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ABSTRACT

Nutrient enrichment in the form of nitrogen and phosphorus is responsible for impairment of more than 5,700 water bodies in the United States (Moore and Kroger, 2011) and is a primary cause of impairment in Florida's lakes, rivers, streams and estuaries. Nutrient fertilizers and human and animal wastewater are considered the main contributors in natural systems as a result of Florida's extensive agriculture and high population density.

Extensive research has been conducted to develop viable, cost-effective technologies to remove nutrients from surface waters and storm and wastewater discharges. One such technology is the Algal Turf Scrubber (ATS™) which was initially developed for treatment of small aquaria, but is currently being used in large-scale stormwater treatment applications.

In this study, six ATS™ pilot studies conducted at sites identified as possible locations for full-scale operations in Florida are compared in an effort to assess nutrient reduction trends under different source water conditions. There is no apparent correlation between water quality characteristics at stormwater sites vs. natural systems, and there was a good deal of variability between and within systems (Table 3).

There is a positive correlation between % TP reduction and influent TN, Mg and Ca concentration (0.61, 0.69 and 0.59, respectively), and a negative correlation with % TP reduction and influent TP and Fe concentration (-0.61 and -0.63, respectively). There is also a correlation between % O-PO₄ reduction and influent Ca concentration (0.65). For total nitrogen, there is a negative correlation between TN concentration reduction and influent total suspended solids concentration (-0.73). Nitrate-nitrite reduction is not well correlated with any routinely measured parameters. While temperature is typically thought to drive nutrient reduction due to the increased productivity of the algal turf, there does not appear to be a significant increase in % nutrient reduction or decrease in effluent concentrations based on temperature among the sites studied.

TP concentration reduction ranged from to 31 percent. Ortho-phosphate reduction ranged from insignificant in an agricultural stormwater canal application to 35 percent at a tidally influenced location receiving primarily urban runoff. Areal removal rate for TP ranged from 3.0 g/m²/yr to 84 g/m²/yr.

There was no significant TN concentration reduction at two of the six sites, and greatest mean TN concentration reduction was 27 percent, observed at a system treating agricultural stormwater runoff. Nitrate-nitrite concentration reduction ranged from insignificant to 59 percent. Areal removal rate for TN ranged from -31.3 g/m²/yr to 698 g/m²/yr.

1 INTRODUCTION

Effects of Elevated Nutrient Levels in Florida

Florida boasts a rich agricultural heritage and is also known as a tourist and retirement destination world-wide. Its tropical and subtropical climates make it ideally located for those looking to escape harsh winters or ailments brought on by less temperate climates. In 2008, Florida was the nation's top producer of sugar cane and most citrus fruits (FDACS, 2011). Additionally, between the years 2000 and 2010, Florida's population growth rate was approximately 18%; almost double the national average.

Of its 54,252 square miles of total land, more than one-fourth is designated as farms and an additional thirty percent of Florida's land is owned by federal, state or local governmental agencies (FDEP, 2011). Perhaps as a result, Florida is ranked tenth in the United States for population density with 350.6 people per square mile, in contrast with the national average of 87.4 people per square mile (U.S. Census, 2010).

Florida's high human density and extensive agricultural production have contributed to water quality degradation, statewide. The Impaired Waters Rule (62-302 and 303, Florida Administrative Code) sets thresholds for pollutants that affect water quality, and was developed in response to the federal Clean Water Act (CWA) of 1972. Under Florida Law, nutrients, including nitrogen and phosphorus are identified as pollutants when found in concentrations that prevent the natural system from functioning within its designated use. For example, most Florida Waters are designated as Category 3 waters, with designated use of "fish consumption, recreation and propagation of a healthy, well-balanced fish and wildlife population". Any pollutant that causes a waterbody to not be viable for these purposes would be considered an impairment. Nutrient impairments are caused by fertilizer, human and animal waste and urban stormwater runoff. In 2010 an estimated eight percent of streams and twenty-six percent of lakes that had been evaluated were designated as impaired for nutrients based on water quality standards for water bodies established by the Florida Department of Environmental Protection (FDEP).

Also in 2010, FDEP analyzed the cost of restoring water quality in impaired waters using its own proposed "numeric nutrient criteria" which were promulgated due to a lawsuit against the Federal Environmental Protection agency, brought on by a consortium of environmental groups claiming that Florida's Impaired Waters Rule was not protective enough or being properly enforced by the state. Using these criteria for nutrient limits, an estimated \$76 million dollars per year would be needed to address nitrogen and phosphorus impacts to Florida's water bodies.

An example land use affects on water quality can be seen in the Florida Everglades. Four of the state's top 10 agricultural producing counties lie south of Lake Okeechobee, which discharges to the historical Everglades. At one time, the Everglades wetland system dominated the entire southern 1/3 of the state from Lake Okeechobee to the Florida Bay and was characterized by extensive sawgrass sloughs, cypress stands and pine flatwoods. This area was drained in the

late 1800's to open the land for development. Rich soils created by centuries of inundation and deposition of organic matter, now exposed some of the most desirable farm land in the southern states. As a result of long-term phosphorus fertilizer applications by agriculture and altered hydrology for urbanization, the Everglades system has suffered from declining water quality, loss of natural bird populations and invasion of exotic species (Bodle et al, 1994). From fiscal years 1999 through 2006, the federal government contributed \$2.3 billion, and Florida contributed \$4.8 billion, for a total of about \$7.1 billion in an effort to restore the Everglades (Highlights of GAO-07-520). Though funding has been reduced in recent years, Florida continues to allocate millions of dollars annually to Everglades restoration.

Aquatic Plant Systems for Nutrient Control

Aquatic plant systems have been implemented and continue to be investigated to mitigate impacts of increased nutrient loads. They range from sub-surface or surface flow treatment wetlands which are considered passive, to more intensively managed and often proprietary technologies such as alum treatment and the Algal Turf Scrubber™. Nutrient removal efficiencies for these systems vary considerably based on the source water quality, local climates, hydraulic retention time and dominant vegetation type.

In Florida, constructed wetlands are commonly utilized for stormwater, and to a lesser extent, wastewater pollution control. Large constructed wetlands known as Stormwater Treatment Areas (STAs) have been extensively used in South Florida as part of Everglades restoration efforts. In the Everglades Agricultural Area (EAA), which lies just south of Lake Okeechobee in Palm Beach, Hendry, Broward and Collier counties, six STAs are in operation. Each STA is unique in size, operational period and dominant vegetation, but they share similarities in that each is composed of individual "cells" which provide for hydrologic control such as residence time and flow pattern.

While not as extensively implemented in Florida, sub-surface constructed wetlands are used in areas where nitrogen is an issue. A recent study comparing subsurface wetland efficiency when treating various wastewater sources for nitrogen reduction showed ranges from 20 percent for landfill leachate treatment to 47% for treatment of agricultural effluent (Vymazal and Kropfelova, 2009). These types of systems are not typically used to target phosphorus removal, as they are generally designed for higher loading rate than is desirable for internal phosphorus processes such as biomass cycling and accretion (Kadec and Wallace, 2009).

Alum (Al_2SO_4) has long been used as a mechanism to reduce phosphorus concentrations in lakes. Mesocosm studies indicate that alum can also be used to enhance phosphorus and nitrogen sequestration in treatment wetlands receiving secondary wastewater effluent, particularly as treatment efficiencies decrease due to accumulation of organic matter, over time (Lindstrom et al., 2011). The degree to which this enhancement occurs depends on influent water quality characteristics, dosage rates and dominant vegetation type but a recent study found that low dosage alum application resulted in an additional 18% reduction in total

phosphorus; and a positive reduction of 8% for vs. -2% for controls for total kjeldhal nitrogen when compared to emergent wetland treatment alone (Malecki-Brown et al., 2009).

A five month study using algal turf scrubbers in Chesapeake Bay tributaries showed mean TN removal rates of 250 mg/m²/day and TP removal of 45 mg/m²/day (Mulbry, et al. 2009) based on tissue analysis. It should be noted that the scale of this study was significantly smaller than those associated with the other technologies described herein.

Algal Turf Scrubber Technology

The Algal Turf Scrubber was developed at the Smithsonian Institution, under direction of Walter Adey, PhD in the 1960s. Early applications were directed toward use for small aquaria, while the commercial license for large scale applications was granted to HydroMentia, Inc. in 1995. Since that time, the ATS™ has been used for wastewater treatment in a commercial aquaculture facility and as tertiary treatment for dairy and industrial wastewater effluent. More commonly, the ATS™ has been applied in the treatment of stormwater runoff in predominantly agricultural areas. The system has been investigated at pilot facilities ranging in size from 3 meters² to 5 acres, with various flow and loading rates (HydroMentia, 2005, 2009). Currently, a full-scale ATS™ is in operation at the Egret Marsh Stormwater Park in Indian River with another facility in design for the same region.

In early commercial applications, the ATS™ was preceded by system in which source water was fed through a lined pond, purposefully stocked with *Eichhornia crassipes* (water-hyacinth) and managed as a monoculture, but data indicated that the ATS could provide sufficient nutrient reduction alone. This was preferable as it reduced treatment costs and eliminated many of the permitting requirements associated with water hyacinth, which is listed as a prohibited aquatic plant in Florida (5B-64.011, F.A.C).

Typical ATS™ systems are constructed over a sloped, graded substrate such as limerock or clean-sand construction fill using polyethylene liner similar to that used under landfills. Over that, a geo-grid material (Apex Mills, Inc.) is secured, which serves as the attachment matrix for filamentous algae assemblages or “algal turf”. The ATS™ headworks is designed to introduce flow over the entire width of the flowway at equal distances and at a constant rate. The flow is pulsed, as this has been shown in many studies to promote productivity and nutrient assimilation by reducing the thickness of the concentration boundary layer and potentially hindering grazers (Barr et al., 2005).

Pilot Studies

Numerous applications of the ATS have been investigated, ranging from wastewater effluent to low-level treatment of constructed wetland effluent in the Everglades Agricultural Area. Studies involving industrial applications are also currently being conducted. After conducting many pilot scale studies using permanently constructed sited, HydroMentia, Inc. recognized the need

to develop a small, mobile pilot unit in order to facilitate fast, cost effective feasibility studies for potential ATS™ applications.

Nitrogen Cycle/Dynamics

A primary purpose of the ATS™ is nitrogen immobilization through biological uptake. The rate at which this occurs depends to some extent upon the physiology of the algal/microbial species present, but is also affected by ambient conditions such as flow rate, water temperature and light availability. Increases in each of these ambient parameters typically result in increased productivity though there is an upper limit, at which time productivity sharply declines (Barr et al., 2008). Water quality parameters such as available carbon and phosphorus, as well as, micro-nutrients also affect nitrogen immobilization as they must be available in sufficient concentration to promote algal growth. Nitrogen can also be removed through ammonia volatilization, which would occur in naturally alkaline waters such as those found in south Florida (high CaCO₃ content) or during periods of high photosynthetic productivity where water column pH is above neutral. While the ATS™ is relatively shallow and the flow regime is designed to maximize dissolved oxygen concentrations, nitrogen removal through denitrification may occur in places along the flowway that have become anaerobic, either through short-circuiting or in the bottom or center portions of a thick algal mat.

Aside from source water, nitrogen may be introduced to the flowway through nitrogen fixation. It has been speculated that this particularly occurs during system start-up when cyanobacteria and diatoms are commonly observed as the first successors. Nitrogen fixation is more likely to occur if available nitrogen (typically NO₃⁻-N) concentration is low, or is low in relation to available phosphorus (low N:P), provided that nitrogen fixing bacteria or periphyton are present. In this process, the nitrogenase enzyme is required to reduce nitrogen gas to ammonia. It should be noted that this phenomena has not been studied on the ATS™, and would not be expected to contribute a significant amount of nitrogen due to the high oxygen content in the sourcewater, and the likelihood that any deficiencies in available nitrogen concentration may be overcome by the high loading rate of the systems, which would supply ample nitrogen on a mass basis to facilitate algal growth (Clark et. al, 2012).

Other, internal nitrogen processes may also occur. For instance, though harvesting is conducted in order to minimize algal turf turn over, there is likely to be some mineralization of organic-N to ammonium (ammonification). This ammonium-N could then be re-immobilized, volatilized or oxidized and discharged in the effluent water as NO_x- N. Because the algal turf is an assemblage of various algal and microbial species; and growth rates, oxygen concentration (redox potential) and nutrients are not homogeneous over the system, it is likely that these processes may occur simultaneously within different micro or macro zones of the ATS™.

Phosphorus Cycle/Dynamics

As with nitrogen, ATS™ systems are optimized for biological uptake of phosphorus as the primary nutrient removal mechanism (HydroMentia, 2005). This optimization is achieved

through surge wave introduction of the source water as a method to introduce adequate supplies of nutrients and disrupt the concentration boundary layer to ease diffusion of nutrients to cellular uptake sites (Adey and Loveland, 1998).

Characterization of phosphorus dynamics is fairly simplified due to the absence of soil substrate and routine biomass. Phosphorus entering the ATS™ may be dissolved or particulate; inorganic or organic as found in a typical wetland watercolumn. Only dissolved inorganic phosphorus in the form of orthophosphate (O-PO₄) is biologically available (Reddy and DeLaune, 2008), however there is sufficient evidence that micro-algae are able to incorporate greater amounts of phosphorus into tissue than is necessary for viability as luxury uptake (Powell et. al, 2011). Phosphorus may also be removed from the water column through co-precipitation of particulate forms with Ca, Mg, Al or Fe also, particularly in pH above neutral which is typical in alkaline systems in south Florida.

Though the systems are routinely harvested in order to minimize necrosis of the algal material and mineralization of assimilated phosphorus, there is indication that organic phosphorus is converted to inorganic forms down the floway, making it biologically available. This may be an important contributor to ATS™ performance as phosphorus removal is achievable down the floway, even under very low phosphorus conditions (HydroMentia, 2005).

Algal Physiology and Nutrient Assimilation

Algal turf is a complex assemblage of simple organisms. While dominated by algal species, bacteria, diatoms and macro invertebrates are important inclusions in ATS™ systems. ATS™ are not “seeded”, but develop algal turf from algal species directly within the source water. Not only is there variability in the succession of the systems from start-up through operation and between seasons, but changes in composition have been noted down the floways, from influent to effluent. This is likely due to changes in nutrient composition; however water quality sampling is typically limited to influent and effluent stations, therefore little data is available to evaluate this theory. Visual examination and identification of algal species is frequently conducted in the field.

Chlorophyta and Rhizochlonium species are commonly dominant in ATS™ systems, though diatoms can be found to dominate at times (HydroMentia, 2010). Because dissolved oxygen is typically in sufficient supply, due to the surge introduction of source water and shallow operation of the system, blue-green algae are not typically observed. There are 21 recognized species of rhizochlonium, which is a filamentous, slender algae growing in fresh, brackish and marine waters, often with other algae species, forming a dense mat. The species at Powell Creek was identified as Rhizochlonium hieroglyphicum, of which 6 variations have been identified and most are found in marine environments. Chlorophyta species are even more prevalent, with over 700 species occurring in almost all aquatic environments. **EXPAND**

3 MATERIALS AND METHODS (FOR THE ANALYTICAL PROCESS)

General Description of Algal Turf Scrubber Treatment System and Monitoring Methods

Six pilot studies were conducted as preliminary assessments of Algal Turf Scrubber treatment efficacy at sites that were identified as having potential for full-scale systems (Table 1). The first study was conducted at the L-62 canal in Okeechobee, FL on a previously existing, modified, full-scale ATS™ system (later referred to as the S-154 pilot). During that study, three ATS™ flowways were operated in parallel at target flow rates of 5 gpm, 10 gpm and 20 gpm to determine the effect of hydraulic loading rates on system performance. Maximum total phosphorus load reduction was observed at the highest loading rate, which was 18.9 gpm based on actual flow measurements. Only the results of the flowway that was loaded at 18.9 gpm are included as part of this report to maintain comparability between systems. The remaining five systems were designed at a flow rate of 18-20 gpm as a result of this finding.

Apart from the S-154 pilot, the other studies were conducted on Mobile Pilot Units (MPU), which were developed specifically for the purpose of preliminary assessment of ATS™ efficacy. The MPU systems were constructed of aluminum with the main components being the influent surger box, flowway and effluent sump station. Water was discharged into the flow-way using an automatic surger unit in order to introduce flow in pulses, which is an integral component of ATS™ operations. The flowways were overlain with a high density polyethylene liner for waterproofing and on top of that, a geotextile mesh netting (Apex Mills), was placed to serve as the growth substrate for the algal turf (Figure 1). The length of each individual MPU flowway varied and was determined using HydroMentia's ATSDem model where available carbon, total phosphorus and total nitrogen within the source water along with temperature and estimated algal turf nutrient content were inputs (HydroMentia, 2005). The ATSDem model uses first order Monod growth kinetics to estimate potential nutrient assimilation based on these inputs (HydroMentia, 2005). Flowway length is a parameter in the output, along with expected nutrient concentration at a the specified length. The point where minimum nutrient concentration is realized was used as the design pilot flowway length.

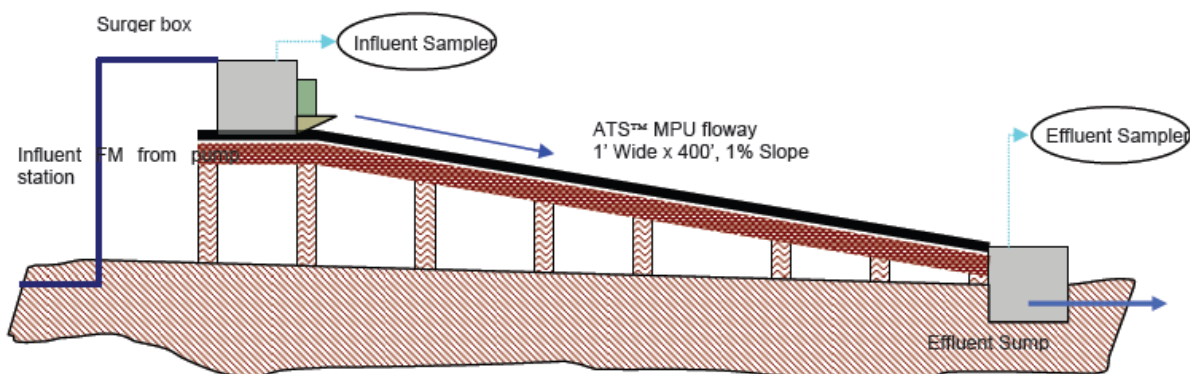


Figure 1. Schematic of Pilot-Scale Lake Lawne ATS™ system. (Not to scale)

Flow measurements were collected using in-line flow meters (Global Water, Inc.) prior to the surger box and for the purposes of this report, effluent flow is considered equal to influent flows with only minimal evaporation losses. Total phosphorus and total nitrogen samples were based on daily samples collected using Sigma900 Autosamplers that were then composited and collected on a weekly bases. Other water quality parameters were collected as grab samples. Ambient water quality parameters were collected using YSI-556 multi-parameter datasonde. Influent samples were collected from within the surger box and effluent samples were collected within the effluent sump. in the same locations.

Routine biomass harvesting is a fundamental operational component of the ATS™. On the pilot systems this was done using a simple squeegee and dustpan to scrape attached algal turf from the grid and collect it prior to weighing. Weights were typically collected at ¼, ½, ¾ and at the distal end of the flowway length, though this varied between sites. A subsample of each incrementally wet weighed portion of harvested biomass was collected and dried for 72 hours. Typically, monthly nutrient and metals analysis was conducted on composited samples from each incremental distance.

Mobil Pilot Unit Site Location and Descriptions

The six pilot ATS™ evaluation sites were located at five geographically distinct locations with six different water sources (Figure 2). There is no apparent correlation between water quality characteristics at stormwater sites vs. natural systems, and there was a good deal of variability between and within systems (Table 3). Site descriptions and the abbreviations used to identify them in the rest of this report are:

S-154 (S-154)

S-154 is a specific sub-basin of Lake Okeechobee. The study site was one mile west of the city of Okeechobee, contiguous to the L-62 canal, which is the primary drainage canal for the sub-basin.

Lake Lawne (LkLn L)

Lake Lawne is a 146-acre lake in highly-urbanized Orlando, FL. Though in an urban area, the entire eastern portion of the lake is surrounded by Barnett Park, and to the north, south and east primarily by residential subdivisions and natural areas. The pilot system received water directly from the lake.

Lake Lawne Stormwater (LkLn St)

The stormwater canal at Lake Lawne which served as the source for this MPU is the primary drainage canal for the urban and industrial areas in the north eastern portion of the watershed. This system experienced more frequent periods of intermittent flows than the other pilot systems.

Stormwater Treatment Area 1 West (STA-1W)

This pilot was situated at the effluent of a large constructed wetland in Palm Beach County in an area known as the Everglades Agricultural Area. The intent of this study was to determine the capability of the ATS™ to achieve an effluent total phosphorus concentration of 10 µg/L.

An evaluation of the potential for further nutrient concentration reduction through solids screening (filtering) was conducted at this site. However, the water quality values used in this report are the pre-screened results, as the screening analysis was not conducted at any of the other pilot sites.

Powell Creek Bypass Canal (PCBP)

The Powell Creek Pilot received water from a stormwater canal discharging to the Caloosahatchee River. This tidally influenced canal is the primary drainage conduit for the 13-square mile Powell Creek watershed.

Santa FeThe Santa Fe River is a natural waterbody in a mixed natural and agricultural watershed in north central FL. This area of Florida is characterized by karst topography, and the Lower Santa Fe River is heavily influenced by springs and sinks along its path. The pilot site was located in northern Alachua County along the middle reach of the Santa Fe River.

Table 1. MPU/site location, period of record evaluated, treatment area, length of flow path and system slope.

Project	Location	Period of Record	Treatment Area (m ²)	Length (ft)	Slope (%)
LKLN_L	Orange County, FL	1/21/2009-6/24/2009	37.2	400	1
LKLN_ST	Orange County, FL	1/21/2009-6/24/09	37.2	400	1
PCBP	North Ft. Myers, FL	12/11/2008-12/10/2009	46.5	500	1
S-154	Okeechobee, FL	5/17/2004-2/7/2005	139	300	1.5
Santa Fe	Alachua, FL	3/16/2010-3/01/2011	46.5	500	1
STA-1W	West Palm Beach, FL	8/20/2008-8/12/2009	111.4	1,200	0.5

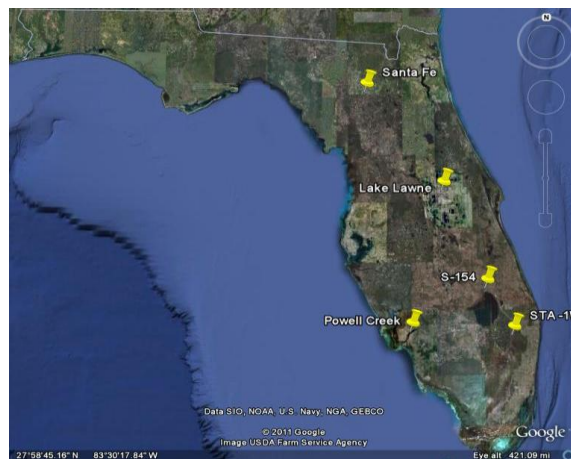


Figure 2. General locations of the six ATSTM pilot studies

4 RESULTS AND DISCUSSION

Evaluation of Influent Water Quality Conditions

Overall, site mean influent TP ranged from 35 μgL^{-1} at STA-1W to 341 μgL^{-1} at S-154. Lowest mean influent concentration for labile phosphorus (O- PO_4) was approximately 2 μgL^{-1} and was observed at three sites: LKLN_L, LKLN_ST and STA-1W. Highest mean O- PO_4 concentration was observed at Santa Fe (21 μgL^{-1}) (Table 2).

Mean influent TN concentrations ranged from 0.91 μgL^{-1} at LKLN_L to 2.49 μgL^{-1} at STA-1W. LKLN_L represented the lowest mean labile nitrogen ($\text{NO}_2 + \text{NO}_3$ or NO_x) concentration (0.02 μgL^{-1}). Highest mean influent NO_x concentration was at Santa Fe (0.13 μgL^{-1}), however this was not significantly different than the mean influent NO_x concentration at STA-1W (0.12 μgL^{-1} , One Way Anova, $P = 0.387$).

In terms of nutrient ratios, lowest mean influent TN:TP was observed at Santa Fe (4:1), while highest N:P was observed at STA-1W (77:1). Ratios of labile nitrogen to phosphorus parameters measured were also shown to be lowest at Santa Fe (0.72:1) and highest at STA -1W (12:1).

Measured macronutrients at all sites were limited to Fe, Mg and Ca. In general, LkLn_L and LkLn_ST showed lowest mean influent concentrations for these parameters (282 $\mu\text{g L}^{-1}$, 2.8 mg L^{-1} and 26.2 mg L^{-1} , respectively at LkLn_L and 722 $\mu\text{g L}^{-1}$, 2.5 mg L^{-1} and 23.4 mg L^{-1} , respectively at LkLn_ST). The exception is mean influent Fe at PCBP, which was 120 $\mu\text{g L}^{-1}$; lower than that observed at the LkLn sites. PCBP showed highest mean concentration for Mg and Ca (115 mg L^{-1} and 228 mg L^{-1} respectively), though these are the result of only one sample event. STA-1W showed consistently higher Mg and Ca than most other sites (23.8 mg L^{-1} and 42.7 mg L^{-1} , respectively) (Table 3).

Mean Influent pH ranged from 6.53 at S-154 to 7.69 at PCBP. S-154 also exhibited the lowest mean influent DO (4.7 mg/L); highest mean influent DO was at LKLN_ST (8.48 mg/L). Lowest mean influent temperature and conductivity were observed at Santa Fe (19.92 $^{\circ}\text{C}$ and 117 $\mu\text{S/cm}$, respectively). Highest mean influent temperature was observed at S-154 (24.42 $^{\circ}\text{C}$) and highest mean influent conductivity was observed at PCPB (10,394 $\mu\text{S/cm}$) (Table 4).

Table 2. Influent mean concentrations for nitrogen and phosphorus species

Project	TP ($\mu\text{g/L}$)	O- PO_4 ($\mu\text{g/L}$)	O- PO_4	TN (mg/L)	TKN (mg/L)	$\text{NO}_2 + \text{NO}_3$ (mg/L)	$\text{NO}_2 + \text{NO}_3$ (as % of TN)	TN:TP	$\text{NO}_x:\text{OPO}_4$
LKLN_L	53 \pm 28	18 \pm 14	34 \pm 17	0.91 \pm 0.10	0.89 \pm 0.10	0.02 \pm 0.01	2.10 \pm 1.53	21 \pm 8	1.54 \pm 1.33
LKLN_ST	203 \pm 207	24 \pm 18	14 \pm 3	1.38 \pm 0.85	1.36 \pm 0.84	0.03 \pm 0.02	2.04 \pm 0.98	9 \pm 3	2.12 \pm 2.04
PCBP	140 \pm 74	109 \pm 68	69 \pm 18	0.96 \pm 0.23	0.91 \pm 0.22	0.05 \pm 0.04	5.56 \pm 3.62	8 \pm 3	0.69 \pm 0.80
S-154	341 \pm	168 \pm 292	28 \pm 29	1.83 \pm	1.77 \pm	0.06 \pm	3.68 \pm	10 \pm 7	5.26 \pm

	323			0.55	0.55	0.06	3.54		16.2
Santa Fe	259 ± 62	211 ± 51	79 ± 19	1.07 ± 0.31	0.95 ± 0.29	0.13 ± 0.07	11.5 ± 6.33	4 ± 1	0.72 ± 0.69
STA-1W	35 ± 12	16 ± 10	45 ± 24	2.49 ± 0.51	2.37 ± 0.49	0.12 ± 0.07	4.66 ± 2.84	77 ± 29	12.3 ± 10.4

Table 3 Influent mean water quality concentrations- ambient and other macro-nutrients

Project	TSS (mg/L)	ALK (mg/L)	TOC (mg/L)	FE (µg/L)	MG (mg/L)	CA (mg/L)
LKLN_L	1.86±1.47	59.83±13.4		282±252	2.83±0.62	26.12±4.23
LKLN_ST	48.64±8.18	4		722±445	2.47±0.14	23.38±2.34
PCBP	6.77±5.39	196.00±41.44	18.45±2.29	20	228	115
S-154	10.54±5.25	44.07±9.87	26.55±4.12	1,319±707	14.0± 8.4	28.38±13.0
Santa Fe	2.61±2.80	30.33±13.9	34.53±14.36	489±237	4.93±1.5	12.84±2.8
STA-1W	3.30±1.96	205.27±58.05	37.64±5.45	443±1,280	23.8±4.11	42.73±19.0

Table 4. Influent mean ambient water quality concentrations

Project	PH	DO (mg/L)	TEMP (°C)	COND (µS/cm)
LKLN_L	7.11± 0.46	7.94±1.3	21.61± 4.76	337±271
LKLN_ST	7.10± 0.64	8.48± 0.97	21.99±4.91	246±82.1
PCBP	7.69± 0.45	7.51± 1.78	24.42±5.05	10,394±12,226
S-154	6.53± 0.27	4.70±1.45	27.78±1.23	
Santa Fe	7.12± 0.42	6.95±1.65	19.92±6.56	117±20.3
STA-1W	7.53± 0.26	6.04±1.99	24.29±4.52	943±151

Evaluation of system performance

2.3 Evaluation of System Performance

An assessment of percent nutrient reduction with influent water quality parameters was conducted to identify whether or not obvious relationships exist that could be used to predict performance. Correlations are based on Residual Maximum Likelihood analysis (REML). The individual datasets were analyzed for normality based on percent reduction of total phosphorus, total nitrogen, ortho-phosphorus and nitrate. Of 239 sample events for all projects, 9 were excluded (2 from STA-1W; 2 from Lake Lawne Stormwater; 1 from Lake Lawne Lake; 1 from Powell Creek and 3 from S-154). In general, events that were outside the 95th

percentile for two or more of these parameters were omitted and corresponded with field data or notations that justified the omission, such as, visible sediments in the sample due to significantly increased or reduced flow during the preceding week, vandalism or sampler error. In instances where Ortho-P values were greater than total phosphorus values for the same week, they were omitted.

Of parameters measured that could be driving nutrient reduction among all sites, there is a positive correlation between % TP reduction and influent TN, Mg and Ca concentration (0.61, 0.69 and 0.59, respectively), and a negative correlation with % TP reduction and influent TP and Fe concentration (-0.61 and -0.63, respectively). There is also a correlation between % O-PO₄ reduction and influent Ca concentration (0.65). For total nitrogen, there is a negative correlation between TN concentration reduction and influent total suspended solids concentration (-0.73). Nitrate-nitrite reduction is not well correlated with any routinely measured parameters. While temperature is typically thought to drive nutrient reduction due to the increased productivity of the algal turf, there does not appear to be a significant increase in % nutrient reduction or decrease in effluent concentrations based on temperature among the sites studied.

Greatest mean TP concentration reduction was observed at STA-1W (31 percent); with a mean effluent concentration of 24 µg L⁻¹. This site also had the the lowest mean effluent TP concentration among all sites. There was no significant reduction of TP concentration at LkLn_L (One-way ANOVA P=0.88 for influent vs. effluent). Overall, TP effluent reduction ranged from insignificant at LkLn_L (-1 percent) to 31 percent at STA-1W. Additionally the difference in percent reduction of TP at Powell Creek and S-154 was 22 and 28 percent, respectively; (Figure 3).

Greatest mean and O-PO₄ reduction was observed at PCBP (35 percent and mean effluent concentration of 78 µg L⁻¹). The lowest mean effluent concentration for O-PO₄ was 0.010 µg L⁻¹, observed at STA-1W. O-PO₄ percent reduction ranged from insignificant at S-154 (-3 percent) to 35 percent at PCBP (Figure 3).

Considering all pilot studies, regression analysis shows a relationship between influent and effluent total phosphorus concentrations ($r^2=0.88$), and an approximate 16% reduction overall. A significant relationship also exists for influent vs. effluent O-PO₄ between projects ($r^2=0.97$) and a mean overall O-PO₄ concentration reduction of 9% (Figure 4).

Though there was no significant reduction of TP concentration at LkLn_L, the site did show a mean O-PO₄ reduction of 32 percent, with an effluent concentration of 14 µg L⁻¹. Conversely S-154 showed a mean TP concentration reduction of 28 percent, although there was no significant reduction of O-PO₄ (One-way ANOVA P=0.445 for influent vs. effluent concentrations). It is possible that conversion of organic phosphorus to more labile forms, and O-PO₄ assimilation into organic or particulate forms could be occurring in different proportions to explain the changes in phosphorus composition between influent and effluent concentrations.

Another explanation for the significant reduction in O-PO₄ with no reduction in TP at LkLn_L may be an artifact of sampling practices at that site, in which large amounts of solids were periodically deposited at the effluent sampling location. The TP samples were collected with an autosampler, while O-PO₄ and NO_x were collected from grab samples. Likewise, TP reduction at the stormwater site may be reflective of solids accumulating at the intake tubing on that flowway.

High TP removal at STA-1W may be attributed to the high mineral content of the source water, which can lead to co-precipitation of phosphorus with calcium during photosynthetic activity as pH increases. Though not a function of direct algal uptake, calcium precipitation resulting from algal photosynthetic activity is likely to be a significant mechanism of O-PO₄ removal in waters where the effluent concentration goals are low and calcium content of influent water is high. Co-precipitation of phosphorus with calcium is further discussed in the section titled “Vegetation Data”.

Greatest mean TN concentration reduction was observed at S-154 (27 percent). There was no significant concentration reduction in total nitrogen at LkLn_L or STA-1W (One Way, P=0.73 and P= 0.9, respectively for influent vs. effluent TN concentrations).

Greatest mean NO_x concentration reduction was observed at Santa Fe (59 %), while NO_x concentration reduction was insignificant at S-154 (P=0.49 when comparing influent to effluent concentration). Project specific NO_x reduction ranged from 2.6% at S-154 to 59% at Santa Fe. Despite no reduction in TN at LkLn_L or STA-1W, significant reductions of NO_x were observed (10 percent and 33 percent, respectively).

Considering all pilot studies, regression analysis shows a relationship between mean influent and mean effluent total nitrogen concentrations ($r^2=0.88$), with a 9% reduction overall relative to influent concentrations. Influent vs. effluent NO_x concentrations between pilot studies are less predictable ($r^2=0.46$); with overall mean concentration reduction estimated at 30% (Figure 4).

In terms of other water quality parameters, only pH showed a significant difference between influent and effluent at each site. Mean temperature, alkalinity and conductivity were not significantly different when comparing influent to effluent at each site. DO was significantly increased at the effluent at both STA-1W and Santa Fe (Table 5).

Table 5. Influent and Effluent Environmental Parameters

Project	Conductivity ($\mu\text{S/cm}$)		Temperature ($^{\circ}\text{C}$)		pH		Dissolved Oxygen(mg/L)		Alkalinity(mg/L)	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
LKLN_L	337	338	21.61	24.24	7.11	8.24	7.94	9.47	60	58
LKLN_ST	246	246	21.99	25.09	7.1	8.02	8.48	8.93	.	.
PCBP	10,400	10,400	24.42	25.99	7.69	8.37	7.51	9.76	196	194
S-154	.	.	27.78	28.26	6.53	8.34	4.7	8.82	44	39.8
SantaFe	117	117	19.92	19.92	7.12	8.07	6.95	11.32	30.3	.
STA-1W	943	929	24.29	26.09	7.53	8.38	6.04	12.69	205	.

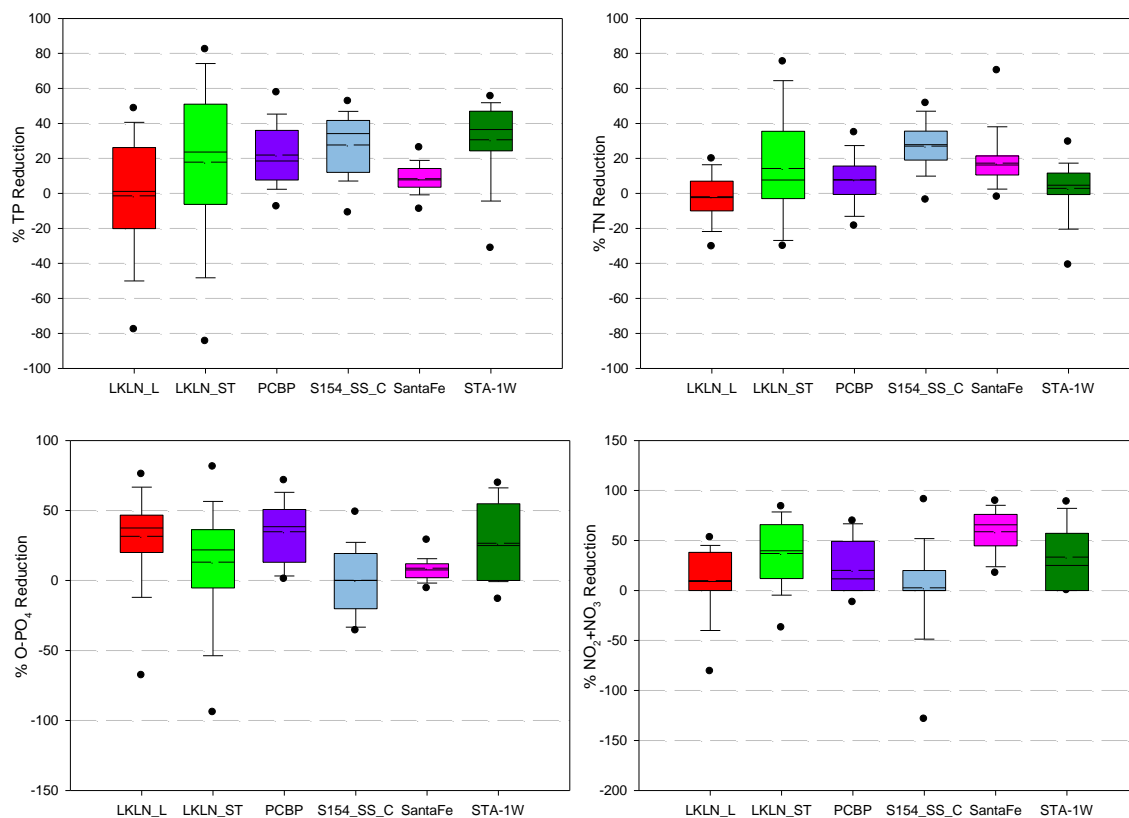


Figure 3. Percent reduction of ATS pilot study measured nutrient parameters. Boxes represent 25th and 75th percentile, while “whiskers” represent 10th and 90th percentile. Lower and upper dots represent 5th and 95th percentile. Dashed line represents mean, while solid line represents median.

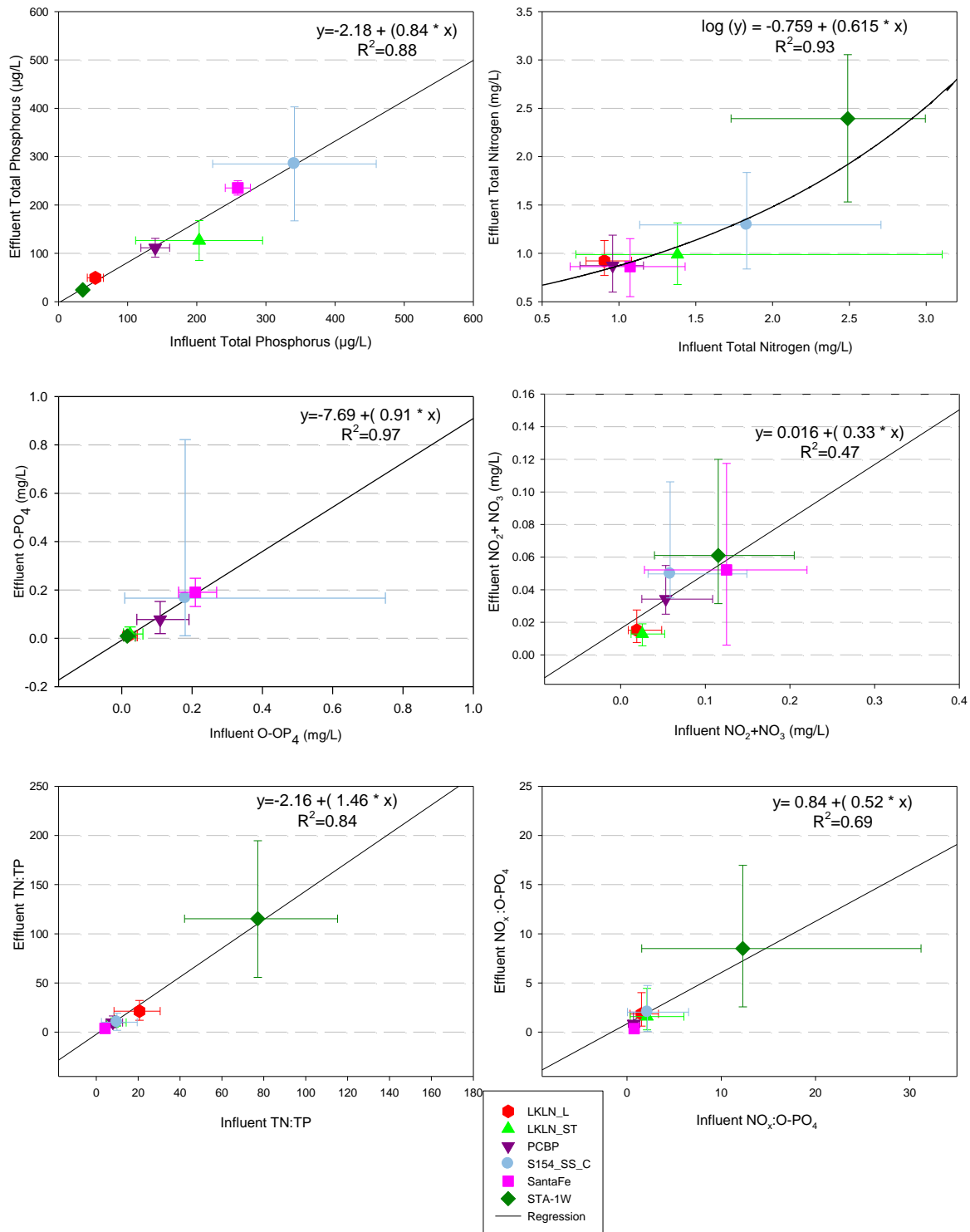


Figure 4. Regression analysis for influent vs. effluent concentration nutrient parameters at 6 ATS pilot studies

Comparison of Removal Efficiencies

In terms of nutrient loading, S-154 received highest mean mass of total phosphorus and total nitrogen ($450\text{g}/\text{m}^2/\text{yr}$ and $2,318\text{g}/\text{m}^2/\text{yr}$, respectively). S-154 also exhibited the highest area removal rate for these parameters ($84.4\text{ g}/\text{m}^2/\text{yr}$ and $698\text{ g}/\text{m}^2/\text{yr}$, respectively).

STA-1W received the lowest loading rate of total phosphorus and total nitrogen ($10.6\text{ g m}^{-2}\text{ yr}^{-1}$, and $673\text{ g m}^{-2}\text{ yr}^{-1}$, respectively) (Table 5). STA-1W also exhibited the lowest area removal rate for TP ($3.0\text{ g m}^{-2}\text{ yr}^{-1}$), while Lake Lawne- Lake exhibited the lowest TN areal removal rate ($-31.2\text{ g m}^{-2}\text{ yr}^{-1}$) (Table 6).

There is an increase in total phosphorus removal efficiency over time for both Lake Lawne floways, and slight increase in efficiency for Powell Creek and STA-1W (Figure 5A decrease in removal efficiency is shown at Santa Fe and S-154). All sites showed similar, though less dramatic trends for total nitrogen removal efficiency over time (Figure 6).

The short duration of the pilot studies, ranging from six months to one year, limits the validity of findings related to trends of removal efficiency over time. However, when comparing monthly influent loading and removal rates, the systems do not seem to be negatively affected by wide variations in influent water quality (Figures 7 and 8).

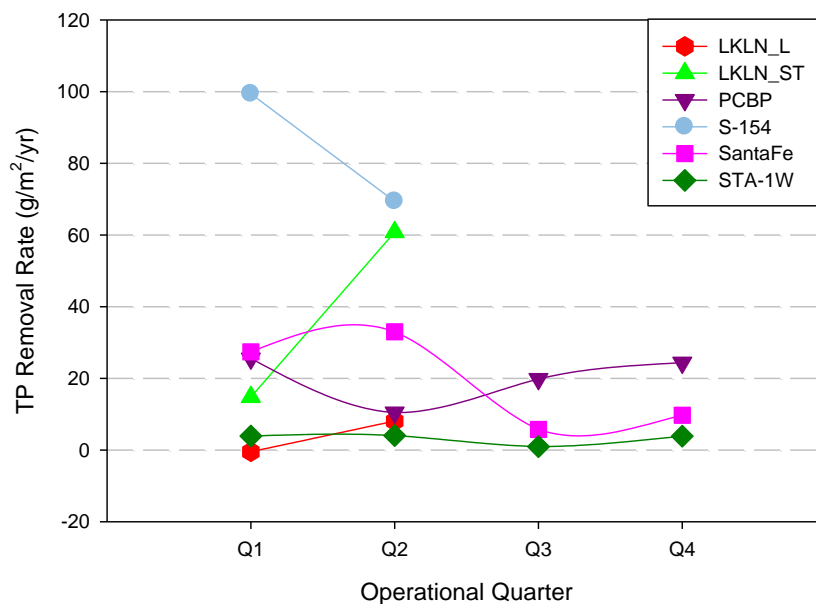


Figure 5. TP removal rate by site and operational quarter

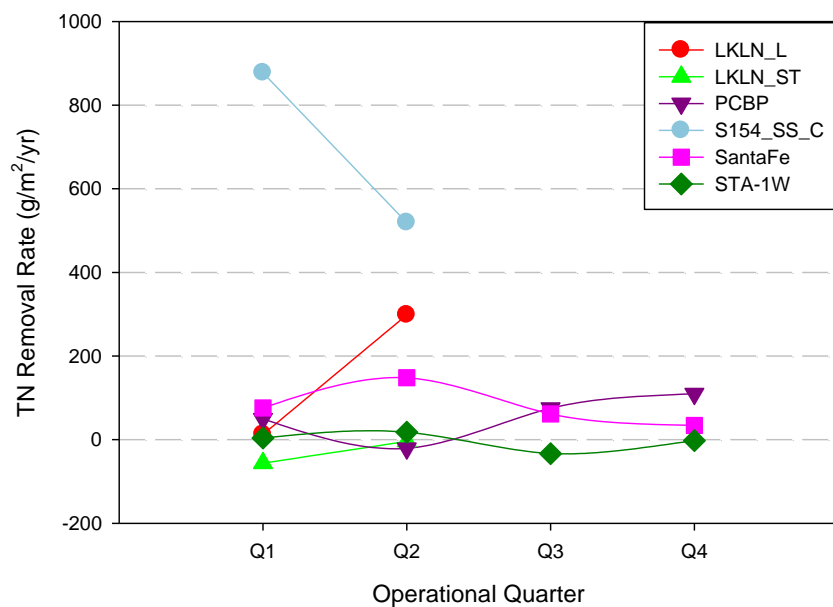


Figure 6. TN removal rate by site and operational quarter

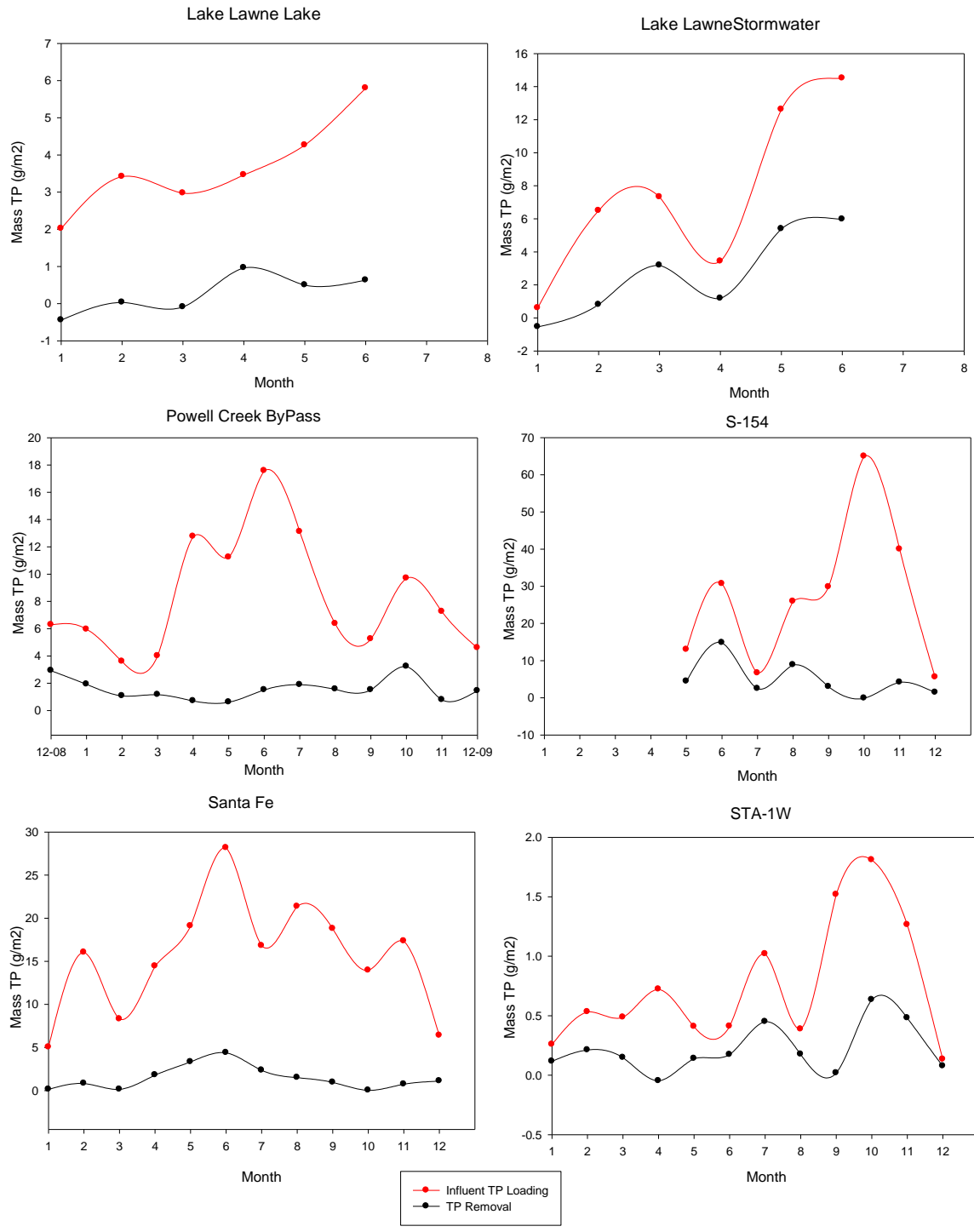


Figure 7. TP influent loading rate and system removal rate by month for the 6 pilots.

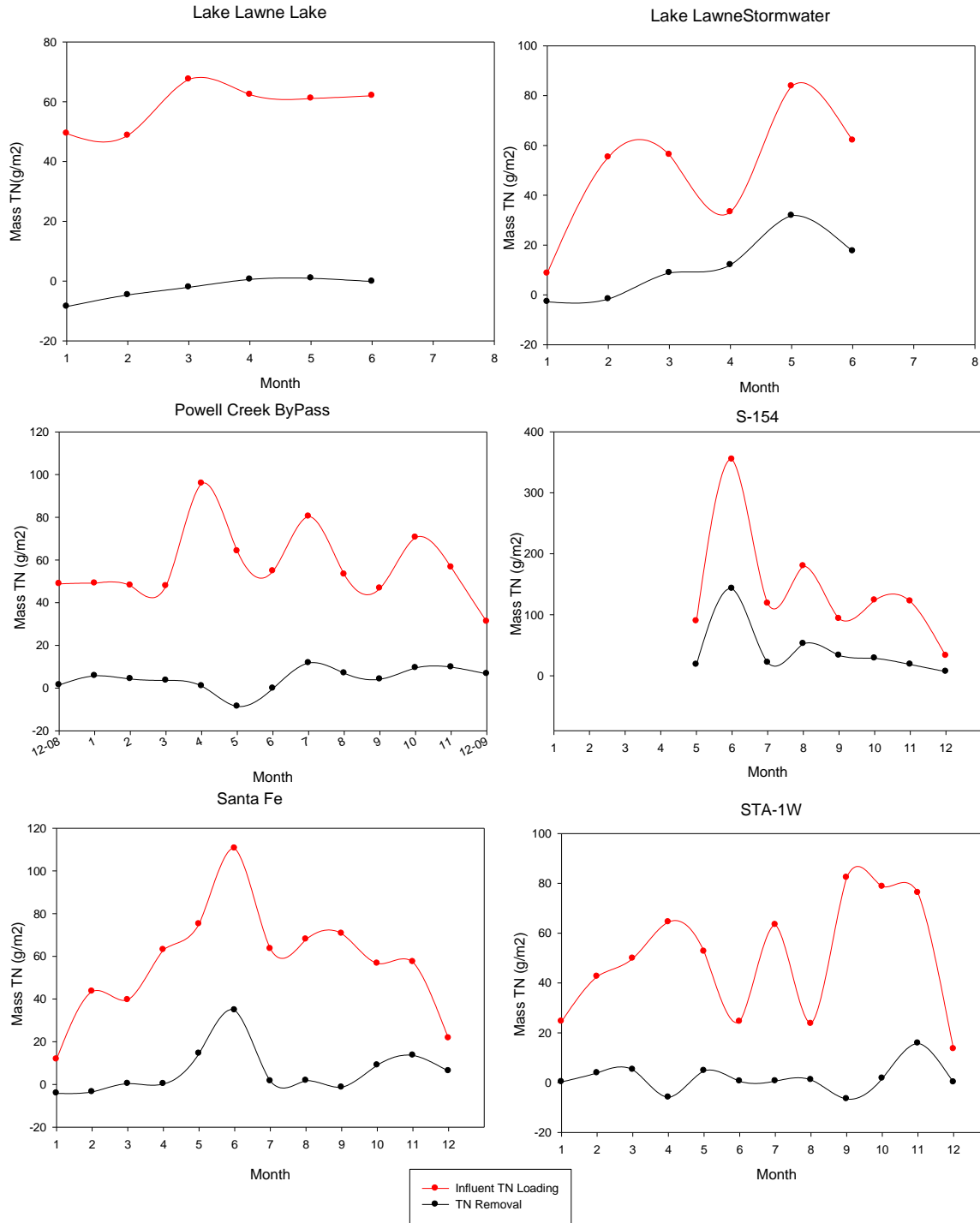


Figure 8. TN influent loading rate and system removal rate by month for the 6 pilots.

Table 6. Mean total phosphorus and total nitrogen loading and removal rates

Project	Influent TP(g/m ² /yr)	TP Removal (g/m ² /yr)	Influent TN (g/m ² /yr)	TN Removal (g/m ² /yr)
LKLN_L	47.5±23	3.44±17.7	794±117	-31.3±106
LKLN_ST	106±117	37.8±89.77	704±473	155±378
PCBP	108±55	20.3±19.68	747±242	56±118
S-154	450±377	84.4±93.29	2,318±882	698±535
Santa Fe	205±64	19.2±23.25	754±283	80.8±226
STA-1W	10.6±5.97	3.0±3.38	704±240	25.22±133

2.4 Vegetation Data

Quantification of vegetation mass and nutrient content are of interest in evaluating how the systems respond to water quality inputs and changes in system performance over time. For most systems, nutrient content was evaluated at 25, 50, 75 and 100 percent of the total length of the floway.

Highest biomass production rate was observed at S-154 (11.8 dry-lbs/m²/yr) while lowest biomass production rate was observed at LkLn_ST (0.48 dry-lbs/m²/yr).

In terms of nutrients, mean biomass total phosphorus content ranged from 0.43% at Powell Creek, to 0.76% at Santa Fe. Mean biomass total nitrogen content ranged from 1.95% at Powell Creek, to 4.68% at STA-1W (Table 6). It may be worthwhile to note that while the lowest and highest percent total phosphorus for algal biomass doesn't correspond with lowest and highest mean influent loading rate for water quality for TP, the sites with lowest and highest total nitrogen biomass content do correspond to lowest and highest influent loading rates for that parameter.

Annual nutrient removal through algal turf production can be estimated based on harvested biomass and nutrient content at each site, normalized for area and time. Estimated annual total phosphorus removal rates for biomass ranged from 1.1 g/m²/yr at LkLn_ST to 31.9 g/m²/yr at S-154. Estimated annual total nitrogen removal rates ranged from 5.5 g/m²/yr at LkLn_ST to 139 g/m²/yr at S-154 (Table 7). Other Algal Turf Scrubber process studies have used biomass nutrient content as the primary indicator of a system's nutrient reduction performance (Mulbry, 2010).

Table 7. Summary of biomass production, nutrient concentration and removal by site

Project	Biomass (g/m ² /yr)	Tissue TP (%)	Tissue TN (%)	Estimated Biomass TP (g/m ² /yr)	Estimated Biomass TN (g/m ² /yr)
LkLn_Lk	518	0.63	2.25	3.09	11.87
LKLN_ST	221	0.52	2.58	1.10	5.44
PCBP	3,840	0.43	1.91	12.64	55.64
S-154	5,350	0.76	3.15	31.92	139.41
Santa Fe	1,400	0.52	4.73	7.33	70.08
STA-1W	3,090	0.76	2.75	23.81	84.89

A general increase in algal production is observed on the pilot studies when looking at quarterly biomass harvests, with the exception of the Santa Fe site, which is consistent with increased trends in removal rate observed for nutrient reduction over time. This trend exists despite different start times for the systems (Table 8). For example, the Powell Creek study began in December while the STA-1W study began in August. It is likely therefore that although there

may be some seasonal variation in algal growth, changes in biomass production during establishment and grow-in are much greater than seasonal differences during the short duration of most of the pilot studies.

Table 8 Total Dry Harvest

Project	Operational Quarter (Harvest lbs)			
	1	2	3	4
LKLN_L	3.43	17.8	.	.
LKLN_ST	4.59	4.48	.	.
PCBP	82.6	75.5	91.1	145
S-154	413.2	270	.	.
SantaFe	31.3	57	44.73	11
STA-1W	140	191	196	232

A decrease in biomass nutrient content is observed with increasing distance from inflow for most parameters (Figure 9). One obvious exception is the Ca content of algae harvested from STA-1W, which almost doubles in concentration at the far reaches of the flowway. This system was the longest of all, at 1,200 ft in length. While effluent pH on this system is consistent with effluent pH observed at the other sites, the influent Ca concentration was considerably higher than most (the exception being Powell Creek). It is likely that the increased Ca concentration is a result of precipitation as pH increased during photosynthetic activity. Note that Mg follows a similar trend at STA-1W, Santa Fe and the Lake Lawne Stormwater site, though not as pronounced.

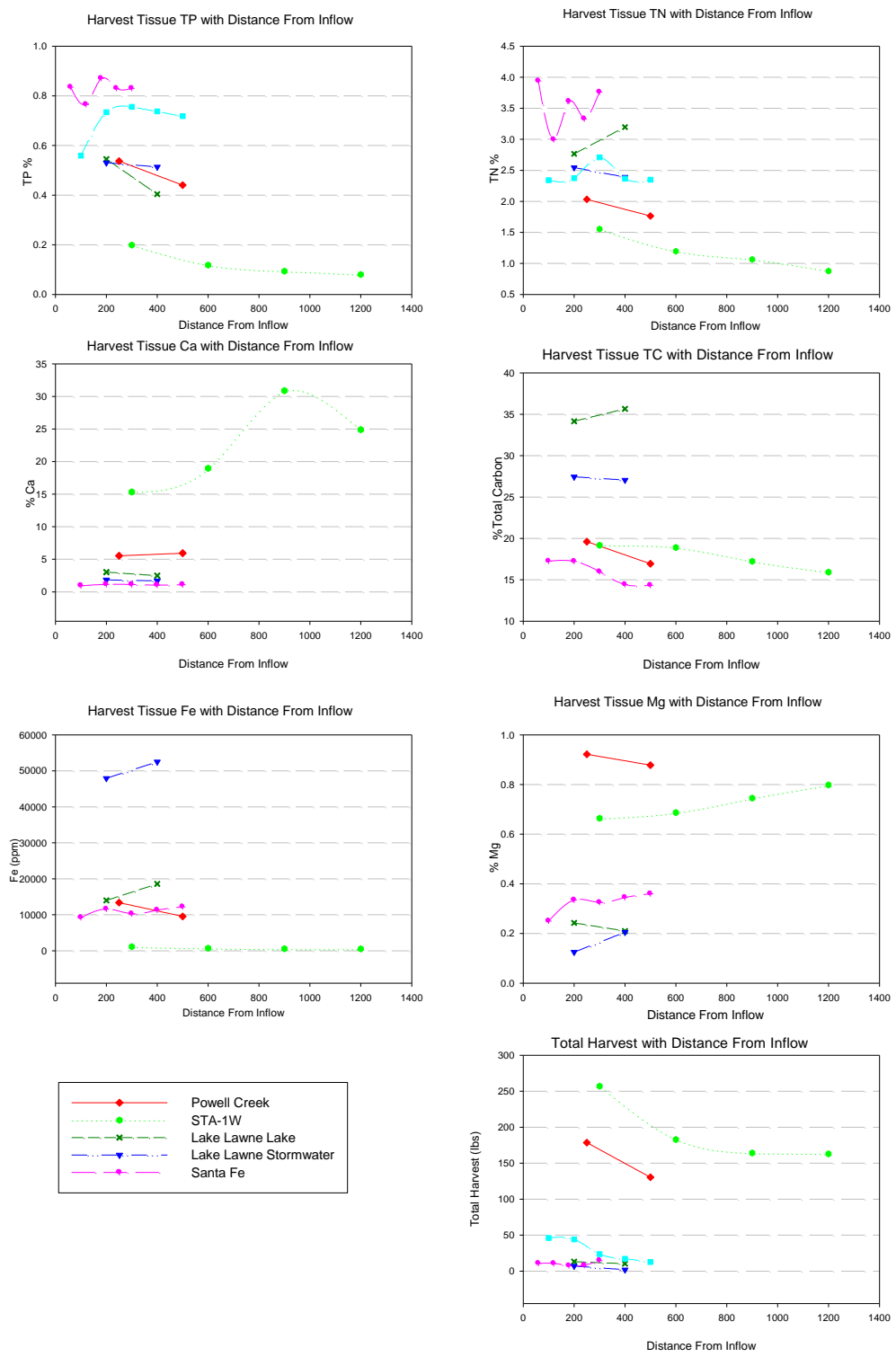


Figure 9. Biomass nutrient content and harvest amount with increasing distance from the influent surge box at 6 ATSTM pilot studies.

Adey WH, Loveland K (1998) *Dynamic aquaria: building living ecosystems*, 2nd edn. Academic Press, San Diego, CA

Barr NG, Kloeppel A, Rees TAV, Scherer C, Taylor RB, Wenzel A (2008) Wave surge increases rates of growth and nutrient uptake in the green seaweed *Ulva pertusa* maintained at low bulk flow velocities. *Aquat Biol* 3:179-186

Bodle M.J., Ferriter, A.P., and Thayer, D.D., 1994, The biology, distribution, and ecological consequences of *Melaleuca quinquenervia* in the Everglades, in Davis, S.M., and Ogden, J.C., *The Everglades—The ecosystem and its restoration*: Delray Beach, Fla., St. Lucie Press, p. 341–355.

Center for Economic Forecasting and Analysis, 2012. The Economic Analysis of the FDEP Proposed Numeric Nutrient Criteria in Florida. FDEP Contract No: SP699

Florida Department of Agriculture and Consumer Services, *Overview of Florida Agriculture*, <http://www.fl-ag.com/agfacts.htm> (March 22, 2012)

Florida Department of Environmental Protection, *Florida's Lands and Water, Brief Facts* http://www.dep.state.fl.us/lands/files/FloridaNumbers_031011.pdf (March, 2011)

Guiry, M.D. & Guiry, G.M. 2012. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>; searched on 02 April 2012

Hydromentia, 2005. S-154 Single Stage Algal Turf Scrubber Final Report. South Florida Water Management District Contract C-13933.

Kadlec, Robert H and Wallace, Scott. *Treatment Wetlands*. 2nd ed. Florida: CRC Press, 2009.

Lindstrom SM, White, JR. (2011) Reducing phosphorus flux from organic soils in surface flow treatment wetlands. *Chemosphere*. 85(4): 625-629

Malecki-Brown LM, White JR, Sees M (2009) Alum application to improve water quality in a municipal wastewater treatment wetland. *J Environ Qual* 38(2):814-21

Powell N, Shilton A, Pratt S, Christi Y(2011) Luxury uptake of phosphorus by microalgae in full-scale waste stabilization ponds. *WATER SCIENCE AND TECHNOLOGY* 63(4): 704-709

Reddy, K. Ramesh and DeLaune, Ronald D. *Biogeochemistry of Wetlands" Science and Applications*. Florida: CRC Press, 2008.

Vymazal J, Kröpfelová K. 2009. Removal of Nitrogen in Constructed Wetlands with Horizontal Sub-Surface Flow:A Review. *Wetlands* 29(4):1114-24