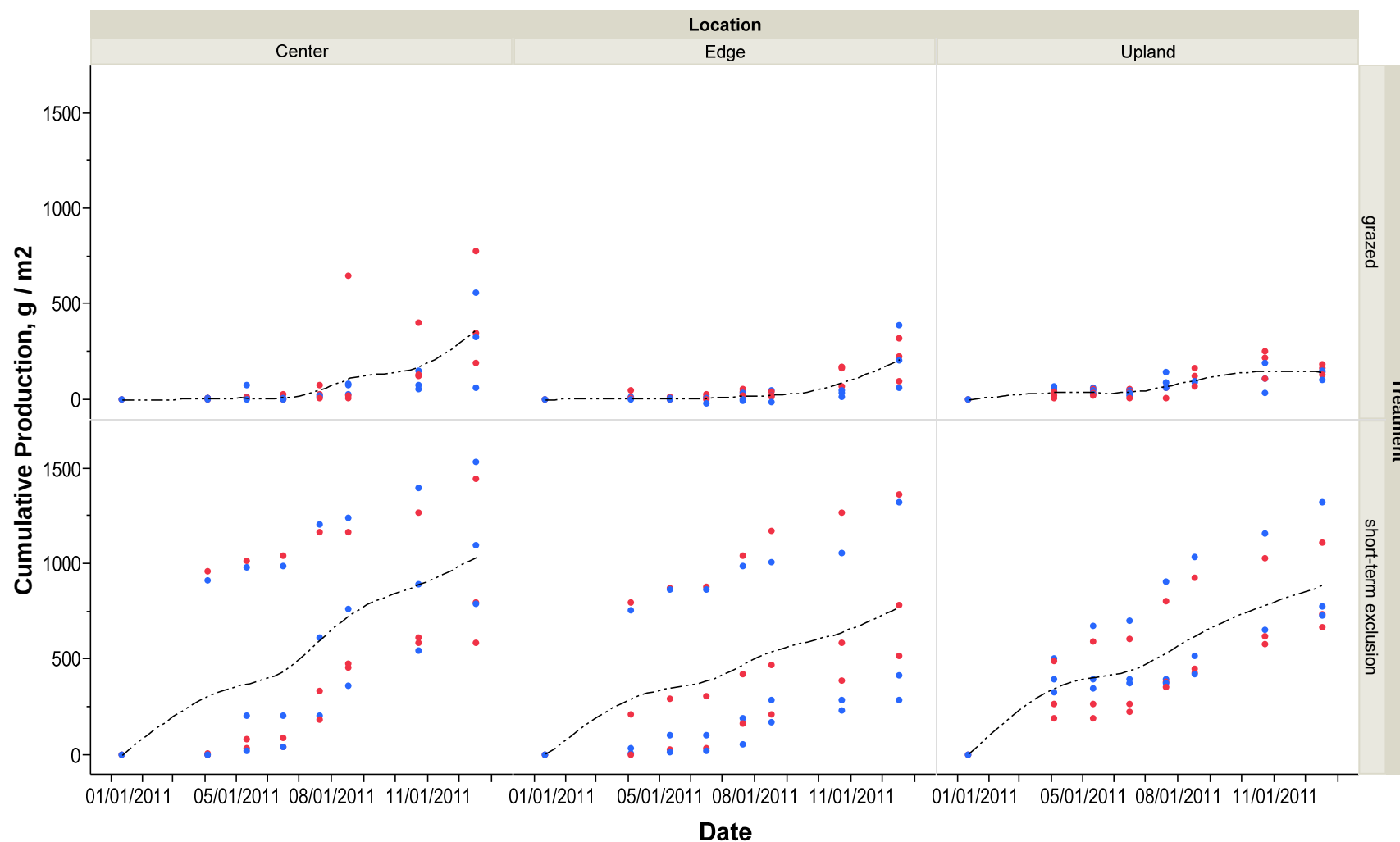


Figure 2-7. Plot of cumulative grazed area production based on change in aboveground standing crop between sampling events (top row) and based on change in standing crop between samplings with short term exclusion used to prevent grazing (bottom row). Red points represent biomass from the Larson East wetland and blue point represent values from the Larson West wetland. Dashed line through points represent least squares fit of data.

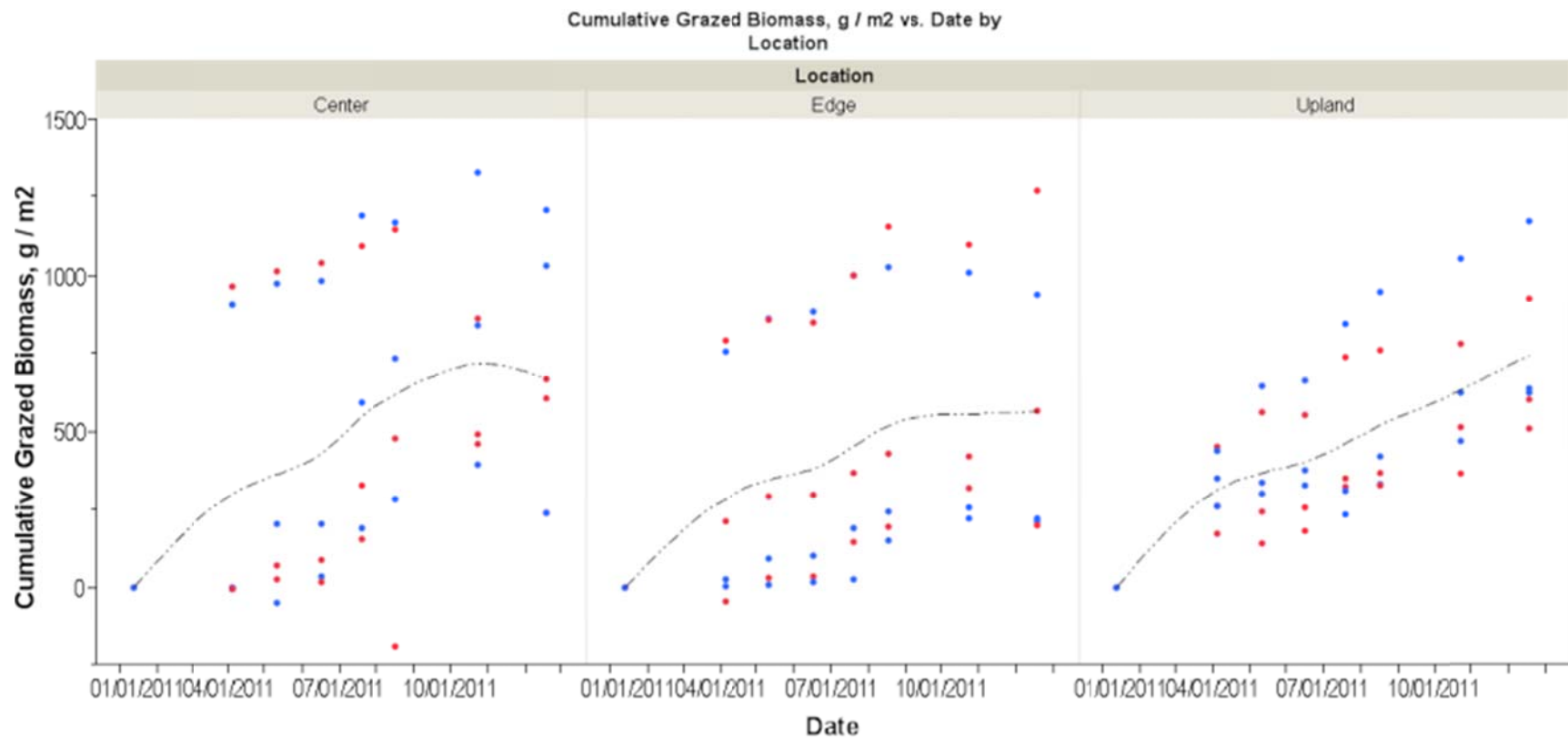


Figure 2-8. Plot of estimated biomass consumed by cows based on difference between short-term enclosure production and standing crop under grazed treatment (Figure 6). Red points represent biomass from the Larson East wetland and blue point represent values from the Larson West wetland. Dashed line through points represent least squares fit of data.

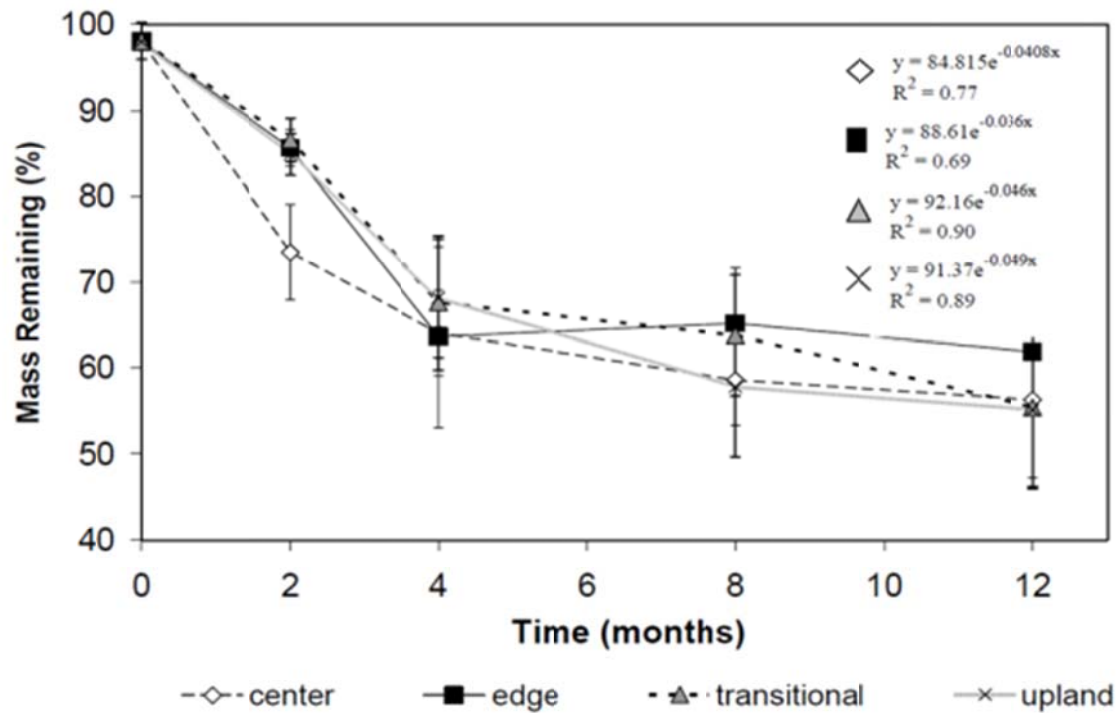


Figure 2-9. Results of litter bag study conducted at Larson Dixie Ranch used to estimate decomposition rates along a hydrologic gradient. Values represent average and one standard deviation of mass loss from four common species present at the site, *Paspalum notatum*, *Polygonum hydropiperoides*, *Juncus effuses* and *Panicum hemitomom*.

Task 3

Composition and Stability of Soil Phosphorus in Wetlands and adjacent Uplands

3.1 Influence of Hydrology on Stability of Soil Organic Phosphorus: Laboratory Experiment

Introduction

Anthropogenic alteration of global phosphorus (P) cycling and its impact upon wetlands has been profound and in many places continues to accelerate. In north central Florida, expansion of cattle ranching since the 1950's led to major landscape modifications, including the drainage of many formerly isolated wetland systems (Steinman and Rosen, 2000). Originally estimated to make up ~13,000 ha (13.8%) of four priority basins north of Lake Okeechobee (McKee, 2005), these small, often shallow, depressional systems acted as surface expressions of the high ground-water level in the region (Lewis et al., 2003; Capece et al., 2007). During historic efforts to both improve pasture drainage and increase accessible range land, many were channelized, which converted them to ephemeral 'head of ditch' wetlands (Flaig and Reddy, 1995). Diffuse and point source loading from agricultural practices (Carpenter et al., 1998; Sharpley et al., 2001) in such a heavily modified landscape threatens the ecological functioning of many downstream waterways and wetland systems (Khan and Ansari, 2005; Verhoeven et al., 2006), yet wetlands also offer a solution, as a means of sequestering both water and nutrients in the agricultural landscape (Paludan et al., 2002; Mitsch and Day, 2006; Moreno et al., 2007).

Florida's Lake Okeechobee Isolated Wetland Restoration Program, a cost-share initiative under the mandate of the Lake Okeechobee Protection Program (Fla. Stat. §373.4595, 2009), has sought to enhance and restore the hydrology of formally 'isolated' wetlands in an attempt to retain water and P within the landscape, thereby reducing P loading to downstream Lake Okeechobee. Although there is evidence of an increase in both soil and total ecosystem P storage with an increasing hydroperiod (Dunne et al., 2007), questions remain as to the efficacy of wetland as a mechanism for long-term P storage. Specifically, the short vs long-term P flux associated with altered hydroperiods (Dunne et al., 2010) and the nature and stability of P associated with developed wetland soils (Cheesman et al., 2010a).

Studies of surface soils in the region have demonstrated that upper horizons consist of uncoated quartz sand grains and very low clay concentrations, dominated by non-crystalline silica (Harris et al., 1994; Harris et al., 1996). Although deeper horizons may interact with P strongly (Graetz and Nair, 1995), mineral components within the surface soils have a very low P binding capacity, resulting in P dynamics driven by an association with organic matter (Cheesman et al., 2010a). In addition, the low topographic relief and limited mineral weathering in surface soils leads to P inputs being dominated by organic matter deposition (Turner, 2005). The functional nature of the P found within this organic matter may be varied (Makarov et al., 2005) and undergo rapid microbial processing (Cheesman et al., 2010b). Yet, the form of biologically derived P has profound implication upon the long-term stability and bioavailability in the environment (Celi and Barberis, 2005; Oberson and Joner, 2005).

There is currently little information on the forms of P entering wetlands as organic matter nor on the direct role abiotic-conditions have in determining the transformation of these functional forms. Several studies looking at terrestrial soils have postulated a reduced decomposition of phosphodiester (including DNA) under wetter and, by extension, more anaerobic conditions (Tate and Newman, 1982; Condon et al., 1990) and this differential phosphodiester decomposition is believed act as a driver in determining

patterns of P forms seen across climatic gradients (Sumann et al., 1998). Indeed, in wetlands typified by anaerobic conditions there seems to be a greater prevalence of phosphodiesteres (Turner and Newman, 2005), yet it is unclear whether this represents decreased decomposition due to biotic hydrolysis, greater or altered standing microbial biomass (Makarov et al., 2002a), abiotic stabilization of DNA (Celi and Barberis, 2005), or a relative increase due to reduced stabilization of other forms (i.e. phosphomonoesters, and inorganic polyphosphates) under fluctuating redox conditions (Suzumura and Kamatani, 1995; Sannigrahi and Ingall, 2005; McKelvie, 2007; Hupfer and Lewandowski, 2008).

The nature of P inputs to wetlands and their transformation via microbial processing under given abiotic conditions will have a direct influence on long-term P dynamics and the efficacy of wetlands as a P control mechanism in the landscape (Sharpley et al., 2001). Although fluctuations in redox conditions has been shown to result in pronounced P fluxes from surface soils and changes in operationally defined P pools (Pant et al., 2002; Dunne et al., 2010) techniques such as ^{31}P nuclear magnetic resonance (NMR) spectroscopy have been applied in lake and marine sediments to show distinct changes in P forms (Sannigrahi and Ingall, 2005; Hupfer and Lewandowski, 2008). This study aims to use standard biogeochemical analysis and solution ^{31}P NMR to track the microbial mediated transformation in surface soils of two organic P inputs characteristic of the agricultural landscape. By tracking the transformation of P associated with organic matter under aerobic and anaerobic conditions we aim to show how management practices including control of hydroperiod could be enacted to improve conversion of P inputs into stable soil P forms.

Materials and Methods

Homogenized surface soils typical of both the upland and marsh wetlands north of Lake Okeechobee were augmented with different P and C sources (cow manure, grass leaf litter, and inorganic phosphate) and held under different simulated hydrologic regimes for up to 150 days (Figure 3-1). Destructive sampling after establishment of experimental conditions at 50 days, and subsequently 150 days, was used to investigate changes in basic biogeochemical characteristics, microbial nutrient biomass, extracellular phosphatase activity, and changes in phosphorus forms as a result of microbial transformations of P inputs.

Initial material

The mesocosms consist of the homogenized surface soil (0-10 cm) collected from a site on the Larson-Dixie ranch north of Lake Okeechobee. The wetland system sampled represents a typical small (<1 ha) shallow depressional head of ditch system within a surrounding matrix of unimproved cow-calf grazing pasture (McKee, 2005; Cheesman et al., 2010a). All soils are mapped as siliceous, hyperthermic Spodic Psammaquents (Basinger series) representing a sandy texture with high infiltration rates, yet low internal drainage given the typically high water table (Lewis et al., 2003). Six sites within the delineated ‘deep marsh’ (Dunne et al., 2007) wetland interior and six from the surrounding pasture were sampled (Plate 1). Sampling consisted of the removal of intact blocks of soil 20 x 20 x 10 cm deep, which were kept at between 10 and 15°C for transport back to the lab. Standing dead leaf litter of *Paspalum notatum* Flugge (Bahia grass) was collected by hand from 10 locations within the surrounding pasture, while fresh manure was collected from 6 representative adult cows in a neighboring field. Given the potential for rapid leaching of P from manure (Wang et al., 1995; Tarkalson and Leytem, 2009) samples were collected only in cases where the deposit had been observed. Fresh manure and leaf litter were also transported back to the lab under temperature controlled conditions (see above). Soils were homogenized, mixed and sieved (2 mm mesh) with all large organic fragments removed by hand. Soils, manure and leaf litter were air dried under conditions of elevated air flow until constant mass was achieved (~ 6 days). Sub samples of the manure and leaf litter, ground to pass through a #20 sieve were used as standard organic amendments.

An inorganic P treatment was also used by applying K_2PO_4 at 1000 mg P L^{-1} . Soils and amendments used were analyzed for both total elemental concentration and P composition by solution ^{31}P NMR.

Mesocosm set up

Study mesocosms consisted of 50 mL pyrex incubation bottles to which $50 \pm 0.001 \text{ g}$ of either upland or wetland soil was added. Individual mesocosms were then either kept as a control or received an additional $1 \pm 0.001 \text{ g}$ of the leaf-litter or manure standard, or 4 mL of 1000 mg P L^{-1} inorganic P standard. Distilled deionized water was then used to bring mesocosms to either field capacity, defined as 18% moisture content or flooded conditions, defined as a 48% water content – enough to result in a completely saturated profile with ~1 cm deep standing water after soil settling. After 50 days, three replicates of each soil-amendment-hydrologic treatment were destructively sampled, three were maintained under the same conditions for a further 100 days. The use of two soil types, four soil amendment treatments, and four hydrologic regimes (i) field capacity for 50 days (ii) flooded for 50 days (iii) field capacity for 150 days (iv) flooded for 150 days, resulted in a total of 96 mesocosms. Mesocosms were loosely capped and held in the dark and at 30°C . Liquid mass lost through evaporation was replaced with distilled deionized water on a weekly basis (Plate 2). At sampling, soils were homogenized and subsampled with half being air dried ($\sim 21^\circ\text{C}$ elevated air flow) and the remainder kept sealed at 4°C – subsequently referred to as ‘fresh’.

Biogeochemical Analysis

Initial air-dried material was analyzed for total carbon (C) and nitrogen (N) using a Flash EA (Thermo-Scientific), total P and P composition was determined parallel to that of mesocosm soils.

Mesocosm soil samples were analyzed for moisture content by gravimetric loss at 75°C after 72 h, and pH using a strict 1:2 soil (dry basis) to DDI ratio and glass electrode. Labile and microbial nutrient pools were determined in four parallel sub-samples ($\sim 0.5 \text{ g}$) weighed into 50 mL HDPE tubes and extracted with either $1 \text{ mol L}^{-1} \text{ KCl}$ or $1 \text{ mol L}^{-1} \text{ NaHCO}_3$, directly or after 24 h chloroform fumigation (Brookes et al., 1982). The KCl extracts were analyzed for total organic carbon (Shimadzu TOC) with the difference between fumigated and non-fumigated samples attributed to release of microbial C. Similarly, both non-fumigated and fumigated soil NaHCO_3 extracts were analyzed for molybdate reactive (MRP) and total P (phosphate determined after persulphate digestion of sample) with the difference in total P between fumigated and non-fumigated attributed to the fumigation release of microbial P. In neither the microbial C nor P data was a correction factor applied.

Fresh samples were also analyzed within 72 h, for two hydrolytic enzymes critical for P cycling (phosphomonoesterase and phosphodiesterase) and one associated with C cycling (β -glucosidase). Enzyme analysis used a fluorogenic substrate and standard microplate assay (Marx et al., 2001) using a modified universal buffer adjusted to individual soils pH and incubated at 30°C for 60 min. Results are reported here in $\mu\text{g MU g}^{-1} \text{ dry soil h}^{-1}$.

Phosphorus composition

Initial materials and dried mesocosm soils were analyzed for total P, and P pool composition. Total P was determined on 0.5 g of finely ground dry material that was combusted at 550°C in a muffle furnace for 4 h. Ash was then dissolved in $6 \text{ mol L}^{-1} \text{ HCl}$ (Andersen, 1976) and the digestate analyzed for P using the automated ascorbic acid method (Method 365.1; USEPA 1993). To determine changes in inorganic and organic P pools, soils were subjected to a modified sequential fractionation scheme based upon Ivanoff et al (1998) and standard solution ^{31}P NMR analysis. Fractionation consisted of extracting 0.5 g of dried soil with 25 mL of $1 \text{ mol L}^{-1} \text{ HCl}$ for 3 h, and subsequently, the residue after centrifugation (10 min, 6000 rpm) with $0.5 \text{ mol L}^{-1} \text{ NaOH}$ for 16 h. Soluble reactive P was determined on HCl (P_{HCl}), samples after

filtration (0.45 μm) while total P was determined in NaOH (P_{NaOH}) extracts after persulphate digestion and standard molybdate colorimetry. Residue from the fractionation scheme is considered as total P – ($\text{P}_{\text{HCl}} + \text{P}_{\text{NaOH}}$). In addition, a standard single step alkaline extraction (Cade-Menun and Preston, 1996; Turner et al., 2007) was applied to initial soils, organic amendments and all sampled mesocosm soils. One gram of each sample was weighed into a 50 mL HDPE centrifuge tube to which 30 mL of 0.25 mol L⁻¹ NaOH 50 mmol L⁻¹ EDTA was added resulting in a 1:30 solid to solution ratio. Samples were shaken at ambient room temperature for 4 hours before being centrifuged at 6500 rpm for 10 min. Resulting alkaline extracts were kept separate for total P determination (NMR_{TP}) using a double acid digest (HNO_3 and H_2SO_4). Residual P (that not extracted by NaOH–EDTA) was defined as a distinct and stable P pool (total P – NMR_{TP}). Subsamples of alkaline extracts were combined within mesocosm replicates and added to a scintillation vial and containing 1 mL of an internal standard methyldiphosphonic acid (MDP) (50 mg P L⁻¹). These combined samples were immediately frozen (-80°C) and lyophilized to await resuspension and NMR spectra acquisition.

Determination of P forms in the lyophilized alkaline extracts of initial material and a subset of mesocosm soils was carried out using a Bruker Avance 500 Console with a Magnex 11.75 T/51mm Magnet, and a 10 mm BBO Probe. Given similarities in the forms of P found within initial material (see 3.1 Initial material), and the desire to highlight potential changes in P forms, deep marsh soils amended with manure were the focus of targeted ³¹P NMR spectroscopy.

Lyophilized alkaline extracts (~300 mg) were re-suspended 2.7 mL (1 mol L⁻¹ NaOH 0.1 mol L⁻¹ EDTA) and 0.3 mL D₂O before vortexing and filtering through a 1 μm glass fiber filter and being transfer to a 10 mm probe. Spectra acquisition was carried out at a stabilized 25°C with a calibrated (~30°) pulse length, a zgig pulse program (Berger and Siegmars, 2004) and a 2 s pulse delay. Results presented here are of up to ~40,000 scans accumulated as sequential experiments with FID's summed post acquisition. Spectra interpretation was carried out using Topspin 3.0 (Bruker Aug 2010). Spectra were referenced against the internal standard (MDP) set as 17.46 ppm and quantified using automatic peak picking and spectral deconvolution of the spectra based upon known spectral assignments (Turner et al., 2003a). Quantification of total P within the sample based upon quantifiable internal standard was within 15% of that determined by NMR_{TP} in all samples (Data not shown).

Statistical Analysis

All statistical analysis was performed using SPSS v 17.0 (2008) soil type (pasture upland, deep marsh) and with sampling periods (50 and 150 days) analyzed separately using a two-way ANOVA with substrate amendment (control, manure, leaf litter, and PO_4) and hydrologic regime in a full factor model. Identification of significant homogeneous subsets within factors was achieved through application of Tukey-b *post-hoc* test. Given positive correlation (Pearson $r > 0.43$ significant at 0.001) between all enzyme activities the decision was chosen to summarize these into a single 'enzyme activity' variable. Dimension reduction was carried out using non-rotated PCA resulting in a single parameter which explained 72% of sample variance, while scoring 0.613 on Kaiser-Meyer-Olkin sampling adequacy test and significant ($p < 0.001$) Bartlett's test of Sphericity. This enzyme activity variable was analyzed using two-way ANOVA as above.

Mesocosms maintained for 50 days at field capacity or under flooded conditions developed distinct biogeochemical characteristics (see 3.3 Sampling at 50 days). Parallel samples containing the same soil (pasture or deep marsh), amendment (manure, leaf litter, inorganic phosphate, or control soil), and subjected to the same hydrologic regime (field capacity or flooded for 50 days) were assumed to develop the same characteristics. Therefore, the comparison of mesocosms held for a further 100 days held at field capacity or under flooded conditions, with those sampled at 50 days provided insight into the impact of hydrologic regime upon pre-established and equilibrated soil systems. Biogeochemical characteristics of

mesocosms sampled at 150 days were therefore compared with averages determined in corresponding samples at 50 days. The resulting delta values were then analyzed by two-way ANOVA, as above, to investigate the role of soil amendment and hydrologic regime upon select soil characteristics over the longer term.

Results

Initial materials

Both pasture-upland and deep marsh soils were shown to be typical of cow-calf grazing landscape north of Lake Okeechobee with elemental concentrations (Table 3-1), and P composition (Table 3-2, Figure 3-2) similar to those determined by Cheesman et al (2010a). The deep marsh soil had substantially higher organic matter content (determined by loss on ignition) and major nutrients N and P than the upland pasture soils. Solution ^{31}P NMR spectra showed both soils to contain orthophosphate (22 and 17% of total P in uplands and wetlands respectively), phosphomonoesters (34 and 27 % of total P), DNA (3 and 1% of total P) and pyrophosphate (6 and 3% of total soil P) with neither soil showing evidence for phosphonates nor long-chain polyphosphates. Unlike previous attempts to apply solution ^{31}P NMR to this study system (Cheesman et al., 2010a) the phosphomonoester region of the deep marsh soil (Figure 3-3) contained peaks indicative of various inositol hexakisphosphates (IP6) although poor spectral resolution prohibits an attempt to quantify them. A peak at 4.20 ppm is assigned to *scyllo*-IP6 (Turner and Richardson, 2004) while peaks at 5.97, 5.05, 4.69, 4.56 are ascribed to *myo*-IP6 (Turner et al., 2003b). Further work using hyperbromate oxidation to remove confounding organic P forms (Irving and Cosgrove, 1981) would be needed to confirm this assignment and allow true quantification.

Analysis of Bahia grass litter and cow manure showed that although manure contained similar levels of organic matter and carbon as Bahia grass it contained 1.5 times the N (19 mg g^{-1}) and approximately five times the concentration of P (4 mg P g^{-1}) (Table 3-1). Phosphorus forms determined by solution ^{31}P NMR (Table 3-2, Figure 3-2) showed both Bahia grass litter and manure to consist of similar P forms although with distinct differences in their relative concentrations. Both organic matter inputs were dominated by inorganic forms of P with substantial concentrations of orthophosphate (36 and 56 % of total P in leaf litter and manure respectively), and inorganic polyphosphates (15 and 3% of total P). Organic P forms included substantial quantities of phosphomonoesters (216 and $379 \text{ } \mu\text{g P g}^{-1}$, 26 and 10% total P in litter and manures respectively) and various phosphodiester (117 and $147 \text{ } \mu\text{g P g}^{-1}$, 14 and 4% total P in litter and manures respectively). There were no distinctive peaks attributable to IP6 (Figure 3), with the majority of the phosphomonoesters region being attributable to the alkaline degradation products of RNA and instable phospholipids (Turner et al., 2003a; Turner and Leytem, 2004).

Mesocosm performance

The use of both organic matter amendments and inorganic P resulted in a range of carbon and nutrient conditions within the study mesocosms (Table 3-3). The addition of 1 g of the cattle manure or Bahia leaf litter standard increased carbon by 7 mg g^{-1} , equivalent to 15 or 5% of native carbon in upland and deep marsh soils respectively. The manure addition also increased total nitrogen by 0.2 mg g^{-1} and P by $\sim 73 \text{ } \mu\text{g g}^{-1}$ (41 and 25% of native P in upland and deep marsh soils) this increase in P was similar to that made by the addition of the inorganic phosphate (45 and 27% of native P) while leaf litter increased total P by $\sim 12 \text{ } \mu\text{g g}^{-1}$ (7 and 4% native soil P in upland and deep marsh soils respectively). Analysis of total P in mesocosm soils showed good replication of soil amendments with of total P averages within $\pm 7\%$ of predicted values (Table 3-2).

Sampling at 50 days

Biogeochemical Characteristics at 50 days

Comparison of soil biogeochemical characteristics after 50 days (Table 3-4) demonstrated clear difference based upon the experimental manipulation. Soil pH, averaging 5.3 and 5.0 in pasture upland and deep marsh soils across all experimental treatments, increased significantly ($p < 0.001$) due to flooding as well as showing a significant ($p < 0.05$) difference as a result of soil amendments (Table 3-4, Figure 3-4). Although the addition of phosphate did not substantially alter pH from that of the control soil mesocosms, the addition of organic matter (both manure and leaf litter) increased soil pH when held at field capacity but buffered (especially in deep marsh soil) changes in soil pH under flooded conditions. This significant ($p < 0.001$) interaction shown between soil amendment and hydrologic regime highlights the role soil organic matter may play in buffering soil pH over short to medium term flooding events.

With labile, NaHCO_3 extractable, P there was a distinct difference when considering molybdate reactive P (often equated to bioavailable P) and non molybdate reactive P (Table 3-4, Figure 3-5). Bioavailable P was found to be higher ($p < 0.001$) in all mesocosms after 50 days of flooding as compared to soils held at field capacity. There was also a substantial ($p < 0.001$) difference seen between all soil amendments, with higher levels of bioavailable P seen in those that received inorganic P or the manure standard. The addition of Bahia leaf litter led to a distinct reduction in bioavailable P ($p < 0.001$, Tukey-b *post-hoc* test) despite the associated addition of total P suggesting a process of P immobilization (Cheesman et al., 2010b) in leaf litter. In soils which received the inorganic P between 65 and 69 $\mu\text{g g}^{-1}$ (81 to 89% of total P added) was recovered as MRP when compared to the corresponding control mesocosm. Similarly, in samples with manure addition between 29 and 34 $\mu\text{g g}^{-1}$ (37 to 44% of total P added) was recovered as labile MRP when compared to their corresponding controls. Extraction of labile non-MRP (often referred to as labile organic P) showed no significant patterns within deep marsh soils as a result of soil amendment or hydrologic regime. While in pasture soils, there was a marginally significant ($p < 0.05$) increase in labile non-MRP as a result of manure amendment (*post-hoc* Tukey-b), although there was no significant effect of flooding. Total microbial P (Table 3-4) showed significant reduction ($p < 0.05$) across all amendments types and in both pasture and deep marsh soils as a result of flooding, averaging 46 $\mu\text{g g}^{-1}$ in all samples held at field capacity and 32 $\mu\text{g g}^{-1}$ in all those held under flooded conditions. There was no significant difference due to soil amendments, although the reduction associated with flooding appeared to be moderated in samples which had received inorganic P.

Extractable, labile C showed a significant ($p < 0.001$) response to flooding in both soils used, averaging an increase of 80% (402 to 723 $\mu\text{g g}^{-1}$) across both soil types and all soil amendments. There was no significant effect of soil amendment in the labile C of pasture soils, while there was a significant effect in deep marsh soils. Interestingly the addition of organic matter (manure and leaf litter) actually caused a decrease ($p < 0.001$) in labile C as compared to both the control and phosphate amended soils (*post-hoc* Tukey-b) indicating a degree of carbon priming, a process by which ‘fresh’ organic matter leads to a utilization of otherwise stable soil organic matter. Microbial C showed the opposite trend to labile C, with a significant ($p < 0.001$) reduction in all samples that were flooded irrespective of soil type or soil amendment. In pasture soils there was no significant difference in microbial C associated with soil amendment used while in deep marsh soils there was an increase ($p < 0.005$) in all amendments over control soils.

Soil hydrolytic enzyme activity at 50 days

Hydrolytic enzyme activities determined at *in-situ* soil pH showed strong variation between mesocosms sampled after 50 days (Table 3-5). Phosphomonoesterase activity ranged from 238 – 544 $\mu\text{g MU g}^{-1} \text{h}^{-1}$ while phosphodiesterase activity ranged from 8.7 – 22.8 $\mu\text{g MU g}^{-1} \text{h}^{-1}$. The only enzyme assayed

associated directly with C metabolism, β -glucosidase, varied from 17.1 –120.6 $\mu\text{g MU g}^{-1} \text{h}^{-1}$. Within samples harvested at 50 days there was a positive correlation (Pearson's $r = 0.56$, $p < 0.001$) between all three enzymes assayed, suggesting a common link to microbial biomass or resource allocation in response to abiotic conditions. The composite parameter 'enzyme activity' derived from considering all mesocosms assayed (see 2.5 Statistical Analysis) was investigated in relationship to soil amendment and flooding regime by application of a two-way ANOVA (Figure 3-6). In both pasture and wetland soils there was a significant ($p < 0.001$) effect of soil amendment and flooding regime. Enzyme activity was reduced under flooded conditions irrespective of soil type or amendment. In pasture soils amended with manure there was a significant increase in enzyme activity over all other amendment types (*post-hoc* Tukey-b), while in deep marsh soil those amended with both manure and litter showed a significantly higher activity than control or PO_4 amended soils.

Soil phosphorus composition

After 50 days there were distinct differences in aspects of soil P composition as a result of soil amendment and flooding regime (Figure 3-7). The addition of inorganic PO_4 resulted in a distinct increase ($p < 0.001$) in HCl extractable inorganic P. In pasture upland soils this increase over control soils represented the entire inorganic P spike while in deep marsh soils it was approximately 90% when averaged across both flooded and field capacity soils. Similarly there was a significant ($p < 0.001$) increase in HCl extractable P in samples that received the manure amendment. With the increase representing approximately 50% of the total P added. This is in contrast with the leaf litter amendment which resulted in no significant increase in HCl extractable P in either pasture or deep marsh soils. Flooding for 50 days had a significant increase on HCl extractable P in both pasture upland ($p < 0.005$) and deep marsh ($p < 0.001$) soils and may represent the release of inorganic P adsorbed to redox sensitive components of the soils matrix. In contrast to the HCl extracted 'inorganic-P' fraction there was only limited alteration of the NaOH extracted 'organic-P' fraction within soils held for 50 days (Figure 6). In upland pasture soils there were no significant effects of soil amendment or flooding regime upon the NaOH-P pool. While in deep marsh soils there was a slight difference ($p < 0.05$) due to soil amendment and between soils held at field capacity and under flooded conditions ($p < 0.005$). With soils that received manure having slightly greater ($\sim 14 \mu\text{g g}^{-1}$) NaOH extractable P than control soils and flooded conditions resulting in a decrease, averaging $13 \mu\text{g g}^{-1}$, across all soil amendments.

Sampling at 150 days

Comparison of mesocosms held for a further 100 days under either field capacity or flooded conditions with sample values at 50 days highlights the potential for further changes in well-established soil systems, see 2.6 Statistical Analysis.

Biogeochemical Characteristics at 150 days

Soil pH in both pasture and deep marsh soils showed further significant ($p < 0.001$) changes based upon both soil amendment and hydrologic regime (Table 3-6, Figure 3-4). Generally mesocosms held at field capacity showed a further reduction in pH (-0.5 averaged over both pasture and deep marsh soil and all amendments) whereas pH under flooded conditions was stable, or in the case of pasture soil showed a slight increase from 50 to 150 days. The larger magnitude of pH change in pasture soils as compared to deep marsh soils and in control and phosphate amended soils as compared to organic matter amended (Table 3-6) highlights how the presence of higher organic matter; either in native soil or due to organic matter amendment buffers changes in pH associated with redox conditions.

In deep marsh soils all treatments showed an increase in labile P from 50 to 150 days (Table 3-6, Figure 3-5) with an average increase of $10.1 \mu\text{g g}^{-1}$ across all experimental manipulations. There was a

significant ($p < 0.001$) difference shown in the magnitude of labile P increase due to both soil amendment and hydrologic treatment. Mesocosms amended with leaf litter showed significantly larger increase in labile P, with a similarly larger increase in the labile P of leaf litter amended pasture soils, suggesting a distinct difference due to organic matter type upon soil P availability. Generally pasture soils showed a similar increase labile P as that seen in marsh soils ($9.2 \mu\text{g g}^{-1}$), but there were no significant effect of hydrology.

Both microbial C and P varied between day 50 and day 150, yet in deep marsh soil at least there was no significant effect of leaf litter or hydrology upon the direction or magnitude of this change after establishment of the initial conditions. In pasture soils there was a significant effect of substrate ($p < 0.001$) with control soils amended seeing a reduction in microbial C between day 50 and day 150 significantly greater than any other treatment.

Changes in P composition after 150 days

In deep marsh soils there was limited changes in HCl extracted P (Figure 3-8) but within these changes there were significant differences between soil amendments ($p < 0.05$). Post hoc analysis showed that while control mesocosms had very limited change in HCl-P there was a significant increase due to leaf litter amendment. This contrasts with changes in pasture soil which showed both a significant reduction in HCl-P under flooded conditions and in those augmented with inorganic phosphate. Given the higher levels of HCl-P in pasture soils augmented with inorganic P at day 50 this decrease, as compared to marsh soils, most likely represents a difference in the temporal interaction of native soils with inorganic P. Changes in P extracted by NaOH showed complex interaction between hydrologic treatment and nature of soils being tested. While pasture upland soils showed a significant decrease in NaOH-P due to flooding (Figure 3-8) deep marsh soils saw a general increase, across all soil amendments.

Solution ^{31}P nuclear magnetic resonance spectroscopy

The single step alkaline (NaOH-EDTA) extraction efficiency averaged approximately 73% of total P in both pasture upland and deep marsh soils, when averaged over all soil amendments and flooding regimes. The P recovered by the single step extraction correlated strongly with that recovered by the sequential fraction scheme (Figure 3-9), suggesting both methods characterized similar soil P pools and that in both the residual P represents a distinct, chemically stable pool. Of the lyophilized extracts, those of deep marsh soil held at field capacity and under flooded conditions for 150 days and marsh soil augmented with manure at both 50 and 150 were run for solution ^{31}P NMR spectroscopy (Figure 3-10, Table 3-8). There was surprisingly little difference in P composition of harvested mesocosms, with all spectra dominated by orthophosphate and phosphomonoesters with evidence for DNA and pyrophosphate but no long chain polyphosphates (as compared to manure standard) and with only one sample showing a trace of phosphonates (deep marsh soil with manure addition held for 50 days at field capacity). In the marsh soils it appeared that flooding resulted in an increase in orthophosphate ($110 \mu\text{g P g}^{-1}$ as compared $77 \mu\text{g g}^{-1}$) at the expense of organic forms with concentrations of both phosphomonoesters and phosphodiester as well as pyrophosphate being reduced. In soils augmented with the manure standard the additional P was found to be present as orthophosphate, inorganic pyrophosphate and the alkali stable, residual pool. There appeared to be little variation in organic P forms identified (Figure 3-10) with phosphomonoesters ranging from 95 to $110 \mu\text{g P g}^{-1}$, phosphodiester from 2.2 to $9.1 \mu\text{g P g}^{-1}$ and the inorganic pyrophosphate 5.6 to $10.3 \mu\text{g P g}^{-1}$ all with no apparent pattern due to hydrologic conditions. There was a substantial interaction of orthophosphate and (reciprocally) residual P to hydrologic condition, with orthophosphate concentrations of $40 \mu\text{g P g}^{-1}$ greater under flooded conditions at 50 days ($20 \mu\text{g P g}^{-1}$ at 150 days) as compared to samples held at field capacity. The lack of distinction between the P composition, and influence of flooding, in native soils and those amended with cow-manure suggest that similar mechanisms may be operating upon native soil organic matter and the additional manure.

Discussion

Initial materials and experimental overview

This study sought to establish the role of hydrologic conditions upon the incorporation of P into surface soils of agricultural wetlands. The use of two organic matter amendments (Bahia grass leaf-litter, and cow manure) were chosen as typical inputs to wetlands in the agricultural landscape, while the use of a control and inorganic phosphate amendment allowed for the investigation of C's role in the incorporation of P into surface soils. Given typically low stocking densities of the study site ~ 1 animal unit ha⁻¹ (Gornak and Zhang, 1999) and assuming an input of 3.31 kg of manure AU⁻¹ day⁻¹ (NRCS, 2010) the level of manure amendment added to each mesocosm equates to approximately 6 times the average annual input, on an aerial basis across the study field. Yet, given the heterogeneous spread of manure inputs as well as the known congregation of cattle to water sources, including wetlands, (Godwin and Miner, 1996; Pandey et al., 2009) it is likely that levels of manure addition used in this study represent a realistic input into surface soils.

Initial analysis of surface soils showed the material used to be typical of surface soils of the study area (Dunne et al., 2007; Cheesman et al., 2010a), although the detection of inositol hexakisphosphates (IP6) within surface soils contrasts with the conclusions drawn by Cheesman et al (2010a). This discrepancy may be attributable to the greater signal to noise ratio in the current studies analysis allowing their detection or may indicate a more dynamic role of IP6 within the active soil P than is traditionally acknowledged (Turner, 2007).

The two organic matter amendments used contained large quantities of DNA, phospholipids, and RNA degradation products the latter perhaps indicative of recently senesced biomass (Makarov et al., 2002b) yet surprisingly, P forms identified in organic matter were largely dominated by inorganic pools, especially in manure where over 60% of total P was found to be either orthophosphate or long-chain polyphosphates. Although this is likely to include a proportion of P from alkali unstable forms degraded to orthophosphate during analysis (Turner et al., 2003a) it is still probable that a large proportion of P is found in manure as readily available P. It therefore appears that grazing and defecation not only localizes inputs of P from across the landscape but potentially modifies P into forms that may be more liable to be lost via leaching (McDowell et al., 2007). The phosphorus composition, and specifically the lack of IP6 in the manure standard was similar to that found in pasture fed beef cattle (Turner and Leytem, 2004) and contrast strongly to the manure of domesticated monogastric animals or ruminants fed high cereal diets which may have manures containing large quantities of IP6 (Leytem and Maguire, 2007).

Although redox measurements were not taken over the course of the experiment, the production and release of reduced sulfurous compounds within flooding treatments confirmed the establishment of anaerobic conditions. In contrast, soils held at field capacity did not produce volatile sulfurous compounds and with weekly homogenization can be assumed to have maintained an aerobic condition.

Establishment of altered biogeochemical conditions

After 50 days of equilibration mesocosms had established distinct biogeochemical characteristics as a result of the applied hydrologic treatment. Flooding generally led to an increase in pH, an increase in labile MRP and C, yet no substantial changes in non-MRP labile P and a reduction in microbial C and P pools. All of these factors showed complex interactions with soil amendments used although, generally, inorganic phosphate mirrored conditions within control soils (with the notable exception of extractable labile P). Both organic matter amendments, buffered changes in pH, reduced labile C, and in the case of leaf litter reduced labile P via nutrient immobilization. Assays at ambient soil conditions showed a

significant reduction in extracellular enzyme activity under flooded conditions (Figure 3-6) and a significant increase in samples that received organic matter. Given, microbial biomass (microbial C) was significantly increased by both organic amendments and inorganic P it appears that net extracellular enzymatic rates were the result of both changes in microbial biomass and the down regulation of enzymatic process due to P availability (Corstanje et al., 2007).

Mesocosms maintained for an additional 100 days at field capacity or under flooded conditions continued to exhibit changes in biogeochemical characteristics, notably a continued divergence in pH between treatments and a general increase in labile P in all soils (Figure 3-4 and 3-5). When considering treatment specific changes in labile C and microbial nutrient content it is hard to establish with the current study design if such trends are indicative of larger temporal trends or may represent sample specific variance. Sampling of a more detailed temporal gradient using a single batch processor from which subsamples are collected would help establish this and help put changes seen in P composition in a better biogeochemical framework.

Influence of biogeochemical conditions upon phosphorus composition

After 50 days of imposed hydrologic treatment there were clear changes in inorganic P pools as demonstrated in the HCl extracted fraction and orthophosphate concentrations of selected solution ^{31}P NMR spectra. It is clear that redox conditions and associated changes in pH, microbial and carbon processes effect inorganic P dynamics. It is also apparent that manure addition to wetland and pasture soils impacts the inorganic P pool to a greater extent than it does the operationally defined organic pool (Figure 3-7). At both 50 and 150 days (Table 3-7) there was only ever limited influence of flooding upon the organic P fraction (there was a slight decrease in deep marsh soils). Indeed NMR spectra acquired in manure amended soils show only very limited difference in the organic P forms present after 150 days. This allows us to suggest that P flux observed after the flooding of similar agricultural wetland soils (Dunne et al., 2010) is due to the influence of redox conditions upon the inorganic P pool and associated binding sites. In contrast organic P appears to represent a relatively stable soil P pool with only limited interaction with site redox conditions, outside of its influence upon the initial accumulation and stabilization of soil organic matter.

Conclusions

Organic matter inputs to agricultural wetlands soils contain a distinct P composition from native soil organic matter. After incorporation and 50 (or 150) days of equilibration in soils held at field capacity or under flooded conditions the distinction in organic P forms is lost, but there continues to be an effect upon the inorganic P fraction. Manure, itself a substantial source of inorganic P, appears to react to flooding in a manner similar to soils native organic matter. Since, although flooding has a pronounced impact upon a range of biogeochemical characteristics there is only limited impact upon organic P composition. Flooding, and presumed anaerobic conditions, leads to an increase in inorganic P due to the release of P bound to redox sensitive components. Yet, there is no evidence to suggest that changes in organic P with redox conditions may impact long term stability. We can conclude that effective long-term sequestration of P in soils is dependent upon the accumulation of organic P as a component of organic matter. And, that once sequestered as organic P it is relatively buffered against changes in redox conditions.

Tables

Table 3-1. Composition of initial materials used in mesocosm experiment

	OM †	Nitrogen	Carbon	Phosphorus
		mg g ⁻¹		µg g ⁻¹
Upland Soil	70	2.8	46	178
Wetland Soil	119	4.9	112	294
Cow Manure	856	18.6	417	3965
Bahia Grass Litter	944	11.7	410	829

† Organic matter, estimated from loss on ignition (550°C, 4 h)

Table 3-2. Phosphorus composition (µg g⁻¹) of initial materials used within mesocosm study

	Ortho-P [†]	Mono-P [‡]	Phosphodiester		Pyro-P [#]	Polyphosphate		Residual ^{††}
			<i>DNA</i>	<i>Other</i>		<i>Mid</i>	<i>End</i>	
Upland Soil	39	61	5	5	10			62
Wetland Soil	51	80	51	1	8			153
Bahia Grass Litter	297	216	117	36	16	111	86	25
Cow Manure	2206	376	147	86	61	55	69	41

† Orthophosphate

‡ Phosphomonoesters

Pyrophosphate

¶ Mid and end chain residue polyphosphates

†† Residual P not identified with alkaline extraction and solution ³¹P NMR

Table 3-3. Predicted and measured total elemental concentrations in mesocosm soils. Based upon, known initial materials and ratio of amendments in mesocosm set up.

Amendment	Soil type							
	Upland soil				Deep marsh			
	C	N	P [†]		C	N	P [†]	
	mg g ⁻¹		μg g ⁻¹		mg g ⁻¹		μg g ⁻¹	
Control	46	2.8	178	(172)	112	4.9	294	(298)
Manure	53	3	252	(257)	118	5	366	(351)
Litter	53	3	190	(198)	118	5	304	(305)
PO4	46	2.8	258	(241)	112	4.9	374	(371)

† Total P predicted, and measured by TP ashing method given in brackets, averaged across all hydrologic treatments.

Table 3-4. Soil biogeochemical characteristics after 50 days, values represent averages (n = 3) ± 1.s.d.

Soil	Amendment [†]	Treatment [‡]	pH [#]	Labile MRP [¶]		Labile non MRP		Microbial P		Labile C		Microbial C	
								µg g ⁻¹					
Pasture upland	Control	FC 50	5.0	10.4	±0.3	22.0	±2.5	45.0	±1.7	455	±107	355	±82
		FL 50	5.6	38.5	±4.4	28.2	±1.5	17.5	±3.6	814	±100	224	±174
	Manure	FC 50	5.2	44.3	±1.2	33.1	±1.0	44.6	±1.0	459	±46	540	±50
		FL 50	5.6	67.6	±8.2	35.8	±7.6	16.0	±13.2	816	±36	93	±89
	Litter	FC 50	5.0	7.7	±0.5	28.4	±3.8	47.8	±4.4	383	±15	491	±51
		FL 50	5.3	25.0	±3.2	33.2	±2.4	25.0	±3.3	652	±60	135	±52
	PO ₄	FC 50	5.1	76.4	±0.2	33.8	±7.2	42.6	±12.6	485	±133	307	±118
		FL 50	5.7	103.4	±3.0	22.3	±5.6	42.3	±37.8	764	±73	197	±18
Deep marsh	Control	FC 50	4.2	23.1	±0.8	52.5	±2.3	39.1	±10.4	333	±4.0	411	±19
		FL 50	5.8	34.1	±3.0	47.1	±6.0	36.5	±7.9	724	±13	170	±18
	Manure	FC 50	4.6	56.2	±1.7	50.8	±1.7	48.3	±6.7	360	±24	474	±117
		FL 50	5.4	66.6	±3.2	50.6	±2.6	40.5	±8.6	660	±43	251	±98
	Litter	FC 50	4.7	14.9	±2.1	50.0	±3.1	50.9	±6.8	380	±17	602	±27
		FL 50	5.3	35.0	±1.2	51.9	±1.1	32.0	±1.4	570	±48	231	±85
	PO ₄	FC 50	4.3	88.8	±1.5	51.9	±2.3	45.4	±5.1	357	±6	484	±24
		FL 50	5.7	101.8	±2.0	45.0	±0.9	45.2	±12.1	783	±51	161	±79
Summary Averages	Soil	Pasture	5.3	46.7		29.6		35.1		604		293	
		Deep marsh	5.0	52.6		50.0		42.2		521		348	
	Amendment	Control	5.1	26.5		37.4		4.5		81		90	
		Manure	5.2	58.7		42.6		7.3		74		39	
		Litter	5.0	20.7		40.9		8.9		96		65	
		PO ₄	5.2	92.6		38.3		3.9		97		87	
	Treatment	FC 50	4.8	40.2		40.3		45.5		402		458	
		FL 50	5.5	59.0		39.3		31.9		723		183	

† Substrate added to mesocosm,

‡ FC50 = maintained at field capacity for 50 days, FL50 = maintained as flooded for 50 days

pH standard deviation all < 0.1

¶ MRP = molybdate reactive P

Table 3-5. Soil extracellular enzyme activities after 50 days, values represent averages (n=3) $\pm$ 1.s.d.

Table 5. Soil extracellular enzyme activities after 56 days, values represent averages ($n = 3$) $\pm$ 1 s.d.										
Soil	Amendment [†]	Treatment [‡]	Phosphomonoesterase		Phosphodiesterase		β - glucosidase		Enzyme Composite	
					$\mu\text{g MU g}^{-1} \text{h}^{-1}$					
Pasture upland	Control	FC 50	379	± 46	17.4	± 2.83	37.9	± 6.80	0.1	0.53
		FL 50	357	± 10	10.7	± 0.27	21.6	± 1.85	-0.9	0.04
	Manure	FC 50	544	± 51	19.4	± 1.78	81.3	± 16.40	1.6	0.62
		FL 50	387	± 18	13.4	± 0.25	37.5	± 3.56	-0.3	0.06
	Litter	FC 50	383	± 39	17.7	± 1.54	72.6	± 11.29	0.7	0.36
		FL 50	272	± 65	12.4	± 3.23	41.0	± 11.07	-0.8	0.72
	PO ₄	FC 50	361	± 31	16.1	± 0.42	30.0	± 4.74	-0.2	0.17
		FL 50	320	± 13	11.1	± 0.19	17.1	± 0.87	-1.1	0.06
Deep marsh	Control	FC 50	292	± 26	20.0	± 1.43	63.0	± 1.22	0.4	0.26
		FL 50	272	± 15	8.7	± 0.41	25.6	± 2.40	-1.4	0.13
	Manure	FC 50	466	± 15	22.8	± 1.39	86.0	± 7.94	1.7	0.31
		FL 50	287	± 26	13.5	± 1.17	43.0	± 1.26	-0.6	0.18
	Litter	FC 50	436	± 19	22.7	± 0.36	120.6	± 1.91	2.2	0.10
		FL 50	281	± 10	13.8	± 0.72	62.2	± 3.79	-0.3	0.10
	PO ₄	FC 50	238	± 32	15.8	± 1.90	51.7	± 1.06	-0.4	0.31
		FL 50	253	± 61	9.7	± 2.6	23.4	± 5.3	-1.4	0.6
Summary Averages	Soil	Pasture	375		14.8		42.4		-0.1	
		Deep marsh	316		15.9		59.5		0.0	
	Amendment	Control	325		14.2		37.0		-0.4	
		Manure	421		17.3		61.9		0.6	
		Litter	343		40.3		45.5		0.5	
		PO ₄	293		39.3		31.9		-0.8	
	Treatment	FC 50	387		19.0		67.9		0.8	
		FL 50	304		11.7		33.9		-0.8	

[†] Substrate added to mesocosm,

[‡] FC50 = maintained at field capacity for 50 days, FL50 = maintained under flooded conditions for 50 days

Table 3-6. Changes in soil characteristics between 50 and 150 days, values represent averages (n = 3) ± 1.s.d.

Soil	Amendment [†]	Treatment [‡]	pH [#]	Labile MRP [¶]		Labile non MRP		Microbial P µg g ⁻¹		Labile C	Microbial C
Pasture upland	Control	FC	-0.7	10.7	±1.8	0.1	±3.9	-7.3	±5.3	-56	±43
		FL	0.4	5.5	±0.8	-5.2	±0.7	-4.4	±2.5	-254	±44
	Manure	FC	-0.6	8.9	±1.7	-10.9	±5.0	4	±9.0	-8	±41
		FL	0.2	15.3	±8.6	-22.6	±3.1	12.7	±21.1	-219	±19
	Litter	FC	-0.4	12.2	±4.7	3	±0.2	-18.5	±10.0	-1	±11
		FL	0.3	16.9	±2.6	-10.3	±4.8	-13	±7.6	-103	±35
	PO ₄	FC	-0.9	6	±2.7	-7.3	±2.8	-8.7	±2.0	-117	±29
		FL	0.3	-2.2	±2.5	3.2	±3.3	-8.7	±10.7	-176	±36
Deep marsh	Control	FC	-0.2	9.6	±0.8	-12.8	±3.8	-4	±4.5	30	±40
		FL	0	4.2	±2.9	-13.7	±9.1	-14.4	±13.7	-195	±18
	Manure	FC	-0.3	11.9	±5.8	-3.9	±4.9	-22.8	±7.4	-156	±21
		FL	-0.2	9.6	±2.5	-11.1	±5.8	-6.7	±24.0	-290	±24
	Litter	FC	-0.5	24.6	±0.3	0.4	±1.6	-36.9	±4.9	-180	±8
		FL	-0.1	7.4	±0.4	-7.6	±3.7	-17.8	±5.1	-247	±30
	PO ₄	FC	-0.2	10.7	±2.0	-7.8	±4.7	-18.5	±8.9	-185	±20
		FL	-0.1	2.6	±4.7	-8.4	±4.4	-28.4	±8.6	-287	±77
Summary Averages	Soil	Pasture	-0.2	9.2		-6.3		-5.5		-116.8	
		Deep marsh	-0.2	10.1		-8.1		-18.7		-188.8	
	Amendment	Control	-0.1	7.5		-7.9		-7.5		-118.8	
		Manure	-0.2	11.4		-12.1		-3.2		-168.3	
		Litter	-0.2	15.3		-3.6		-21.6		-132.8	
		PO ₄	-0.2	4.3		-5.1		-16.1		-191.3	
	Treatment	FC	-0.5	11.8		-4.9		-14.1		-84.1	
		FL	0.1	7.4		-9.5		-10.1		-221.4	

[†] Substrate added to mesocosm,

[‡] Hydrologic regime of mesocosm over first 50 days (T1) and subsequent 100 days (T2). FC50 = maintained at field capacity for 50 days, FL = maintained under flooded conditions for 150 days FC = maintained at field capacity for 150 days

[#] pH standard deviation all < 0.1

[¶] MRP = molybdate reactive P

Table 3-7. Phosphorus composition summarized across all factors

			Sequential fractionation			NMR TP	
		<i>n</i>	HCl	NaOH	Residue		
			$\mu\text{g g}^{-1}$			$\mu\text{g g}^{-1}$	%
Soil	Pasture	48	76	93	47	158	73
	Deep marsh	48	82	154	96	246	74
Amendment	Control	24	54	120	61	170	74
	Manure	24	90	133	81	226	74
	Litter	24	50	125	76	175	69
	PO ₄ -P	24	122	116	68	236	78
Treatment	FC – 50 days	24	70	132	71	200	74
	FC – 150 days	24	76	126	70	197	72
	FL – 50 days	24	83	115	71	204	76
	FL – 150 days	24	87	121	73	205	73

Table 3-8. Phosphorus composition determined in deep marsh soils amended with manure and at field capacity and under flooded conditions for up to 150 days.

Substrate	Hydrologic regime		Phos-P [†]	Ortho-P [‡]	Mono-P [#]	Diesters	Pyro-P [¶]	Residual ^{††}
Deep marsh soil	Field capacity 150 days			77	116	14.5	5.2	89
	Flooded 150 days			110	97	1.9	3.	86
Deep Marsh soil with Manure	Field capacity	50 days	trace	131	111	9.1	10.3	82
		150 days		149	95	2.2	7.0	93
	Flooded	50 days		171	104	7.9	7.1	72
		150 days		170	106	3.3	5.6	64

† Phosphonate

‡ Orthophosphate

Phosphomonoesters

¶ Pyrophosphate

†† Residual P not identified with alkaline extraction and solution ³¹P NMR

Figures

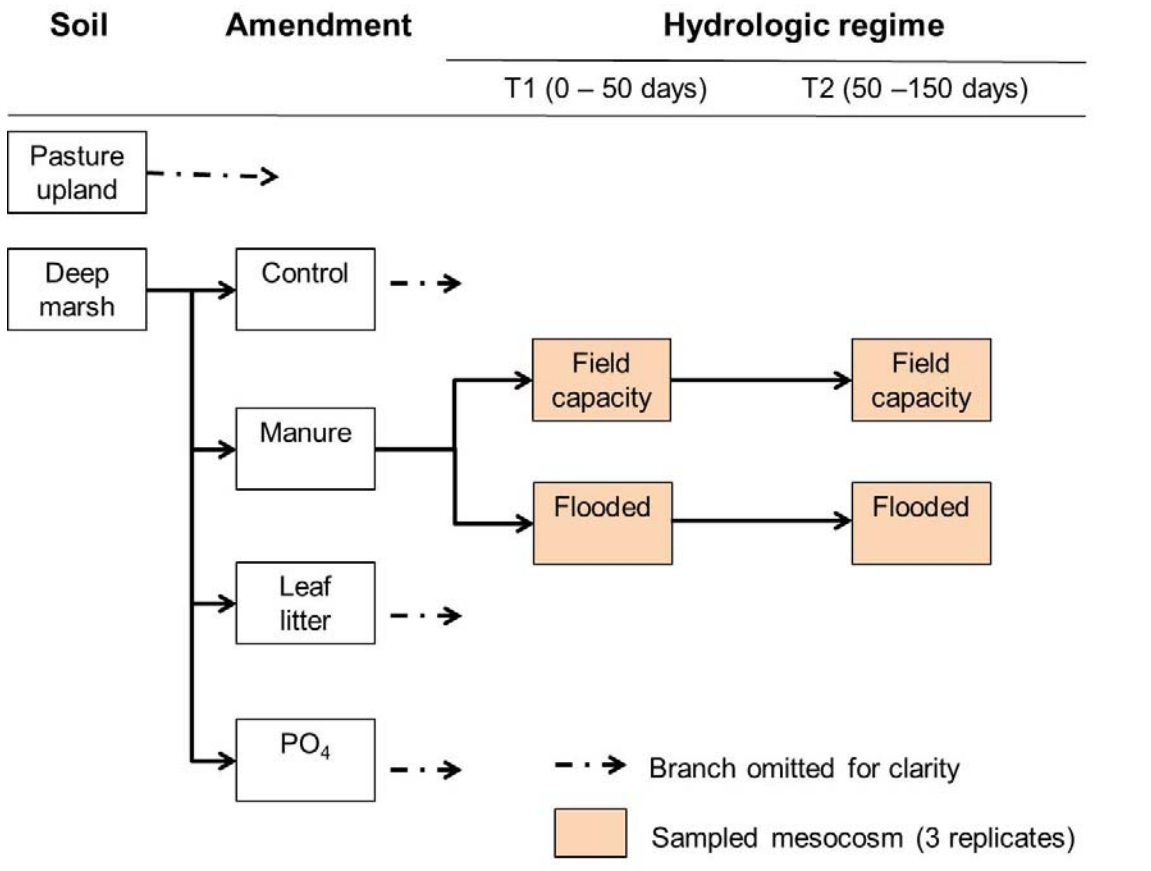


Figure 3-1. Experimental overview. Showing two soil types, four soil amendments and hydrologic regime applied to mesocosms for up to 150 days.

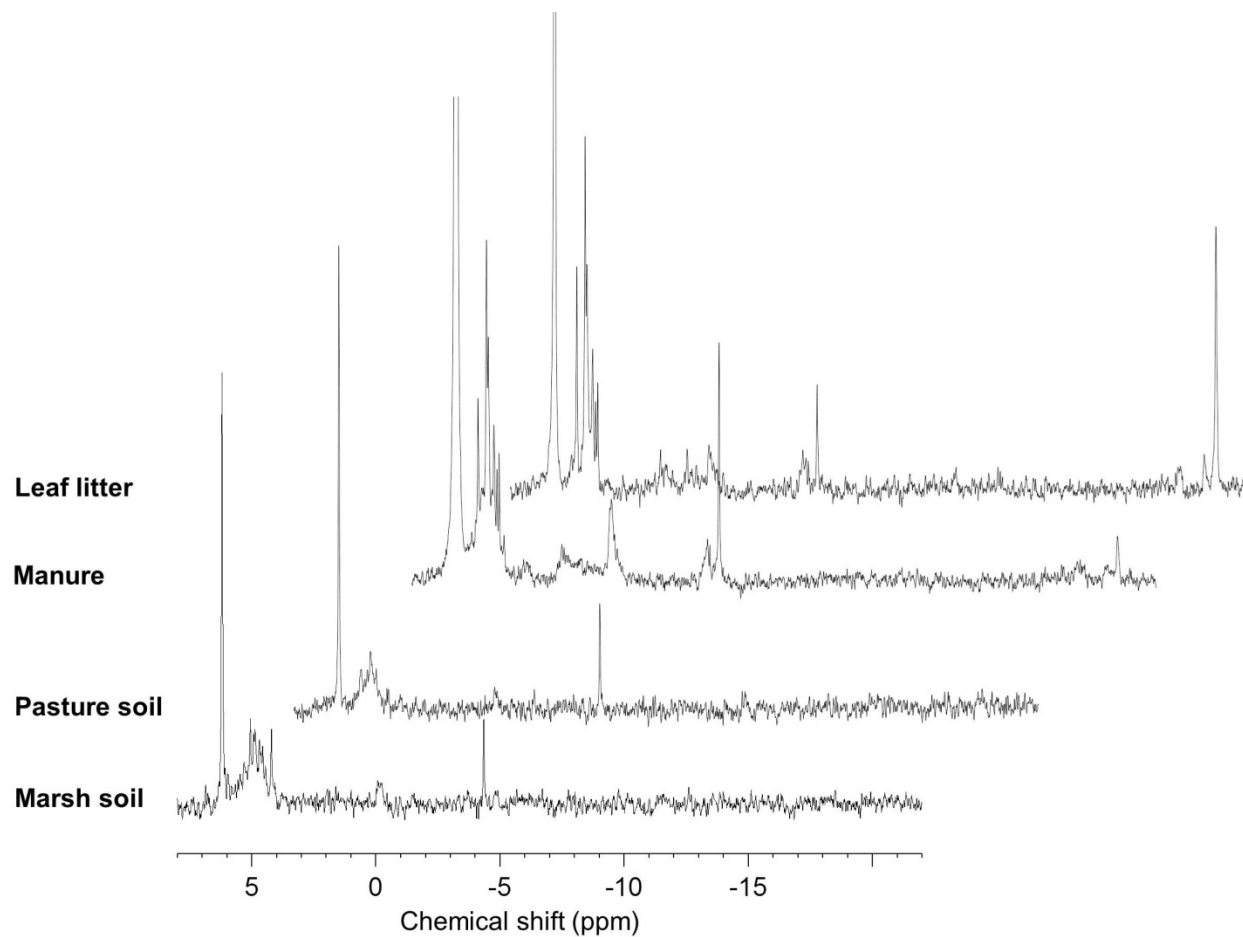


Figure 3-2. Solution ^{31}P NMR spectra of initial material used in mesocosm study. All spectra scaled to internal standard methylenediphosphonic acid (MDP $\delta = 17.46$ ppm). Note orthophosphate peak (~ 6.20 ppm) on both manure and leaf litter spectra are curtailed to allow for clear depiction of other components.

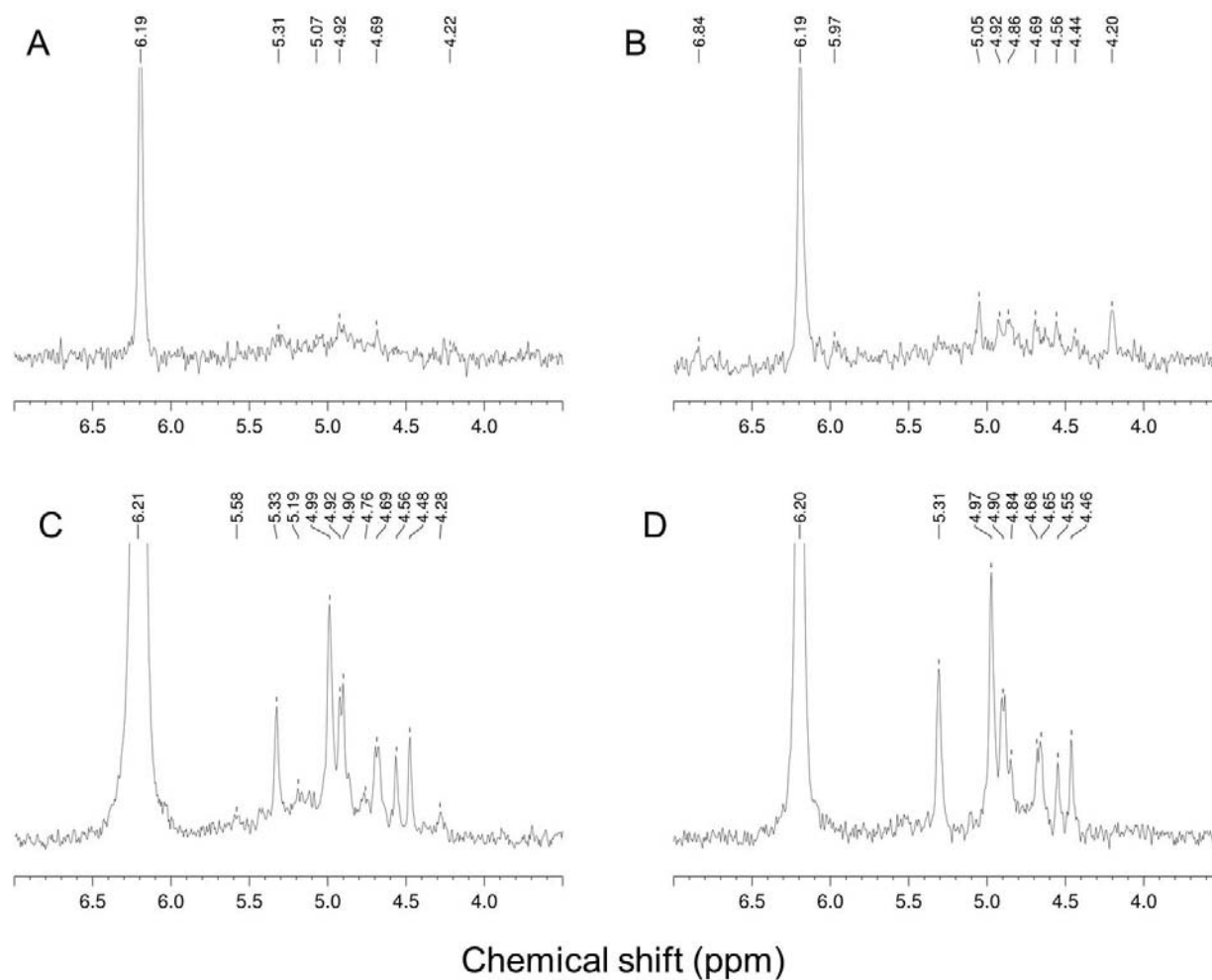


Figure 3-3. Detail of ^{31}P NMR spectra of initial materials used in mesocosm study A) pasture soil B) deep marsh soil C) cow manure D) Bahia leaf litter. Spectra represent ~30,000 scans plotted using 1 Hz line broadening scaled and referenced against internal standard methyldiphosphonic acid.

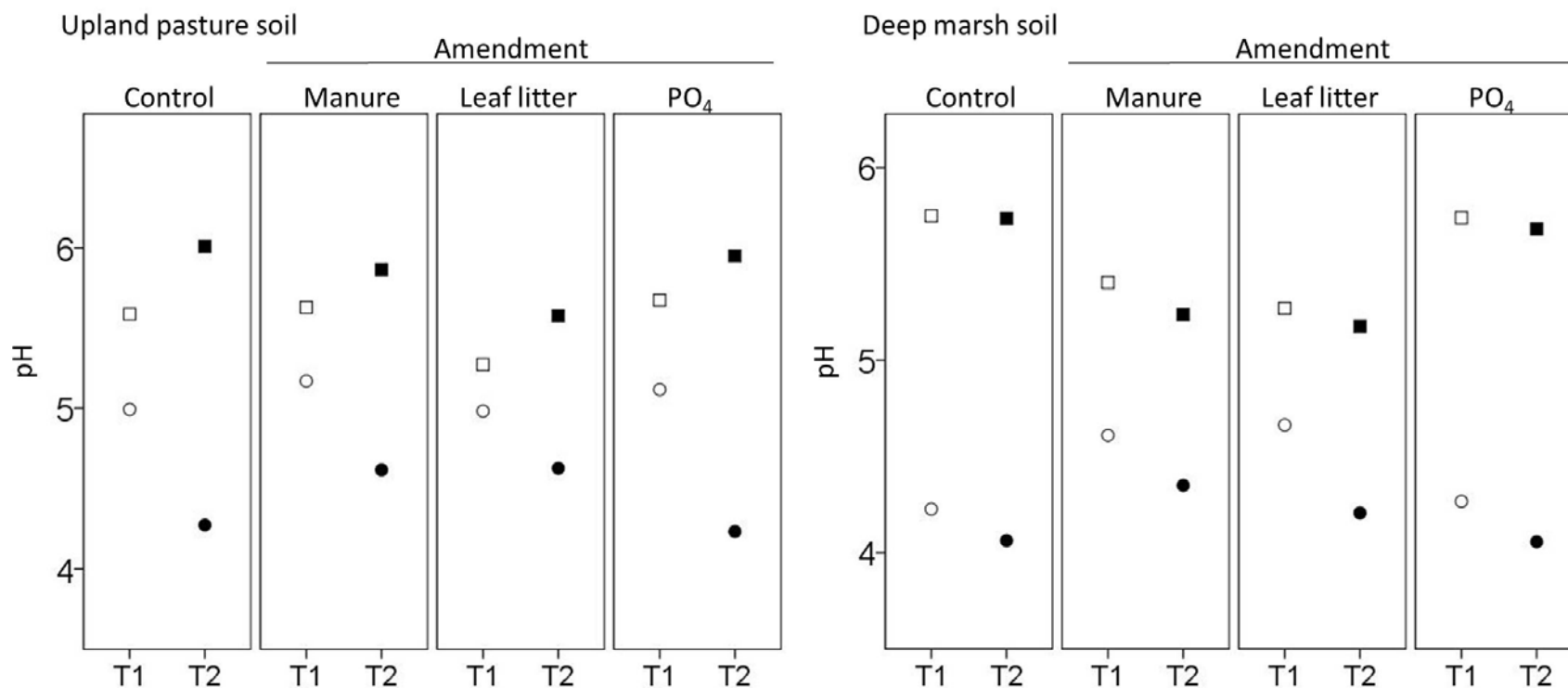


Figure 3-4. Soil pH determined in mesocosm soils subjected to various flooding regimes at T1) 50 days and T2) 150 days. Unfilled square= flooded for 50 days, unfilled circle = field capacity for 50 days, black square = flooded for 150 days, black circle = field capacity for 150 days. Values represent averages (n = 3), error bars omitted for clarity.

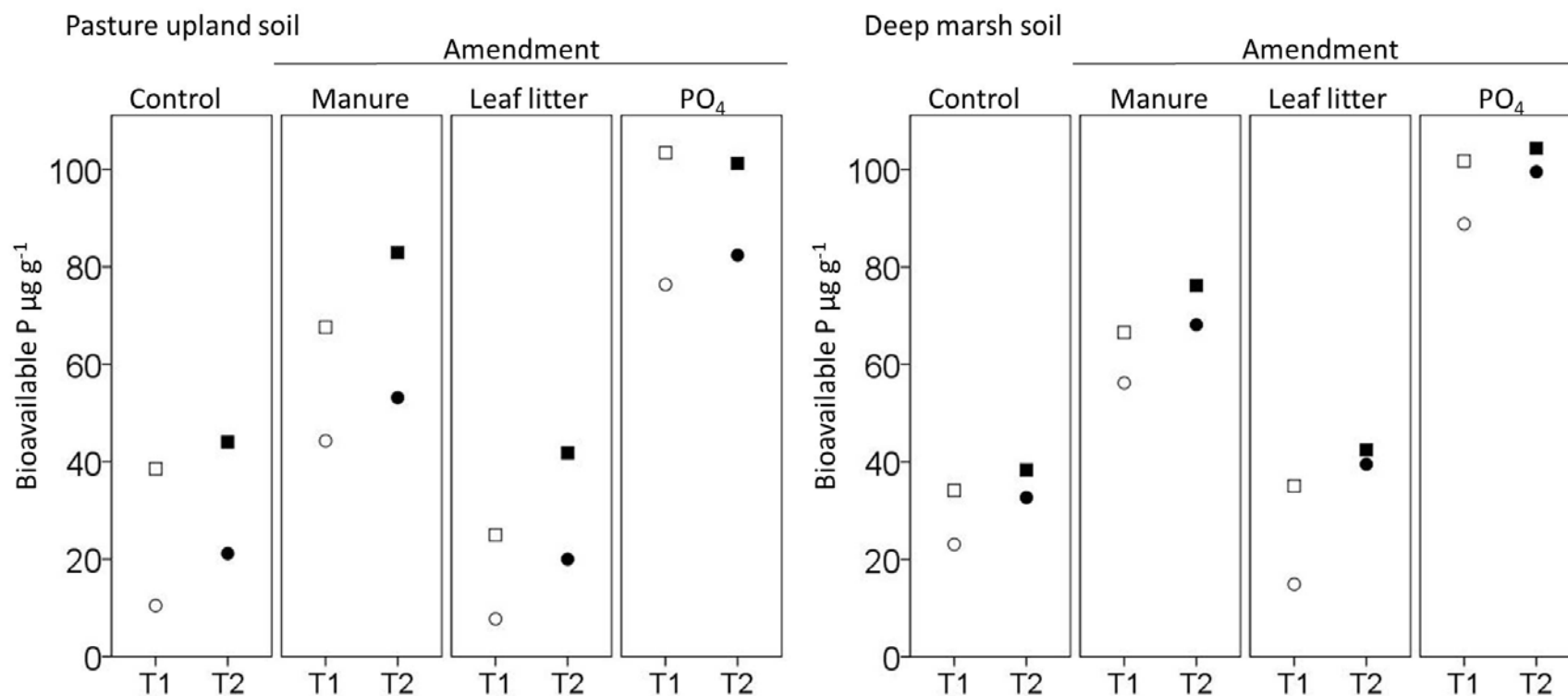


Figure 3-5. Labile molybdate reactive P determined in mesocosm soils subjected to various flooding regimes at T1) 50 days and T2) 150 days. Unfilled square= flooded for 50 days, unfilled circle = field capacity for 50 days, black square = flooded for 150 days, black circle = field capacity for 150 days. Values represent averages ($n = 3$), error bars omitted for clarity.

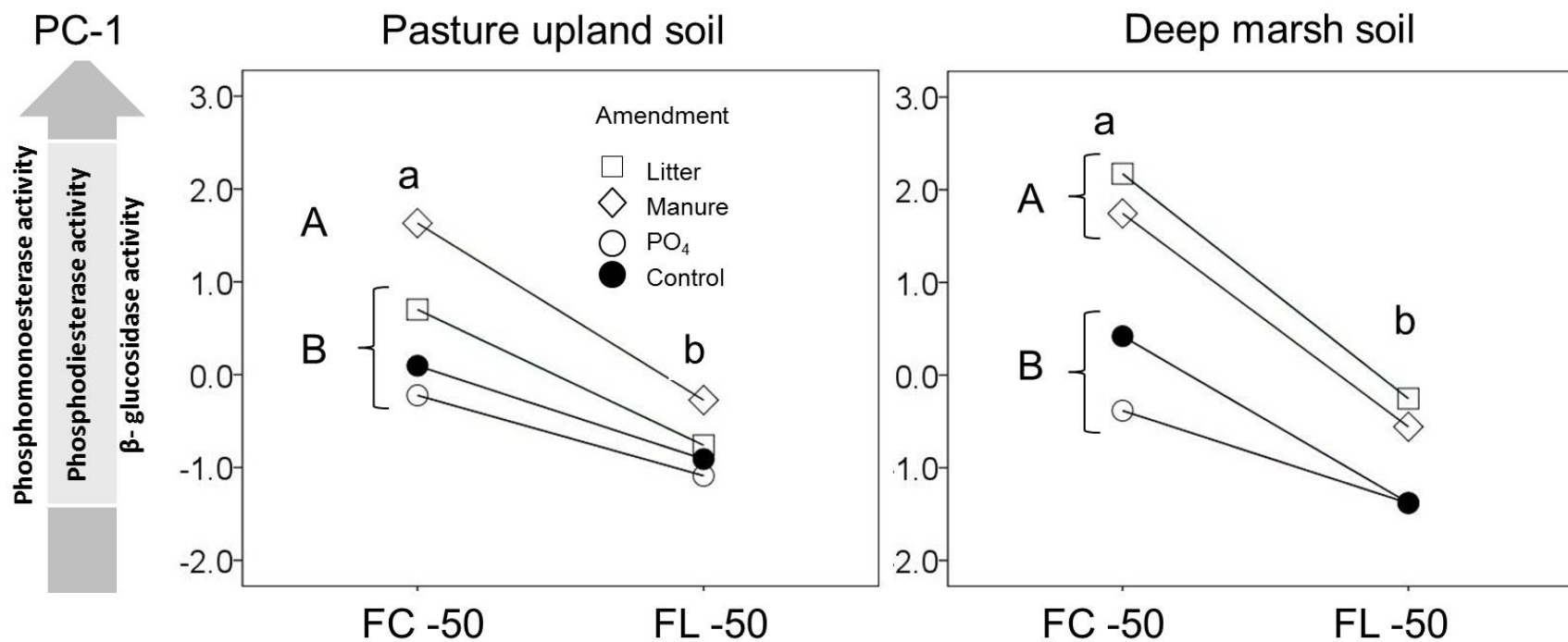


Figure 3-6. Estimated marginal means of composite enzyme activity axis (unit less) in mesocosm soils sampled at 50 days (FC-50 = field capacity, FL-50 = flooded). Significant homogeneous subsets (Tukey-b) highlighted with the same suffix (lower case flooding regime, upper case soil amendment).

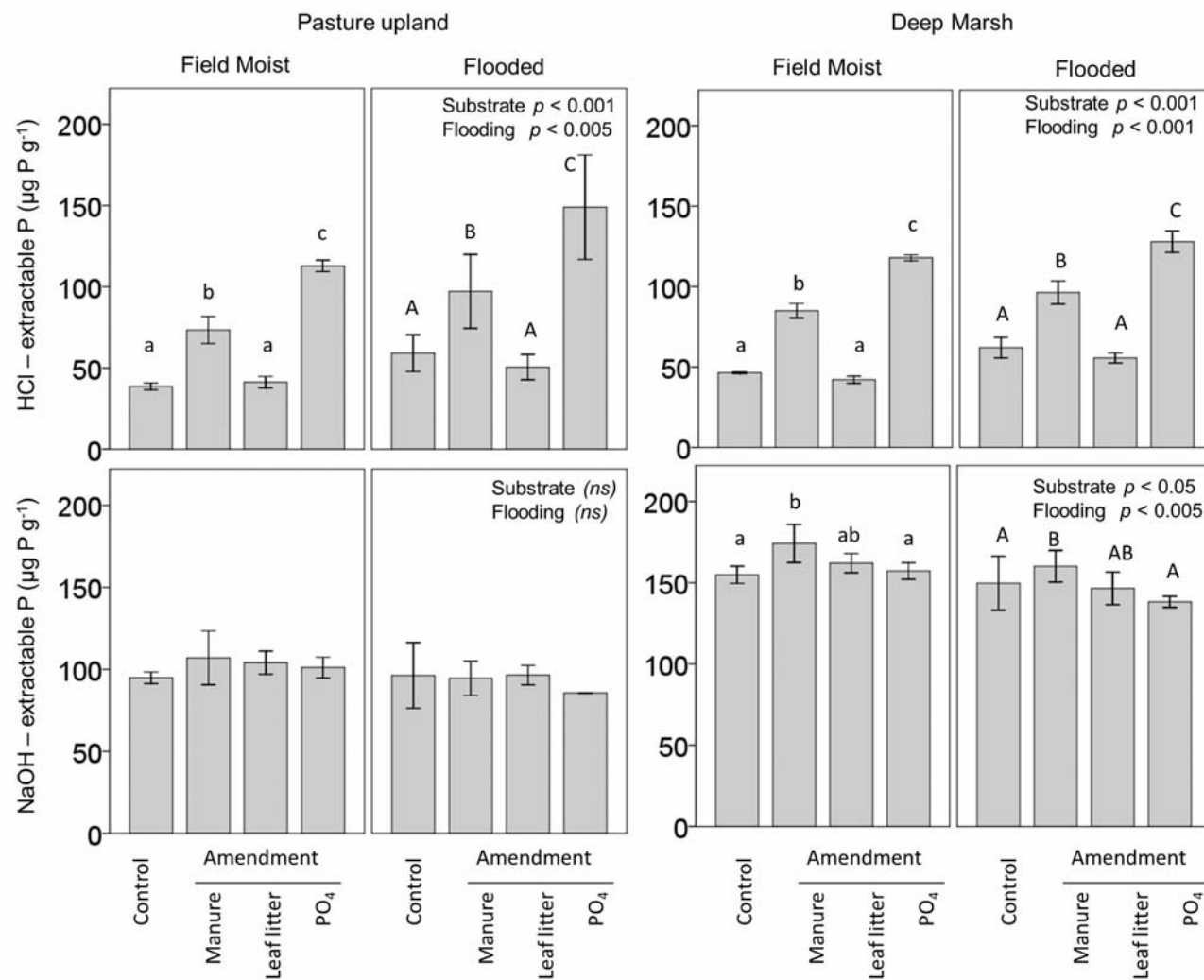


Figure 3-7. Phosphorus composition of mesocosm soils after 50 days of field capacity or flooded conditions.

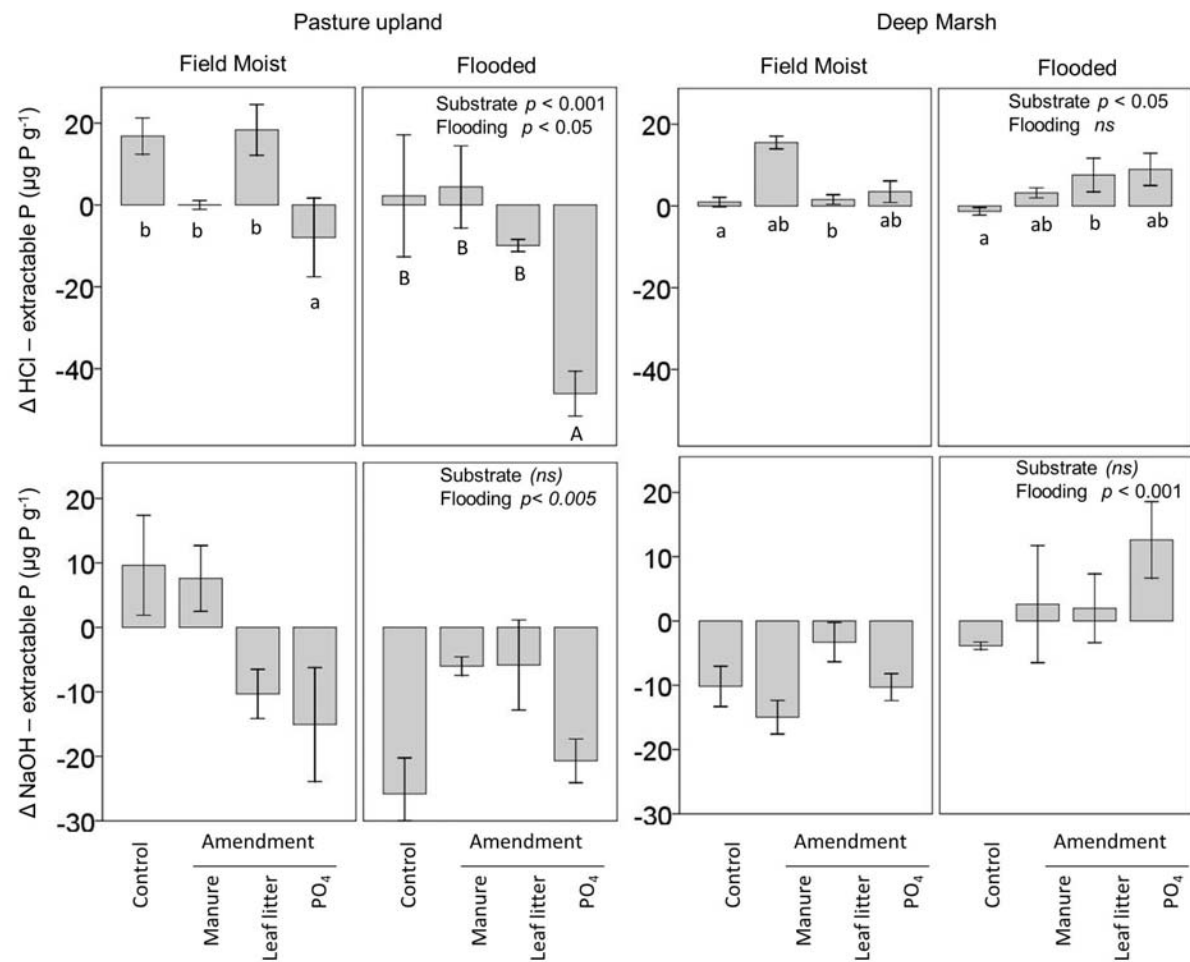


Figure 3-8. Change in P fractionation of mesocosm soils between 50 and 150 days when held at field capacity of under flooded conditions.

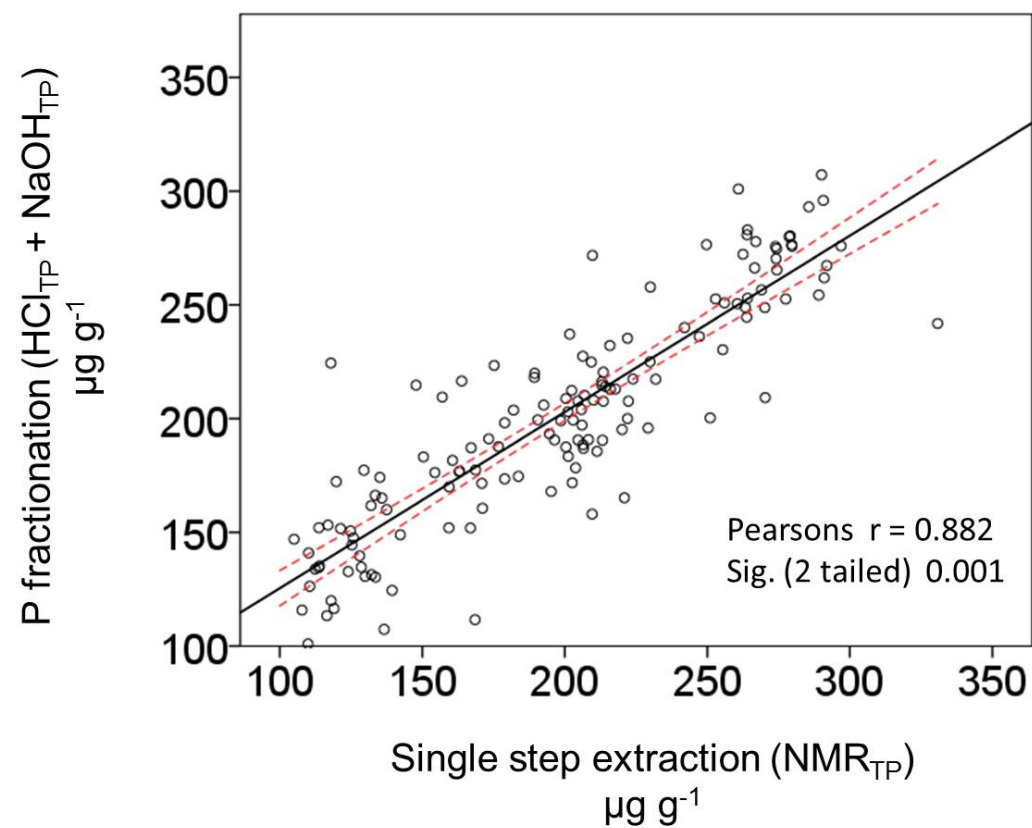


Figure 3-9. Correlation between single step alkaline extraction and P recovered by sequential fractionation in all mesocosm soils.

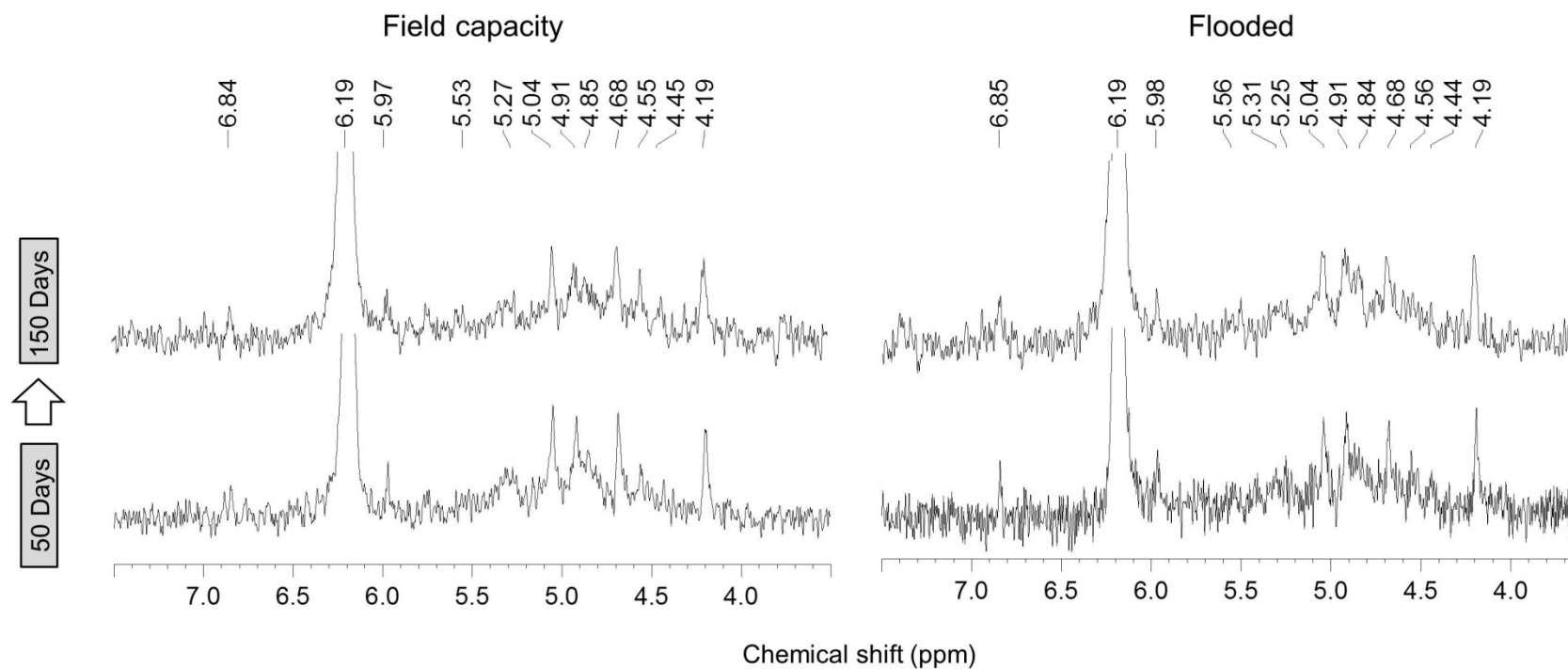


Figure 3-10. Solution ^{31}P NMR spectra of deep marsh soils augmented with manure and held at field capacity or under flooded conditions for up to 150 days.

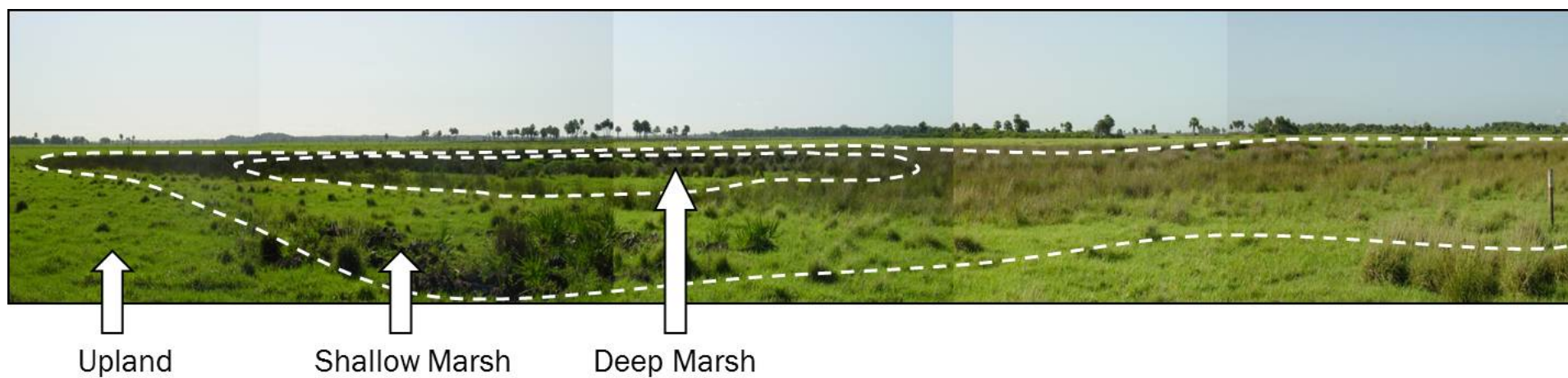


Plate 1. Overview and landscape delineation of study wetland within the Larson Dixie ranch north of Lake Okeechobee, based upon Dunne et al (2007)

A



B



Plate 2. Mesocosm study set up. A) Samples held at 30 °C in the dark. Containers were randomly rotated within incubator and maintained at correct water content by the addition of distilled deionized water on a weekly basis. B) Detail of deep marsh soil with Bahia leaf litter amendment held under flooded (left) and field capacity (right) conditions.

3.2 Legacy Phosphorus in the Okeechobee Basin- Synthesis

We have reviewed all available data on soil phosphorus in the Okeechobee Basin and other hydrologic units of the south Florida ecosystems and determined the longevity of the legacy phosphorus in regulating loads to Lake Okeechobee. Results of this study are presented in the follow two papers.

- Dunne, E.J., M.W. Clark, R. Corstanje, and K.R. Reddy. 2011. Legacy phosphorus in subtropical wetland soils: Influence of dairy, improved and unimproved pasture land use. *Ecological Engineering* 37:1481-1491.

This study suggests that the physicochemical and P characteristics, P fractions, and P indices of dairy pasture wetland soils were very different from improved and unimproved pasture wetland soils. These differences were mostly related to soil characteristics like soil metal content, TC and inorganic P fractions. Typically, the greatest nutrient concentrations occurred in surface soils (0-10 cm).

Dairy wetland soils had the greatest concentrations of soil total nutrients along with WEP, most of the P fractions, and soil metals. Further, dairy wetland soils also had greatest P sorption capacities, probably related to soil metal content. However, using the soil P indices, we found that dairy wetland soils were P saturated relative to improved and unimproved pasture wetland soils and they had no ability to retain additional amounts of P. The legacy of P in soils, particularly soils collected from dairy pastures wetlands, combined with effective BMPs to reduce nutrient loading to receiving systems, could indirectly contribute to a scenario for P impacted soils to flux P from soil to overlying waters. We hypothesize that this scenario is transient, until there is a new long-term equilibrium established between underlying soil and overlying water.

One approach to decrease dairy wetland soil P saturation and to increase the soils ability to retain additional loadings and/or to mitigate the loss of legacy P already stored in dairy pasture wetland soils is to use soil amendments (Pant et al., 2002; Leader et al., 2008; Bruland et al., 2009; Malecki-Brown and White, 2009). To store P in soils on a long-term basis, soils have to accrete and accumulate organic matter (Rybczyk et al., 2002; Kadlec, 2009). Accumulating new organic matter is the only long-term soil sink for P (Craft and Richardson, 1993). We found that both improved and unimproved pasture wetland soils stored the majority of their P in organic and residual P fractions. For example, improved pasture wetland soils tended to store P in slowly available organic P fractions (FAP and HAP), whereas unimproved pasture wetland soils stored P in more recalcitrant forms (ResP). To control and mitigate for P release and increased P storage it will be important to undertake active management of wetland water regimes and hydroperiods (Aldous et al., 2005; Dunne et al., 2007). Increasing wetland hydroperiod contributes to decreased organic matter decomposition, increases organic matter accretion rates, directly affects soil water content, soil nutrient content, nutrient dynamics between underlying soil and overlying water (Aldous et al., 2005; Leeds et al., 2009), and vegetation composition and overall character of the wetland biota (Aldous et al., 2005).

The presently used P indices, are indices that are typically used for mineral terrestrial, agricultural soils that have been loaded with inorganic and organic fertilizers (Nair and Graetz, 2002; Nair et al., 2004) for many years. We found that these indices worked well for P impacted dairy pasture wetland soils; however, the use of these indices in improved and unimproved pasture wetland soils, where the majority of P is in organic P fractions, may not be appropriate. We suggest that where soil P is composed predominantly of organic and/or residual P fractions that an additional factor (like FAP, HAP or Residual P) be incorporated.

- Reddy, K. R., S. Newman, T. Z. Osborne, J. R. White, and H. C. Fitz. 2011. Phosphorus cycling in the Everglades ecosystem: Legacy phosphorus implications for management and restoration. *Crit. Rev. Env. Sci. Technol.*, 41, pp. 149–186.

Phosphorus (P) retention in wetlands is an important function of watershed nutrient cycling, particularly in drainage basins with significant non-point nutrient contributions from agriculture and urban sources. Phosphorus storage involves complex inter-related physical, chemical, and biological processes that ultimately retain P in organic and inorganic forms. Both short-term storage of P mediated by assimilation into vegetation, translocation within above- and below-ground plant tissues, microorganisms, periphyton, and detritus, and long-term storage (retention by inorganic and organic soil particles and net accretion of organic matter) need to be considered. Here, we review and synthesize recent studies on P cycling and storage in soils and sediments throughout the Greater Everglades Ecosystem and the influence of biotic and abiotic regulation of P reactivity and mobility as related to restoration activities in south Florida.

Total P storage in the floc/detrital layer and surface soils (0-10 cm) is estimated to be 400,000 metric tons (mt) within the entire Greater Everglades Ecosystem, of which 40% is present in the Lake Okeechobee Basin (LOB), 11% in sediments of Upper Chain of Lakes, Lake Istokpoga, and Lake Okeechobee, 30% in the Everglades Agricultural Area (EAA), and 19% in the Stormwater Treatment Areas (STAs) and the Everglades. Approximately, 35% of the P stored is in chemically non-reactive (not extractable after sequential extraction with acid or alkali) pool and is assumed to be stable. Phosphorus leakage rates from LOB and EAA are approximately 500 and 170 mt P per year, respectively, based on on long-term P discharges into adjacent ecosystems. The estimated reactive P in the LOB soils is 65% of the total P, of which only 10 to 25% is assumed to leak out of the system. Under this scenario, legacy P in LOB would maintain P loads of 500 mt per year to the lake for the next 20 to 50 years. Similarly, surface soils of the EAA are estimated to release approximately 170 mt P per year for the next 50 to 120 years.

The role of the STAs in reducing loads to downstream regions, is critical and requires effective management of P forms to ensure the P is stabilized in these systems by the addition of chemical amendments or by dredging of accumulated soils. Also, additional efforts to minimize leakage of the legacy P from the northern regions should also be evaluated to reduce external P loading loads to the STAs.

3.3 On-going Field Studies – Stability of Soil Phosphorus under Grazed and Ungrazed Conditions

Introduction

Landscape units such as wetlands both natural and constructed have an inherent ability to retain incoming nutrients such as P by accumulating organic matter, which comprises about 50%-70% of total P. At present, the role of historically isolated wetlands to store and retain P is being evaluated in the LOB. Within the four priority basins, it is estimated that historically isolated wetlands soils (0-10 cm) store about 290 kg P ha⁻¹ (McKee, 2005). However, there is no information at the landscape-scale on the composition and stability of stored P and organic P fractions. Ditch soils, which tend to be similar to upland pasture soils, have low to medium levels of P (< 600 mg P kg⁻¹) with organic matter and metals being important for soil P storage. Dairy and improved pasture ditch soils have the potential to impact water quality in the four priority basins (Dunne et al., 2007). There is no information on the stability and characteristics of organic P in ditch soils and sediments; further, there is no information on how changing

water levels affect the composition and stability of organic P and soil P storage. There is little information on organic P stability in canal and stream sediments in the basin. Previous studies south of Okeechobee Basin (canals in Water Conservation Areas) suggest that the organic P fractions may account for 20% of soil total P, which ranges between 660 and 1,100 mg kg⁻¹ (Daroub et al., 2003).

As well as storing P these landscape units can also transform P between biologically available and unavailable forms (Reddy et al., 1999). In particular, P stored during high pollutant loading may be relatively unstable and easily remobilized following changes in nutrient status or hydrological regime. This is important because remobilization of stored P could maintain eutrophic conditions and contribute to downstream P pollution for many years after cessation of pollutant inputs. Also, the stored P can be affected by changes in hydrology such as seasonal water level change. The long-term stability of organic P in soil following decomposition of plant and microbial litter depends, in part, on the recalcitrance of the organic material.

At the landscape-scale most of the P that is lost from source areas, such as dairies and improved pasture, is transported in water through wetlands, ditches, streams, sloughs and canals and is ultimately conducted to Lake Okeechobee. As water and P are transported through these systems, processes between P in overlying water and underlying soil are ongoing. In the past, when P loads leaving source areas were high, P dynamics in these systems were dominated by inorganic processes. However, now that loads leaving upland areas may be decreasing with time, due to implementation of BMPs in source areas, phosphorus dynamics in wetlands, ditches, streams, sloughs, and canals are likely to be dominated by: (i) the composition and stability of organic P in the soil and (ii) the legacy of stored P in the soil relative to incoming P loads (Reddy et al., 2011).

A key outstanding issue concerns the composition and long-term stability of P stored in soils and sediments of different landscape units in the LOB, as the stability of organic P can affect regional water quality within the LOB. This lack of knowledge can also confound the ability to determine the effectiveness of soil P BMPs. Very little information exists on the chemical forms and stability of organic P in soils and sediments, which is surprising, as accretion of organic P is one of the most important mechanisms for P storage (Reddy et al., 1998). This information gap on the composition and stability of organic P limits our ability to predict long-term P storage within the LOB.

Materials and Methods

We collected soil cores (0-10 cm and 10-20 cm depth) inside and outside exclosures at the Larson Ranch and analyze samples for reactive and non-reactive P (Figure 3-11). A total of 36 samples were collected representing center of wetland, edge of the wetland, and upland during September 2010 and January 2011 (prior to establishing exclosures) and August 2011 (6 months after exclosures were established). Project funding period ended in December 2011. Using internal UF funding resources and with the assistance of graduate students we conducted an additional sampling in January 2012 (12 months after exclosures were established). This effort was not funded by the current contract with FLDACS. This additional data is included in the project.

Soil physicochemical characteristics such as pH, water content, bulk density, nutrients (total phosphorus, total nitrogen, and total carbon), and the different fractions of phosphorus (composition and stability) will be measured in the depth specific soil sections. Phosphorus fractions will also be determined in these samples on a yearly basis using the standard sequential phosphorus fractionation. This scheme fractionates inorganic and organic phosphorus in three pools: (1) acid-extractable (reactive inorganic P), (2) alkali-extractable (reactive organic P), and (2) residual (non-reactive inorganic and organic P). All samples were analyzed according to the Wetland Biogeochemistry Laboratory (WBL) Standard

Operational Procedure (SOP). The WBL has NELAP certification E72949 by the Department of Health, Bureau of Laboratories. All metals were analyzed by the UF-IFAS Analytical Research Laboratory, which is also NELAP-certified (E72850).

Soil pH: Soil pH was determined using soil:water ratio of 1:1 and glass electrode.

Bulk density (BD): A subsample of wet soil was dried at 70°C to determine dry weight and moisture content. The bulk density was determined by calculating the dry weight of the sample and dividing it by the volume of the corer.

Loss on ignition (LOI): Loss on ignition was determined by ignition a known amount of (oven-dried soil) at 550°C. Loss in weight after ignition corresponds to organic matter content of the soil. Results are expressed on percentage of over-dried basis.

Total carbon and nitrogen (TC and TN): Total carbon and nitrogen were determined on dried, ground samples using Flash EA 1112 Elemental Analyzer (CE Instruments, Saddlebrook, NJ). Results are expressed on an over-dried basis. Based on long-term data (2004 -2011 soil samples from the Okeechobee Basin) we have developed predictive equations to estimate total C and N content of soil samples using LOI as an indicator (Figure 3.12).

$$\begin{aligned} \text{TC} &= 0.506 [\text{LOI}] - 1.74; & R^2 &= 0.955; & n &= 521 \\ \text{TN} &= 0.038 [\text{LOI}] - 0.018; & R^2 &= 0.933; & n &= 521 \end{aligned}$$

Where TC = total carbon, g C/kg of soil; LOI = Loss on Ignition of soil organic matter, g/kg of soil; TN = total nitrogen, g N/kg of soil. These equations were validated using independent soils data collected from wetlands of the Kissimmee River Basin (Osborne et al., 2012). Strong relationships between predicted and estimated values shown in Figure 3.13 suggests that these equations predict reliable estimates of total C and N in soils. Total C and N data presented for soil samples collected during January 2012 is based on the estimates using above equations.

Total phosphorus (TP): Total P represents the amount of organic and inorganic P in soil samples. Total phosphorus was determined by a combination of ignition at 550°C and acid digestion to dissolution convert organic P into inorganic P, followed by analysis for inorganic P in digests by ascorbic acid techniques using an autoanalyzer (U.S. EPA, 1993; Method 365.1).

Water extractable phosphorus (WEP): Water-soluble P concentrations provide an estimate of the amount of P that is subject to vertical and/or lateral flow within the soil profile. Water extractable phosphorus in soil was extracted with deionized water using soil to water ratio of 1:10). Filtered solutions (0.45 µm filter) were analyzed for P using an autoanalyzer (U.S. EPA, 1993; Method 365.1).

Acid extractable inorganic phosphorus (HCl-TP_i): Total inorganic P in soil was extracted with 1 M HCl (soil to solution ratio = 1:50; 3 hours extraction time). Filtered solutions (0.45 µm filter) are analyzed for P using an autoanalyzer (U.S. EPA, 1993; Method 365.1).

Alkali (NaOH) extractable P: The residual soil was then treated with 0.5 M NaOH and allowed to equilibrate for a period of 17 h on a mechanical shaker, followed by centrifugation and filtration as described above. Filtered solutions were analyzed for both SRP and a separate portion extract is digested and for TP (U.S. EPA, 1993, Method 365.1). These fractions are referred to as NaOH-P_i and NaOH-TP, respectively, with NaOH-P_i considered to represent Fe- and Al-bound P. Extraction with 0.1 M NaOH also removes the P associated with humic and fulvic acids. The difference between NaOH-TP and NaOH-P_i was assumed to be organic P (NaOH-P_o) associated with fulvic and humic acids.

Residual P and Total P: The residue from the above extraction was combusted at 550°C for 4 h. The ash was dissolved in 6 M HCl followed by analysis using an autoanalyzer (Andersen, 1976; U.S. EPA, 1993, Method 365.1). A similar method was also used to analyze total P of the original soil.

Results and Discussion

Soil moisture of samples collected reflects the hydrologic conditions at the time of sampling (Table 3.9). Significant differences were noted between sampling sites (center, edge, and upland) and season ($p = 0.05$). No significant differences were observed between Larson East or West wetland sites. Soil bulk density was generally lower in the center of wetland and increased towards upland (Table 3.10). Bulk density was not influenced by grazing or by wetland location.

Soil organic matter content (expressed as Loss on Ignition, LOI) was higher in the center of wetland as compared to edge and upland sites ($p=0.05$, Table 3.11). This was expected because center of the wetland has the longest hydroperiod and maintained anaerobic conditions, resulting in accumulation of organic matter. Other variables such as grazing, wetland location, and season showed no significant differences. However, soils collected after 12 months in ungrazed sites (inside the exclosures) showed elevated levels of organic matter, as compared to grazed sites. Total C and N content followed similar trends as soil organic matter content of soils (Table 3.13 and 3.14). Both C and N content of soils from center of both wetland sites were significantly higher than soils in the edge and upland areas ($p = 0.05$).

Total phosphorus content of soils was significantly different between two wetlands, with higher concentrations observed in Larson West ($p=0.05$) (Table 3.15). This pattern was not consistent with the previous observations which showed no significant difference between two sites. Surface soil phosphorus concentrations measured during 2004 to 2012 suggests no significant difference between these two wetland sites. Limited number of samples and high spatial and temporal heterogeneity probably masked the effect of hydrologic restoration on long-term sequestration of P in these wetlands. Total phosphorus content was significantly higher in surface soils and decreased with depth ($p=0.05$). Total P content of soils collected along hydrologic gradient (wetland center, edge, and upland) showed significant differences with high concentrations measured in the wetland center ($p=0.05$).

Operationally defined chemical fractionation schemes have been routinely used to identify labile and non-labile pools (Reddy et al., 2011). Key chemical extractants used in identification of organic and inorganic pools are 0.1 to 0.5 M NaOH and 0.5 to 1 M HCl. We found no statistical difference in organic P extracted with 0.1 to 0.5 M NaOH. Soil P not extracted by either acid or alkali is considered as residual P or operationally defined non-reactive P, while the P extracted with acid and alkali is defined as reactive P. For all practical purposes, non-reactive P is considered essentially unavailable for biotic or abiotic transformations. Approximately 65% of the total P is accounted by organic P, 23% is in inorganic P pool, and 12% is in residual pool (Figure 3.14). Total P content is strongly correlated with organic matter content, suggesting that P accumulation is directly linked accretion of organic matter (Figure 3.15).

Water extractable P, extractable inorganic P, and extractable organic P pools were significantly higher in the wetland center than edge and upland sites (Tables 3.16, 3.17, and 3.18). Grazed and ungrazed plots showed no difference in total P and P forms including water extractable P, extractable inorganic P and organic P. This is not surprising given short-term (12 months) study period. Residue P (non-reactive P) in soils is considered to be not available and potentially stable pool. Residue was higher in the wetland center as compared to edge and upland sites (Table 3.19). Residue P was not affected by grazing activities. Residue P increased significantly during the 12 month period. This was probably due to prolonged saturated soil conditions.

Total P, N, and C storage in soils were significantly higher in the wetland center as compared to edge and upland sites (Tables 3.21, 3.22, and 3.23). Carbon to phosphorus ratio (based mass basis) was 289 (average of all soils collected from both soil depths) and C/N ratio was 13. These ratios suggest that the system may be nitrogen limited indicating that nitrogen availability may regulate overall P storage in soils.

Data presented in Figure 3.17 suggests that P and N storage is strongly linked to C storage. The empirical relationships presented below indicates that 0.0032 g P and 0.074 g N are sequestered per g of C.

$$\text{Total P Storage (g/m}^2\text{)} = 2.9 + 0.0032 \text{ TC; } R^2 = 0.981; n = 216$$

$$\text{Total N Storage (g/m}^2\text{)} = 7.4 + 0.0742 \text{ TC; } R^2 = 0.981; n = 216$$

Similar values were reported for the N and P sequestration per unit of C in the Everglades Water Conservation Area (0.0035 g P/g C and 0.064 g N/g C) (Reddy and Delaune, 2008).

Data on change in P, N, and C storage during the one study period (January 13, 2011 to January 21, 2012) are presented in Figures 3.18, 3.19, 3.20). Results presented are inconclusive at this time. However, the data obtained that during the study period, the wetland center accumulated P, with substantial accumulation in grazed plots of Larson west wetland site. Similarly, change in both C and N storage followed similar patterns. We hypothesized that ungrazed will result greater accumulation of P, C, and N as compared to grazed areas. It is likely that below ground biomass and turnover are probably different between grazed and ungrazed plots and contributing to this compounded effects. During the same time period, both edge and upland sites showed significant loss of P, N and C, suggesting drought and flooding influenced the export these maco-elements from the system.

The mechanisms that remove P in wetlands are in three categories: (1) sorption on antecedent substrates, (2) storage in an increased biomass of vegetation, and (3) accretion of new sediments involving detrital organic matter. Inorganic P retention is primarily due to sorption and precipitation reactions. Thus soils typically have finite capacity and therefore cannot contribute to long-term, sustainable P removal. Once the soils are saturated, equilibrium shifts can potentially increase surface water P concentrations. Thus, the long-term retention of P in wetlands is determined by accretion of organic matter produced by plant litter material. The cycling of organic P and soil organic matter in wetland soils is largely mediated by microbial metabolism and their role should be studied in detail for understanding the long-term stability of phosphorus in treatment wetlands.

Conclusions

This study shows that P and N sequestration in isolated wetlands is strongly linked organic matter accumulation. Results of this study indicate that soils with longer hydroperiod (wetland center) tend accumulate P, N, and C as compared to sites with shorter hydroperiod (edge and upland sites). Approximately 80 to 85% of total P was present in reactive pools with most of it present as organic P. Increasing the hydroperiod can significantly slow down the mineralization of organic P, suggesting hydrologic restoration can potentially increase the P retention. Results also suggest that grazing may not have any negative impact on P retention in wetlands.

Table 3.9. Moisture content of soil samples collected from isolated wetlands of Larson Ranch in the Okeechobee basin. Exclosures were installed immediately after January 13, 2011. Means represent n= 6 for samples collected during 9/8/2010 and n =3 for samples collected from January 14, 2011 to January 21, 2012.

Date	Location	Soil Moisture % (0-10 cm)				Soil Moisture % (0-10 cm)			
		Larson East				Larson West			
		Not Grazed (NG)		Grazed (G)		Not Grazed (NG)		Grazed (G)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
9/8/2010	Center			47	8			50	8
	Edge			42	11			40	8
	Upland			31	5			38	8
1/13/2011	Center	38	8	32	9	30	11	29	12
	Edge	30	11	29	8	25	3	28	9
	Upland	29	5	23	3	23	5	26	2
8/17/2011	Center	28	2	34	10	36	9	34	3
	Edge	24	3	26	3	21	9	23	2
	Upland	23	8	24	1	22	6	27	10
1/21/2012	Center	43	2	41	4	40	2	43	2
	Edge	29	3	33	5	34	5	35	10
	Upland	23	7	26	4	22	2	26	1
		Soil Moisture % (10-20 cm)				Soil Moisture % (10-20 cm)			
9/8/2010	Center			26	6			31	5
	Edge			18	3			19	4
	Upland			16	1			19	7
1/13/2011	Center	25	4	23	5	23	4.9	22	3
	Edge	11	1	12	5	9	3.1	13	5
	Upland	9	1	8	1	9	2.5	7	2
8/17/2011	Center	18	3	24	9	24	3.5	31	6
	Edge	12	1	21	14	8	1.9	10	2
	Upland	13	1	17	4	13	5.0	11	3
1/21/2012	Center	29	3	25	4	27	2.0	29	4
	Edge	18	4	22	6	17	4.7	19	4
	Upland	12	1	14	2	11	0.7	10	1

Table 3.10. Bulk density of soil samples collected from isolated wetlands of Larson Ranch in the Okeechobee basin. Exclosures were installed immediately after January 13, 2011. Means represent n= 6 for samples collected during 9/8/2010 and n =3 for samples collected from January 14, 2011 to January 21, 2012.

Date	Location	Bulk Density g cm ⁻³ (0-10 cm)				Bulk Density g cm ⁻³ (0-10 cm)			
		Larson East				Larson West			
		Not Grazed (NG)		Grazed (G)		Not Grazed (NG)		Grazed (G)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
9/8/2010	Center			0.74	0.2			0.67	0.2
	Edge			0.86	0.2			0.89	0.2
	Upland			0.99	0.1			0.89	0.2
1/13/2011	Center	0.82	0.2	1.00	0.2	0.93	0.2	0.93	0.2
	Edge	0.91	0.3	0.94	0.1	1.00	0.0	0.97	0.2
	Upland	0.95	0.1	1.00	0.1	1.01	0.0	0.84	0.1
8/17/2011	Center	0.84	0.0	0.88	0.2	0.94	0.2	0.86	0.0
	Edge	0.99	0.1	0.99	0.1	1.08	0.3	1.09	0.0
	Upland	0.99	0.1	1.02	0.1	0.94	0.1	0.96	0.0
1/21/2012	Center	0.80	0.0	0.89	0.1	0.91	0.0	0.86	0.1
	Edge	0.99	0.1	0.99	0.0	0.81	0.1	0.87	0.1
	Upland	0.94	0.1	0.93	0.1	0.92	0.1	0.82	0.0
		Bulk Density g cm ⁻³ (10-20 cm)				Bulk Density g cm ⁻³ (10-20 cm)			
9/8/2010	Center			1.28	0.1			1.13	0.2
	Edge			1.47	0.1			1.59	0.2
	Upland			1.51	0.1			1.56	0.3
1/13/2011	Center	1.23	0.1	1.30	0.1	1.25	0.1	1.27	0.2
	Edge	1.51	0.0	1.50	0.1	1.51	0.0	1.43	0.1
	Upland	1.46	0.0	1.47	0.1	1.51	0.0	1.47	0.0
8/17/2011	Center	1.31	0.1	1.21	0.2	1.21	0.1	1.06	0.2
	Edge	1.53	0.0	1.48	0.2	1.48	0.1	1.59	0.0
	Upland	1.47	0.1	1.50	0.0	1.45	0.1	1.51	0.0
1/21/2012	Center	1.15	0.1	1.22	0.1	1.22	0.1	1.17	0.1
	Edge	1.41	0.2	1.35	0.1	1.48	0.1	1.41	0.0
	Upland	1.46	0.0	1.49	0.0	1.44	0.1	1.39	0.2

Table 3.11. Soil pH of samples collected from isolated wetlands of Larson Ranch in the Okeechobee basin. Exclosures were installed immediately after January 13, 2011. Means represent n= 6 for samples collected during 9/8/2010 and n=3 for samples collected from January 14, 2011 to January 21, 2012.

Date	Location	pH (0-10 cm)				pH (0-10 cm)			
		Larson East				Larson West			
		Not Grazed (NG)		Grazed (G)		Not Grazed (NG)		Grazed (G)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
9/8/2010	Center			5.4	0.1			5.8	0.2
	Edge			5.2	0.4			5.1	0.4
	Upland			4.9	0.3			4.8	0.6
1/13/2011	Center	4.9	0.0	5.0	0.1	4.9	0.0	4.9	0.3
	Edge	4.9	0.3	4.8	0.1	4.7	0.2	4.9	0.2
	Upland	5.0	0.3	4.5	0.3	4.3	0.3	4.5	0.3
8/17/2011	Center	4.9	0.2	4.9	0.3	5.2	0.1	4.9	0.1
	Edge	5.1	0.1	4.8	0.0	4.8	0.1	5.0	0.1
	Upland	5.3	0.3	4.7	0.2	4.8	0.1	4.6	0.1
1/21/2012	Center	4.8	0.1	4.9	0.3	5.4	0.2	5.2	0.1
	Edge	4.7	0.1	4.9	0.2	4.8	0.1	4.9	0.1
	Upland	4.8	0.2	4.9	0.3	4.7	0.3	4.7	0.4
		pH (10-20 cm)				pH (10-20 cm)			
9/8/2010	Center			4.9	0.2			5.4	0.4
	Edge			5.1	0.2			5.4	0.2
	Upland			5.6	0.4			5.3	0.6
1/13/2011	Center	4.7	0.1	4.9	0.3	4.8	0.0	4.8	0.2
	Edge	5.0	0.2	5.0	0.2	5.2	0.2	5.1	0.1
	Upland	5.4	0.2	5.1	0.4	5.0	0.3	5.3	0.4
8/17/2011	Center	4.9	0.1	5.1	0.3	4.9	0.1	4.8	0.2
	Edge	5.2	0.1	5.0	0.3	5.4	0.3	5.2	0.2
	Upland	5.5	0.6	4.8	0.4	5.3	0.2	5.3	0.2
1/21/2012	Center	4.7	0.2	4.7	0.3	5.1	0.2	4.9	0.2
	Edge	4.9	0.0	4.9	0.2	5.2	0.2	5.4	0.5
	Upland	5.4	0.4	5.4	0.6	5.7	0.3	5.7	0.4

Table 3.12 . Organic matter content (expressed as Loss on Ignition, LOI) of soil samples collected from isolated wetlands of Larson Ranch in the Okeechobee basin. Exclosures were installed immediately after January 13, 2011. Means represent n= 6 for samples collected during 9/8/2010 and n =3 for samples collected from January 14, 2011 to January 21, 2012.

Date	Location	Loss on Ignition % (0-10 cm)				Loss on Ignition % (0-10 cm)			
		Larson East				Larson West			
		Not Grazed (NG)		Grazed (G)		Not Grazed (NG)		Grazed (G)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
9/8/2010	Center			14.1	4.3			14.9	5.9
	Edge			13.7	6.8			13.9	4.3
	Upland			11.8	7.0			13.0	2.9
1/13/2011	Center	16.3	4.0	12.0	6.0	15.1	6.7	14.0	5.6
	Edge	11.6	7.0	12.2	3.6	11.0	1.7	13.0	5.6
	Upland	13.1	3.8	8.8	0.1	10.3	2.9	11.9	1.0
8/17/2011	Center	16.8	3.0	19.6	9.4	16.4	4.2	20.2	2.9
	Edge	11.7	1.4	12.2	5.8	10.2	3.3	9.4	1.5
	Upland	9.0	1.2	8.9	1.1	12.8	5.8	11.3	1.1
1/21/2012	Center	15.2	1.8	14.7	4.0	13.3	1.6	16.9	2.3
	Edge	7.8	1.9	8.8	3.8	12.7	3.8	12.5	3.7
	Upland	7.3	3.1	7.9	1.1	7.6	1.3	11.0	1.4
		Loss on Ignition % (10-20 cm)				Loss on Ignition % (10-20 cm)			
9/8/2010	Center			7.3	2.0			10.5	3.0
	Edge			2.8	1.7			4.0	3.8
	Upland			1.5	1.4			3.6	3.8
1/13/2011	Center	7.7	2.5	6.2	2.9	8.3	2.9	7.5	1.7
	Edge	1.2	0.2	1.9	2.0	1.2	0.9	3.2	2.5
	Upland	1.2	0.8	1.1	1.0	1.1	0.4	1.3	0.8
8/17/2011	Center	7.8	1.8	10.5	7.5	11.0	3.0	12.1	1.2
	Edge	1.8	0.7	4.3	5.2	1.4	1.3	1.6	0.8
	Upland	1.6	0.8	1.0	0.2	1.5	0.8	0.9	0.2
1/21/2012	Center	9.6	2.0	7.4	2.6	7.7	0.4	10.4	3.0
	Edge	1.8	2.5	4.2	3.1	2.9	1.7	3.4	3.5
	Upland	1.2	0.5	1.2	0.4	1.8	0.9	1.4	0.3

Table 3.13. Total carbon content of soil of samples collected from isolated wetlands of Larson Ranch in the Okeechobee basin. Exclosures were installed immediately after January 13, 2011. Means represent n= 6 for samples collected during 9/8/2010 and n =3 for samples collected from January 14, 2011 to January 21, 2012.

Date	Location	Total C g kg ⁻¹ (0-10 cm)				Total C g kg ⁻¹ (0-10 cm)			
		Larson East				Larson West			
		Not Grazed (NG)		Grazed (G)		Not Grazed (NG)		Grazed (G)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
9/8/2010	Center			71.4	22			70.3	27
	Edge			65.7	34			63.6	24
	Upland			52.6	36			60.5	16
1/13/2011	Center	87.9	23	64.3	30	75.4	31	67.4	31
	Edge	61.4	38	63.9	18	51.8	13	64.1	29
	Upland	68.2	26	48.2	1	49.8	13	56.0	10
8/17/2011	Center	81.1	20	101.5	46	71.6	17	91.6	11
	Edge	51.2	6	58.6	27	48.8	20	50.2	7
	Upland	40.9	6	39.2	5	60.6	30	56.5	3
1/21/2012	Center	75.4	9	72.6	20	65.4	8	84.0	12
	Edge	37.6	10	42.8	19	62.3	20	61.6	19
	Upland	35.1	16	37.9	6	36.7	6	54.0	7
		Total C g kg ⁻¹ (10-20 cm)				Total C g kg ⁻¹ (10-20 cm)			
9/8/2010	Center			34.5	10			45.3	15
	Edge			11.3	8			15.3	17
	Upland			4.3	4			13.9	19
1/13/2011	Center	40.0	11	33.3	16	40.8	14	36.2	8
	Edge	4.7	2	9.5	11	5.2	6	15.1	12
	Upland	5.1	4	4.2	4	5.1	2	5.4	3
8/17/2011	Center	34.4	10	51.4	39	47.9	14	54.1	6
	Edge	3.1	1	15.2	21	4.7	6	3.7	2
	Upland	4.3	3	1.8	0	5.0	5	2.4	1
1/21/2012	Center	47	10	36	13	37.0	2	50.7	15
	Edge	7	13	19	16	12.8	9	15.2	18
	Upland	4	3	4	2	7.1	4	5.4	1

Table 3.14. Total nitrogen content of soil of samples collected from isolated wetlands of Larson Ranch in the Okeechobee basin. Exclosures were installed immediately after January 13, 2011. Means represent n= 6 for samples collected during 9/8/2010 and n =3 for samples collected from January 14, 2011 to January 21, 2012.

Date	Location	Total N g kg ⁻¹ (0-10 cm)				Total N g kg ⁻¹ (0-10 cm)			
		Larson East				Larson West			
		Not Grazed (NG)		Grazed (G)		Not Grazed (NG)		Grazed (G)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
9/8/2010	Center			5.45	1.7			5.27	2.1
	Edge			5.19	2.3			5.03	2.0
	Upland			4.04	2.9			4.31	1.3
1/13/2011	Center	6.78	2.2	4.90	2.2	5.88	2.6	5.19	2.5
	Edge	4.62	2.7	4.92	1.4	4.03	1.1	5.58	2.9
	Upland	5.14	2.3	3.50	0.2	3.52	1.1	4.22	0.8
8/17/2011	Center	6.21	1.3	7.45	3.2	5.40	1.3	7.10	0.7
	Edge	3.97	0.4	4.53	1.9	3.86	1.7	3.97	0.4
	Upland	3.07	0.4	2.79	0.3	3.87	1.2	4.15	0.3
1/21/2012	Center	5.84	0.7	5.62	1.5	5.08	0.6	6.49	0.9
	Edge	2.96	0.8	3.36	1.5	4.85	1.5	4.79	1.4
	Upland	2.78	1.2	2.99	0.4	2.90	0.5	4.21	0.5
		Total N g kg ⁻¹ (10-20 cm)				Total N g kg ⁻¹ (10-20 cm)			
9/8/2010	Center			2.36	0.7			3.19	1.2
	Edge			0.81	0.6			1.08	1.2
	Upland			0.32	0.3			0.96	1.3
1/13/2011	Center	2.73	0.8	2.25	1.1	2.86	1.1	2.50	0.6
	Edge	0.36	0.1	0.67	0.7	0.40	0.4	1.08	0.8
	Upland	0.33	0.2	0.33	0.3	0.37	0.2	0.45	0.3
8/17/2011	Center	2.39	0.7	3.43	2.6	3.31	1.1	3.69	0.1
	Edge	0.35	0.2	1.05	1.4	0.38	0.4	0.35	0.2
	Upland	0.38	0.3	0.18	0.0	0.34	0.1	0.22	0.1
1/21/2012	Center	3.65	0.8	2.81	1.0	2.92	0.2	3.96	1.2
	Edge	0.64	1.0	1.57	1.2	1.08	0.7	1.26	1.3
	Upland	0.42	0.2	0.42	0.1	0.65	0.3	0.52	0.1

Table 3.15. Total phosphorus content of soil of samples collected from isolated wetlands of Larson Ranch in the Okeechobee basin. Exclosures were installed immediately after January 13, 2011. Means represent n= 6 for samples collected during 9/8/2010 and n =3 for samples collected from January 14, 2011 to January 21, 2012.

Date	Location	Total P mg kg ⁻¹ (0-10 cm)				Total P mg kg ⁻¹ (0-10 cm)			
		Larson East				Larson West			
		Not Grazed (NG)		Grazed (G)		Not Grazed (NG)		Grazed (G)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
9/8/2010	Center			252	67			312	123
	Edge			201	100			258	82
	Upland			216	225			217	68
1/13/2011	Center	311	100	231	90	301	128	302	119
	Edge	183	106	188	78	184	45	244	132
	Upland	240	161	148	16	167	26	251	62
8/17/2011	Center	272	39	370	159	312	80	399	14
	Edge	151	34	169	100	172	77	185	73
	Upland	144	53	99	28	168	50	198	40
1/21/2012	Center	309	19	280	61	301	53	394	42
	Edge	120	34	165	74	223	57	289	94
	Upland	116	45	145	39	143	18	221	62
		Total P mg kg ⁻¹ (10-20 cm)				Total P mg kg ⁻¹ (10-20 cm)			
9/8/2010	Center			126	30			194	58
	Edge			55	38			90	61
	Upland			31	29			87	54
1/13/2011	Center	138	29	125	36	147	53	141	34
	Edge	24	6	48	47	40	44	155	158
	Upland	25	18	24	24	65	28	72	49
8/17/2011	Center	124	32	183	122	182	56	198	17
	Edge	19	7	54	63	28	29	40	14
	Upland	34	25	9	3	50	18	59	54
1/21/2012	Center	189	34	142	51	157	19	201	41
	Edge	39	45	90	69	77	28	70	61
	Upland	19	11	24	20	55	23	41	1

Table 3.16. Water extractable phosphorus content of soil of samples collected from isolated wetlands of Larson Ranch in the Okeechobee basin. Exclosures were installed immediately after January 13, 2011. Means represent n= 6 for samples collected during 9/8/2010 and n =3 for samples collected from January 14, 2011 to January 21, 2012.

Date	Location	Water Extractable Pi mg kg ⁻¹ (0-10 cm)				Water Extractable Pi mg kg ⁻¹ (0-10 cm)			
		Larson East				Larson West			
		Not Grazed (NG)		Grazed (G)		Not Grazed (NG)		Grazed (G)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
8/17/2010	Center	2.94	2.3	1.80	0.9	2.36	1.3	8.12	8.0
	Edge	0.28	0.3	2.05	1.9	5.77	4.7	6.07	4.7
	Upland	1.47	1.2	0.13	0.1	2.14	2.5	8.94	9.2
1/21/2012	Center	1.09	0.8	2.52	1.2	3.61	1.6	2.76	1.1
	Edge	1.11	1.1	1.73	0.9	2.05	0.8	4.31	5.2
	Upland	0.12	0.1	0.25	0.2	1.08	0.6	3.61	6.0
		Water Extractable Pi mg kg ⁻¹ (10-20 cm)				Water Extractable Pi mg kg ⁻¹ (10-20 cm)			
8/17/2011	Center	0.83	0.6	1.15	0.7	0.96	0.4	3.26	3.0
	Edge	0.13	0.1	1.18	1.0	0.71	0.9	0.72	0.4
	Upland	0.38	0.2	0.10	0.0	0.97	0.8	1.03	1.0
1/21/2012	Center	0.89	0.6	2.42	2.0	0.93	0.2	1.25	0.4
	Edge	0.26	0.4	2.09	2.7	0.73	0.5	1.05	0.8
	Upland	0.08	0.0	0.08	0.1	0.28	0.2	2.06	3.1

Table 3.17. Inorganic phosphorus (acid extractable) content soil of samples collected from isolated wetlands of Larson Ranch in the Okeechobee basin. Exclosures were installed immediately after January 13, 2011. Means represent n= 6 for samples collected during 9/8/2010 and n =3 for samples collected from January 14, 2011 to January 21, 2012.

Date	Location	Total inorganic P mg kg ⁻¹ (0-10 cm)				Total Inorganic P mg kg ⁻¹ (0-10 cm)			
		Larson East				Larson West			
		Not Grazed (NG)		Grazed (G)		Not Grazed (NG)		Grazed (G)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
9/8/2010	Center			24.0	6			25.4	8
	Edge			18.7	8			34.0	10
	Upland			42.6	72			23.7	17
1/13/2011	Center	18.6	2	12.5	3	15.8	3	18.2	8
	Edge	9.1	5	8.7	4	22.1	13	29.3	26
	Upland	29.8	37	12.6	5	19.1	10	56.3	62
8/17/2011	Center	18.6	4	19.7	9	19.3	6	32.7	19
	Edge	9.6	2	15.1	3	38.6	42	60.3	62
	Upland	41.1	41	8.6	4	27.0	11	43.1	32
1/21/2012	Center	12.3	6	18.8	8	25.8	15	21.2	5
	Edge	6.5	3	12.1	3	16.1	5	68.8	87
	Upland	7.8	4	17.7	18	17.9	8	47.4	39
		Total inorganic P mg kg ⁻¹ (10-20 cm)				Total inorganic P mg kg ⁻¹ (10-20 cm)			
9/8/2010	Center			16.6	6			21.6	7
	Edge			10.4	17			29.8	25
	Upland			6.6	9			23.0	27
1/13/2011	Center	21.0	9	24.6	5	14.2	6	14.8	4
	Edge	2.1	1	8.8	13	14.2	21	85.6	115
	Upland	5.6	8	4.0	5	29.3	34	28.5	26
8/17/2011	Center	12.8	8	41.8	40	11.1	1	16.9	4
	Edge	3.1	0	10.0	6	6.7	5	14.0	8
	Upland	16.6	15	3.1	0	35.5	31	48.5	54
1/21/2012	Center	12.5	6	23.4	11	8.7	1	11.7	3
	Edge	2.8	3	18.9	22	17.0	23	15.4	5
	Upland	1.7	1	2.6	2	20.6	22	9.3	4

Table 3.18. Organic phosphorus (alkali extractable) content of soil of samples collected from isolated wetlands of Larson Ranch in the Okeechobee basin. Exclosures were installed immediately after January 13, 2011. Means represent n= 6 for samples collected during 9/8/2010 and n =3 for samples collected from January 14, 2011 to January 21, 2012.

Date	Location	Extractable Organic P mg kg ⁻¹ (0-10 cm)				Extractable Organic P mg kg ⁻¹ (0-10 cm)			
		Larson East				Larson West			
		Not Grazed (NG)		Grazed (G)		Not Grazed (NG)		Grazed (G)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
9/8/2010	Center			174	46			190	79
	Edge			126	70			149	60
	Upland			126	99			137	48
1/13/2011	Center	214	81	155	68	205	104	198	96
	Edge	127	84	128	63	118	38	164	82
	Upland	148	81	106	18	105	16	157	28
8/17/2011	Center	208	24	234	102	208	61	282	2
	Edge	131	49	153	60	107	94	113	29
	Upland	111	27	89	13	115	37	112	24
1/21/2012	Center	208	11	177	30	185	23	242	45
	Edge	72	30	114	17	162	30	148	50
	Upland	82	38	100	18	96	17	125	14
		Extractable Organic P mg kg ⁻¹ (10-20 cm)				Extractable Organic P mg kg ⁻¹ (10-20 cm)			
9/8/2010	Center			74	28			108	40
	Edge			27	17			34	30
	Upland			15	13			35	36
1/13/2011	Center	86	33	65	29	93	43	77	31
	Edge	13	3	21	19	15	13	42	33
	Upland	15	11	15	15	19	4	20	12
8/17/2011	Center	68	27	109	72	109	43	111	48
	Edge	13	4	36	28	29	.	15	6
	Upland	23	7	9	.	12	5	11	1
1/21/2012	Center	108	22	73	33	93	29	125	25
	Edge	20	26	32	21	19	10	28	25
	Upland	9	8	13	13	19	11	10	6

Table 3.19. Residual phosphorus (non-reactive phosphorus) content of soil of samples collected from isolated wetlands of Larson Ranch in the Okeechobee basin. Exclosures were installed immediately after January 13, 2011. Means represent n= 6 for samples collected during 9/8/2010 and n =3 for samples collected from January 14, 2011 to January 21, 2012.

Date	Location	Residue P mg kg ⁻¹ (0-10 cm)				Residue P mg kg ⁻¹ (0-10 cm)			
		Larson East				Larson West			
		Not Grazed (NG)		Grazed (G)		Not Grazed (NG)		Grazed (G)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
9/8/2010	Center			33	9			35	12
	Edge			25	10			30	10
	Upland			22	11			26	10
1/13/2011	Center	36	12	28	10	40	23	36	15
	Edge	9	10	24	7	23	5	31	16
	Upland	21	12	18	4	17	6	25	4
8/17/2011	Center	31	5	43	13	35	11	43	3
	Edge	21	2	26	11	22	14	21	5
	Upland	18	2	13	2	21	2	16	0
1/21/2012	Center	54	2	48	11	44	14	62	14
	Edge	23	3	32	11	44	18	35	14
	Upland	16	6	25	2	19	3	29	5
		Residue P mg kg ⁻¹ (10-20 cm)				Residue P mg kg ⁻¹ (10-20 cm)			
9/8/2010	Center			19	4			22	6
	Edge			11	4			11	5
	Upland			8	2			12	6
1/13/2011	Center	15	6	12	2	14	7	15	4
	Edge	3	0	5	3	4	3	7	4
	Upland	4	2	3	2	6	1	5	4
8/17/2011	Center	17	6	27	16	22	7	26	2
	Edge	8	1	13	6	11	.	7	2
	Upland	10	3	7	.	7	2	7	2
1/21/2012	Center	33	7	25	8	25	7	40	12
	Edge	9	10	15	10	10	3	11	5
	Upland	4	1	5	3	7	2	7	2

Table 3.20. Total phosphorus storage in surface soil of samples collected from isolated wetlands of Larson Ranch in the Okeechobee basin. Exclosures were installed immediately after January 13, 2011. Means represent n= 6 for samples collected during 9/8/2010 and n =3 for samples collected from January 14, 2011 to January 21, 2012.

Date	Location	TP Storage g m ⁻² (0-10 cm)				TP Storage g m ⁻² (0-10 cm)			
		Larson East				Larson West			
		Not Grazed (NG)		Grazed (G)		Not Grazed (NG)		Grazed (G)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
9/8/2010	Center			17.8	2			19.3	4
	Edge			15.5	4			22.0	5
	Upland			19.6	17			18.4	2
1/13/2011	Center	24.3	5	21.8	4	26.1	7	26.4	7
	Edge	14.7	4	17.0	4	18.3	4	22.4	9
	Upland	22.7	15	14.7	2	16.9	3	21.5	8
8/17/2011	Center	22.9	3	30.2	6	28.3	1	34.4	0
	Edge	14.8	2	16.0	7	17.6	6	20.2	8
	Upland	14.1	4	10.0	2	15.8	5	19.2	5
1/21/2012	Center	24.8	1	24.4	2	27.1	4	33.8	2
	Edge	11.8	3	16.1	6	17.7	3	25.5	10
	Upland	10.6	4	13.4	3	13.1	2	18.0	4
		TP Storage g m ⁻² (10-20 cm)				TP Storage g m ⁻² (10-20 cm)			
9/8/2010	Center			15.8	2			21.1	3
	Edge			8.0	6			13.8	8
	Upland			4.5	4			13.1	7
1/13/2011	Center	16.8	3	16.0	3	18.0	5	17.6	2
	Edge	3.6	1	6.9	7	6.1	7	21.4	21
	Upland	3.7	3	3.5	3	9.7	4	10.6	7
8/17/2011	Center	16.0	3	20.1	9	21.6	5	21.1	5
	Edge	2.9	1	7.3	8	4.3	5	6.3	2
	Upland	5.0	3	1.4	0	7.4	3	8.9	8
1/21/2012	Center	21.5	3	16.9	4	19.1	3	23.0	2
	Edge	5.1	5	11.5	8	11.3	4	9.9	9
	Upland	2.8	2	3.5	3	8.0	4	18.0	21

Table 3.21. Total nitrogen storage in surface soil of samples collected from isolated wetlands of Larson Ranch in the Okeechobee basin. Exclosures were installed immediately after January 13, 2011. Means represent n= 6 for samples collected during 9/8/2010 and n=3 for samples collected from January 14, 2011 to January 21, 2012.

Date	Location	TN Storage g m ⁻² (0-10 cm)				TN Storage g m ⁻² (0-10 cm)			
		Larson East				Larson West			
		Not Grazed (NG)		Grazed (G)		Not Grazed (NG)		Grazed (G)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
9/8/2010	Center			380	61			326	65
	Edge			399	67			425	122
	Upland			376	211			369	66
1/13/2011	Center	532	112	456	83	510	158	453	179
	Edge	369	136	451	54	400	87	512	175
	Upland	482	211	350	47	354	109	351	21
8/17/2011	Center	519	79	609	122	489	16	612	58
	Edge	393	9	435	132	386	74	431	48
	Upland	304	22	285	26	359	77	400	38
1/21/2012	Center	467	35	489	71	459	39	557	76
	Edge	289	55	328	127	384	79	409	100
	Upland	256	108	277	25	266	52	346	51
		TN Storage g m ⁻² (10-20 cm)				TN Storage g m ⁻² (10-20 cm)			
9/8/2010	Center			294	68			343	80
	Edge			117	81			154	148
	Upland			46	34			129	138
1/13/2011	Center	331	82	284	108	350	111	312	43
	Edge	55	20	97	93	61	66	151	105
	Upland	49	24	48	38	57	27	66	40
8/17/2011	Center	309	74	373	205	392	88	390	51
	Edge	54	25	141	173	57	65	55	32
	Upland	55	35	27	2	48	12	33	10
1/21/2012	Center	414	58	334	83	357	35	452	79
	Edge	80	120	201	146	158	91	179	190
	Upland	61	29	62	19	92	44	72	20

Table 3.22. Total carbon storage in surface soil of samples collected from isolated wetlands of Larson Ranch in the Okeechobee basin. Exclosures were installed immediately after January 13, 2011. Means represent n= 6 for samples collected during 9/8/2010 and n =3 for samples collected from January 14, 2011 to January 21, 2012.

Date	Location	TC Storage g m ⁻² (0-10 cm)				TC Storage g m ⁻² (0-10 cm)			
		Larson East				Larson West			
		Not Grazed (NG)		Grazed (G)		Not Grazed (NG)		Grazed (G)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
9/8/2010	Center			4994	785			4367	799
	Edge			4991	1010			5376	1462
	Upland			4903	2628			5221	1032
1/13/2011	Center	6932	1042	5981	1313	6559	1807	5903	2281
	Edge	4875	1741	5860	714	5144	1105	5923	1641
	Upland	6395	2296	4810	351	5042	1409	4655	245
8/17/2011	Center	6770	1249	8247	1968	6501	217	7902	863
	Edge	5053	258	5616	1870	4919	755	5446	704
	Upland	4048	426	3992	234	5551	2167	5438	380
1/21/2012	Center	6025	463	6304	944	5905	515	7211	1004
	Edge	3661	730	4176	1681	4936	1054	5264	1325
	Upland	3233	1423	3517	335	3373	677	4438	672
		TC Storage g m ⁻² (10-20 cm)				TC Storage g m ⁻² (10-20 cm)			
9/8/2010	Center			4319	929			4891	958
	Edge			1622	1180			2172	2162
	Upland			624	534			1844	2111
1/13/2011	Center	4865	1053	4199	1537	5004	1341	4520	582
	Edge	707	298	1380	1505	793	973	2112	1581
	Upland	744	529	602	548	781	291	785	487
8/17/2011	Center	4429	1056	5593	2967	5682	1092	5675	419
	Edge	484	230	2011	2676	719	896	591	392
	Upland	627	438	276	18	713	668	357	106
1/21/2012	Center	5286	772	4219	1110	4523	449	5776	1059
	Edge	904	1561	2446	1939	1871	1206	2148	2495
	Upland	592	388	605	249	999	583	748	254

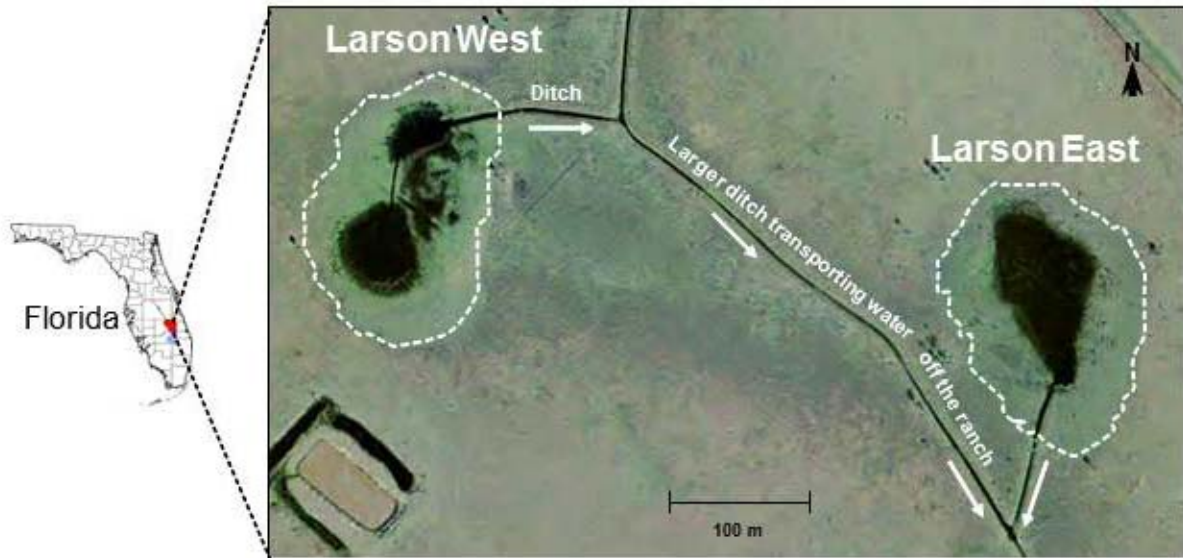


Figure 3.11. Map showing the study sites in the Okeechobee Basin

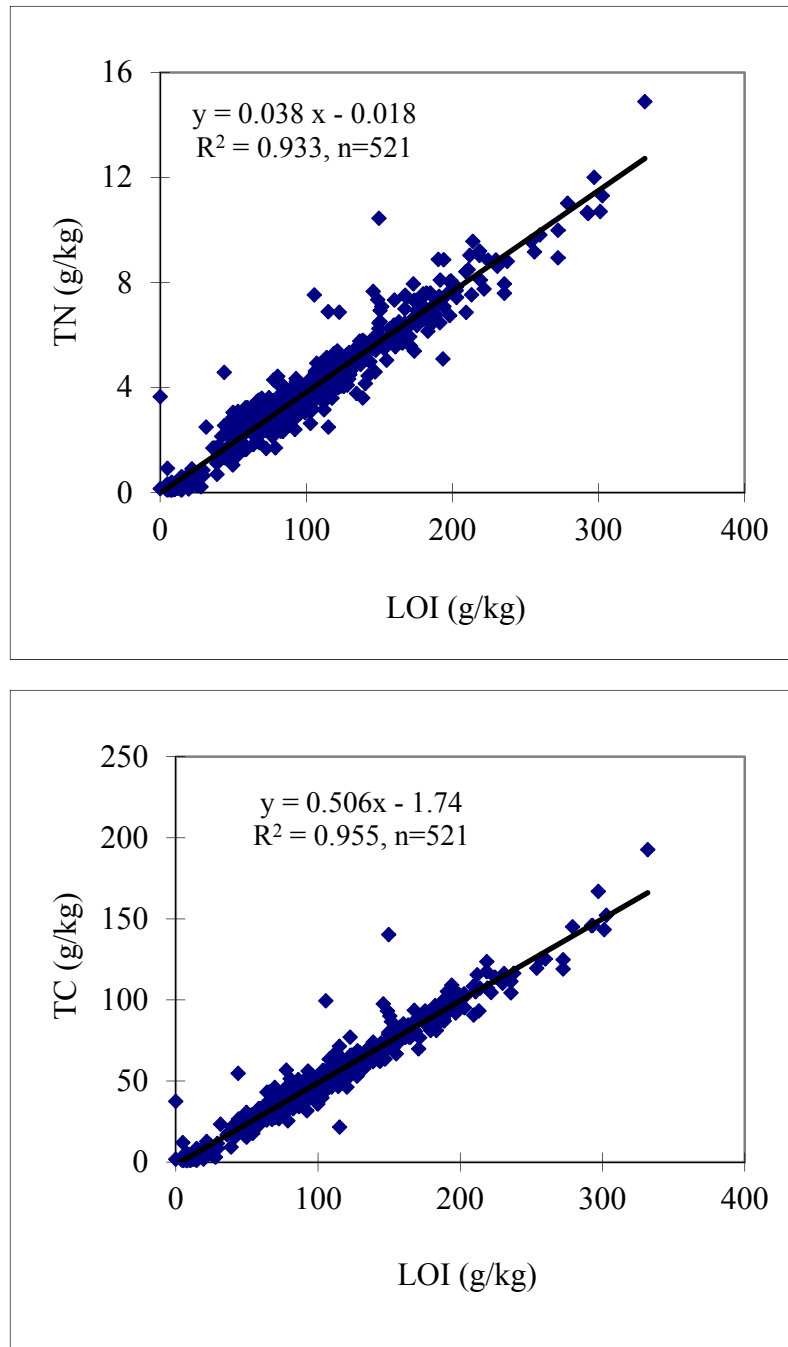


Figure 3.12. Relationship between loss on ignition (LOI or organic matter content) and total carbon and total nitrogen content of soils collected from two isolated wetlands located in Larson Ranch

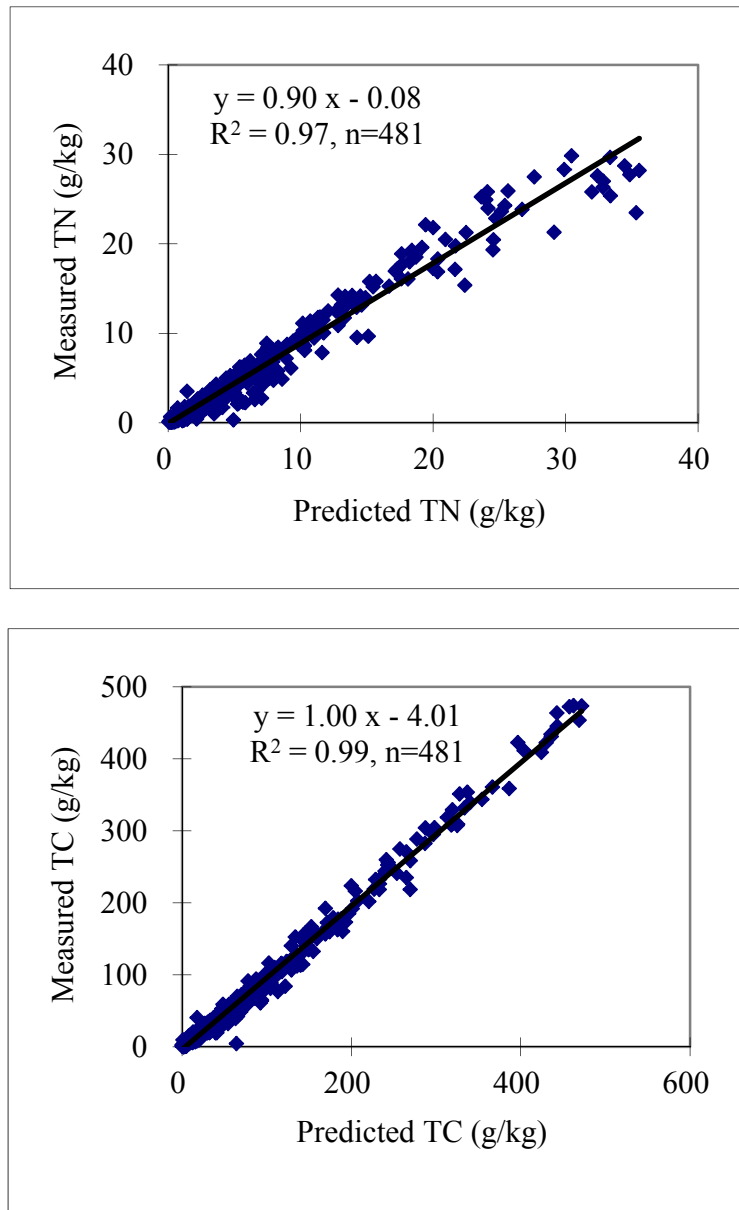


Figure 3.12. Relationship between predicted and measured values of independent data (T. Z. Osborne et al., 2012) obtained from soil samples collected from the Kissimmee River Basin.

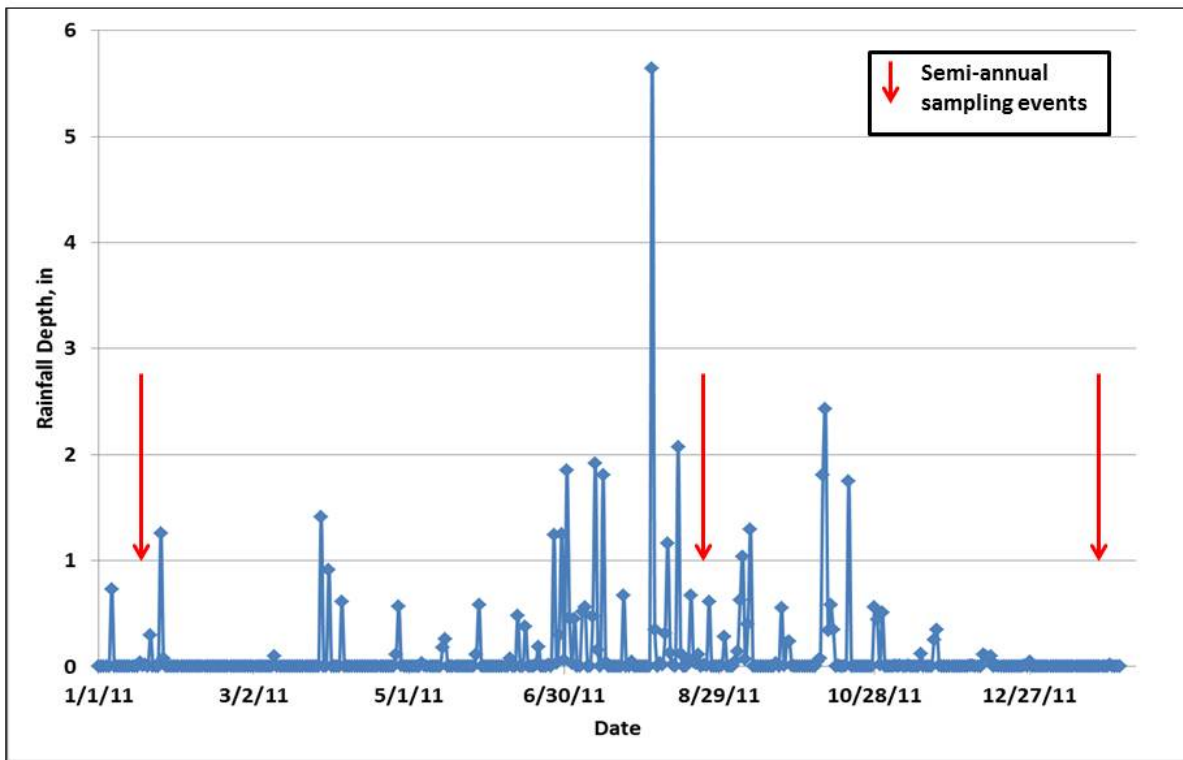


Figure 3.14. Annual rainfall patterns in the Okeechobee Basin during the soil sampling period

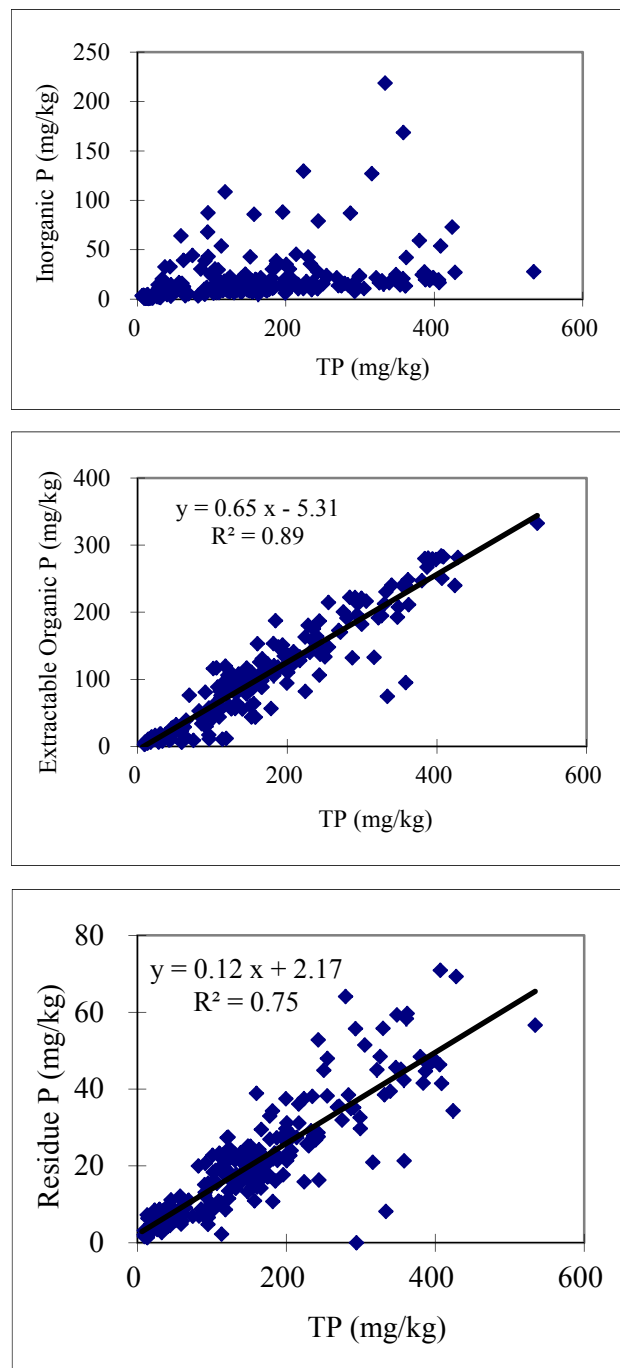


Figure 3.15. Relationship between reactive phosphorus (inorganic and organic) and non-reactive phosphorus and total phosphorus soils.

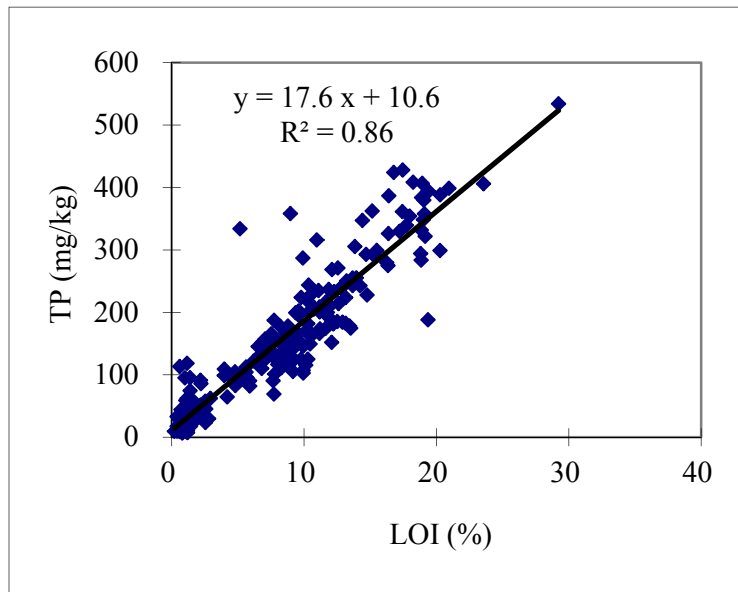


Figure 3.16. Relationship between total phosphorus and organic matter content (expressed as Loss on Ignition, LOI) of soils.

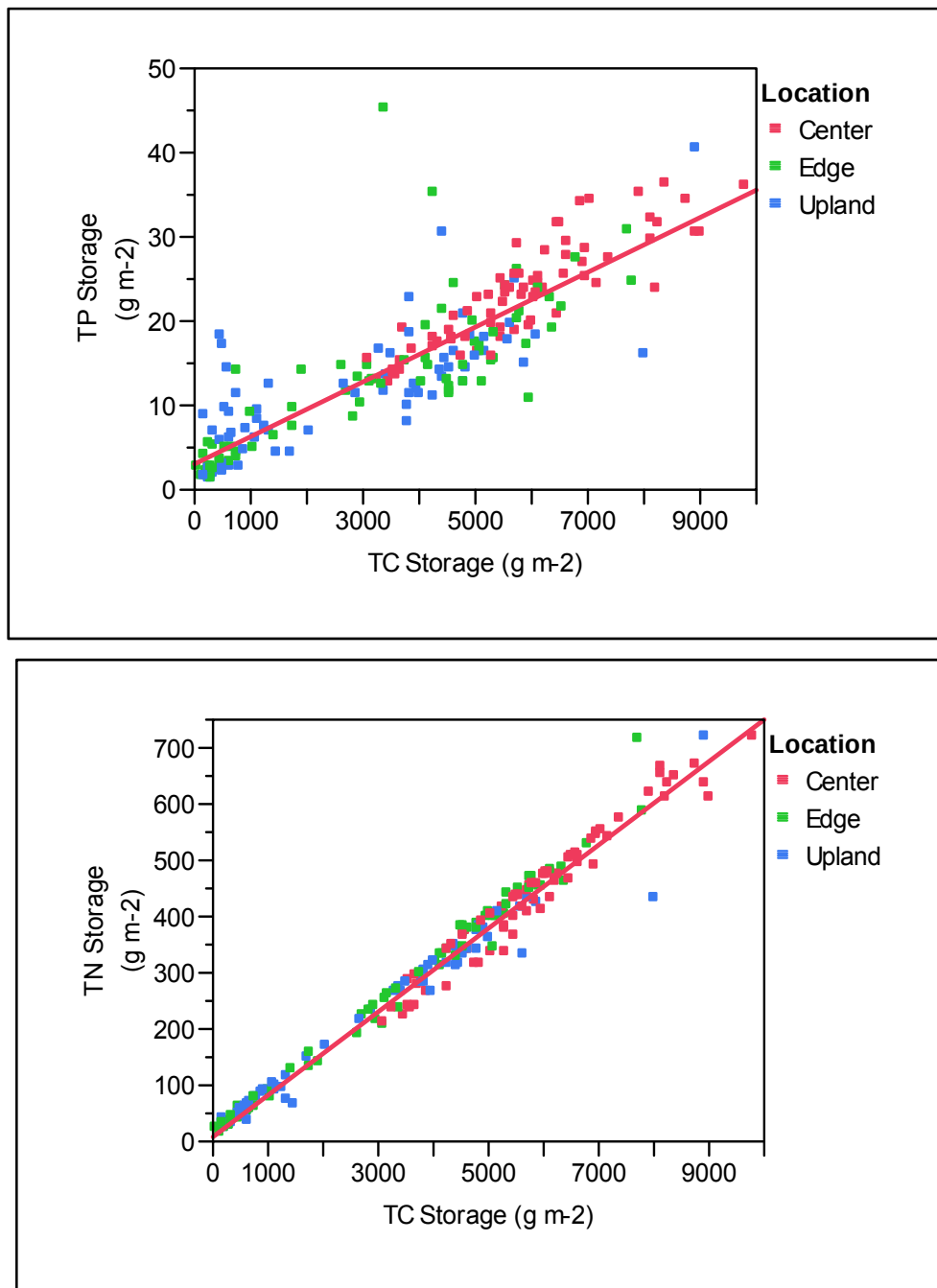


Figure 3.17. Relationship between soil phosphorus (TP), nitrogen (TN), and carbon (TC) storage in 0-20 cm soil depth. Data includes soil samples from all experimental treatments and sampling periods (2011-2012).

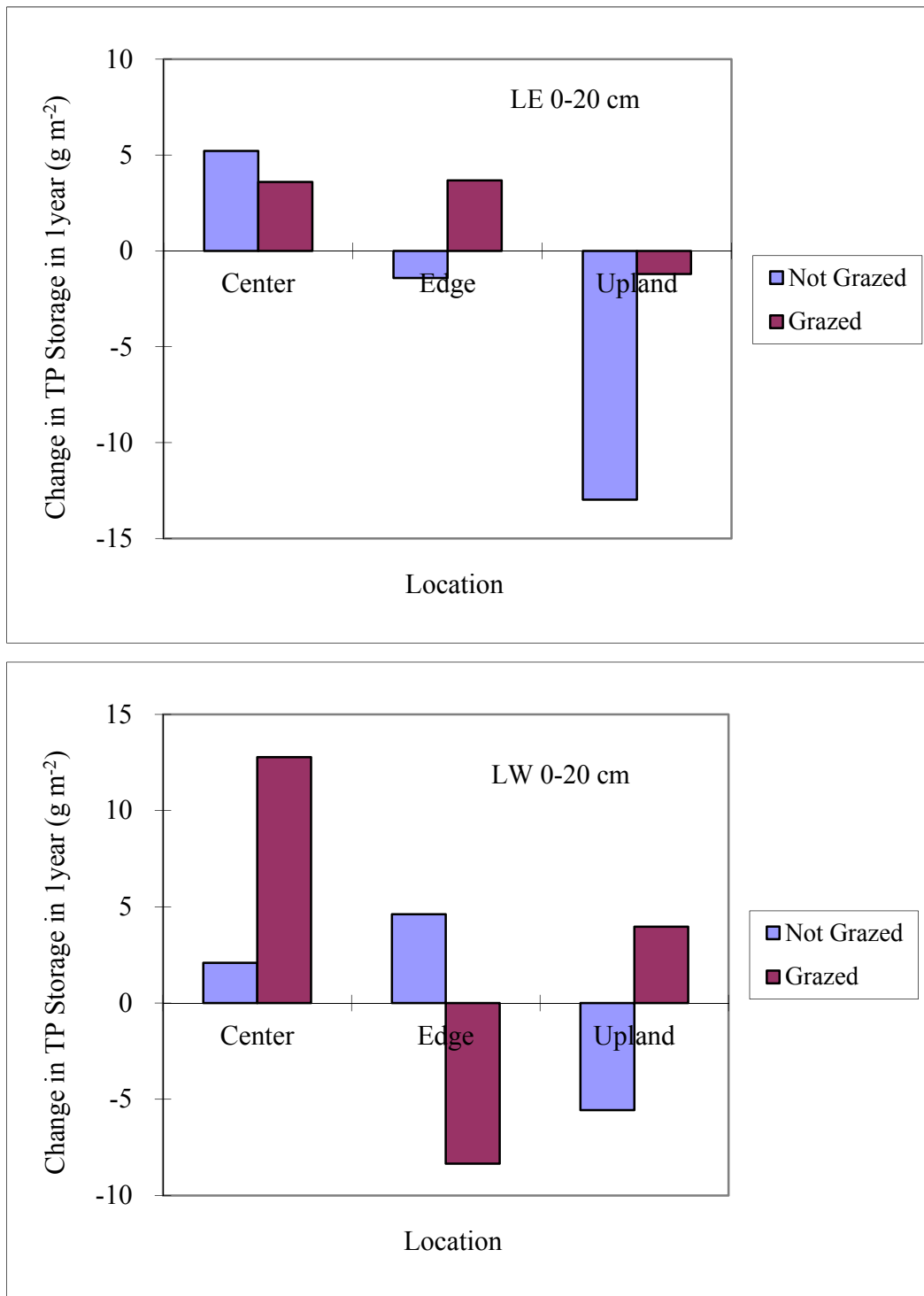


Figure 3.18. Change in soil phosphorus storage during one year sampling period under grazed and ungrazed conditions.

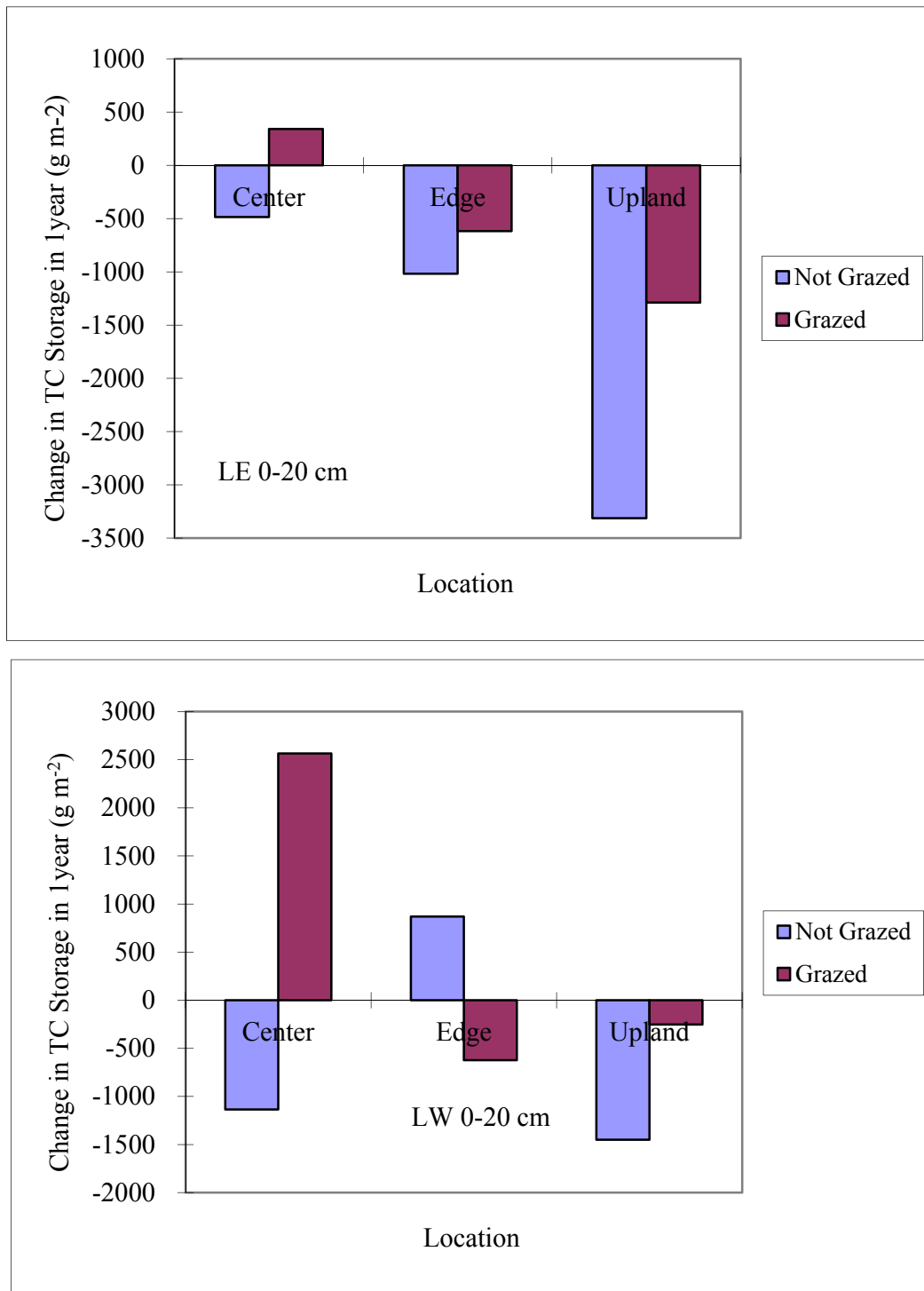


Figure 3.19 Change in soil carbon storage during one year sampling period under grazed and ungrazed conditions

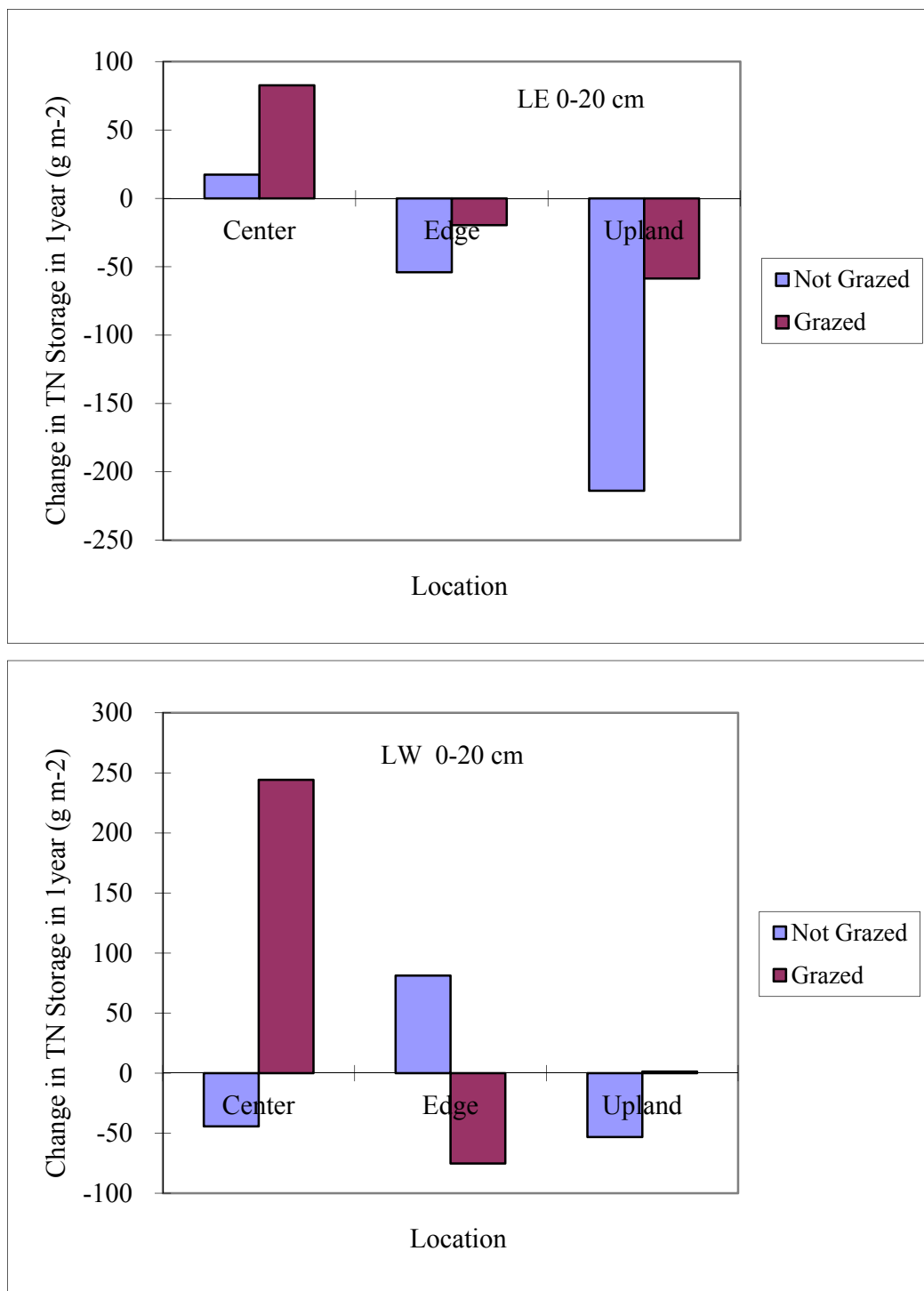


Figure 3.20. Change in soil carbon storage during one year sampling period under grazed and ungrazed conditions

Task 4:

Validate hydrologic and Phosphorus Models for Adaptation to Isolated Wetlands

Introduction

Assessment of the temporal variability of river flows is important from the human perspective of water supply, flood protection, and hydropower generation [e.g., *Vogel and Fennessey*, 1995; *Barnett and Pierce*, 2008], as well as from the ecological context of preservation and restoration of aquatic habitat [e.g., *Richter et al.*, 1996; *McMahon and Finlayson*, 2003; *Olden and Poff*, 2003]. Of emerging, and related, interest is the temporal variability in solute loads carried with river discharge [*Vogel et al.*, 2005; *Godsey et al.*, 2009; *Basu et al.*, 2010]. Important motivations for concern about solute loads are the ecological effects of solutes on receiving water bodies, such as the link between nitrate discharge from the Mississippi River basin and hypoxia in the Gulf of Mexico [*Turner and Rabalais*, 1994; *McIsaac et al.*, 2001], and phosphorus discharge from the Kissimmee River basin and eutrophication in Lake Okeechobee, FL [*Flaig and Reddy*, 1995; *Hiscock et al.*, 2003]. Temporal variability in these loads at inter- and intra-annual scales can be critical factors in assessing ecological impacts [*Horwitz*, 1978; *Poff and Ward*, 1989], effectiveness of best management practices (BMPs) for reducing solute loads [*Rice et al.*, 2002], and in designing downstream treatment systems such as constructed wetlands [*Mitsch et al.*, 2001].

A wide variety of river flow regime characteristics have been used by investigators from different disciplines to quantify flow variability, with differing degrees of emphasis on high flow events, low flow events, mean flow, and recurrence interval. For example, five flow regime components have been suggested as critical for regulation of ecological processes in river ecosystems: the magnitude of flow, timing of occurrence of particular hydrologic conditions, frequency of occurrence of specific hydrologic conditions, duration of time associated with the specific conditions of interest, and the rate of change of flows [*Richter et al.*, 1996]. Within these categories, hundreds of different flow variables have been evaluated [*Horwitz*, 1978; *Poff and Ward*, 1989; *Clausen and Biggs*, 2000; *Kennard et al.*, 2010]. Studies addressing the question of which hydrologic indices to choose to characterize river flow regime have grown in complexity, perhaps culminating in the *Olden and Poff* [2003] analysis of 171 flow indices using long-term records from 420 sites. Most of these measures are based on variants of traditional general distribution statistics such as maxima, minima, mean, variance, coefficient of variation, or skewness applied to different data intervals, but other examples include constancy and predictability as measures of flow periodicity [*Colwell*, 1974; *Gan et al.*, 1991].

An important graphical tool that captures the entire distribution of flows in a river is the flow duration curve (FDC). The FDC describes the relationship between flow magnitude and frequency by plotting discharge against the proportion of the observation period in which that discharge has been equaled or exceeded. Commonly, daily average flows are used for the entire period of record. Reviews of FDC applications are available for water resource planning [*Vogel and Fennessey*, 1994; 1995], characterizing river low-flow regimes [*Smakhtin*, 2001; *McMahon and Finlayson*, 2003], and assessing groundwater contributions to streamflow [*Winter*, 2007]. The FDC methodology is extensible to other water resource indices, such as hydropower generation potential or solute loads, through combination of a FDC with a rating curve for the index of interest [*Vogel and Fennessey*, 1995]. The US EPA recommends construction of load duration curves (LDC) as part of the process for developing total maximum daily load (TMDL) regulations as part of the Clean Water Act requirements [*USEPA*, 2007]. In this case, LDCs are constructed by multiplying the ordered stream flow values in the FDC by a numeric water quality

target for the pollutant of concern.

Here it is suggested that the quantitative information about temporal variability of discharge that is embedded in FDCs (and LDCs) can be complemented and extended by Lorenz curves that show exceedance probability for cumulative discharge (and load), rather than just for a specific discharge (or load). Despite the broad range of tools and metrics that have been used to evaluate hydrologic variability, the authors are not aware of any studies that have considered Lorenz rankings and the associated quantitative measure, the Gini coefficient, G , which are well-known measures of inequality, particularly in economics [e.g., *Aitchison and Brown*, 1957; *Bendel et al.*, 1989; *Sen*, 1997].

The Lorenz curve shows the cumulative percent of a quantity (commonly income; here flow and load) against the cumulative proportion of observations (commonly households; here temporal measurements) [*Lorenz*, 1905]. The upper and lower limits of a Lorenz curve are perfect equality where the quantity is equally distributed among all observations, and perfect inequality where all of the quantity is allocated to a single observation. An example Lorenz curve for daily discharge measured from 1933 to 2010 in the Peace River, FL (further site information is provided below) is compared in Figure 4-1 to the condition of perfect equality (the diagonal). In this example, 80% of the time accounts for only 40% of the discharge (and conversely, approximately 60% of the discharge occurs in 20% of the time). All Lorenz curves must lie on or below the diagonal, with the curvature increasing with inequality. The Gini coefficient is defined based on the Lorenz curve as the ratio of the area between the line of perfect equality and the Lorenz curve (shaded in Figure 4-1) to the triangular area under the line of perfect equality.

Four aspects of Lorenz ranking have particular appeal for consideration for characterizing flow and load variability. First is the intrinsically expressive nature of the Lorenz curve which shows the entire distribution of values on dimensionless ordinate and abscissa for easy comparison between basins. Second, G is a standard measure to characterize the shape (sometimes referred to as the ‘bendiness’) of the Lorenz curve. The range of G is zero to one, which also has intuitive appeal. Third, the Lorenz curve and G are easily parameterized with probability density functions (pdfs) such as the lognormal distribution, which has been shown to reasonably represent distributions of flows and loads [e.g., *LeBoutillier and Waylen*, 1993; *Vogel et al.*, 2005]. Finally, the effects of intermittency (zero flow or load conditions) are transparent, and easily incorporated with other pdfs through mixed models, as described below.

Flow intermittency is recognized as one of three key components (in addition to overall flow variability and flood regime patterns) for characterizing the ecological importance of river flow variability [*Poff and Ward*, 1989]. Intermittency is more prevalent in lower-order streams, which not only exhibit more temporal variability than those of higher order but also have profound influence on downstream flow and loads. For example, headwater areas of the northeastern US contribute approximately 70% of the flow and 65% of the nitrogen load to second-order streams, with only marginal declines to 55% and 40% in fourth order and higher [*Alexander et al.*, 2007]. As shown below, intermittency contributes significantly to the temporal inequality of river flows and loads. Similarly, in a suite of sediment transport experiments, *Radice* [2009] found that the majority of the observed non-uniformity in sediment load variability between experiments was attributed to differences in the intermittency ratio of the observation period.

Variability in solute load, L [MT^{-1}], can be directly related to variability of flow, Q [L^3T^{-1}], if solute concentration, C [ML^{-3}], can be related to discharge. That is, since $L = CQ$, if $C = f(Q)$ then $L = f(Q)$ only. For example, many studies have found the power law relationship $C = \alpha Q^\beta$ to be a reasonable representation [e.g., *Manczak and Florczyk*, 1971; *Stow and Borsuk*, 2003; *Vogel et al.*, 2005; *Godsey et al.*, 2009; *Siebert et al.*, 2009], indicating favorable prospects for a direct relationship between L and Q statistics under a wide range of conditions. Further, recent work has suggested that in many cases solutes of interest with both geogenic [*Godsey et al.*, 2009] and anthropogenic [*Basu et al.*, 2010] influences are

functionally chemostatic with near-constant concentrations. Moreover, because load is defined in terms of discharge, the self- (or spurious) correlation between them can be significant for the common case where the variability in discharge is greater than that of concentration [Kenney, 1982; Vogel *et al.*, 2005]. Thus, it is suggested that temporal variability in solute load is commonly equal to flow variability.

The specific objectives of this paper are to: 1) demonstrate application of Lorenz curve rankings and G to evaluate temporal inequality of flow and solute load, 2) develop analytic Lorenz and G relations in terms of parameters of the lognormal distribution under general conditions that include periods of intermittent flow, and 3) extend the framework for linking flow and load variability considering the effects of spurious correlation, intermittency, and Lorenz ranking. The discharge and load data used here are from 22 rivers in Florida (Figure 4-2), including four of the primary tributaries to Lake Okeechobee, which has received annual loads of total phosphorus (TP) that have averaged more than double the regulatory TMDL for more than 10 years [McCormick *et al.*, 2010]. The observed relationships are compared to those from sodium (Na^+) and nitrate (NO_3^-) loads from 9 of the catchments of the Hubbard Brook, NH long-term study site [e.g., Likens, 2004]. By incorporating these data into the developed theoretical framework, we illustrate how observed patterns in temporal variability of loads can be explained by concentration and flow variability.

2. Metrics of inequality

The relative merits of the following common measures of inequality have been reviewed by Bendel *et al.* [1989] and Sen [1997]: skewness, g , coefficient of variation, CV , and the Gini coefficient. All three are direct functions of shape parameters of common models such as the lognormal and gamma distributions. Thus, when the data approximately follow one of these models, all three metrics are highly correlated and conclusions about inequality would be the same regardless of which metric was used [Bendel *et al.*, 1989]. For characterizing inequality of plant size distributions that are not well described by standard distributional forms, Bendel *et al.* [1989] recommended g or CV in favor of G because the former are more sensitive measures of the tail (high values) of the distribution. However, while CV and G are measures of spreading relative to the mean (or relative precision), g is a measure of asymmetry, which is not the same as inequality. An un-skewed symmetric distribution need not be an equal one [Sen, 1997]. Further, as a temporal measure, g requires longer data records for accurate quantification than measures of relative precision. For example, Kennard *et al.* [2010] used 15 and 30 years of discharge data to estimate the ‘true’ values (based on 75 years of data) of 120 metrics of river flow for 16 Australian rivers. The accuracy of the indicators increased steadily with record length such that 90% were within 30% of the true mean using 15 years of data, and were within 20% using 30 years of data. Skewness of annual flows was among the least accurate of 120 metrics evaluated, approximately one-third the accuracy of CV of annual flows. Thus, we focus on CV and G , with an emphasis on the latter because unlike CV , G is not a commonly used hydrologic measure. We also emphasize G here because it has the advantage of being directly tied to the Lorenz diagram, which is in itself an expressive diagnostic. Lastly, it is suggested that a metric such as G that has an intrinsic range of $[0,1]$ has intuitive appeal.

2.1 The Lorenz curve and Gini coefficient

For a probability density function $p(x)$ with cumulative distribution function (CDF) $X(x)$ and range $[0,\infty)$, the Lorenz curve $Y(X(x))$ is

$$Y(X(x)) = \frac{\int_0^x xp(x)dx}{\int_0^\infty xp(x)dx} = \frac{m_{1,x}(0,x)}{m_{1,x}(0,\infty)} \quad (1)$$

where $m_{n,x}(u,l)$ is the n th absolute moment of the x distribution calculated over the range from upper and lower limits u and l , respectively. The numerator and denominator of the right side of Equation 1 can be

recognized as, respectively, the truncated and complete first moments [Jawitz, 2004]. Based on the definition of G as the ratio of the areas between the line of perfect equality and the Lorenz curve and under the line of perfect equality, G can be expressed as

$$G = 1 - 2 \int_0^1 Y(X) dX \quad (2)$$

For lognormally distributed x , X and Y can then be expressed as [Jawitz, 2004]

$$X(x) = m_{0,x}(0, x) = \frac{1}{2} \left[\operatorname{erf} \left(\frac{\ln x - \mu_{\ln x}}{\sigma_{\ln x} \sqrt{2}} \right) + 1 \right] \quad (3)$$

$$Y(x) = \frac{m_{1,x}(0, x)}{m_{1,x}(0, \infty)} = \frac{1}{2} \left[\operatorname{erf} \left(\frac{\ln x - \mu_{\ln x}}{\sigma_{\ln x} \sqrt{2}} - \frac{\sigma_{\ln x}}{\sqrt{2}} \right) + 1 \right] \quad (4)$$

The Lorenz plot can be produced from Equations 1, 3, and 4 where x is an internal variable. The single-parameter formulation for determining Y directly from X for a lognormal distribution can also be expressed

$$Y(X) = \Phi(\Phi^{-1}(X) - \sigma) \quad (5)$$

where $\Phi(z)$ is the standard normal cumulative distribution function

$$\Phi(z) = \frac{1}{2} \left[\operatorname{erf} \left(\frac{z}{\sqrt{2}} \right) + 1 \right] \quad (6)$$

and Φ^{-1} is the inverse. The Gini coefficient for a lognormal distribution is a simple function of $\sigma_{\ln x}$ [e.g., Schader and Schmid, 1994]

$$G = 2\Phi\left(\frac{\sigma_{\ln x}}{\sqrt{2}}\right) - 1 = \operatorname{erf}\left(\frac{\sigma_{\ln x}}{2}\right) \quad (7)$$

The CV is the standard deviation divided by the mean, which for a lognormal distribution is

$$CV = \left[\exp(\sigma_{\ln x}^2) - 1 \right]^{1/2} \quad (8)$$

Note that Equations 7 and 8 are valid for non-zero values of x . Methods for incorporating periods of zero flow are described in the following section.

2.2. Accounting for intermittency

The pdf for the record of discharges, $p(Q)$, for intermittent streams can be viewed as the superposition of pdfs of the zero flow, Z , and non-zero, NZ , flow periods:

$$p(Q) = f_Z + (1 - f_Z) p_{NZ} \quad (9)$$

where f_Z is the fraction of the record that is zero flow. An analogous relation can be constructed for concentration pdfs. While flow and concentration distributions have been observed to be generally lognormal, superpositions of different distributions, as in Equation 9, which can be multimodal, are not lognormal. Here, the statistics of the total distributions are recognized as the bimodal sum contributions of the zero and non-zero flow periods, and the statistics for only the (approximately) lognormally distributed non-zero flow periods can then be determined [e.g., Haan, 1977]. The finite probability of encountering a zero flow is f_Z , and a continuous distribution of probability is established for $Q > 0$. The inequality metrics described above can be determined for continuous periods or only for the times of non-zero flow. The metrics for these periods are related as described below.

For a superposition of distributions, the n th moments are additive such that

$$m_{n,T} = f_Z m_{n,Z} + (1 - f_Z) m_{n,NZ} \quad (10)$$

where the subscript T indicates the total distribution. Note that for zero flows, $m_{n,Z} = 0$ for $n > 0$. Equations 1 and 2 can be used to construct Lorenz diagrams for either the total record or only the non-zero flows, with corresponding Gini coefficients, G_T and G_{NZ} which are related follows. The Lorenz vertical axis Y is based on first moments and from Equation 10, $m_{1,T} = (1-f_Z)m_{1,NZ}$. But as shown in Equation 4, Y is a ratio of first moments (truncated and complete), so $Y_{NZ} = Y_Q$. Substituting this and the derivative (based on Equation 10) $dX_T = (1-f_Z)dX_{NZ}$ into Equation 2 results in

$$G_T = f_Z + (1-f_Z)G_{NZ} \quad (11)$$

The effect of intermittency on CV can be similarly determined. Substituting Equation 10 with $n=\{1,2\}$ into the definition $CV = [m_2 / m_1^2 - 1]^{1/2}$ results in

$$CV_T = \left[\frac{CV_{NZ}^2 + 1}{(1-f_Z)} - 1 \right]^{1/2} \quad (12)$$

Equations 11 and 12 enable quantitative linkage between degree of intermittency and flow distribution inequality metrics. Examination of these relations shows that for a given degree of inequality in the non-zero flows, both G_T and CV_T increase with f_Z , as illustrated in Figure 4-3. Because the upper limit for G is bounded (i.e., =1) while CV is unbounded, the magnitude of the effect of increasing f_Z is larger for CV . These results are generalized and do not require an assumption of distributional form.

As noted above, entire records for intermittent streams are likely to be less well-described by lognormal distributions because of non-zero flow periods. But when the total flow record (including zero flows) is characterized with a lognormal distribution, the shape parameter can be related to $\sigma_{\ln NZ}$ as follows. For lognormally distributed x , the complete moments are

$$m_{n,x} = \exp(n\mu_{\ln x} + n^2\sigma_{\ln x}^2 / 2) \quad (13)$$

Expanding Equation 13 for $n = \{1,2\}$, and solving for $\sigma_{\ln x}$ results in

$$\sigma_{\ln x} = \left[\ln(m_{2,x}) - 2 \ln(m_{1,x}) \right]^{1/2} \quad (14)$$

By combining Equations 10, 13 and 14, the total record lognormal distribution shape parameter can then be expressed in terms of f_Z and $\sigma_{\ln NZ}$:

$$\sigma_{\ln T} = \left[\sigma_{\ln NZ}^2 - \ln(1-f_Z) \right]^{1/2} \quad (15)$$

One can then determine the shape parameter for either the total record or only the non-zero flows, and coupled with an estimate of f_Z , the inequality metrics (G_T, G_{NZ}) or (CV_T, CV_{NZ}) can be interchangeably calculated from Equations (7,11) or (8,12).

3. Discharge control of loads

In this section, solute loads are described in terms of concentration and flow statistics, which then allows quantification of load inequality in terms of flow inequality. The effect of spurious or self-correlation between load and discharge is also evaluated.

3.1 Concentration as a function of discharge

As described above, the relation between concentration and discharge has been commonly fitted with a power law of the type $C = \alpha Q^\beta$. A general equation of the following form results from inclusion of a random error term, W [e.g., Cvetkovic *et al.*, 1998; Jawitz *et al.*, 2003; Vogel *et al.*, 2005]:

$$\ln C = \ln \alpha + \beta \ln Q + \gamma \ln W \quad (16)$$

where $\square$ here]: al..al.tion C , Q , and W are assumed to be lognormally distributed. Note that this

assumption indicates that statistics used to describe the flow distribution in the treatment that follows should be from non-zero flow periods. Introducing normally distributed variables $c = \ln C$, $q = \ln Q_{NZ}$, and $w = \ln W$, Equation 16 is now

$$c = \alpha + \beta q + \gamma w \quad (17)$$

where $\alpha = \ln a$. The w distribution is zero mean and uncorrelated to q . Because w and q are normal, c is also normal with

$$\begin{aligned} \mu_c &= \alpha + \beta \mu_q \\ \sigma_c^2 &= \beta^2 \sigma_q^2 + \gamma^2 \sigma_w^2 \end{aligned} \quad (18)$$

For $\gamma \neq 0$, c and q approach perfect correlation as $\gamma \rightarrow 0$, while $\gamma = 0$ results in the uncorrelated case. The degree of correlation is measured with Pearson's correlation coefficient:

$$\rho_{c,q} = \frac{\text{cov}(c,q)}{\sigma_c \sigma_q} = \frac{E[cq] - E[c]E[q]}{\left(E[c^2] - E[c]^2\right)^{1/2} \left(E[q^2] - E[q]^2\right)^{1/2}} \quad (19)$$

where $E[\]$ is the expected value with $E[q] = \mu_q$, $E[c] = \alpha + \beta \mu_q$, and $E[cq] = \alpha \mu_q + \beta E[q^2]$. Substitution of these definitions into Equation 19 results in

$$\rho_{c,q} = \frac{\beta \sigma_q}{\sigma_c} \quad (20)$$

Note from combination of Equations 18 and 20 that $\sigma_w^2 = \left(\frac{1}{\rho_{c,q}} - 1\right) \frac{\beta^2 \sigma_q^2}{\gamma^2}$. Equation 20 shows that the

power law exponent β is the proportionality constant between the strength of correlation between log-concentration and log-discharge and their variance ratio. This relation is also used below to quantify the correlation between the logarithms of load and discharge.

3.2 Load as a function of discharge

As the product of flow and concentration, solute load can be expressed

$$\lambda = c + q = \alpha + (\beta + 1)q + \gamma w \quad (21)$$

where $\lambda = \ln L$. Because c and q are normally distributed and related by Equation 17, λ is also normally distributed with

$$\begin{aligned} \mu_\lambda &= \alpha + (\beta + 1)\mu_q \\ \sigma_\lambda^2 &= \sigma_c^2 + \sigma_q^2 + 2\rho_{c,q}\sigma_c\sigma_q = \sigma_c^2 + (2\beta + 1)\sigma_q^2 \end{aligned} \quad (22)$$

The tendency of discharge to be more variable than solute concentrations has been noted as a contributing factor in high correlations observed between load and discharge [Godsey *et al.*, 2009; Basu *et al.*, 2010].

Therefore, the ratio $r = \sigma_c^2 / \sigma_q^2$, is of interest and is introduced to simplify Equation 22 to

$$\sigma_\lambda^2 = (2\beta + r + 1)\sigma_q^2 \quad (23)$$

As with q , it is reminded that λ refers most properly to non-zero periods and that $\square_\square$ should be coupled with f_Z for computation of the total period inequality metrics G_T or CV_T from Equations (7,11) or (8,12). The effects of r and the power law exponent β on the variance of log load (scaled to variance of log

discharge, $\frac{\sigma_\lambda^2}{\sigma_q^2}$) from Equation 23 are illustrated in Figure 4-4. Note that for the many cases where β is

close to zero [Godsey *et al.*, 2009; Basu *et al.*, 2010], low relative variability in concentration corresponds to near equivalence between load and flow variance.

This result is also conveniently described using the correlation between load and discharge. Because load is itself determined from discharge, the correlation between them is fundamentally spurious [e.g., *Haan*, 1977]. But since loads and their variability with time (or discharge) are often of interest, the strong correlation is also useful. Following the general treatment by *Kenney* [1982], substituting the definitions of Equation 21 into Equation 19 leads to the expression for the correlation between the logarithms of load and discharge, $\rho_{\lambda q}$

$$\rho_{\lambda q} = \frac{1 + \rho_{cq} r^{1/2}}{\left[1 + r + 2\rho_{cq} r^{1/2}\right]^{1/2}} \quad (24)$$

Equation 24 allows quantification of the self-correlation between load and discharge based on the correlation between concentration and discharge and the relative variability of these (Figure 4-5a). As r approaches zero, $\rho_{\lambda q}$ approaches 1. Further, $\rho_{\lambda q} < 0.5$ only when both $r > 0.8$ and $\rho_{cq} < -0.5$. That is, as flow variability becomes much greater than concentration variability, load and discharge approach perfect correlation.

When the power law model is used to relate C and Q , Equation 20 can be substituted into Equation 24 resulting in

$$\rho_{\lambda q} = \frac{1 + \beta}{\left[1 + \left(\beta / \rho_{cq}\right)^2 + 2\beta\right]^{1/2}} \quad (25)$$

This relation provides the same results as the somewhat more complicated result provided by *Vogel et al.* [2005]. The effects of ρ_{cq} and β on $\rho_{\lambda q}$ are shown in Figure 4-5b. The range of β values shown is consistent with the range measured for 21 large catchments in the Mississippi River basin [*Basu et al.*, 2010]. We see that $\rho_{\lambda q} > 0.9$ for approximately one-half of the plane space shown, and $\rho_{\lambda q} < 0.5$ only for a relatively narrow range of parameter values.

For completeness it is noted that Equations 24 and 25 enable exact quantification of $\rho_{\lambda q}$, but for the potentially even more desirable quantity ρ_{LQ} , the following solution is only approximate [*Haan*, 1977; *Kenney*, 1982]:

$$\rho_{LQ} = \frac{1 + \rho_{CQ} (CV_C / CV_Q)}{\left[1 + (CV_C / CV_Q)^2 + 2\rho_{CQ} (CV_C / CV_Q)\right]^{1/2}} \quad CV_C, CV_Q < 0.05 \quad (26)$$

This solution is close to exact when both C and Q exhibit low variability, with decreasing accuracy as the variability increases. The threshold value of CV for which Equation 26 is most accurate is from the approximation by *Kenney* [1982]. While this value may be low compared to phenomena such as catchment discharges that tend to have high variability, it is noted from Equation 26 that when $\rho_{CQ} = 0$ and $CV_C = CV_Q$, $\rho_{LQ} = 0.71$ and increases towards perfect (spurious) correlation as $CV_Q > CV_C$.

The final consideration evaluated here is the relative inequality of loads compared to flows, as measured by G . Assuming lognormally distributed data, the ratio of total-record $G_{L,T}$ and $G_{Q,T}$ can be expressed in terms of r and β based on Equations 7, 11, and 23:

$$\frac{G_{L,T}}{G_{Q,T}} = \frac{f_Z + (1 - f_Z) \operatorname{erf} \left[\sigma_q (2\beta + r + 1)^{0.5} / 2 \right]}{f_Z + (1 - f_Z) \operatorname{erf} \left[\sigma_q / 2 \right]} \quad (27)$$

Because this ratio is a function of four variables, graphical illustration of solutions to Equation 27 is constrained. Here, the effects of r and β on $G_{L,T}/G_{Q,T}$ are shown in Figure 4-6 for an example case of

fixed $f_Z = 0.5$ and $\sigma_q^2 = 2$. This graphic highlights that for the commonly observed phenomena of low r and σ_q^2 and This graphic highlights that for the commonly observed phenomena of low solutions to Equation 27 plane space shown in Figure 4-6, $G_{L,T}$ is within 15% of $G_{Q,T}$. Generally, the sensitivity of Equation 27 to σ_q is weak but relatively stronger for f_Z .

In summary, the relations described in this section all illustrate how the correlation between load and discharge can be quantified. Collectively, the relations developed above show that the combined effects of high variability in discharge relative to concentration and near-chemostatic conditions (σ_q values close to zero) create conditions where load and discharge are highly correlated. The important implications of this for the present analysis are that for many solutes the temporal inequality of loads is likely to be similar to that of discharge. In the next section, multi-decadal flow and discharge data from rivers in Florida and Hubbard Brook, NH are used to illustrate this conclusion.

4. Application to 22 rivers in Florida and 9 in Hubbard Brook

4.1 Data collection and analysis

Flow data from 22 gaging stations across Florida were obtained from the U.S. Geologic Survey (USGS, <http://waterwatch.usgs.gov>, Table 4-1). Four of these stations are on one river (St. Johns) to evaluate the effect of increasing catchment size, but three additional tributaries to Lake Okeechobee are also included, for a total of 22 rivers evaluated. The entire available period of record was used from each USGS station, with only one record of less than 30 years. Daily flows were determined by the USGS from flow and stage rating curves. Rivers of multiple flow classes were examined to describe how the Lorenz diagram and G are influenced by basin area and groundwater contribution to base flow.

Temporal inequality metrics were determined for flow data from all the rivers, but to evaluate the relationship between flow and load inequality, this work focuses on the Lake Okeechobee Basin (LOB) and nine of the Hubbard Brook watersheds. Lake Okeechobee (1,890 km²) is the largest lake in Florida, and is contained within a basin of approximately 14,000 km². For management purposes the South Florida Water Management District (SFWMD) has divided the LOB into 61 sub-basins. Emphasized here are 4 sub-basins (Table 4-2) that account for 55% of the LOB area and 61% of the average annual TP load to the lake between 1991 and 2009. These basins were selected because they covered a large portion of the landscape, contributed significant loads to the lake, and represented a range of flow magnitudes and variability. The climate in south central Florida is sub-tropical with mean annual rainfall from 1972 to 2008 of 132 cm [Florida climate division 4, National Climatic Data Center, <http://www7.ncdc.noaa.gov/CDO/CDODivisionalSelect.jsp>]. The LOB has a distinct rainy season (June-October) in which 65 percent of annual rainfall occurs. Annual flows and loads were determined based on a water year of May 1 to April 30.

Flow from three of the selected sub-basins into the lake is managed for drainage and flood control through control structures [McCormick *et al.*, 2010]. For the naturally flowing Fisheating Creek, USGS flow data only from water years 1975 to 2009 were used for load estimates, based on water quality data availability. For the other sub-basins daily flows monitored at control structures were obtained from the publicly accessible online database maintained by SFWMD (DBHYDRO, http://www.sfwmd.gov/dbhydroplsql/show_dbkey_info.main_menu). Taylor Creek/Nubbin Slough discharge data were monitored at spillway S-191 (27:11:31.168N, 80:45:45.201W) from 1975-2009. While there is a USGS gaging station on the Kissimmee River, these data do not account for periods when flow is controlled because of the regional drainage and flood protection management schedule. Thus, Kissimmee discharge data were based on monitoring by SFWMD at the S-65E spillway (27:18:49.287N, 81:01:25.296 W) from 1989 to 2009. Discharge for S-154 was monitored in a culvert, S-154C (27:12:38.876N, 80:55:12.09W) from 1978 to 2009.

Regular monitoring of streamflow and solute concentrations in the Hubbard Brook Experimental Forest began in the 1950s, and the Hubbard Brook Ecosystem Study [HBES, *Likens*, 2004] maintains an online archive of data (http://www.hubbardbrook.org/data/dataset_search.php). Monthly stream flow and sodium (Na^+) and nitrate (NO_3^-) concentrations from all nine of the HBES watersheds were used to determine monthly loads from each watershed from June 1995 to December 2007. The power law model $C = \square = \square$ was fit using the method of least squares to monthly average concentrations and corresponding monthly average flow.

The bimodal, or mixed, model of Equation 9 was fitted to the flow records for the LOB sub-basins with a finite probability, f_z , for the zero flows and the lognormal distribution for non-zero flows. Observed $G_{Q,T}$ values were compared to modeled values determined from Equations 15 and 7. However, records with a large number of days with non-zero, but very low flow are not as well fit by the lognormal distribution. Yet, these low flow days contribute very little to the total discharge which is the quantity of interest in a Lorenz framework. Therefore, here we defined f_z as the fraction of the record with flows at or below a minimum value that sum to less than 1% of the total discharge. This approach resulted in significantly improved fits to the data by the lognormal model, as described below.

The power law model $C = \square = \square$ was fit using the method of least squares to bi-weekly TP concentrations measured in surface water grab samples (also available in DBHYDRO) and the corresponding average daily flows. Loads were determined from the sum of average daily flows multiplied by interpolated daily concentrations. It is recognized that in many cases regression methods are preferred for estimating annual loads and that averaging may result in underestimates [*Preston et al.*, 1992; *Robertson and Roerish*, 1999; *Stow and Borsuk*, 2003]. However, averaging and regression methods provide comparable estimates when there is little relationship between concentration and flow [*Preston et al.*, 1992], as is the case for these LOB sub-basins. Furthermore, it was desired to maintain accordance with the averaging method used by SFWMD [*McCormick et al.*, 2010]. Note that the loads used here were compared to those determined from regression techniques using LOADEST [*Runkel et al.*, 2004], and the resulting values differed by an average of only 3.75%.

Flow inequality

The inequality metrics G and CV were calculated from the rank sorted daily flows for all rivers (Tables 4-1 and 4-2) and Lorenz diagrams were constructed for selected rivers (Figure 4-7). The selected rivers exhibit a wide range of flow variability, from the nearly egalitarian Rainbow River ($G_{Q,T} = 0.09$), through the relatively unequal Ochlockonee ($G_{Q,T} = 0.57$), to the flashy and highly unequal Fisheating Creek ($G_{Q,T} = 0.78$). Streamflows that are naturally near-uniform are indicative of steady groundwater input, and the lowest- G rivers listed in Table 4-1 are indeed spring-fed. The gaging stations at Rainbow and St. Mark's Rivers ($G_{Q,T} = 0.09$ and 0.23) are respectively 8 and 1 km downstream from eponymous first-magnitude headwater springs (flow greater than $2.8 \text{ m}^3/\text{s}$). The Suwannee, Santa Fe, and Withlacoochie Rivers ($G_{Q,T} = 0.36, 0.37$ and 0.45) traverse karst terrain and are also heavily fed by springs from the Upper Floridan Aquifer. Conversely, highly variable (flashy) streamflows indicate relatively un-damped response to precipitation. Traditional FDCs are shown for the four Lake Okeechobee sub-basins evaluated here in Figure 4-8. Flatter FDCs indicate relatively damped response to precipitation and in the low-flow region low slopes indicate significant subsurface flow contribution, while steep slopes indicate flashiness and small baseflow contributions [*Smakhtin*, 2001; *Winter*, 2007]. The FDCs here are presented in order of increasing slope (or decreasing flow uniformity), with the Kissimmee River (Figure 4-8a) the most uniform and S-154 (Figure 4-8d) the least. The discharge Lorenz curves for these sub-basins are shown in the same order in Figure 4-9. The increasing flow inequality from the Kissimmee to S-154 is apparent in the increased concavity, as quantified by G_Q (Table 4-2). The Kissimmee $G_{Q,T}$ value (0.66) is lowest and

S-154 (0.89) is highest, with Fisheating Creek and Taylor Creek/Nubbin Slough exhibiting similar mid-range values.

An important characteristic of these LOB sub-basins is the high proportion of zero-flow days (Table 4-2). As illustrated in Figure 4-3, increasing f_Z increases the total-record inequality. Note that for the LOB sub-basins the total-record $G_{Q,T}$ values were significantly greater than $G_{Q,NZ}$ from only the times with streamflow. For example, $G_{Q,NZ}$ for S-154 was the lowest (most equal) of the four, but because it was the most intermittent (highest f_Z), its $G_{Q,T}$ was the highest (most unequal, Table 4-2). The primary reason for the high degree of intermittency in these catchments is their relatively small areas (i.e., low-order streams). However, the Kissimmee basin is much larger than the other three and has greater $G_{Q,T}$ than similar-size basins listed in Table 4-1. When only the flowing periods are considered, $G_{Q,NZ}$ for the Kissimmee is consistent with that of Ochlockonee, which drains a basin that is 20% larger, but greater (more unequal) than the $G_{Q,T}$ values for the next largest basins examined, Ocklawaha and Withlacoochie, both of which are significantly influenced by first-magnitude springs (Silver and Rainbow, respectively). Lack of spring input explains the Kissimmee $G_{Q,NZ}$, but the high f_Z is from active management. A naturally flowing Kissimmee would have lower intermittency and more-equal discharge regime but flows are periodically restricted based on drainage, flood protection, and water supply management priorities for Lake Okeechobee. Note that rainfall patterns and local geomorphology also contribute. For example, inflows to Lake Okeechobee from tributaries in the basin are particularly sensitive to decadal climatic patterns, varying by 40% between Atlantic multi-decadal oscillation (AMO) warm and cool phases [Enfield *et al.*, 2001].

The flow records for the LOB sub-basins were well described by the mixed-lognormal model. The average relative difference between the fitted and measured $G_{Q,T}$ values was 2% (Table 4-2). Note that when f_Z was determined strictly for $Q = 0$ only, this relative difference was 7%. Most of the range of observed flows was well described by the lognormal distribution, but the Kissimmee and Fisheating Creek had significant periods of very-low flow days (Figure 4-8) that were not well captured by the lognormal model. However, because the contribution of these periods to the total discharge was minimal, the discharge Lorenz curves for these sub-basins are well described by the mixed lognormal model (Figure 4-9).

Also shown in Table 4-1 are the ratios of flows exceeded 20 and 80 percent of the time ($Q_{20/80}$), which is an indicator of the range of flows [e.g., Olden and Poff, 2003]. Overall this metric is not well correlated to G . However, the two rivers with the highest Q ratios (Hillsborough and Fisheating Creek) have high degrees of intermittency. When these two are excluded, the correlation $\rho_{G, Q_{20/80}} = 0.85$, further indicating the importance of specifically accounting for intermittency. Finally, recall that for the lognormal distribution G and CV are both direct functions of the shape parameter (Equations 7 and 8). Thus, because the data presented here are well described as lognormal, the $G_{Q,T}$ and $CV_{Q,T}$ values for all 25 stations in Tables 4-1 and 4-2 are strongly correlated with $\square_{G, CV} = 0.91$.

Load inequality reflects flow inequality

The TP load Lorenz diagrams for the LOB sub-basins are shown in Figure 4-9 and the corresponding $G_{L,T}$ values are listed in Table 4-2. First it is noted that the loads were well-described using the mixed-lognormal model with average relative difference between the fitted and measured $G_{L,T}$ values of 3%. But the most important result that is emphasized here is the similarity between the load and discharge Lorenz curves and G values. For the four LOB sub-basins, the mean ratios of $G_{L,T}$ to $G_{Q,T}$ based on observations and best fit from the mixed-lognormal model were 1.03 and 1.06. That is, the degree of inequality in the loads closely matched that of the flows. This result can be explained within the framework developed

above using the extent of intermittency, the power law model, and the log concentration and flow variances.

For the four LOB sub-basins, the mean $\sigma_{\ln Q, NZ}$ was 0.99 with mean $f_Z = 0.51$ (Table 4-2), and using these values in Equations 7 and 11 (illustrated in Figure 4-3a) results in model fit $G_{Q, NZ}$ and $G_{Q, T}$ mean values of 0.52 and 0.76, which compare favorably to 0.53 and 0.77 determined respectively from observed non-zero flows and the entire flow record. Using the mean f_Z and $\sigma_{\ln Q, NZ}$ values in Equation 15 results in mean $\sigma_{\ln Q, T} = 1.3$, which is equal to the mean value determined from the total flow records. However, it is cautioned that use of this value in Equation 7 results in an underestimated mean $G_{Q, T} = 0.64$, because it is only the non-zero flows that are well approximated as lognormal, not the entire flow record. Thus, it is reminded that for intermittent flows the mixed-model method of Equation 11 is the appropriate approach for determining the modeled $G_{Q, T}$.

The mean power law exponent β for the four LOB sub-basins was 0.04 and the mean $r (= \sigma_c^2 / \sigma_q^2)$ was 0.50 (Table 4-2). That is, the solute concentrations were nearly chemostatic ($\beta \approx 0$) and the flow variability was significantly greater than the concentration variability. From Equation 23 (illustrated in Figure 4-4) these β and r values correspond to $\sigma_\lambda^2 / \sigma_q^2 = 1.58$ ($\sigma_\lambda / \sigma_q = 1.26$), or mean $\sigma_\lambda = 1.25$. Then, from Equations 7 and 11 (with the mean observed f_Z), mean lognormal-estimated $G_{L, NZ}$ and G_L are 0.62 and 0.80. Thus, using the mean parameters for these four basins results in the lognormal-estimated ratio $G_L / G_Q = 1.07$, which again compares favorably to the observed value. Note that this model-estimated ratio can also be obtained directly from Equation 27 (illustrated in Figure 4-6). Finally, using the mean β and r values in Equation 20 results in mean $\rho_{c, q} = 0.06$ (from Equation 20 and the values in Table 4-2). This value used in turn in either Equations 24 or 25 (illustrated in Figure 4-5) results in $\rho_{\lambda, q} = 0.83$. In summary, these results illustrate how low β and low variance of concentration relative to flow produce strong correlation between load and discharge. This strength is enhanced by self- or spurious correlation with a net effect of highly similar temporal inequality in loads and flows.

For comparison in a setting where solute concentrations may not be constant, Na^+ and NO_3^- data from nine of the Hubbard Brook catchments were evaluated. For Na^+ and NO_3^- , the mean values for $\square$, r , and $G_{L, T} / G_{Q, T}$ were $\{-0.13, 0.33\}$, $\{0.03, 1.64\}$, and $\{0.90, 1.58\}$, respectively. Thus, neither solute exhibited truly chemostatic behavior, and NO_3^- was more chemodynamic, with an absolute value of $\square$ more than double that for Na^+ , and also greater variability in concentration than in flows. In contrast, flow variability overwhelmed the variability in Na^+ concentrations ($r \sim 0$). The combined influences of low values for $\square$ and r led to Na^+ load inequality being similar to flow inequality, whereas for NO_3^- high $\square$ and r corresponded to higher inequality in load than in flows. The quantitative link between these parameters is described by Equation 27, and the $G_{L, T} / G_{Q, T}$ values computed from this relation differed from the observed values by an average of 7%, as illustrated in Figure 4-10. This figure compares the Na^+ and NO_3^- $\square$, r , and $G_{L, T} / G_{Q, T}$ values for all nine Hubbard Brook catchments to Equation 27. In addition to showing that the analytical relation matches the observations well, these results also indicate that a range of load inequality behaviors (not just chemostatic) for different solutes of interest can be readily understood based on $\square$ and the ratio of variability in concentration and discharge.

Discussion

When considering quantitative descriptors of inter-period variability it is useful to recall that the magnitude of the metric of choice is affected by the degree of intra-period averaging. The effects of differing degrees of internal averaging in both space and time are illustrated for G in Figure 4-11. Flows monitored at increasing distances downstream in gaining rivers are supported by greater contributing watershed area, and thus increase in magnitude. But the increased areal averaging also reduces inequality of flows in downstream reaches. For example, of four gaging stations along the St. Johns River shown in

Figure 4-11a, the station nearest the headwaters has the greatest inequality, with G decreasing with distance downstream. Note that the middle two stations with nearly identical Lorenz curves have comparable contributing areas (Table 4-1). Similarly, coarser temporal granularity also results in decreased measures of inequality. In the example case shown in Figure 4-11b, flows averaged at the daily scale were the most unequal with G decreasing for monthly and annual averages. Likewise, consideration of finer resolution temporal data, such as hourly or less, would be expected to result in greater inequality than for the daily scale. A final consideration when comparing magnitudes of metrics such as G is that variability measures are sensitive to record length [e.g., Kennard *et al.*, 2010]. Measurement durations that are “long enough” depend on the management purpose as well as regional or local sensitivity to climatic patterns of different periodicity, such as the AMO effect in the Lake Okeechobee area [Enfield *et al.*, 2001].

The flow regime of the LOB sub-basins examined here can be characterized as the third type described McMahon and Finlayson [2003]: high CV of annual flows with flow every summer (wet season) and cease-to-flow every winter (dry). Even though the tributaries flow every summer, there are substantial differences between successive summers. Of the nine flow regime categories of Poff and Ward [1989], the Okeechobee catchments are ‘intermittent flashy’ and ‘intermittent runoff’, as determined by high variability of annual runoff and substantial periods of zero flow. Note that for 78 streams these authors found CV of annual flow and average annual number of zero flow days to be strongly correlated ($\rho = 0.92$), as would be expected based on Equation 12 (illustrated in Figure 4-3b). In contrast to these more narrative, categorical approaches the emphasis of this work is to describe flow (and load) regimes within a continuous, dimensionless framework (Lorenz curve) with quantitative inequality metrics (G and CV) under general conditions of intermittency.

Various physical mechanisms have been proposed to relate the spatial distribution of solute sources in the landscape to an observed ρ [e.g., Haygarth *et al.*, 2004; Siebert *et al.*, 2009; Godsey *et al.*, 2009; Basu *et al.*, 2010]. One hypothesis is that a large reservoir of solute mass distributed ubiquitously in the landscape translates to chemostatic behavior. This conceptualization is consistent with the nearly chemostatic behavior noted for export of a variety of solutes of geologic origin from a wide range of relatively unimpacted natural catchments [Godsey *et al.*, 2009], and for nutrients (total nitrogen and phosphorus) exported from managed catchments in the Mississippi River and Baltic Sea basins [Basu *et al.*, 2010]. In the latter case, the ubiquity of large solute reservoirs was attributed to the accumulation of nutrient sources from decades of excess fertilizer applications. The low ρ values observed here (mean = 0.04) for TP in the LOB indicate nearly chemostatic behavior, which may also be attributed to the accumulation of legacy sources in the soil. Phosphorus has been imported into the LOB in large quantities in the form of fertilizer and animal feed for decades, and import-export budgets have indicated that the majority has been retained within the basin [Boggess *et al.*, 1995; Hiscock *et al.*, 2003], likely to be slowly released over a long period of time.

In the Hubbard Brook catchments, the geogenic constituent Na^+ is expected to be more uniformly distributed than NO_3^- , which is not expected to be ubiquitous in the soils of the Hubbard Brook forested catchments that have not been subjected to long-term nutrient additions common in agricultural settings. Consistent with the conceptual model of Basu *et al.* [2010], the lower ρ and r values for Na^+ than for NO_3^- are considered to result from the more homogeneous spatial distribution of the former. The correlation between load and flow can then be shown from Equation 25 to be much stronger for the more-uniformly distributed Na^+ (mean $\rho_{\lambda q} = 0.99$) than for NO_3^- (mean $\rho_{\lambda q} = 0.73$).

Finally, we emphasize that consideration and quantification of temporal inequality in flows and loads has important implications for watershed management. High decadal-scale variability in flow in the LOB translates to near-complete reversals in water management policies over the same time period from flood protection during the (wetter) positive phase of the AMO, to precariously balancing water supply

demands from humans and natural systems during the (drier) negative phase [Enfield *et al.*, 2001]. The moderate degree of inter-annual variability of flows and loads is recognized in regulatory and management policies by considering load targets based on a five-year moving average [McCormick *et al.*, 2010]. However, as described above, intra-annual variability is greater than inter-annual variability. For the LOB sub-basins considered here, the relatively high intermittency of flow (mean $f_Z = 0.51$), contributes significantly to the high degree of temporal inequality of load export (mean $G_L = 0.80$). The corresponding Lorenz curve for this value is similar to that shown for Fisheating Creek in Figure 4-7. This curve also approximately intersects the colloquial “80:20 rule” (also known as the Pareto principle) wherein 80% of a quantity such as production or income is attributed to 20% of the population. In this case, approximately 80% of the annual TP load to Lake Okeechobee occurs in only 20% of the year. While regulatory policy and management activities are currently based on annual loads [e.g., Hiscock *et al.*, 2003; McCormick *et al.*, 2010], it is suggested here that the degree of intra-annual inequality should also be explicitly considered. For example, the TP load delivered to Lake Okeechobee for the year 2009, reported as 656 MT [McCormick *et al.*, 2010], could instead be considered, based on the mean G_L , as 525 MT (80% of the load) delivered in only 73 days (20% of the year), with the remaining 131 MT delivered over 292 days. The inequality for sub-basin S-154 is even more extreme: the Lorenz curve for this basin (Figure 4-9, $G_L = 0.89$) shows that 85% of the load is delivered in only 10% of the time. A variety of load interdiction measures, including vast treatment wetlands, are in various stages of planning and implementation in the LOB [McCormick *et al.*, 2010] and the design and operation details of these could be critically influenced by the extent of temporal flow and load inequality.

In conclusion, we suggest that Lorenz curves and the associated Gini coefficient are intrinsically expressive descriptors that may be useful tools when considering river flows and loads to receiving waters. We have described simple relations between lognormal model parameters, extent of non-zero flow, and the Gini coefficient. These relations together with the connection of the quantitative measure G to the visual display of the Lorenz curve and its intuitive [0,1] range contribute to the appeal of the Gini coefficient as a metric of flow and load inequality. While we have focused on lognormal distributions, the Lorenz and Gini framework are suggested as useful tools for nonparametric analysis and interpretation of data that may not be well described by simple distributions.

We have illustrated a broad range of observed flow variability with a synthesis of flow data from 22 rivers in Florida using both Lorenz curves and G , and we have examined the relation between flow and load variability with a focus on four of the primary tributaries to Lake Okeechobee and nine of the watersheds at Hubbard Brook. The Okeechobee basin examples emphasized the importance of intermittency and relative contribution of groundwater to streamflow as contributors to flow variability. We note that when comparing absolute values of variability metrics, it is important to contextualize data granularity in both space and time. As demonstrated with data from the Lake Okeechobee and Hubbard Brook catchments, the correspondence between load and flow inequality can be understood based on two aspects of the concentration-discharge relationship. The ratio of the variances of C and Q exerts primary influence, with the strength of their correlation also contributing. Finally, we conclude that flow variability is likely a strong surrogate for load variability because of the combined effects of high correlation between concentrations and flows and higher variance of flows than concentrations for many solutes.

Table 4-1. Daily flow statistics from USGS gaging stations on selected rivers in Florida. Mean annual flow (Q_{avg}), CV , $G_{Q,T}$, and the ratio of flows exceeded 20 and 80 percent of the time ($Q_{20/80}$) were calculated from daily flows.

River	ID	USGS station	Period of record	Area (km ²)	Q_{avg} (mm/yr)	$G_{Q,T}$	CV	$Q_{20/80}$
Perdido	1	2376500	1941-2010	1020	685	0.42	1.33	2.75
Blackwater	2	2370000	1950-2010	531	590	0.50	1.78	3.70
Shoal	3	2369000	1938-2010	1228	805	0.38	1.05	2.67
Chipola	4	2359000	1943-2010	2022	644	0.35	0.77	2.88
Apalachicola	5	2359170	1977-2010	49728	440	0.37	0.74	3.42
Ochlockonee	6	2330000	1933-2010	7115	213	0.57	1.43	10.6
St. Mark's	7	2326900	1956-2010	1386	452	0.23	0.51	1.90
Santa Fe	8	2322500	1933-2010	3559	375	0.28	0.61	2.09
Suwannee	9	2323592	1999-2010	25830	265	0.36	0.75	2.84
St. Mary's	10	2231000	1933-2010	531	307	0.69	2.08	14.4
Northfork Black	11	2246000	1933-2010	458	369	0.66	2.37	6.65
Ocklawaha	12	2243960	1969-2010	4403	255	0.36	0.74	2.96
St. Johns	13	2236000	1954-2010	7940	350	0.39	0.74	3.57
St. Johns	14	2232500	1954-2010	3986	282	0.54	1.11	9.44
St. Johns	15	2232400	1954-2010	3447	261	0.55	1.16	9.64
St. Johns	16	2232000	1954-2010	2507	236	0.61	1.40	11.5
Rainbow	17	2313100	1965-2010	190	3233	0.09	0.16	1.33
Withlacoochie	18	2313000	1933-2010	4727	175	0.45	0.87	3.99
Hillsborough	19	2304500	1938-2010	1616	230	0.73	1.97	960
Manatee	20	2299950	1966-2010	169	391	0.74	2.66	11.0
Peace	21	2295637	1933-2010	2139	258	0.58	1.53	6.27
Fisheating Creek	22	2256500	1933-2010	805	283	0.78	2.48	150

Table 4-2. Daily flow statistics for the period of record from selected sub-basins of Lake Okeechobee, FL: Fisheating Creek (FEC), Kissimmee River (KR), S-154, and Taylor Creek/Nubbin Slough (TC/NS). Mean annual flow (Q_{avg}), CV and G were calculated from daily flows. Flow and load G were determined from observed data and best fits of lognormal distributions, and are shown for the total period of record, as well as only non-zero (NZ) days. For this analysis, zero flow periods were classified as the values that sum to less than 1% of the total discharge.

Basin	ID	Area (km ²)	Period of record	Q_{avg} (mm/yr)	CV	f_z	σ_c^2	σ_q^2	$\square$	$\sigma_{lnQ,T}$	Observed				Estimated			
											$G_{Q,NZ}$	$G_{Q,T}$	$G_{L,NZ}$	$G_{L,T}$	$G_{Q,NZ}$	$G_{Q,T}$	$G_{L,NZ}$	$G_{L,T}$
FEC	22	1170	1975-2009	181	2.19	0.43	0.51	1.21	-0.04	1.33	0.62	0.78	0.73	0.85	0.56	0.75	0.69	0.83
KR	23	5872	1989-2009	228	1.50	0.31	0.69	0.83	0.002	1.08	0.51	0.66	0.50	0.66	0.33	0.64	0.56	0.70
S-154	24	137	1978-2009	250	1.90	0.75	0.55	0.79	0.13	1.47	0.47	0.87	0.54	0.89	0.45	0.85	0.52	0.88
TC/NS	25	488	1975-2009	260	2.32	0.53	0.23	1.12	0.07	1.36	0.52	0.77	0.52	0.77	0.54	0.79	0.57	0.79

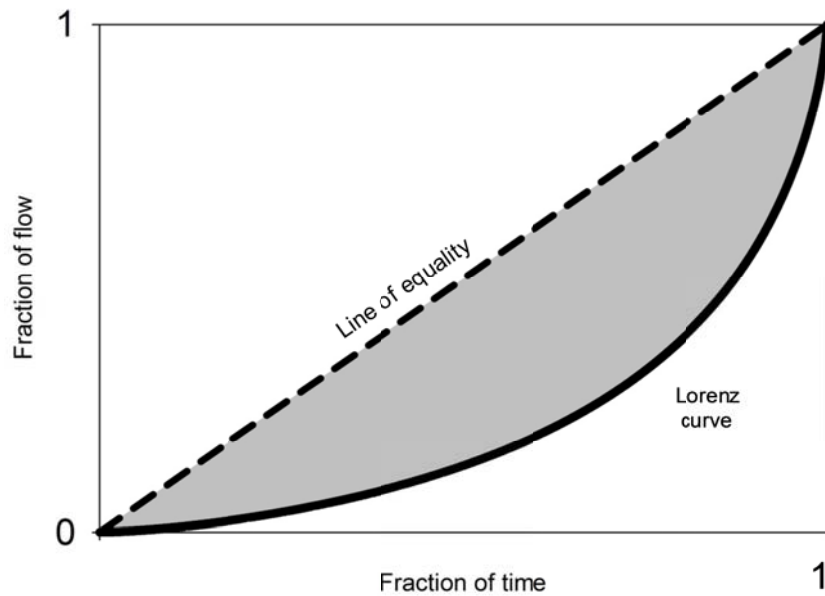


Figure 4-1. Illustration of the Lorenz diagram, defined as cumulative proportion of the variable (here daily average flow) plotted against cumulative proportion of the population (here days). Data for this example are daily flows measured in Peace River, FL from 1933 to 2010. The Gini coefficient, G , is the ratio of the area between the line of equality ($y=x$) and the Lorenz curve for the data (the shaded area in the figure), and the total area under the line of equality. For this example $G = 0.58$.

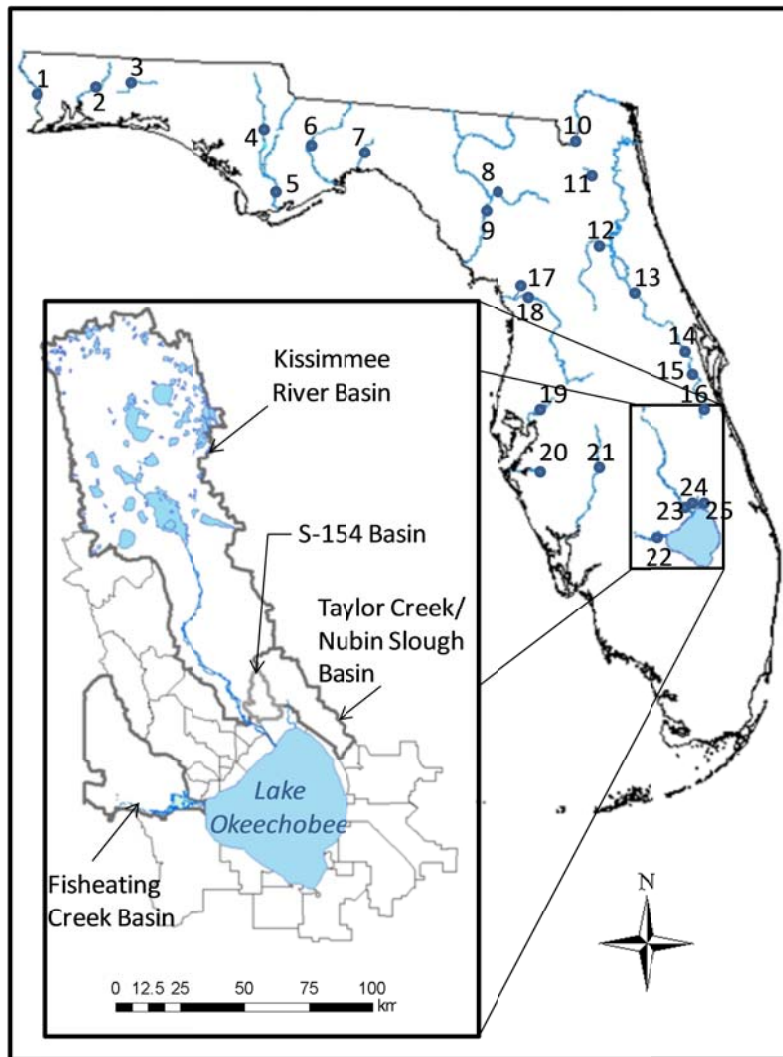


Figure 2. Map illustrating locations of 25 gaging stations on 22 rivers in Florida used in this analysis. Data from 4 stations on the St. Johns River were used, as described in the text. Inset is the Lake Okeechobee basin, with all sub-basins delineated and those emphasized here indicated.

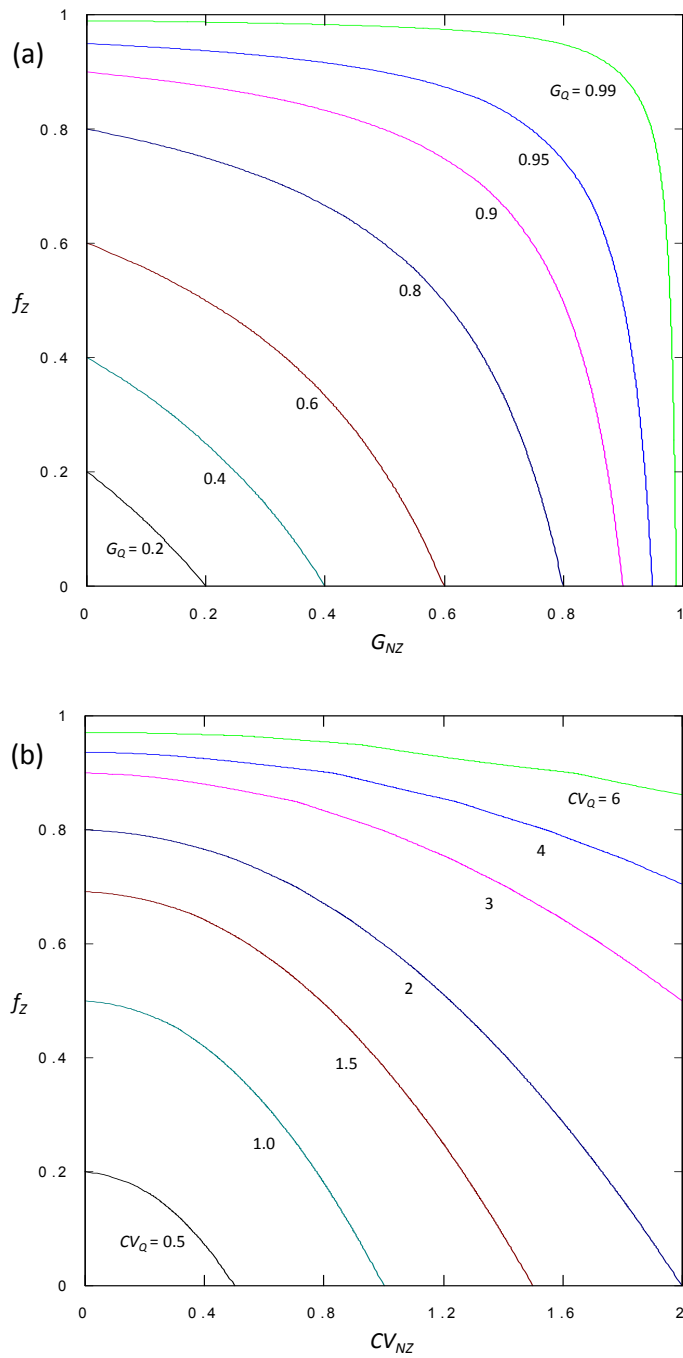


Figure 4-3. As the fraction of the monitoring period that is zero flow, f_Z , increases for a given degree of inequality in the non-zero flow record, as measured by Gini coefficient, G_{NZ} (a) and coefficient of variation, CV_{NZ} (b), the corresponding total-record inequality metrics (G_Q , CV_Q) also increase. Graphs based on Equations 11 (a) and 12 (b).

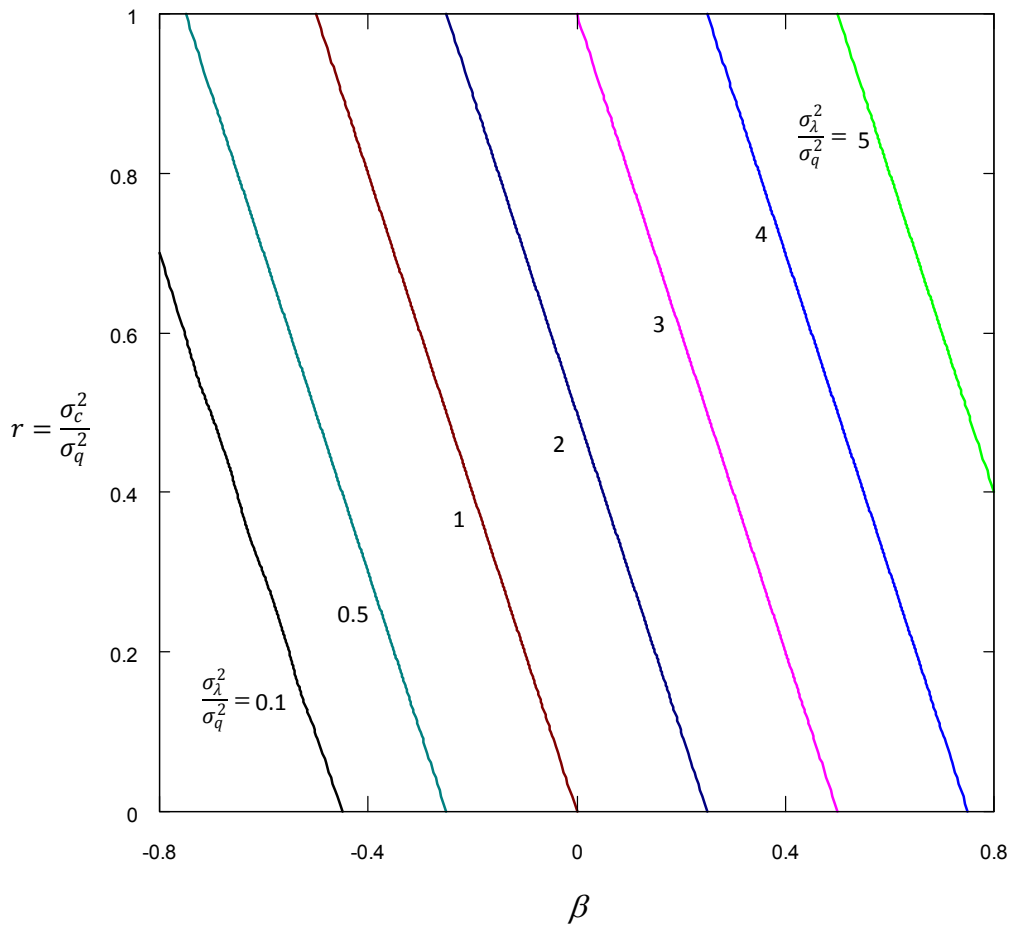


Figure 4-4. Variance of log load (scaled to variance of log discharge), $\frac{\sigma_\lambda^2}{\sigma_q^2}$, as a function of log concentration and log flow variances, $r = \frac{\sigma_c^2}{\sigma_q^2}$, and exponent α (from $C = \alpha Q^\alpha$). Graph based on Equation 23.

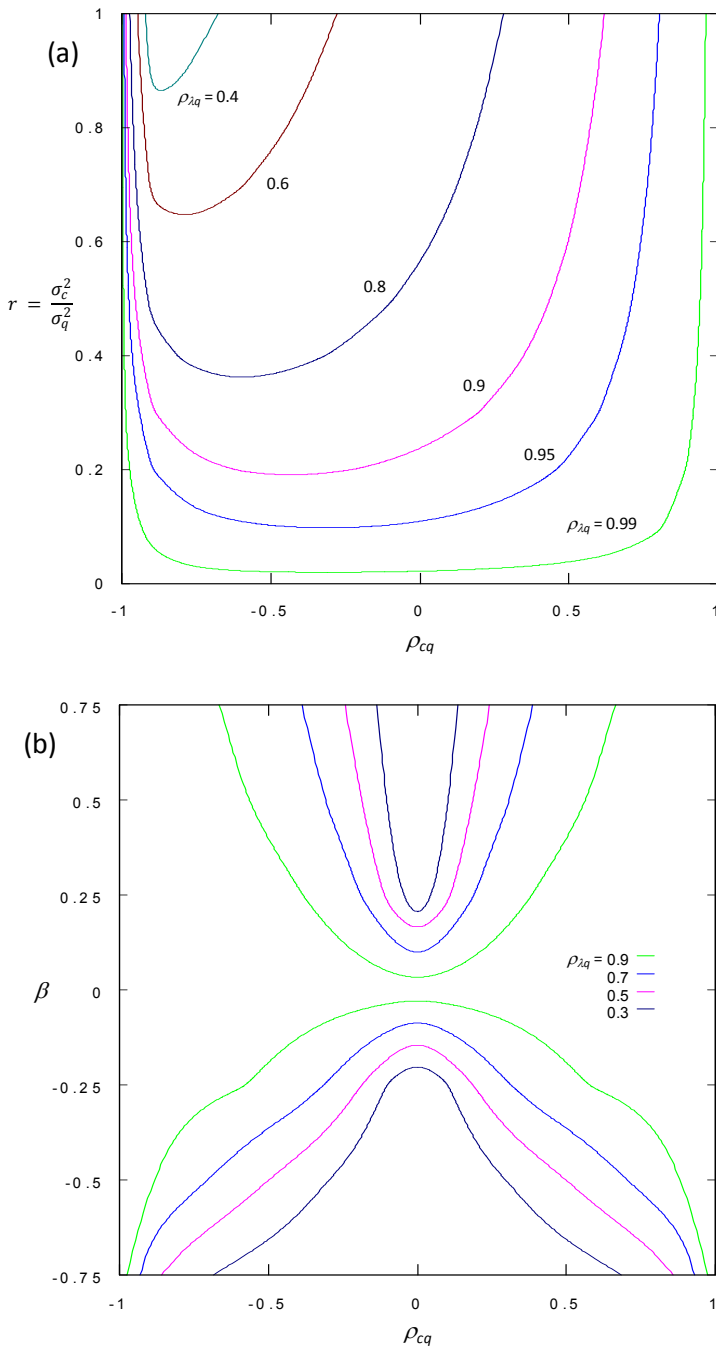


Figure 4-5. Self-correlation between load and discharge based on the correlation between concentration and discharge and (a) the relative variability of these, and (b) β from $C=aQ^\beta$). Graphs based on Equations 24 and 25.

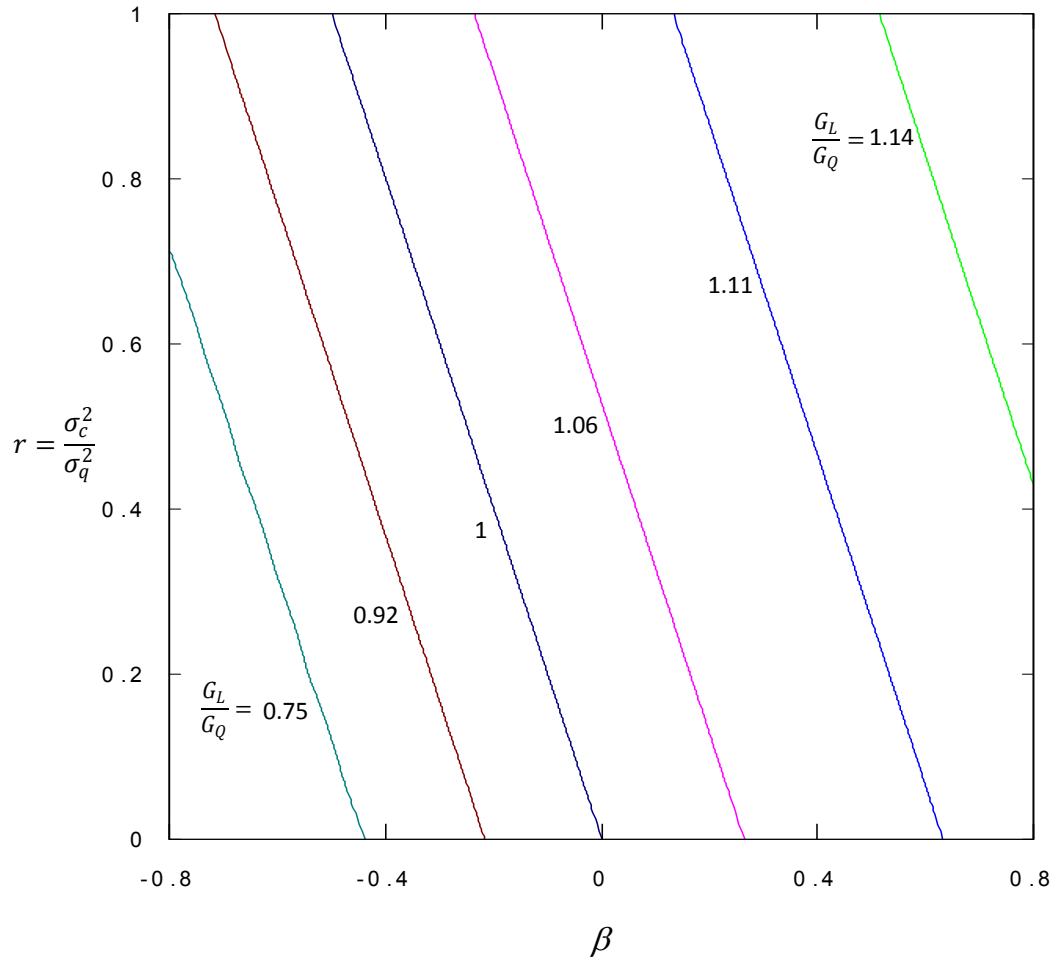


Figure 4-6. The relative inequality of loads compared to flows, as measured by G . The ratio of G_L to G_Q is shown as a function of the relative variability of concentration and discharge, and β is shown as a function of $f_z = 0.5$ and $\sigma_{\ln Q}^2 = 2$. Graph based on Equation 27.

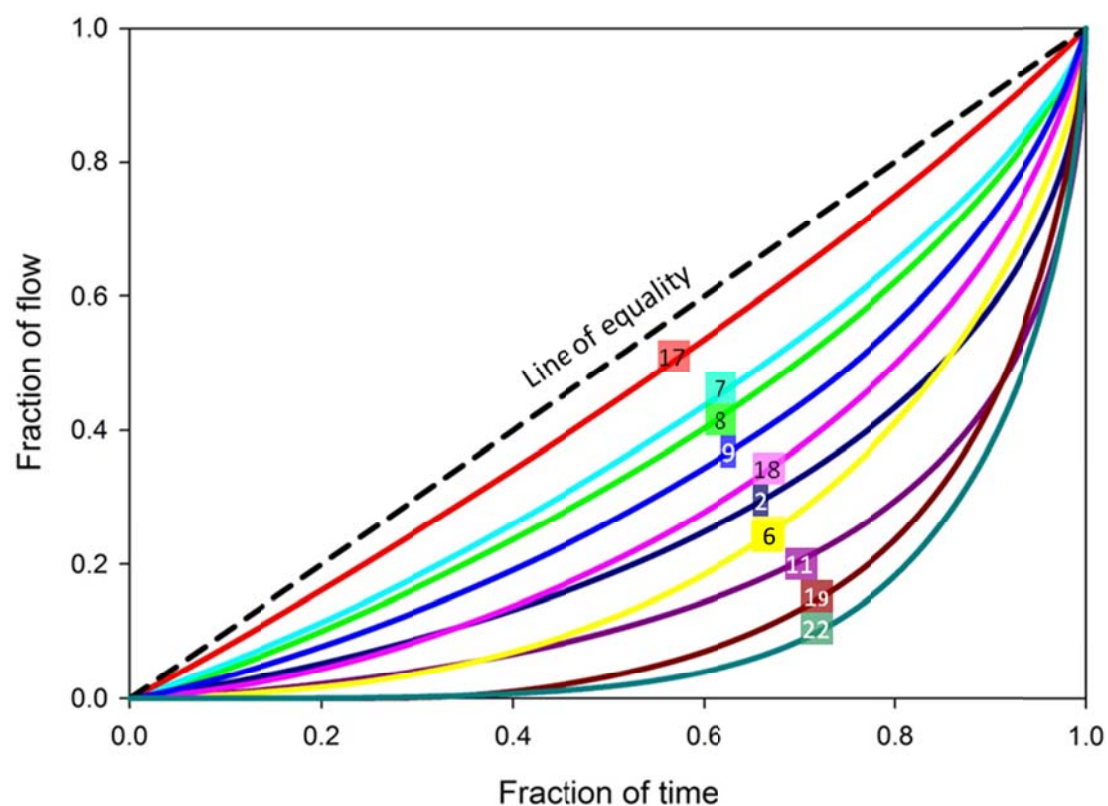


Figure 4-7. Lorenz diagrams for daily flows of nine rivers across Florida. Identification numbers refer to rivers listed in [Table 4-1](#): (2) Blackwater, (6) Ochlockonee, (7) St. Marks, (8) Santa Fe, (9) Suwannee, (11) Northfork Black, (17) Rainbow, (18) Withlacoochee, (19) Hillsborough, and (22) Fisheating Creek.

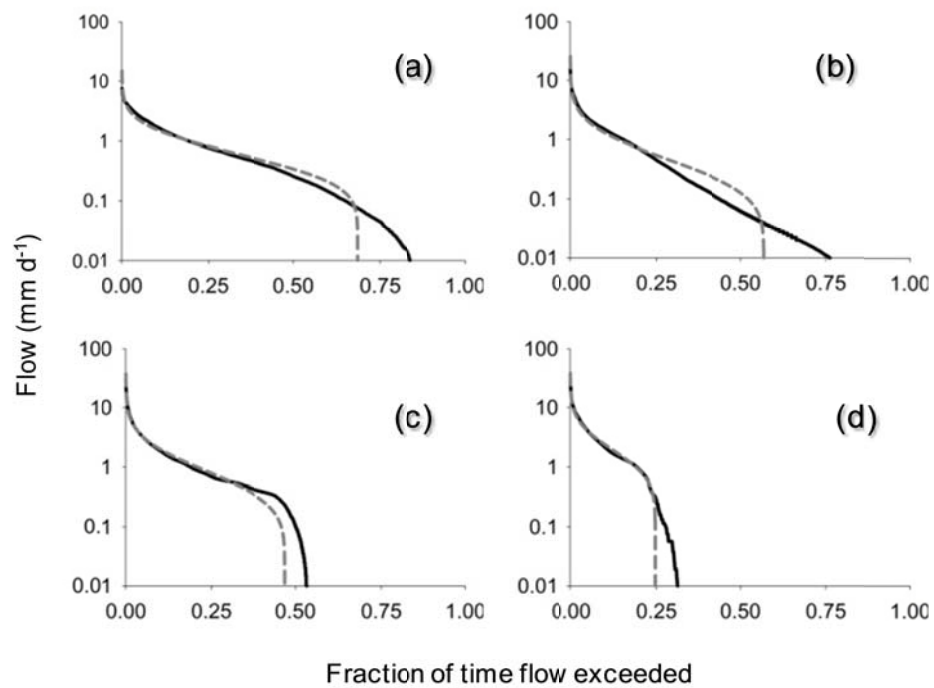


Figure 4-8. Daily average flow duration curves for observed data (solid lines) and lognormal fits (dashed lines) for (a) Kissimmee River, (b) Fisheating Creek, (c) Taylor Creek/Nubbin Slough, and (d) S-154.

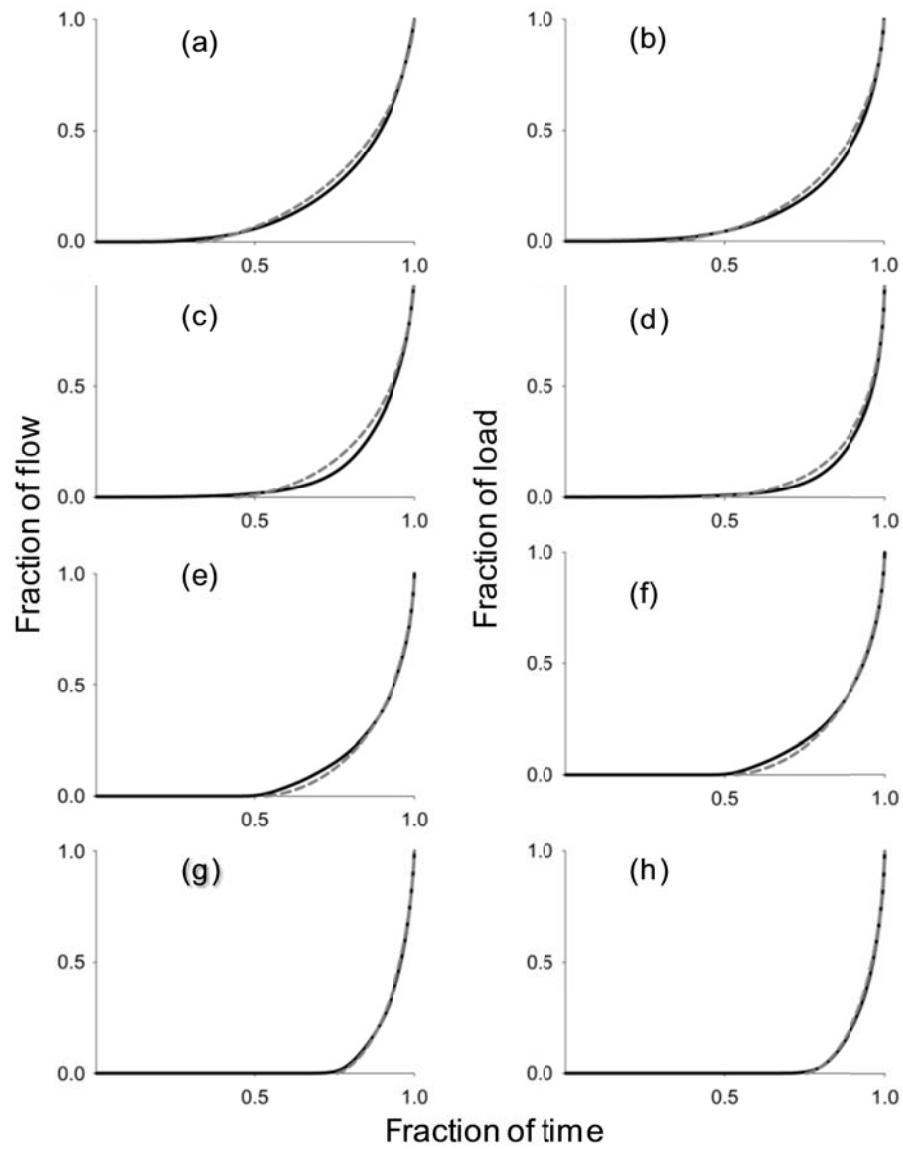


Figure 4-9. Lorenz diagrams for flow and loads for observed data and lognormal fits for the Kissimmee River (a and b), Fisheating Creek (c and d), Taylor Creek/Nubbin Slough (e and f) and S-154 (g and h).

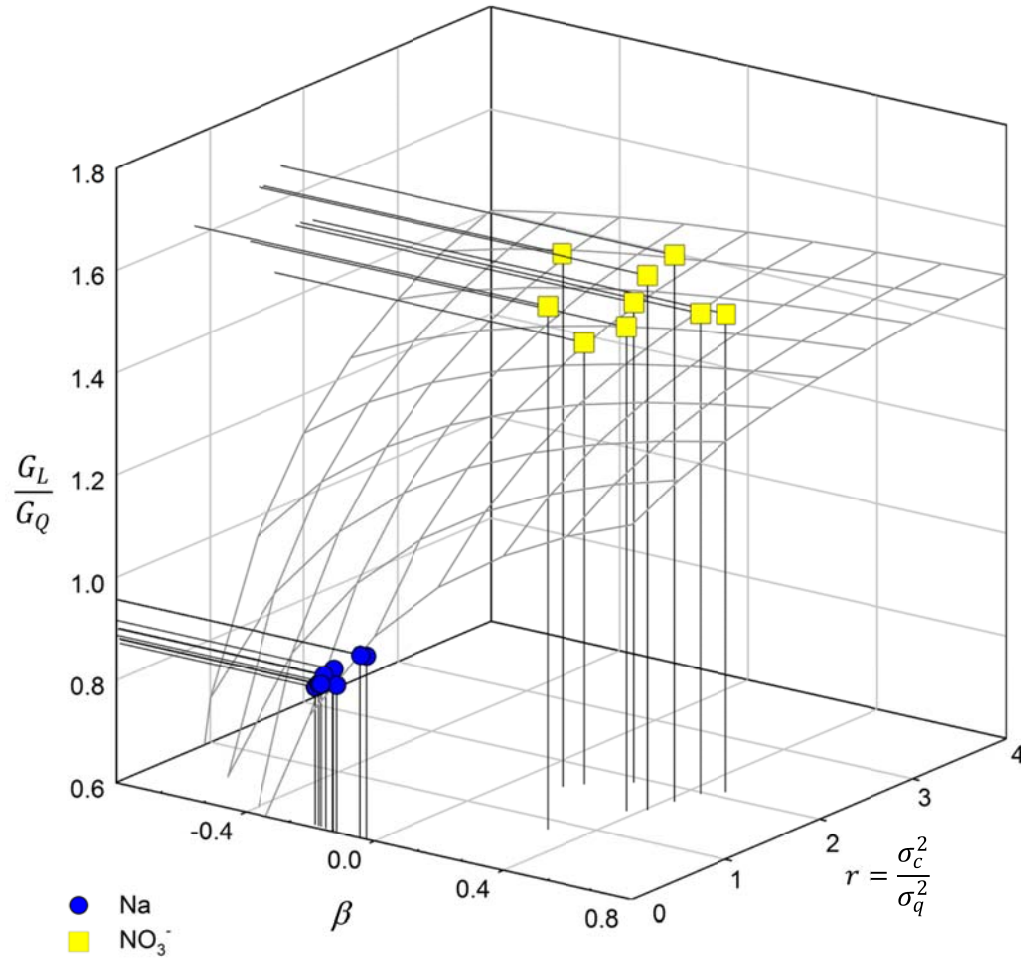


Figure 4-10. The relative inequality of loads compared to flows, the ratio of G_L to G_Q , is shown as a function of the relative variability of concentration and discharge, and β . One data point is shown for each of 9 catchments in the Hubbard Brook Experimental Forest, NH for sodium (Na^+) and nitrate (NO_3^-) based on monthly measurements from 1995 to 2007. The gray curved plane was computed from Equation 27.

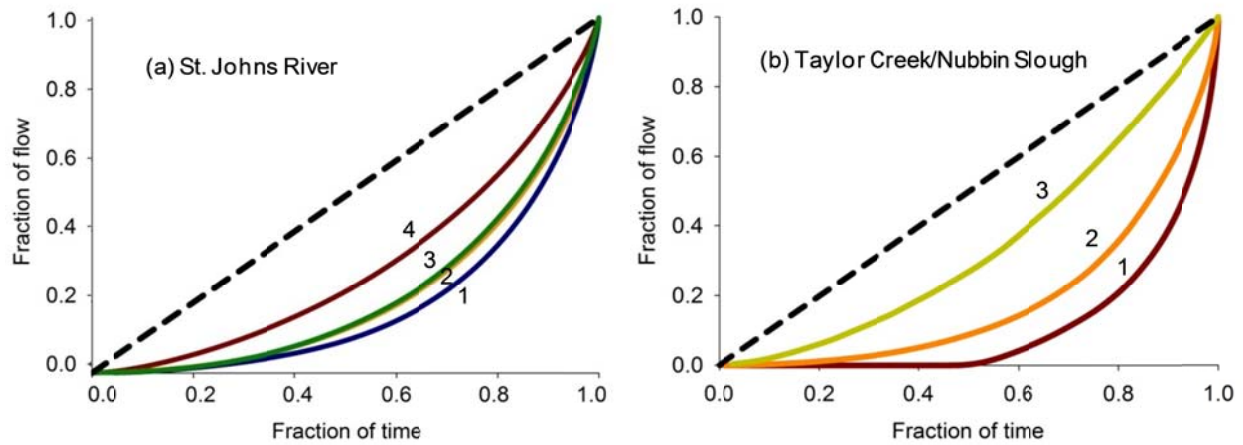


Figure 4-11. Lorenz curves for discharge showing the effects of (a) river reach contributing catchment area, and (b) different time interval averaging. Flows in the St. Johns River (a) were measured at the following USGS gaging stations at increasing distances downstream from the headwaters: (1) 2232000 (near Melbourne, FL), (2) 2232400 (near Coco, FL), (3) 2232500 (near Christmas, FL), and (4) 2236000 (near Deland, FL). Flows in Taylor Creek/Nubbin Slough (b) were averaged at (1) daily, (2) monthly, and (3) yearly intervals.

Additional results from this work published during this fiscal year.

- Min, J-H., Paudel, R., and Jawitz, J.W., 2011. Mechanistic biogeochemical model applications for Everglades restoration: A review of case studies and suggestions for future modeling needs. *Critical Reviews in Environmental Science and Technology*, 41: 6, 489 – 516, doi: 10.1080/10643389.2010.531227.

Abstract: Mechanistic biogeochemical model applications for freshwater wetland ecosystems are reviewed with an emphasis on applications in the Florida Everglades. Two significant human impacts on the Everglades have been hydrologic alteration and phosphorus (P) enrichment. Thus, it is important for research conducted in support of Everglades restoration to integrate understanding of the coupled effects of hydrologic and biogeochemical processes. Models are tools that can facilitate such integration, but an important challenge in model development is determining the appropriate level of model complexity. Previous wetland biogeochemical and flow modeling efforts are categorized here across the spectrum of complexity from empirical and spatially aggregated to mechanistic and spatially distributed. The focus of this review is on mercury and P, as these two elements represent major environmental concerns in this ecosystem. Two case studies of coupled hydrologic and biogeochemical modeling for P transport are described in further detail to illustrate the implications of different levels of model complexity. The case study simulation results on time series TP data revealed that the mechanistic biogeochemical model with more complexity did not guarantee significantly better simulation accuracy compared to the simpler one. It is concluded that the level of model complexity should be represented appropriately based on the modeling objectives, hypotheses to be tested, and data availability. Finally, better integration between data collection and model development is encouraged as cross-fertilization between these processes may stimulate improved system understanding.

- Basu, N.B., Destouni, G., Jawitz, J.W., Thompson, S.E., Loukinova, N.V., Darracq, A., Zanardo, S., Yaeger, M., Sivapalan, M., Rinaldo, A., and Rao, P.S.C., 2010. Nutrient loads exported from managed catchments reveal emergent biogeochemical stationarity, *Geophysical Research Letters*, 37, L23404, 1-5, doi:10.1029/2010GL045168.

Abstract: Complexity of heterogeneous catchments poses challenges in predicting biogeochemical responses to human alterations and stochastic hydro-climatic drivers. Human interferences and climate change may have contributed to the demise of hydrologic stationarity, but our synthesis of a large body of observational data suggests that anthropogenic impacts have also resulted in the emergence of effective biogeochemical stationarity in managed catchments. Long-term monitoring data from the Mississippi-Atchafalaya River Basin (MARB) and the Baltic Sea Drainage Basin (BSDB) reveal that inter-annual variations in loads (LT) for total-N (TN) and total-P (TP), and for geogenic constituents exported from a catchment are dominantly controlled by discharge (QT), leading inevitably to temporal invariance of the annual, flow-weighted concentration. Emergence of this consistent pattern across diverse managed catchments is attributed to the anthropogenic legacy of accumulated nutrient sources generating memory, similar to ubiquitously present sources for geogenic constituents. These responses are characteristic of transport-limited systems. In contrast, in the absence of legacy sources in less-managed catchments, concentration values were highly variable and supply limited. We offer a theoretical explanation for the observed patterns at the event scale, and extend it to consider the stochastic nature of rainfall/flow patterns at annual scales. Our analysis suggests that: (1) expected inter-annual variations in LT can be robustly predicted given discharge variations arising from hydro-climatic or anthropogenic forcing, and (2) water-quality problems in receiving inland and coastal waters would persist until the accumulated storages of nutrients have been

substantially depleted. Our analysis has notable implications on catchment management to mitigate adverse water-quality impacts, and on acceleration of global biogeochemical cycles.

Project Publications

- Basu, N.B., Destouni, G., Jawitz, J.W., Thompson, S.E., Loukinova, N.V., Darracq, A., Zanardo, S., Yaeger, M., Sivapalan, M., Rinaldo, A., and Rao, P.S.C., 2010. Nutrient loads exported from managed catchments reveal emergent biogeochemical stationarity, *Geophysical Research Letters*, 37, L23404, 1-5, doi:10.1029/2010GL045168.
- Bhadha, J. H., Jawitz, J. W. (2010). Characterizing deep soils from an impacted subtropical isolated wetland: Implications for phosphorus storage. *Journal of Soils and Sediments*, 10: 514-525.
- Bhadha, J. H., Harris, W. G., Jawitz, J. W. (2010). Soil phosphorus release and storage capacity from an impacted subtropical wetland. *Soil Science Society of America Journal*, 74, 1816-1825.
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- Dunne, E. J, J. Smith*, D. B. Perkins, M. W. Clark, J. W. Jawitz, and K. R. Reddy. 2007. Phosphorus storages in historically isolated wetland ecosystems and surrounding pasture uplands. *Ecological Engineering*. 31:16–28.
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- Dunne EJ, Clark MW, Mitchell J, Jawitz JW, Reddy KR. 2010. Soil phosphorus flux from emergent marsh wetlands and surrounding grazed pasture uplands. *Ecological Engineering* 36: 1392-1400.
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- Reddy, K. R., S. Newman, T. Z. Osborne, J. R. White, and H. C. Fitz. 2011. Phosphorus cycling in the Everglades ecosystem: Legacy phosphorus implications for management and restoration. *Crit. Rev. Env. Sci. Technol.*, 41, pp. 149–186.

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