1-1-2007

Performance and microbial evaluation of an artificial wetland treatment system for simulation model development.

Lesley A. Spokas
University of Massachusetts Amherst

PERFORMANCE AND MICROBIAL EVALUATION OF AN ARTIFICIAL WETLAND TREATMENT SYSTEM FOR SIMULATION MODEL DEVELOPMENT

A Dissertation Presented

by

LESLEY A. SPOKAS

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2007

Department of Plant and Soil Sciences

PERFORMANCE AND MICROBIAL EVALUATION OF AN ARTIFICIAL WETLAND TREATMENT SYSTEM FOR SIMULATION MODEL DEVELOPMENT

A Dissertation Presented

by

LESLEY A. SPOKAS

Approved as to style and content by:

Peter L.M. Veneman, Chair

Stephen C. Simkins, Member

Sharon C. Long, Member

Peter L.M. Veneman, Department Head
Department of Plant, Soil, and Insect Sciences

PERFORMANCE AND MICROBIAL EVALUATION OF AN ARTIFICIAL WETLAND TREATMENT SYSTEM FOR SIMULATION MODEL DEVELOPMENT

A Dissertation Presented

by

LESLEY A. SPOKAS

Approved as to style and content by:

Peter L.M. Veneman, Chair

Stephen C. Simkins, Member

Sharon C. Long, Member

Peter L.M. Veneman, Department Head
Department of Plant, Soil, and Insect Sciences

DEDICATION

This work and all that it represents is dedicated

first to the amazing women who have shaped me by setting the bar high with their accomplishments:

my grandmother Corinne who got a Bachelor's Degree and a job because she was not the "pretty one"; and

my mother Louise, who curtailed her own career to raise a family and in so doing raised me to believe that I could do and be whatever I choose;

second to my father who would have been pleased beyond his ability to express it;

and third, but not last to Eric, Melissa, Avery, and Ella may this be your inspiration and may you always feel free to make your own choices.

ACKNOWLEDGMENTS

I would like to express my gratitude to the following people without whose encouragement and support this milestone would not have come to pass.

Dr. Peter Veneman, mentor, major professor, "Dutch Uncle", thank you for your patience and guidance; for knowing when to be supportive and when to leave me alone; but mostly for the opportunity to test my mettle.

Dr. Steve Simkins whose office door was always open when I needed to talk, who faithfully checked my equations, and even helped inject acetylene into my samples so I wouldn't bend all the needles the first day.

Dr. Sharon Long who helped to open my eyes to the engineering world and had amazing editing suggestions.

Emily K.D. Stockman (dirt girl) and Dr. Elizabeth Johnson (dirt lady), my fellow graduate students, who shared the ups and downs along the way, thank you for reminding me to exhale.

Dr. Ron Lavigne thank you for introducing me to Artificial Wetlands, inspiring me to be the best, and for giving me the space to follow the dream.

ABSTRACT

PERFORMANCE AND MICROBIAL EVALUATION
OF AN ARTIFICIAL WETLAND TREATMENT SYSTEM
FOR SIMULATION MODEL DEVELOPMENT

MAY 2007

LESLEY A. SPOKAS, B.S., UNIVERSITY OF MASSACHUSETTS

M.S., UNIVERSITY OF MASSACHUSETTS

Ph.D., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Professor Peter L.M. Veneman

The current research was undertaken to evaluate the performance of a top loading vertical flow submerged bed treatment system (TLVFSBTS) treating primary sewage effluent. Both pollutant elimination and microbial processes were measured. The wetland system is located just west of the Hudson River in upstate New York. The system consists of four 232-m^2 wetland cells, currently operating in series. Two cells are planted with *Phalaris arundineacea* and two with *Phragmites communis*. The data collected were used to evaluate an existing mechanistic compartmental simulation model and to develop an new simulation model for TLVFSBTS wetlands.

Treating $CBOD_5$ to permit levels (< 4 mg L^{-1}) was not difficult to accomplish and occurred in the first wetland cell during much of the study period. At no time during the 17-month evaluation period did measurable $CBOD_5$ leave the wetland, although lack of carbon source ($CBOD_5$) for microorganisms in subsequent wetland cells (cells 3 and 4) may have been a limitation for denitrification. Ammonium oxidation in the first wetland cell was greater than in the second wetland cell (70.8%

and 39.2%, respectively). Nitrite accumulation in the first wetland cell (2.204 mg L^{-1} maximum value) appeared to be seasonal, and not directly related to nitrite oxidation. Both nitrite and nitrate leave the wetland system at levels below primary drinking water standards (1 and 10 mg L^{-1}, respectively. The ammonium concentration leaving the system was at or below permit level (2.2 mg L^{-1}) during much of the study period.

Nitrification potential and denitrification enzyme activity in the wetland system, especially the first wetland cell, exceeded published values for natural wetlands, tropical soils, and both marine and freshwater sediments. These findings, however, demonstrate the ability of TLVFSBTS wetlands to remove the various nitrogen constituents once the microbial population becomes acclimated to the influent wastewater.

The original simulation model evaluated was determined to be inappropriate for TLVFSBTS wetlands. A new TLVFSBTS model was developed, parts of which worked quite well. A computational problem with the chosen simulation software, however, made it impossible to determine the applicability of the current model. Future work will continue to pursue development of the current model, perhaps with different software.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Treatment Wetlands

Artificial wetlands for wastewater treatment are a sustainable, green technology that has been employed in Europe since the 1940's (Seidel, 1970). Following the Clean Water Act of 1972, artificial wetland wastewater treatment (AWWT) technology was adopted in the United States on a limited basis. The majority of AWWT systems built in the United States are free water surface systems (FWS) (Fig. 1). These systems are comprised of open water bodies with distinct flow channels and areas of emergent macrophytic vegetation such as cattails (*Typha* spp.), bulrush (*Scirpus* spp.), rushes (*Juncus* spp.) and sedges (*Carex* spp.) (Kadlec, 2003). FWS systems in the United States are predominately used in conjunction with conventional concrete and steel treatment facilities to polish secondary effluent to meet stringent discharge limits (Knight *et al.*, 1993).

The original European submerged bed treatment (SBT) systems, as well as the few SBT systems currently in use in the United States, were horizontal flow (HF) systems. European countries are now experimenting with vertical flow (VF) systems (Fig 2) (Weedon, 2003; Winter and Goetz, 2003). Both SBT configurations are composed of some type of media, usually gravel or small stones, with hydrophytic vegetation, usually common reed (*Phragmites sp.*), supported by the liquid waste that is kept several centimeters below the surface (Kadlec and Knight, 1996; Reed *et al.*,1995). Vertical flow systems may be either top-loading or up-flow and may be submerged

constantly, or of the flood and drain type (von Felde and Kunst, 1997; Ottová *et al.*, 1997; Brix, 1993; Guang *et al.*, 2002).

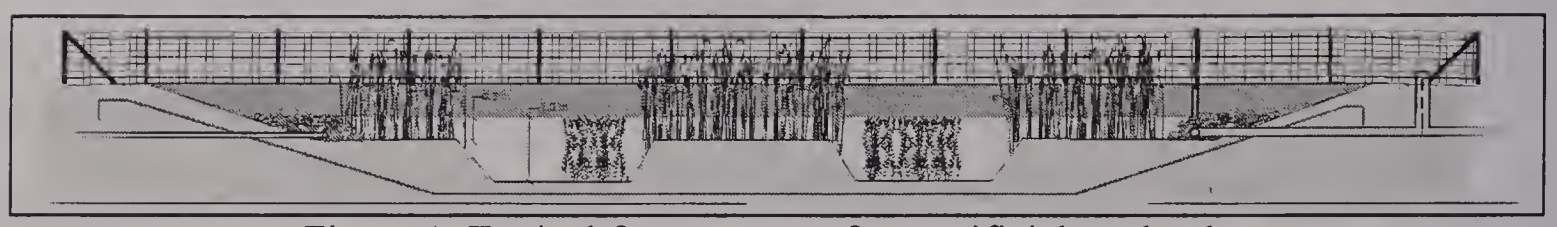


Figure 1. Typical free water surface artificial wetland.

FWS systems require a treatment time of 30 d or longer (Reed *et al.*, 1995; Kadlec and Knight, 1996), thus they require larger land area to construct. The prime advantage of these systems is that they are inexpensive to build, so long as land need not be purchased, and inexpensive to operate. The disadvantages are that they provide a place for mosquitoes and other disease vectors to breed, they are prone to "secondary biochemical oxygen demand (BOD_5) and total suspended solids (TSS)" production due to algae growth, and they may have odor problems dependant on the quality of the influent (Reed *et al.*, 1995).

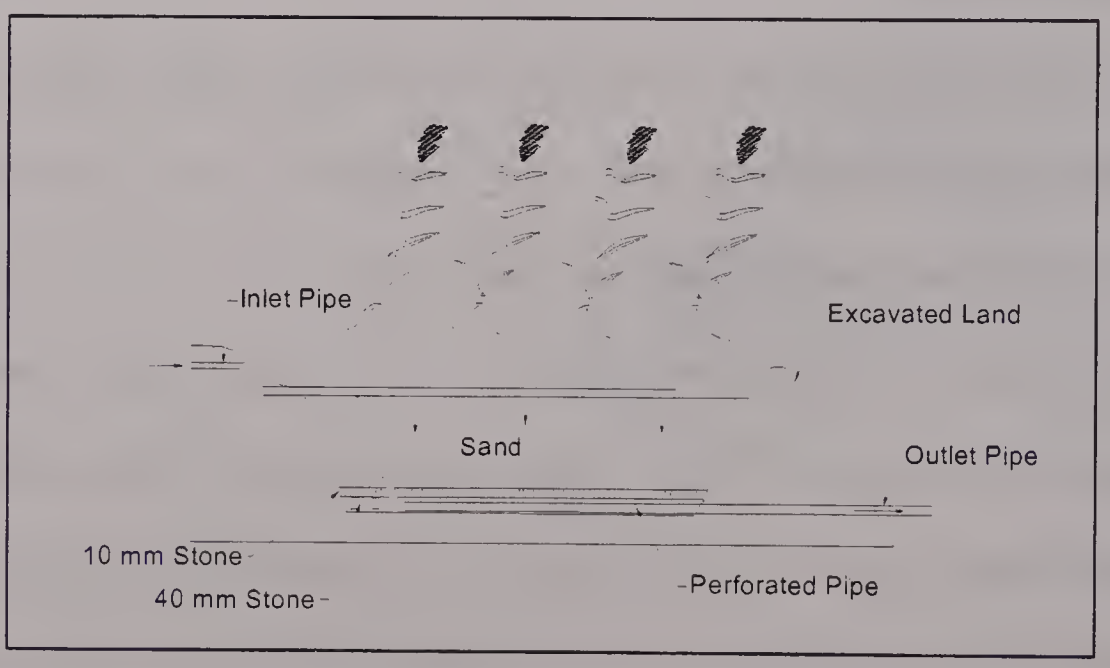


Figure 2. Cross-section through a typical European vertical flow artificial wetland (from Weedon, 2003).

Subsurface flow submerged bed systems are typically constructed 70-80-cm deep, with some type of liner material and dikes high enough to provide a measure of

"free board" for rain events as well to prevent surface water from entering during storm and associated run-off events. The advantages of submerged bed systems are that they are designed to be dry (phreatic level ≥ 5-cm below the surface), thus there will be no disease or odor problems. The disadvantage to these systems is that they are more expensive to build (excavation, liner, media, pipes, etc) than FWS systems (excavation, liner). These costs, however, need to be weighed against the smaller footprint required due to shorter treatment time in the VF bed.

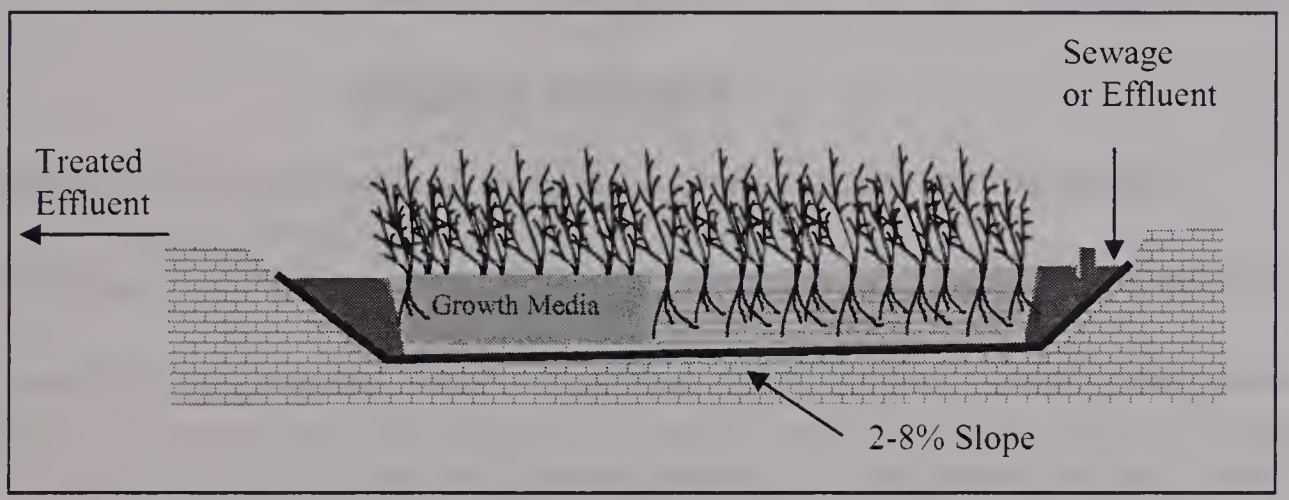


Figure 3. End loading (horizontal flow) submerged bed artificial wetland treatment system.

Figure 3 illustrates a typical "end loading" system that requires the wastewater to travel the full length of the bed. End loading systems perform well when there are little or no suspended solids in the waste stream. If suspended solids exist, or if dense bacterial growth at the head-works/media interface are not compensated for, the systems are prone to clogging (Blazejewski and Murat-Blazejewski, 1997; Breen and Chick, 1995; Tanner *et al.*, 1998; EPA, 1993). Once the hydraulic conductivity at the inlet end falls below the application rate, wastewater surfaces and flows overland. When this occurs, treatment efficiency is significantly reduced, and the system is in failure (Breen and Chick, 1995; Blazejewski and Murat-Blazejewski, 1997). A second limiting factor

of horizontal flow systems is the minimal cross sectional area available for loading (i.e. width x depth).

An alternative operational mode is vertical-flow. For vertical-flow systems $H \cdot L^{-1}$ is typically unity, clogging is reduced due to the large surface area available for loading (i.e. greater K), and the cross sectional area (A_{xs}) becomes the surface area of the entire top of the bed rather than the area of the end of the bed. The required detention times are maintained by a "control box" which is designed to provide variable heads ranging from 0 to unity (Lavigne and Jankiewicz, 2000).

Treatment Processes

Wastewater treatment processes require both aerobic and anaerobic conditions for complete treatment, but anaerobic conditions are not typically part of conventional wastewater plants, leaving many complex organic compounds untreated. In a SBT system both environments exist within close proximity to each other. Figure 4 illustrates a typical root cross section within an SBT system. As illustrated, oxygen gas diffuses and or is "pumped" out of the root creating an aerobic annulus around it (Mitch and Gosselink, 2000; Good and Patrick,1987). These aerobic microsites will be surrounded by anaerobic microcosms where oxygen concentrations have been reduced to zero due to the demand created by aerobic biochemical activity. Biological and chemical processes will remain anaerobic until the waste passes into another aerobic microsite. The diameter of the oxygen-rich annulus will be controlled by the degree of the oxygen-demanding forces at work, and the ability of the plant species to provide oxygen (Sorrell and Brix, 2003; Teal and Kanwisher, 1966; Weisner and Granéli, 1989). Changes in the ratio of aerobic to anaerobic micro-sites typically occur naturally with

variations in wastewater quality, but the ratio can also be manipulated by such factors as loading rates, bed and root depths, re-circulation (aeration), and plant choice (Gersberg *et al.*, 1989b; Guang *et al.*, 2002; Szogi *et al.*, 2003).

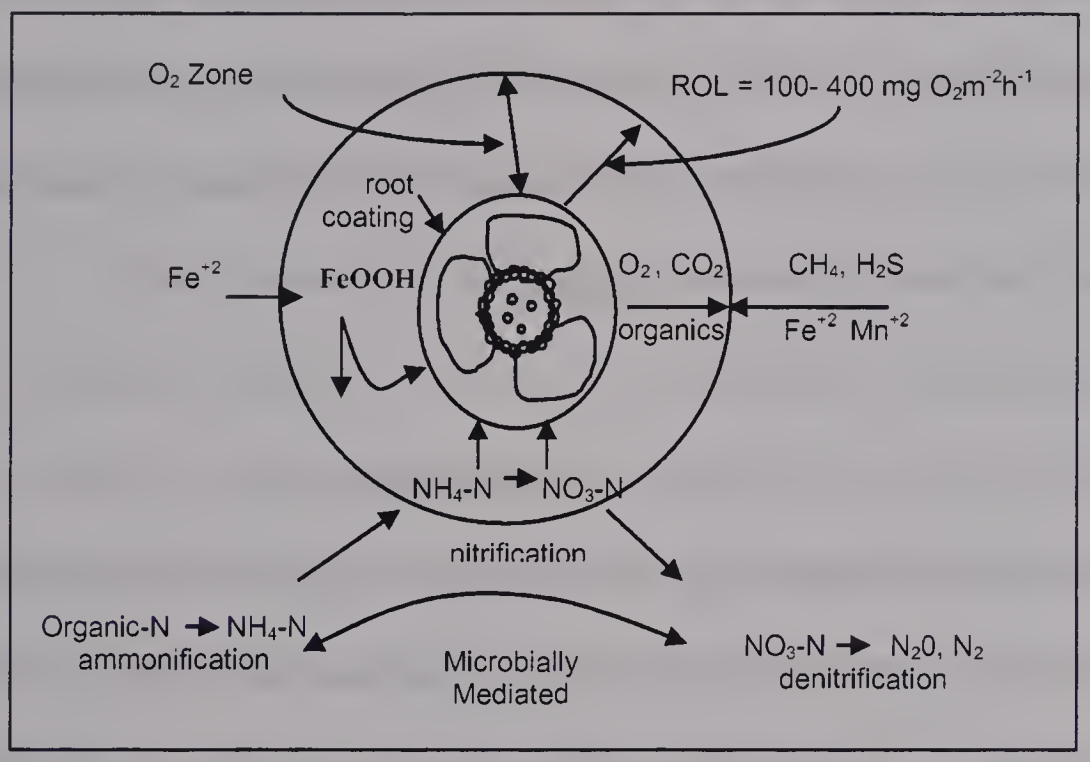


Figure 4. Dynamics of the root-growth media interface. (Adapted from Good and Patrick, 1987).

Settling tanks typically remove about 65% of the suspended solids in wastewater (Metcalf and Eddy, 2003). The remaining 35% (both organic and inorganic) will be removed, generally at the stone/sand interface around the distribution lines with VF or at the head works/media interface of HF systems (Tanner *et al.*, 1998). This biological "scum layer" is also the primary location for removal of bacteria (total coliform, fecal coliform) and other microscopic organisms (Brix, 1993; Gersberg *et al.*, 1989a).

One of the primary differences between SBTS units and other wetland treatment types is the large surface area associated with the growth media. The matrix material not only filters solids, and supports fixed bacterial films, but it also enhances removal of contaminants by a variety of surface adsorption, complexation, and chelation processes

(Brix, 1993; Ottová *et al.*, 1997). As the media particle size decreases, surface treatment processes are augmented by the increased surface area. Unfortunately the hydraulic conductivity decreases as the pore size decreases and it can reach a point where reduced flows through the media are unacceptable as pore velocity decreases (Wynn and Liehr, 2001). Conversely, if growth media particle size is increased to enhance flow through the system (e.g. pea gravel) surface areas are decreased exponentially as are the associated treatment efficiencies (Lavigne and Jankiewicz, 2000).

Justification

All vegetated submerged bed artificial wetland treatment systems are not the same and should not be "lumped" together as free water surface or submerged bed wetland systems as has been done throughout the literature. AWWT systems are a complex interaction of microorganisms and higher plants in a submerged environment. Media type and size are an integral part of the whole. The microorganisms are the "heart" of the system, but these organisms do not simply exist in the wastewater as with conventional treatment processes. The bacteria form a complex living system with the plants and the media, thus the systems must be studied in their entirety. Consequently there is a need for improved understanding of the microbial ecosystem within each specific AWWT type. This research evaluated a Top Loading Vertical Flow Submerged Bed Treatment System (TLVFSBTS) with specific attention to the interactions of the microbial populations with each other and within their ecosystem.

Hypothesis

Any model used for sizing AWWT systems or predicting their performance needs to be written specifically for the type of system being built, and that wetland type (FWS, HV, VF) and physical construction components are important factors in system sizing and performance. Parameters for microbial processes and interactions need to be included in future models, rather than mere rate constants.

Objectives

This research evaluated a vertical flow submerged bed artificial wetland system and a mechanistic, compartmental simulation model for wetland performance (Wynn and Liehr, 2001). The model was found to be unnecessarily complicated and inappropriate for the actual conditions in a TLVFSBTS treating primary effluent.

Specific objectives for this study were:

1) To collect system performance data including: influent and effluent concentrations of biochemical oxygen demand, ammonium, nitrite and nitrate;
2) To collect local weather data including temperature and precipitation;
3) To collect redox potential data at different depths within the wetland cells;
4) To collect microbial data including: heterotrophic potential, nitrification potential, denitrification rate and total microbial biomass, and
5) To develop a compartmental simulation model for TLVFSBTS.

CHAPTER 2

LITERATURE REVIEW

The Classic Literature

In 1993, Knight *et al.* published a summary article of findings from the USEPA Data Base of AWWT systems in North America. At that time there were 126 artificial wetland systems mentioned in the article, of which, only 26 were vegetated submerged bed (VSB) treatment systems. The most recent update of the Database (November, 2000) listed 334 wetland systems, of which 51 were VSB systems and 13 were a combination of free water systems and VSBs. Of the 334 systems, only 10 were treating primary effluent. Most of the AWWT systems were treating secondary effluent (BOD_5 < 50 mg L^{-1}; TSS < 80 mg L^{-1}; NH_3 < 20 mg L^{-1}). By comparison, in 2000 there were approximately 5000 constructed wetlands in Germany alone, although most of these (95%) were for systems treating the sewage of 20 or fewer people (Luederitz *et al.*, 2001)

Knight *et al.* (1993) concluded that loading rate and land area do not have much of an effect on BOD_5 removal efficiency except at very high hydraulic loading rates (HLR). This conclusion was based on the FWS systems that are prone to secondary BOD_5 and TSS problems from algae growth in warm weather. The larger the surface area of the system, the more algae growth, therefore, the higher the "good" BOD_5 and TSS. This article, along with the EPA evaluation of Artificial Wetlands (EPA, 1993) became the foundation of the "literature" on AWWT systems, and the basis of the opinion held by many State Regulators that, "Artificial Wetlands do not work". During the intervening years (1993 to present), many research studies have been conducted on

artificial wetlands. Some systems were bench scale, some pilot scale, and some full scale. Media type and size varied, plant species varied, waste stream varied, AWWT system type varied. As late as the late 1990's, studies were still being conducted that compared planted beds to unplanted beds (Kemp and George, 1997) and the exact role of the plants - nutrient sink or microbial aerator, was still being discussed.

Gersberg *et al.* (1989a) is the classic review of pathogen removal in constructed wetlands, although it is more of an introduction to microbial contaminant levels than a review of system performance. The minimal infective dose (MID) for bacteria was high, ranging from 10^4 for *Shigella* sp. to between 10^6 and 10^9 for *Salomnella* sp., *Vibrio* sp., or toxic *Escherichi coli*. All of the wetlands being discussed in the study were able to meet this level of treatment. However, the MID for most viruses may be 1 and the ability of AWWT systems to meet this level of treatment has yet to be demonstrated. At hydraulic residence times of 3-6 days, SBT artificial wetlands are more effective at removal of disease-causing bacteria and viruses than conventional treatment systems (Gersberg *et al.* 1989a) (HRT usually < 0.5 d). In addition to pathogen removal, AWWT systems also remove suspended solids and ammonia, both of which interfere with efficient disinfection. Disinfection is the means by which conventional wastewater treatment plants prevent pathogen dispersal. Disinfection practices, however, have been shown to be "more effective at killing the predators to cysts and viruses" than in killing the actual pathogens (Davis and Masten, 2004), and physical removal of cysts and viruses during the activated sludge process is limited.

Wetland Design

To design any wastewater treatment system it is necessary to know the strength of the wastewater and what effluent limits will be. Typical National Pollution Discharge Elimination System (NPDES) discharge limits for secondary treatment plants were designed to return the waters of the United States to a fishable and swimable condition (Clean Water Act, 1972). Thus, the discharge limits for secondary treatment plants discharging to a medium to large waterway are typically 30 mg/L BOD_5 and 30 mg/L TSS.

Primary domestic effluent (following settling) has BOD_5 values ranging from 110–400 mg L^{-1}; TSS ranging from 100-350 mg L^{-1}; total nitrogen (TN) ranging from 20 – 85 mg L^{-1}; total organic carbon (TOC) ranging from 80 – 290 mg L^{-1}; and total phosphorous (TP) ranging from 8-35 mg L^{-1}. Of the TN present, the dominant forms are organic nitrogen compounds and free ammonium (NH_4^+) with values of 8-35 mg L^{-1}, and 12 – 50 mg L^{-1}, respectively (Metcalf and Eddy, 2003).

Early design models were based on first-order kinetics decay models with plug-flow hydraulics and a single design parameter. The following is an example of this simplistic approach to sizing calculations for BOD_5 reduction, TSS, and nitrogen.

Biochemical Oxygen Demand

Using the integrated form of the first order decay model for BOD_5 reduction Eq.[1,2] detention time may be calculated.

$$C_e = C_o e^{-kt} \qquad [1]$$

or

$$t = \frac{1}{k} \ln \frac{C_o}{C_e} \qquad [2]$$

where C_o= influent BOD_5 concentration
C_e= effluent BOD_5 concentration
t = detention time (days)
k = decay rate constant ($days^{-1}$)

The desired detention time for BOD_5 based on a presumed influent concentration of 350 mg L^{-1} and desired effluent concentration of < 5 mg L^{-1} with a decay rate constant of 1.104 $days^{-1}$ (Reed, 1995; Kadlec and Knight, 1996) at 20°C can be calculated by substituting into Eq. [3].

$$t = \frac{1}{1.104} \cdot \ln \frac{350}{5} \qquad t = 0.906 \text{ x } \ln 70 \qquad t = 3.85 \text{ days}$$

Bed Sizing

Once the desired detention time is calculated, the size of the beds can be calculated using Eq. [3].

$$V_T = Q \cdot t = L^2 \cdot h \cdot f \qquad [3]$$

where: V_T = total bed volume in m^3
Q = flow rate in $m^3\ d^{-1}$ (39 $m^3 d^{-1}$)
t = detention time in days (3.85 d)
L^2 = bed area in m^2
h = height of the porous media (0.6 m)
f = porosity of the porous media (0.35)

After substituting the above values into Eq. [3], the required bed volume V_T may be calculated:

$$V_T = 39\ m^3\ d^{-1} \cdot 3.85\ d = 150\ m^3$$

Once the bed volume has been calculated the required bed area (L^2) may be calculated:

$$150\ m^3 = L^2 \cdot h \cdot f = L^2 \cdot 0.6\ m \cdot 0.35 \qquad L^2 = 715\ m^2$$

Total Suspended Solids

Using the simple model derived for BOD removal and substituting into Eq. [2]

$$t = \frac{1}{k} \ln \frac{C_o}{C_T} = \frac{1}{1.25} \cdot \ln \frac{150}{9} = 0.8 \cdot \ln 16.67 = 2.25 \text{ days}$$

where C_o= influent TSS concentration (150 mg/L)
C_T= effluent TSS concentration (9 mg/L)
k = TSS removal rate constant (1.25 d^{-1} Crites and Tchobanoglous, 1998)

gives a detention time of 2.25 days. This value when substituted into Eq.[3]

$$V_T = 39\ m^3\ d^{-1} \cdot 2.25\ d = 88\ m^3 = L^2 \cdot h \cdot f = L^2 \cdot 0.6\ m \cdot 0.35$$

yields a total bed volume of less than 88 m^3 and a bed area of 418 m^2.

Nitrogen Reduction Model

The third parameter of interest is nitrogen reduction, specifically ammonia removal by nitrification. Reed (1995) used a temperature dependent rate constant k_{NH} to represent the nitrification rate as a function of *rz*, the portion of the wetland cell that is occupied by the root zone. The relation is expressed by Eq. [4].

$$k_{NH} = 0.01854 + 0.3922\ rz^{2.6077} \qquad [4]$$

K_{NH} ranges from 0.01854 in an unplanted wetland cell to 0.4107 with a fully developed root zone (Reed, 1995). The temperature dependence of the rate constant k_T is given by Reed (1995) as k_{NH} (0.4103) d^{-1} at T = 1°C, and k_{NH} $(1.048)^{(T-20)}$ d^{-1} at T > 1°C. Therefore, at 15°C with full rooting

$$k_T = .4107 \cdot 1.048^{(15-20)} = 0.325\ d^{-1}$$

following the same steps as before, this gives t = ln (60/5) ÷ 0.325, t = 7.6 d

$$V_T = 39\ m^3\ d^{-1} \cdot 7\ d = 298\ m^3$$

yields a total bed volume of 298 m^3 and a bed area of 1420 m^2.

$$273\ m^3 = L^2 \cdot h \cdot f = L^2 \cdot 0.6\ m \cdot 0.35 \qquad L^2 = 1420\ m^2$$

At 20°C, t drops to 6 days and L^2 to 1124 m^2.

The values Reed (1995) used to determine *rz* and k_{NH} are based on data from the Santee California wetland (Gersberg *et al.* 1989b). The assumption was that it takes 5 g of oxygen to nitrify 1 g of ammonium. By using the ammonium removal numbers from

the Santee wetland Reed (1995) derived an average available oxygen value for the different plant species, which was then used to derive the k_{NH} equation.

Using the simple models discussed above, the AWWT system would need to be twice as large for NH_4^+ removal (1420 m^2) as for BOD_5 reduction (713 m^2). An unplanted wetland cell receiving 7.5 m^3 d^{-1} of wastewater with the "typical" inflow concentration of 60 mg L^{-1} NH_4^+ (Metcalf and Eddy, 2003) would require 184 d to meet a 2.2 mg L^{-1} NPDES limit using Reed's k_{NH} of 0.0184 d^{-1} at 20°C. An AWWT system with a mean influent NH_4^+ concentration of 127 mg L^{-1} would require a total bed area of 2317 m^2 at a flow rate of 7.5 m^3 d^{-1} to meet permit at 15°C and yet a TLVFSBTS in Upstate New York receiving that influent concentration had an effluent ammonium concentration for the first month of operation that averaged 3.6 mg L^{-1} with a bed area of only 950 m^2. Over the course of the 6½ years this system has been in operation, the mean ammonium influent concentration has been 124 mg L^{-1} with a mean effluent concentration of 6.06 mg/L while effluent temperatures have ranged from 0 – 30°C (unpublished data). Clearly, the simplistic model, even with corrections for temperature and rooting depth, does not adequately predict TLVFSBTS performance.

Data from various wetland treatment systems, primarily FWS wetlands have been used to add various "correction factors" to the first order kinetics plug flow decay model. Most of these design models ignore the importance of microbial growth and metabolism. As seen in the previous discussion, reactions are lumped together in "black box" method with zero- or first-order kinetics models and plug-flow hydraulics. The best fit for the hydraulics of TLVFSBTS at the Upstate NY facility may be sequencing batch reactors rather than plug flow or continuously stirred tank reactors (CSTR).

Gidley (1995) developed a mechanistic, compartmental simulation model consisting of 6 linked sub-models representing various wetland treatment processes including: autotrophic and heterotrophic microbial growth components, carbon and nitrogen cycle components, and oxygen and water balance components. Wynn and Liehr (2001) calibrated and evaluated the model using data from the treatment wetland at the Mayo Water Reclamation Facility (Anne Arundel County, MD). The Mayo wetland is a subsurface horizontal flow system and Wynn and Liehr used CSTR flow for their calibration. Microbial growth constants were taken from "pure cultures" and needed to be decreased orders of magnitude in the calibration process. The use of CSTR hydraulics for this model may not have been the best choice as end-loading horizontal flow wetlands are usually modeled with plug flow hydraulics. This compartmental simulation model has yet to be verified with additional wetland data.

Current Literature

An AWWT system should be considered a specialized version of a fixed film reactor and that the microorganisms within the submerged bed are the major source of treatment within the system. The literature that has been reviewed for this research, therefore, has focused on the microbial processes occurring within AWWT systems. Comparing the systems discussed in the literature and their performance, however, is akin to comparing bacteria and fungi (Table 1).

Microbial Processes

Discussion of microbial processes in the literature generally falls into two categories, pathogen removal efficiencies, and discussion of microbially mediated

Table 1. An example of the diversity of the literature for AWWT systems, including system type, media type, plant species, system scale and waste being treated.

Author(s)	Year	Type	Media	Plant species	Loading	Scale	Waste
von Felde and Kunst	1997	VF	Sand & gravel	unknown	Load, drain, load	Full (h)	PDS
Green *et al.*	1997	VF	Gravel	None	Slow fill, fast drain	Bench	Nut. Sol.
Tanner *et al.*	1998		Gravel	*Scirpus validus*		Pilot	Dairy Process Water
Guang *et al.*	2002	All	Gravel	*Typha angustifola;Typha latifolia; Scirpus validus; Scirpus fluviatilis Spartanium eurycarpum*	Various	Full	PDS
Gersberg *et al.*	1989b	HF	Gravel	*Typha* sp.; *Scirpus* sp; *Phragmites* sp.	5 cm d^{-1}	Pilot	PDS
Breen and Chick	1995	HF	Gravel	*Eleocharis sphacelata*	C 43 m^3 d^{-1}	Pilot	SDS
Breen and Chick	1995	HF	Gravel	*Eleocharis sphacelata*	Batch, 1L d^{-1}	Bench	PDS
Shannon *et al.*	2000	HF, SF, FWS	Gravel	*Typha sp.; Scirpus sp;*			PDS
Kantawanichkul *et al.*	2001	VF/HF	Sand & gravel	*Cyperus flabilliformis*	Load, drain, load	Bench	Liquid Pig manure (set.)
Szogi *et al.*	2003	FWS	Loamy sand	*Scirpus cyperinus, Schoenoplectus americanus, S. tabernaemontani*, and *Juncus effuses*; or *Typha augustifolia ,T. latifolia* and *Sparganium americanum*	Varied	Pilot	Swine lagoon waste
Kemp & George	1997	HF	Gravel	*Scirpus validus* or none	Varied	Pilot	PDS
Weedon	2003	VF	Sand	*Phragmites* sp.	Load, drain, load	Full (h)	PDS

Abbreviations: System Type: Horizontal flow (HF), Vertical flow (VF), Free water surface(FWS), Sand Filter (SF); Scale: Single Family House (h); Waste: Primary domestic sewage (PDS), Secondary domestic sewage (SDS); Nutrient solution (Nut. Sol.)

pollutant removal (typically nitrogen). Pathogen removal is discussed in terms of the specific articles incorporated in this review, but was not a focus this research.

Ottová *et al.* (1997) summarized performance of 5 fully operational AWWT systems in Poland. Of specific interest to these researchers were the microbial communities of the various wetland systems, their extent, performance, and the influence of the plant community on the microbial community. Table 2 summarizes the systems (location, age, plant species and hydrology). All five systems were treating primary domestic sewage. Microbial reduction was attributed to physical factors: filtration, sedimentation, aggregation, UV ray action; biological factors: antibiosis, ingestion by nematodes, protozoans, cladocera; attacks by lytic bacteria and bacteriophages; natural death; and chemical factors: oxidation, adsorption, and exposure to toxic exudates from other microbes and plants. These researchers reported 99% removal of total coliforms in all but the Chmelná system. Ottová *et al.* (1997) attribute the lower performance in the Chmelná system to reduced rooting of the *Phalaris arundinacea* (20-30-cm depth) as compared to the *Phragmites* sp. beds where roots were found to a depth of 60-80-cm. The influent ratio of aerobic:anaerobic organisms was greater than 1, but the effluent ratio was less than 1 for all systems. On roots and rhizomes, bacterial colonies were reported as: denitrifying bacteria $10^6 - 10^8$ g^{-1} dry matter, ammonifying bacteria $10^7 - 10^8$ g^{-1} dry matter, and nitrifying bacteria $10^3 - 10^5$ g^{-1} dry matter.

Table 2. Summary data of operational parameters for 5 artificial wetland treatment systems in Poland (from Ottová *et al.*, 1997).

Location	Date started (mo/yr)	Area (m^2)	Media	Vegetation	HRT* (d)	Loading Rate cm d^{-1}
Doksy	4/93	300	CSH** + gravel	*Glyceria* sp.	10.7	2.2
Onšov	11/93	2 beds (100)	gravel	*Phragmites* sp.	4.7	4.5
Ondřjov	7/91	806	gravel	*Phragmites* sp.	4.5	6.6
Koloděje	10/93	4 beds (493)	sand	*Phragmites* sp.	4.5	4.0
Chmelná	11/92	706	gravel	*Phalaris arundinacea*	1.7	11.0

* HRT – Hydraulic retention time.
** CSH – Crushed soil heap

Role of Plants

The role of plants in AWWT systems has been discussed for many years. Plants have been touted as both the most important part of the system for nutrient/pathogen removal (Guang *et al.*, 2002) and as the cause of hydraulic short-circuiting (EPA, 1993; Breen and Chick, 1995; Tanner, 2001). Many studies still compare vegetated beds to unplanted beds (Gersberg *et al.*, 1989b; Kemp and George, 1997). While plants may increase nutrient removal from the waste these nutrients would be subject to return to the AWWT beds if plants are not harvested and removed.

Nutrient and pathogen removal in surface flow (free water surface), subsurface flow (submerged bed), and floating aquatic plant systems has been compared by Guang *et al.* (2002). All three systems showed statistically significant reductions in total coliform and *E. coli.* Influent *E. coli* was 12,292 cfu 100-ml^{-1} while the effluent from the SBTS bed was 193 cfu 100 ml^{-1}. The lowest dissolved oxygen (DO) was reported in the SBT system suggesting a greater amount of biodegradation and oxygen

consumption. Only the SBT system showed significant reduction in BOD_5 or NH_4^+. The authors cited Kadlec & Knight (1996) as evidence that a sufficient population of *Nitrosomonas* sp. and *Nitrobacter* sp. should be present within 6 – 12 months of planting to allow complete microbial degradation of ammonium. The authors also cited Reddy and Patrick (1983) when discussing the concept that the limiting O_2 concentration for nitrification was 0.3 mg L^{-1} dissolved oxygen, this amount of DO was measured in all three systems, yet only the SBT showed significant ammonium removal. Neither of the other systems showed statistically significant reductions in any of the wastewater parameters.

The performance of an end-loading vegetated submerged bed system followed by a sand filter, and free water surface wetland at a Pennsylvania campground and conference center was reported by Shannon *et al.* (2000). The system was designed for BOD_5 removal with influent BOD_5 concentration set at 150 mg L^{-1}, and a desired effluent concentration of 20 mg L^{-1}. The mean influent ammonium concentration in 1998 was 33 mg L^{-1}, with a range of 25-45 mg L^{-1}. The effluent concentration was initially 5 mg L^{-1} but the concentration increased to 12 mg l^{-1} by the end of the 1998 season. During the 1999 season the mean influent ammonium concentration increased to 40 mg L^{-1} with a range of 30-65 mg L^{-1} while effluent concentration averaged 25 mg L^{-1}. These authors concluded that the more important role of deeply rooted emergent macrophytes (i.e. cattail and bulrush) was in oxygenating the rhizosphere for aerobic decomposition and nitrification. It has been reported that nutrient assimilation by plants never exceeds 20 –25% of total nitrogen and that the amount assimilated is inversely proportional to the load (Gersberg *et al.*, 1986; Tanner *et al.*, 1998).

Szogi *et al.* (2003) reported on a FWS wetland treating swine lagoon wastewater. The system consisted of four cells 3.6-m x 33.5-m x 0.1-m water depth. One pair of cells had three bulrush species *Scirpus cyperinus, Schoenoplectus americanus,* and *S. tabernaemontani* and one rush *Juncus effuses* while the other two cells were planted with two cattail species *Typha angustifolia* and *T. latifolia* and bur-reed *Sparganium americanum*. Loading rate varied with the strength of the lagoon waste, which was diluted with fresh water to obtain desired N concentrations. The authors report that the cattail/bur-reed cells had a better root/soil condition for microbial removal of NH_4^+-N suggesting that they "leak more air".

Tanner *et al.* (1998) reported on the relationship between loading rate and pollutant removal during maturation of gravel-bed constructed wetlands. Results were from a 5-year study period. The loading rate increased from 15 to 70 mm d^{-1} during the coarse of the study. The influent was dairy process water with influent ammonium concentration of 40± 21.4 g m^{-3}. Effluent ammonium was 24.2 – 33.2 g m^{-3}, which represented a 60% to 34% reduction depending on loading rate. As loading rate increased, ammonium removal decreased. Five years of data for these gravel beds show a sustained performance level for carbonaceous biochemical oxygen demand (CBOD), carbonaceous and nitrogenous biochemical oxygen demand (CNBOD), total nitrogen and fecal coliform, but a decrease in removal efficiency for total solids. Tanner *et al.* (1998) attribute the increase in TSS to clogging, which was shown to have occurred at the higher treatment levels. Tanner and Sukais (1995) measured organic solids within the media, reporting accumulation closest to the inflow in end-loading beds.

In a related study, Halley (1990) used ^{15}N-labeled ammonium tracer in one of the end-loading pilot-scale gravel beds reported to have a theoretical hydraulic retention time of 7 d. The mean time for the pulse to travel through the wetland was found to be about 60 d. Based on these results, Tanner *et al.* (1998) suggest that N fluctuations in the influent may not be seen for anywhere between 1 and 3 months. These authors especially caution against the use of simultaneous inflow/outflow pollutant concentrations as a measure of treatment performance.

Breen and Chick (1995) reported on a 22-month old pilot scale end-loading vegetated submerged bed system receiving 4.3 kL d^{-1} (43 m^3 d^{-1}) continuous flow of secondary treated domestic sewage. The system had a theoretical retention time of 7 d. The hydraulics of the system were checked with eriochrome acid red dye as a tracer. A 20 L slug of dye solution (0.1g L^{-1}) was added to the wastewater over the course of 1 hour. The dye tracer indicated major hydraulic differences between the upper and lower portions of the trench system. For the first 12.5 m the tracer peaked at essentially the same time (1 day) but the majority of the flow was in the upper portion of the bed. At the mid point of the bed (25 m from the head) dye appeared in the bottom portion about 24 hours after application and peaked at 2 days. The upper portion of the section of bed did not receive the tracer until 2 days after application. The tracer arrived in the lower portion of the bed, ¾ of the way down the trench, 2 days after application and peaked at 3 days, although dye was still detectable 6 days after application. In the upper portion of this section of the bed the dye arrived 3.25 days after application and the concentration was still rising at the end of 6 days. For a trench system with a theoretical detention time of 7 days and plug flow, the calculated times for peaks would be 1.75, 3.5, and

5.25 days, respectively. Breen and Chick (1995) suggest that the root density in the upper half of the bed was responsible for channeling of flow to the lower half of the trench as the path of least resistance. The authors suggest a "critical root biomass" of 112 – 251 g/m^2 for maximum plant mediated nutrient removal. Thus vertical flow maximizes the wastewater/root-zone contact in the upper portion of the trench, while the horizontal flow in the lower portion of the trench minimizes partitioning and short-circuiting. This root density configuration maximizes both spatial and temporal wastewater/root-zone contact.

Breen and Chick (1995) reported the presence of plants as the major factor determining profile variability in a greenhouse study that was conducted using 20-L pots 0.35-m deep with 3-7-mm washed gravel to simulate vertical up-flow vegetated submerged bed systems. The pots were planted with *Schoenoplectus validus* (Vahl, A. & D. Love) and were 6 months old at the start of the experiments. Pots were sampled at 0.1 and 0.25-m depths. Primary domestic sewage was batch loaded at 1 L d^{-1} and the system had a theoretical retention time of 6 days. Significant differences occurred between different vertical positions in the profile of the planted pots, but no differences were reported in the unplanted controls. In the experimental cells, root biomass was found to be 70% in the upper half of the vessels and 30% in the lower half. This ratio was the same for both winter and summer sampling, although overall biomass was greater in the summer sample. The overall level of performance for the planted experimental systems measured as a percentage reduction was very similar in the winter and summer trials, suggesting that temperature *per se* was not the major factor for differences in the concentrations of variables within the profile.

Oxygen Balance

Measurement of dissolved oxygen (DO) in effluent from AWWT systems is temperature dependant, and is not an accurate measure of aerobic microcosms within the system. The DO of the effluent is an integration of all aerobic and anaerobic sites within the bed (Lavigne and Jankiewicz, 2000). The stoichiometric ratios for O_2 required for the reduction of influent constituents such as BOD_5 (1 g O^2 g^{-1} C) and NH_4^+ (4.5 mg O_2 per mg N) are probably a more accurate measure of the oxygen balance.

The performance of a compact vertical-flow constructed wetland system, during the first 2 years of operation, was reported by Weedon (2003). Of particular interest in this paper was the calculation of an oxygen transfer rate (OTR) as follows:

$$OTR = 0.7\ g\ O_2/gCOD\ degraded + 4.3g\ O_2/gTKN\ degraded - 2.9\ g\ O_2/g\ TON\ denitrified$$

(TON = total organic nitrogen). This equation assumes 0 mg L^{-1} DO in the influent and takes no account of biological N assimilation, therefore the author suggested recalculating assuming 10% denitrification. Other researchers have expressed OTR rates on an area basis. Luederitz *et al.* (2001) calculated that based on the German specific area requirement (50 m^2 m^{-3} d^{-1}) 8.8 g O_2 m^{-2} would be required for satisfy the COD, with an additional 4.3 g O_2 m^{-2} necessary for nitrification. Amounts of oxygen available within the root zone remain a source of controversy, however, with calculated ranges from as low as 20 mg O_2 m^{-2} (Brix *et al.*, 1996) through 2-12 g O_2 m^{-2} (Gries *et al.*, 1990; Armstrong *et al.*, 1990), 10 – 33 g O_2 m^{-2} (Platzer, 1998) to a high of 23-64 g O_2 m^{-2} (Cooper, 1999). Variation in measurement techniques and calculation formulas were the most probable source of the range listed above.

As early as 1966, researchers were measuring gaseous diffusion rates and oxygen concentrations in the roots of hydrophytic plants. Teal and Kanwisher (1966) noted that the amount of oxygen transported to the roots of *Spartina alterniflora* plants was from 1/3 to twice the requirement for root respiration. Weisner and Graneli (1989) measured mean oxygen concentrations of 16% of the gas content of the rhizome and 20 – 21% of the gas content of the shoot base.

Comprehensive studies of several wetland plant species by Wiesner *et al.* (2002) linked oxygen diffusion from the roots to the redox status of the rhizosphere. The rate at which the plants released oxygen differed with species, and with the redox potential of the rhizosphere. All species tested (*Typha latifolia, Phragmites australis, Juncus effuses,* and *Iris pseudacorus*) had an absolute release maximum in the range of -250mV to -190 mV. Oxygen release was also measured for all species over the entire range of redox values studied (-300 to 200 mV). The authors concluded that the plants released oxygen continuously, albeit at different rates, until the immediate rhizosphere was highly oxidized.

The Nitrogen Cycle

It is often assumed that nitrification can only be achieved in vertical flow wetlands by aeration of some type, which is difficult to achieve in a submerged bed system. Most studies show fill and drain systems, with the draining process used to draw air into the media, or effluent recycling to increase oxygen content and/or to dilute influent ammonium concentration. In aerated systems, however, lack of anaerobic micro-sites is often the reason for excess nitrate in the effluent (Hammersley *et al.*, 2003; Hammersley and Howes, 2002). These findings are comparable to Luederitz *et al.*

(2001) citing the work of von Felde *et al.* (1997) that in a comparison of horizontal flow and vertical flow wetlands in Saxony (Germany) there was a 3-fold higher effluent ammonium concentration from horizontal flow systems, but that vertical flow systems had a 25% higher effluent total N concentration.

In the horizontal flow pilot system mentioned previously, Breen and Chick (1995) sampled influent and effluent 4 times a day for 3 consecutive days in mid and late winter. A buildup of nitrite/nitrate and a decrease in ammonium in the mid winter sample (water temp. 9.7°C) was reported, with more ammonium and less nitrite/nitrate in the bottom samples than in top samples. These authors concluded that since nitrite and nitrate are usually denitrified as quickly as they are produced a build up of either was unusual.

von Felde and Kunst (1997) studied nitrogen and chemical oxygen demand (COD) in a two-bed vertical flow AWWT system. Ninety percent removal of NH_4^+-N was reported but only 5% of total-N was removed. The researchers reported nitrification activity to a depth of 60 cm, but most of the activity was in the 0-15-cm depth interval. Nitrate reductase activity was reported to be 10x greater in the 0-15-cm depth than from 15-60 cm.

In a bench scale study designed to mimic a full scale vertical flow system in use in Israel, Green *et al.* (1997) built 1-m^2 square (0.3-m x 0.32-m) boxes 75-cm deep with gravel layers. Ten centimeters of coarse sand with a 1-cm aeration pipe was underlain by 18-cm of small gravel (2.5 – 4-mm), above 18-cm of coarse gravel (4 – 6-mm). Five centimeters of coarse stone (10 – 25-mm) was used as under-drain. The loading rate varied with the unplanted boxes ponded, drained, and ponded again on a 6-hour

rotation. Application was for "slow fill, fast drain" Drainage was by siphon since the fast drain causes suction of air into air pipes, reoxygenating the units. The experimental system was loaded with simulated wastewater containing 40 – 100 mg L^{-1} NH_4^+-N. Using the stoichiometric equation that oxidation of each mole of NH_4^+ to NO_3^- requires 2 moles of O_2. The maximum calculated ammonium removal was 60 mg L^{-1} while maximum observed removal was 74 mg L^{-1}. The difference between calculated values and observed values was reported to be ammonium oxidation to nitrite, which lowers O_2 consumption per ammonium ion removed, and thus enables higher ammonium removal.

Nitrogen removal in a combined system (vertical flow vegetated bed over horizontal flow sand bed) was discussed by Kantawanichkul *et al.* (2001). The pilot scale system was 1.2-m x 1.2-m x 1.2-m with the vegetated portion 0.8-m deep and the horizontal flow section 0.3-m deep. The system had intermittent loading, 4 hours loaded, 4 hours rest, with 3.7-cm d^{-1} of liquid pig manure (after 5 days of settling). The average influent concentrations were COD 2,800 mg L^{-1} (1,725-3,210 mg L^{-1} range), total Kjeldahl nitrogen (TKN) 240 mg L^{-1}, and NH_4^+ 168 mg L^{-1}. Effluent concentrations averaged 43 mg L^{-1} for COD and 6.6 mg L^{-1} for TKN. The ammonium concentration leaving the vertical flow portion of the system was 1.9 mg L^{-1} while the effluent concentration leaving the horizontal flow section was 3.1 mg L^{-1}. Recycling 50% of the effluent increased total nitrogen reduction, but the NO_3^- leaving the system was 57 mg L^{-1}.

Kemp and George (1997) studied subsurface flow constructed wetlands treating municipal wastewater for nitrogen transformation and removal. A total of 77% of NH_4^+ was removed, with the first stage cells reducing the "organic load" (BOD) and the

second stage cells nitrifying the NH_4^+. In the second stage cells the increase in NO_3^- accounted for 10% of the NH_4^+ lost. The BOD_5/TN influent ratio in the second stage cells was approximately 0.7, which the authors report would favor the growth of nitrifying bacteria citing EPA (1975).

Gersberg *et al.* (1989b) reported on the results of a pilot scale end-loading vegetated submerged bed wetland system at the Water Reclamation Center, Santee, California. The beds for the nitrogen removal study were 18.5 x 3.5 x .76m-deep with an unspecified size gravel (Darcy's K $\langle 10^{-5}$ m/sec). Beds were planted to one of three hydrophytic species *Scirpus* sp., *Typha* sp., or *Phragmites* sp. Primary effluent with a BOD_5 in of 113 mg L^{-1}, NH_4^+ of 70 mg L^{-1} was applied at a rate of 5 cm d^{-1}. Mean effluent values were 11 mg L^{-1} for BOD_5 and 6.8 mg L^{-1} for NH_4^+. Total N removal with bulrush and reeds was 80% while ammonium removal varied with plant species: *Scirpus* sp. 94%, *Phragmites* sp. 78%, *Typhia* sp, 28%, and unplanted beds 11%.

Most papers suggest that there is insufficient oxygen in a wetland environment to sustain nitrification, and the effluent NH_4^+ concentrations reported support this assumption. Gersberg *et al.* (1989b), however, calculated that the bulrush plants in their study had supplied the bed with 7.2 g of oxygen per square meter of bed, more than enough to remove both BOD and NH_4^+. It is possible that the low NH_4^+ removal efficiencies reported (Sikora *et al.*, 2005; Neralla *et al.*, 2000; Morris and Herbert, 1997; White, 1995; and Zhu and Sikora, 1995) are due to low influent concentrations (20 – 40 mg L^{-1}) favoring organisms adapted to oligotrophic conditions, and that at higher influent concentrations removal efficiencies are greater due to a consistent substrate source.

Wastewater treatment N removal is by: i) degradation of organic compounds; ii) oxidation of NH_4^+ to NO_3^-; and iii) reduction of NO_3^- to N_2. Efficient oxidation of ammonium (nitrification) is typically considered to require aerobic conditions, while denitrification is thought of as an anaerobic process. Mechanized wastewater treatment plants are aerobic treatment plants, with aeration provided by mechanical means (i.e. bubble diffusers, pumping to trickling filters, rotating biological contactor, etc). Aeration in any form is expensive, and if nitrate accumulates it is difficult to treat in the aerobic environment, but the accumulation of nitrite has been shown to have inhibitory effects on nitrification, denitrification, and biological phosphate uptake; and accumulation of NO_2^- may promote the release of N_2O (a greenhouse gas) from wastewater (Nielson *et al.*, 2004).

The current focus in wastewater treatment is on partial nitrification, (oxidation of NH_4^+ to NO_2^-) followed by the anammox process, as way to reduce energy requirements and therefore the economic costs of wastewater treatment. In the anammox process bacteria utilize ammonium and nitrite in a 1:1 ratio to form N_2 gas

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$$

Bacteria capable of carrying out the anammox reaction are a new, deep branching group within the Planctomycetales including: *Candidatus Brocadia anammoxidans* which shows less than 80% 16S rRNA sequencing similarity to other planctomycetes; and *Candidatus Kuenenia stuttgartiensis* (<91 % sequencing similarity) and "several" close relatives of K. stuttgartiensis (>98% sequencing similarity) (Pynaert *et al.*, 2003). Anammox bacteria have a specific anaerobic oxidation rate 50 times faster than that of aerobic ammonia oxidizing bacteria and K_s values for ammonia and nitrite less than 5

uM (Sliekers *et al.*, 2005). These bacteria have been identified in biofilms from wastewater treatment plants (Nielson *et al.*, 2005), but have also been identified in natural sediments (Carter *et al.*, 1995; Thamdrup and Dalsgaard, 2002; Dalsgaard *et al.*, 2003).

Thamdrup and Dalsgaard (2002) observed a nitrite accumulation at two sites along the Baltic-North Sea transition. These researchers considered that it was likely that the nitrite was the oxidant for ammonium while the reduction of nitrate to nitrite was coupled to the oxidation of organic matter in the sediments. This would be analogous to the transformations occurring in wastewater reactors exhibiting the anammox process. Using ^{15}N-labeled nitrate (90% labeled) and no labeled ammonium, these researchers determined that up to 70% of the labeled N was in $^{29}N_2$; and that this distribution deviated strongly from the expected ratio for conventional denitrification which would have produced less than 10% $^{29}N_2$ through random isotope pairing. Most of the ^{14}N originated from ammonium (Thamdrup and Dalsgaard, 2002).

In a similar study in the anoxic water column of Golfo Dulce, Costa Rica, Dalsgaard *et al.* (2003) used ^{15}N labeled $^{15}NH_4^+$ but not labeled NO_2^- or NO_3^-. The bacteria produced $^{14}N^{15}N$ but essentially no $^{15}N^{15}N$. When $^{15}NO_3^-$ was used, however, both $^{14}N^{15}N$ and $^{15}N^{15}N$ were produced, with production rates increasing with increased incubation time due to the gradual build up of $^{15}NO_2^-$ in the nitrite pool. These experiments clearly demonstrate that nitrite is the electron acceptor for anammox and is a free intermediate in N_2 formed by denitrification while added $^{15}NO_3^-$ must be transferred into the nitrite pool before ^{15}N-labeled N_2 can be produced (Dalsgaard *et al.*, 2003).

In wastewater treatment the anammox process is being studied either in the CANON reactor (Completely Autotrophic Nitrogen removal Over Nitrite) or the SHARON process (Single-reactor High-activity Ammonium Removal Over Nitrite). CANNON reactors are operated at O_2 limiting conditions in combination with high ammonium concentrations (5 mM), which prevent the complete oxidation of NO_2^- to NO_3^- (Nielson *et al.*, 2005). The SHARON process is run at temperatures higher than ambient (30-35°C), at pH 7-8, with cell residence time of 1 day. These conditions create an environment where AAOB (aerobic ammonia oxidizing bacteria) can maintain themselves, and NOB (nitrite oxidizing bacteria) are washed out, resulting in the production of nitrite only (Pynaert *et al.*, 2003). The combination of the SHARON process and anammox would mean complete autotrophic nitrogen removal. To demonstrate that these processes could be combined, anaerobic oxidizing bacteria (ANAOB) were added to a continuously operation SHARON process in a rotating biological contactor (RBC). The RBC had a total reactor load of 52.32 g N/day with 89% removal (N loss of 46.56 g N/day). Ammonia was not detected (i.e. no volatilization), N_2O production was only 3.1% of N loss and NO was even lower (0.0003% of N loss). Batch tests under anoxic conditions showed simultaneous removal of ammonium and nitrate, and 99.7% of the N loss was production of N_2. The remaining 0.3% was N_2O production that occurred during the first hour, and then no more was produced. The RBC was removing N at an average of 7.4 g N/m^2 d^{-1} and translates into a ammonium surface loading rate of 8.3 g N m^2 d^{-1} attributed to autotrophic organisms, because no NH_3 volatilization occurred and because the synthetic wastewater had no carbon source. The AAOB and NOB compete for oxygen; the ANAOB and the NOB

compete for nitrite and the AAOB and the ANAOB compete for ammonium. When ammonium concentrations are high the AAOB produce nitrite, which the ANAOB combine with additional ammonia to produce nitrogen gas and a small amount of nitrate. NOB activity under these conditions was negligible. (Pynaert *et al.*, 2003).

Robertson *et al.* (1988) reported that many of the common denitrifying bacteria in soil are also heterotrophic nitrifiers. For example *Thiosphaera pantotropha* simultaneously converts ammonium to nitrite, nitrite to nitrogen gas and oxygen to water (as shown below), provided a suitable electron donor (i.e., acetate, succinate, etc.) is available as shown below (Robertson *et al.*, 1988).

$$NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^-$$
$$NO_2^- \rightarrow N_2O \rightarrow N_2$$
$$O_2 \rightarrow H_2O$$

This means that under fully aerobic conditions, these organisms convert ammonium to nitrogen gas without accumulating nitrite, and therefore not making nitrate. Rates are even faster under low DO conditions (<30% air saturation) Based on these results, Robertson *et al.* recalculated heterotrophic nitrification from published studies (Table3), showing that in some cases heterotrophic nitrification rates were only an order of magnitude less than the autotrophs. When combined with the fact that growth rates of heterotrophs may be as high as an order of magnitude greater than growth rate of nitrifiers, the significance of heterotrophic nitrification becomes much greater.

Table 3. Nitrification rates calculated from published results of batch culture experiments. For ease of comparison results were recalculated for ammonia if the original experiments used other nitrogen compounds. (from Robertson *et al.*, 1988)

Organism	Activity nm of NH_3 min^{-1} mg dry wt^{-1}	Reference
Pseudomonas aeruginosa	70-90	Ralt *et al.*, 1981
P. denitrificana	2.6	Castignetti and Hollocher, 1984
P. aureofaciens	2.8	Castignetti and Hollocher, 1984
Alcaligenes faecalis	11.9	Castignetti and Hollocher, 1984
Alcaligenes sp.	33	Castignetti *et al.*, 1985
Arthrobacter sp.	0.8	Verstraete and Alexander, 1972
Thiosphaera pantotropha	35.4	van Niel *et al.*, 1987
Nitrosomonas europae	50-100	Drozd *et al.* 1978
Nitrosomonas sp	590-2,300	Goreau *et al.* 1980

CHAPTER 3

SYSTEM PERFORMANCE

Traditionally, the nitrogen cycle has been considered to have two basic components; nitrification, the conversion of ammonium to nitrate in a two step process in the presence of oxygen; and denitrification, the anaerobic conversion of nitrate to nitrogen gas by as many as five steps. Incomplete denitrification has been identified as a source of the greenhouse gases referred to collectively as NO_x. As advances in microbiology have occurred it has come to light that the organisms associated with nitrification and denitrification are also capable of nitrification by the nitrite pathway (Wyffels *et al.*, 2003), anaerobic ammonium oxidation (Dalsgaard *et al.,* 2003), aerobic denitrification (Carter *et al.,* 1995), and anammox. Anammox is the conversion of ammonium and nitrite directly to nitrogen gas ($NH_4^+ + NO_2^- \rightarrow N_2 + 2\ H_2O$) (Dalsgaard *et al.,* 2003).

For *Nitrosomonas europaea*, as with other nitrifying bacteria, ammonium oxidation (nitrification) is the preferred mechanism to gain energy. These bacteria can grow, however, under oxic or anoxic conditions using hydrogen or organic compounds as electron donors, and nitrite as electron acceptor (Schmidt *et al.,* 2004). In natural systems, as well as in wastewater treatment processes, oxygen is often limited, and current studies are focusing on the presence of bacteria that oxidize ammonium to nitrogen gas by the anammox process (Carter *et al.,* 1995, Thamdrup and Dalsgaard, 2002, Dalsgaard *et al.,* 2003). Nitrification-anammox ($NH_4^+ \rightarrow NO_2^-$; $NH_4^+ + NO_2^- \rightarrow N_2$) results in up to 62.5% reduction in the oxygen requirement for nitrification because only half of the ammonium needs to be oxidized and the carbon requirement is

completely removed because NH_4^+ is the electron donor for nitrite reduction (Wyffels *et al.*, 2003). For wastewater treatment plants, this could represent a huge decrease in cost (electrical and chemical) as well as reduced greenhouse gas production (NO, and N_2O). Several bacteria in the order *Planctomycetales*, that are capable of carrying out the anammox process, have been identified in biofilms from wastewater treatment plants (Nielson *et al.,* 2005). Similar bacteria have also been identified in natural sediments (Dalsgaard *et al.,* 2003, Kuypers *et al.,* 2003). While the focus of this study was not to determine if the anammox process was occurring in the wetland cells, the recent research brings that possibility to the table for discussion.

The classic literature of wetland assessment indicates that nitrogen removal in artificial wetlands has been of limited success, usually in the range of 70 to 80% removal of ammonium with an increase in nitrate (Reed, 1995; Kadlec and Knight, 1996, Gersberg *et al.*, 1989b). The artificial wetland, which was the focus of this study, has performed much better than literature values would predict (95-99% reduction in ammonium, 1-3% increase in nitrate) since it became operational in 2001. There have, however, been periods when nitrogen removal was not at acceptable levels. The current study was undertaken to determine the probable cause(s) of these lapses in performance. Wastewater parameters measured for this study included ammonium, nitrate, nitrite, and $CBOD_5$. Additionally, redox probes were placed at three depths within the wetland cells to determine if the probes could be used as a diagnostic tool in assessing plant and or microbial health and activity.

Methods and Materials

Site

The Zumtobel Staff Wetland is located in the town of Highland, NY on the western bank of the Hudson River approximately 90 miles north of New York City. The Staff system began receiving wastewater in June 2001. This system was constructed using Top Loading Vertical Flow Submerged Bed Treatment System (TLVFSBTS) technology (Fig. 5).

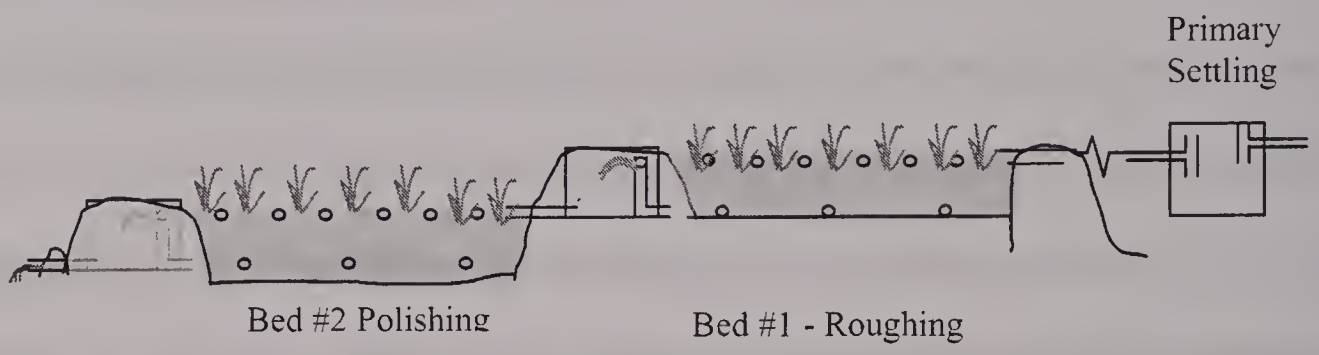


Figure 5. Top loading vertical flow submerged bed treatment system (TLVFSBTS, Pat. Pending) cross sectional view showing control boxes.

The system consists of 4 treatment cells, two trains (parallel operation) of 2 beds in series. Each bed is 237 m^2 (950 m^2 total). Two beds (1 train) are planted with Reed Canarygrass (*Phalaris arundinacea*) and the other train with common reed (*Phragmites communis*). The design flow rate was 39 $m^3\ d^{-1}$. The current mean flow rate is 7.5 $m^3 d^{-1}$. The current flow pattern is four beds in series, with all wastewater passing through the two Canarygrass beds before being pumped to the reed beds.

The treatment system is a part of the Highland, NY Sewer District and discharges to two small man-made ponds, the second of which discharges to an intermittent stream. The wetland system is subject to a NY SPDES permit. Permit levels for the Staff wetland system are BOD_5: 5 mg L^{-1}; TSS: 10 mg L^{-1}; NH_4^+: 2.2 mg L^{-1}; and PO_4^{3-}: <1 mg L^{-1}, respectively.

Media Type

The media is processed black shale coarse sand with a uniformity coefficient of 5.3 and no fines (Fig. 6). Mean bulk density at the surface, as tested in November 2006 is 1.08 ± 0.01 Mg m^{-3}. The mean water content at 15 cm-depth is 0.15 ± 0.01 g g^{-1} (water: soil).

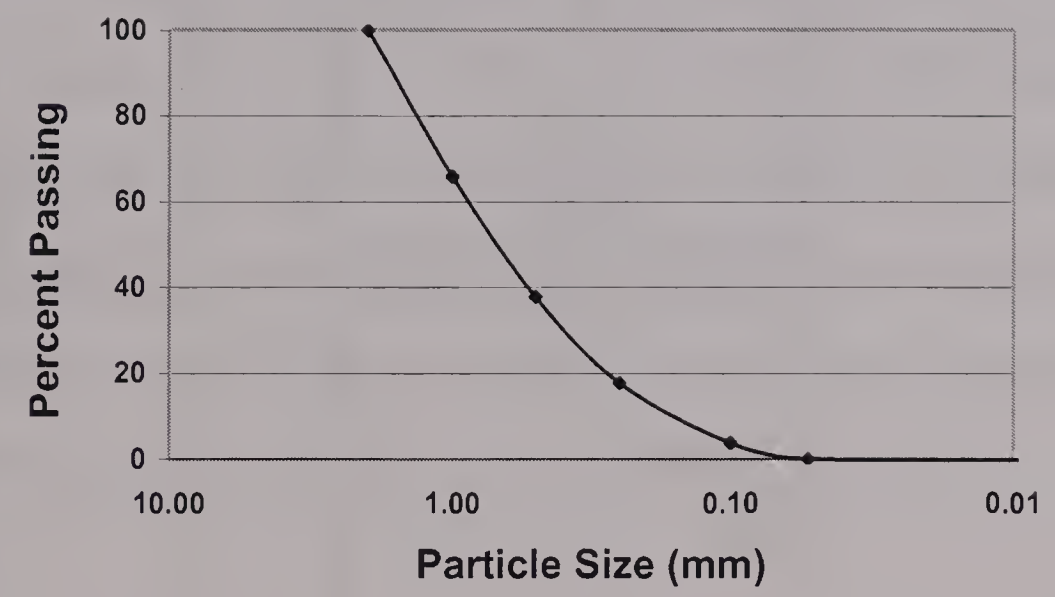


Figure 6. Particle size distribution for wetland media, Zumtobel Staff Lighting, Highland, NY.

Monitoring Data

Samples were collected weekly (April - December) or bi-weekly (January – March) and routine wastewater parameters were measured. These consisted of $CBOD_5$ by Standard Method SM 507 (American Public Health Association *et al.*, 2003); and ammonium, nitrite, and nitrate by HACH methods 8075 (Nessler method), 8507 (diazotization NED rapid liquid method), and 8171 (cadmium reduction method), respectively (HACH Company, Loveland, CO). $CBOD_5$ samples were replicated 5 times, while all nitrogen measurements were done in triplicate.

Influent samples were taken from the "distribution" box (D-Box) at the head of the four-wetland cells (Figure 7). This D-Box allows the influent to be: 1) split and sent

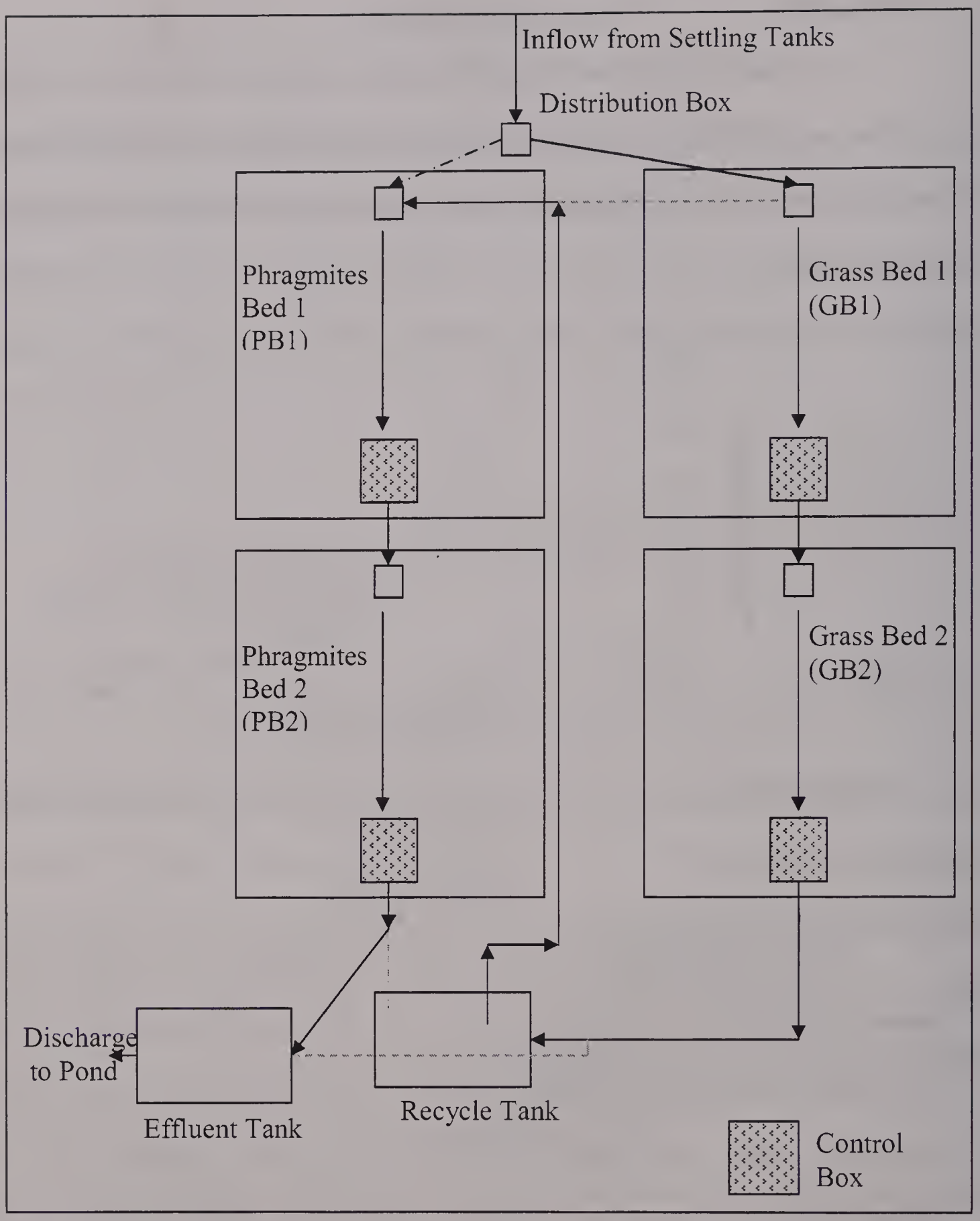


Figure 7. Schematic diagram of the Staff wetland after spring 2006 recycle tank installation. Arrows indicate direction of flow; dashed gray lines indicate alternative flow paths; and dash-dot-dash arrow indicates overflow pathway.

to both GB1 and PB1 in approximately equal portions, or 2) for all flow to be sent to either GB1 or PB1. During the first four years (2001 – 2004) the system operated as two parallel systems, with the effluent being recycled through the system or sent to the existing leachfield. System flow was GB1 to GB2 to effluent tank and PB1 to PB2 to effluent tank. In May 2005 system operation was converted to four beds in series, using the "effluent tank" to recycle effluent to PB1 (system flow was GB1 to GB2 to effluent tank; effluent tank to PB1 to PB2 to effluent tank, with the effluent tank receiving inflow from both GB2 and PB2). During the spring of 2006, a new recycle tank was added to the system so that system flow was from GB1 to GB2 to recycle tank, to PB1, to PB2, to effluent tank to discharge. Monitoring samples were also taken from the control box of each bed and are referred to as GB1, GB2, PB1 and PB2.

Redox Probes

Redox probes were constructed by the method of Vepraskas and Bouma (1976) by soldering 1.25 cm platinum wire (20 gauge) to copper wire (12 gauge). The copper wire was sealed inside a 0.67 cm PVC pipe using epoxy (Loctite, Corp.) to make the probes watertight and to seal the platinum/copper junction in epoxy. Five replicate probes were installed at 15-, 30-, and either 45- or 60 cm-depths within each wetland cell and within 25 cm of a salt bridge. Salt bridges were made from 1.25-cm PVC pipe, filled with saturated KCl in 3% agar (Veneman and Pickering,1983). Holes were drilled in the salt bridges at 15, 30, 45, and 60 cm. Redox potentials were measured with a calomel electrode connected to a digital multi-meter (Radio Shack, #22 - 813). Redox potentials were taken weekly during the growing season, biweekly during the remainder of the year and were corrected according to Bates (1973).

Statistical Analysis

All system performance data were subjected to an analysis of variance (ANOVA) using either PROC ANOVA or PROC GLM of the SAS System, Version 9.1.3 (SAS Institute, Inc., 2004). When indicated by the ANOVA F-Test, means were separated by use of Duncan's New Multiple Range Test.

Results and Discussion

Wetland influent and effluent samples from each cell were analyzed for ammonium, nitrate, nitrite (Appendix A), and $CBOD_5$. Table 4 provides summary data for these measurements. The additional nitrogen analyses were performed for use with calibrating the model (Chapter 5) but they also increase the understanding of the nitrogen transformations within each bed and may shed light on the possible microbial processes occurring within the wetland cells.

Table 4. Influent and effluent nitrogen concentrations in samples from August 2005 through December 2006.

Parameter	Sample	Average	Maximum	Minimum
		---------- mg L^{-1} ----------		
Ammonium[†]	Influent	170.8 ±26.0	265.0	94
	GB1 Effluent	49.9 ±17.4	93.3	13
	GB2 Effluent	30.4 ± 9.2	58.0	8
	PB1 Effluent	10.8 ± 7.8	34.7	0
	PB2 Effluent	2.2 ± 1.8	10.0	0
Nitrate[‡]	Influent	3.1 ±2.0	11.2	0
	GB1 Effluent	11.2 ±5.1	29.2	1.2
	GB2 Effluent	10.2 ±5.1	25.3	0
	PB1 Effluent	8.25 ±4.6	24.2	0
	PB2 Effluent	4.13 ±2.5	25.7	0
Nitrite[†]	Influent	0.015 ±0.008	0.102	0.000
	GB1 Effluent	0.380 ±0.364	2.204	0.010
	GB2 Effluent	0.023 ±0.024	0.191	0.002
	PB1 Effluent	0.021 ±0.016	0.135	0.002
	PB2 Effluent	0.002 ±0.002	0.019	0.000

[†] n = 153 for influent, GB1 and GB2; 105 for PB1; and 120 for PB2
[‡] n = 150 for influent, GB1 and GB2; 102 for PB1; and 117 for PB2

Carbonaceous Biochemical Oxygen Demand

Influent $CBOD_5$ ranged from 103-421 mg L^{-1} with a mean concentration of 259 mg L^{-1}. Low values correspond to times when the settling tanks had been pumped, and extreme high values correspond to shortly before pumping occurred. Influent samples and samples from GB1 were diluted to obtain repeatable and valid measurements. Samples from GB2, PB1 and PB2 were run undiluted, and valid results were obtainable from GB2 only. During the fall and early winter of 2005-2006 samples from both *Phragmites* beds failed to exhibit the 2 mg L^{-1} decrease in DO required for a valid $CBOD_5$ test, so analysis of these samples was discontinued. Effluent from GB1 exhibited a seasonal trend in $CBOD_5$ reduction (Fig. 8), with concentrations below 5 mg L^{-1} (Permit level) during the growing season, but rising to as high as 18 mg L^{-1} during the winter and early spring. Effluent from GB2 showed the same seasonal trend, but remained below 4 mg L^{-1} throughout the monitoring period.

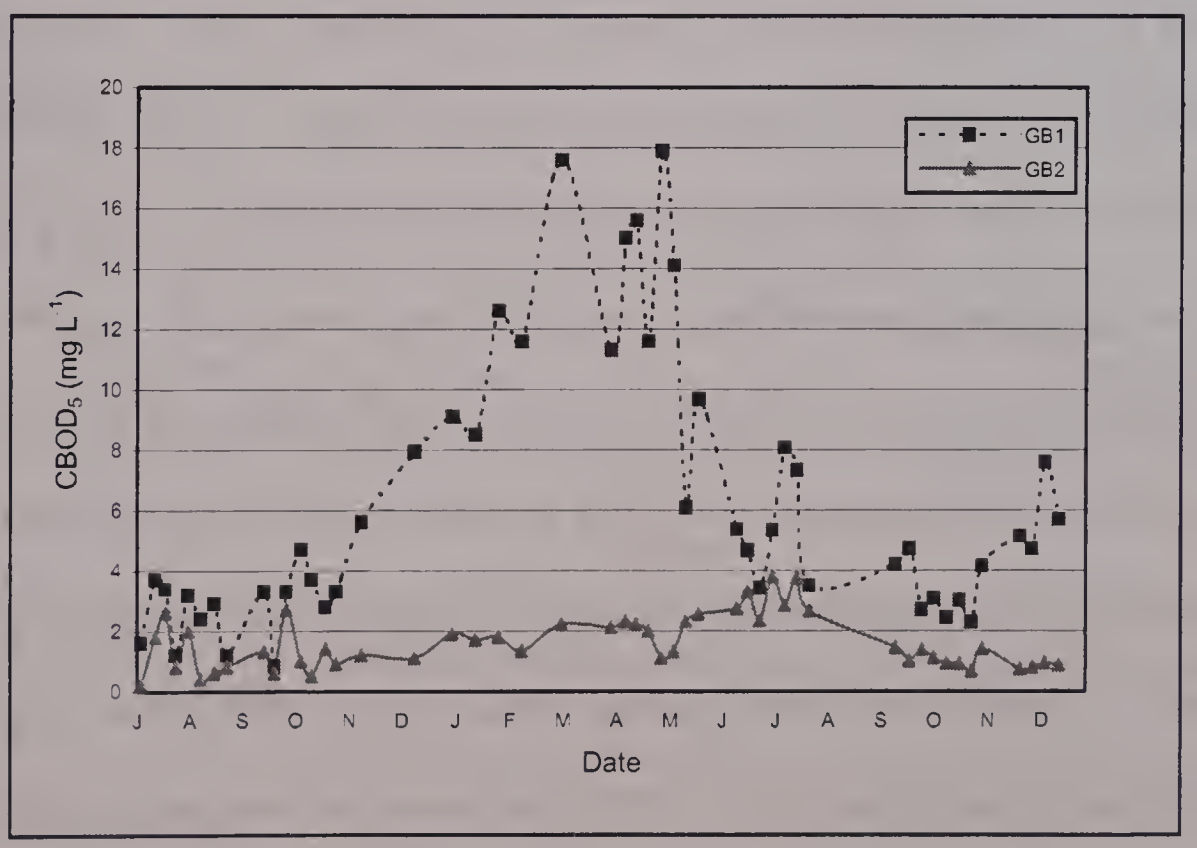


Figure 8. Mean five-day carbonaceous biochemical oxygen demand for effluent from GB1 and GB2 between July 14, 2005 and December 14, 2006.

Ammonium

Influent and effluent ammonium concentrations for GB1 and GB2 are presented in Figure 9. It was possible to collect a sample from these two beds every week that sampling occurred. Data for PB1 and PB2 are presented in Appendix A because during most of the summer and fall it was not possible to obtain an adequate sample from PB1. It is clear that during some of this time there was little or no outflow from either bed. At other times it was possible to collect a sample from PB2, so obviously flow was going through PB1 into PB2, just not at the time of sampling. Additionally, since overflow from the D-Box at the head of the system was shunted to PB2 during periods of peak flow, an accurate method for establishing what the PB1 influent ammonium-N was not attainable. This was not considered to be a problem with nitrate or nitrite, as any influent bypass would not have introduced appreciable amounts of either.

Regardless of inflow ammonium concentration, effluent from GB1 was less than 50 mg L^{-1} during the late growing season, early winter (August – February) but rose to 70–80 mg L^{-1} during the spring and summer (March – July). Effluent from GB2 followed the same seasonal trend with fall/winter concentration ranging from 20–30 mg L^{-1} and spring/summer concentrations remaining between 40 and 50 mg L^{-1}. This seasonal pattern can be attributed to the increased oxygen available in the root zone from actively growing plants. The delay in return to fall/winter performance levels can be explained by the growth habit of cool season grass species. During times when top growth is favored over root growth, performance suffered. The large decrease in ammonium concentration in GB1 (70.8% reduction) as compared to GB2 (39.2%) may be due to a smaller microbial population in GB2 (see Chapter 4). Evapotranspiration

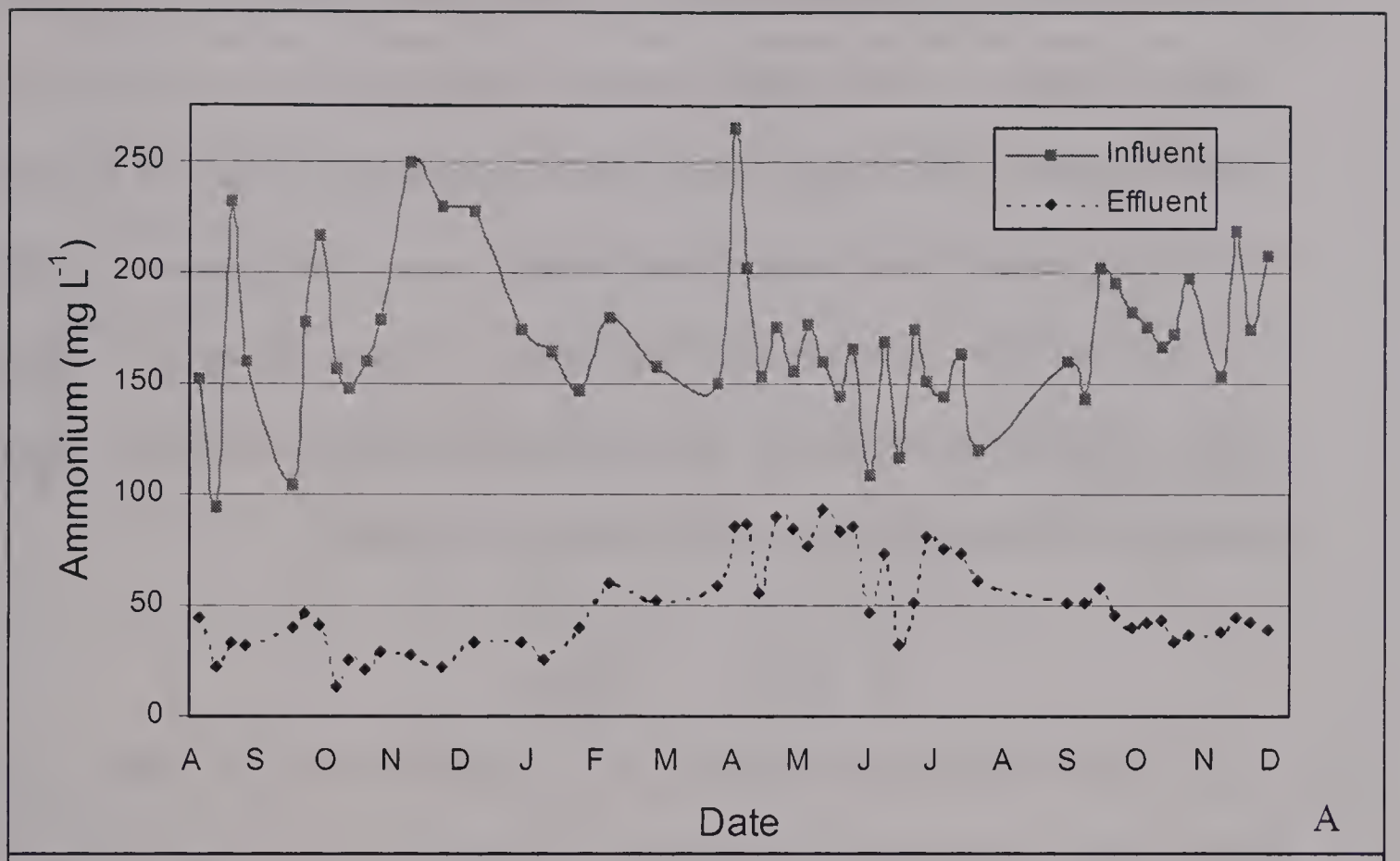


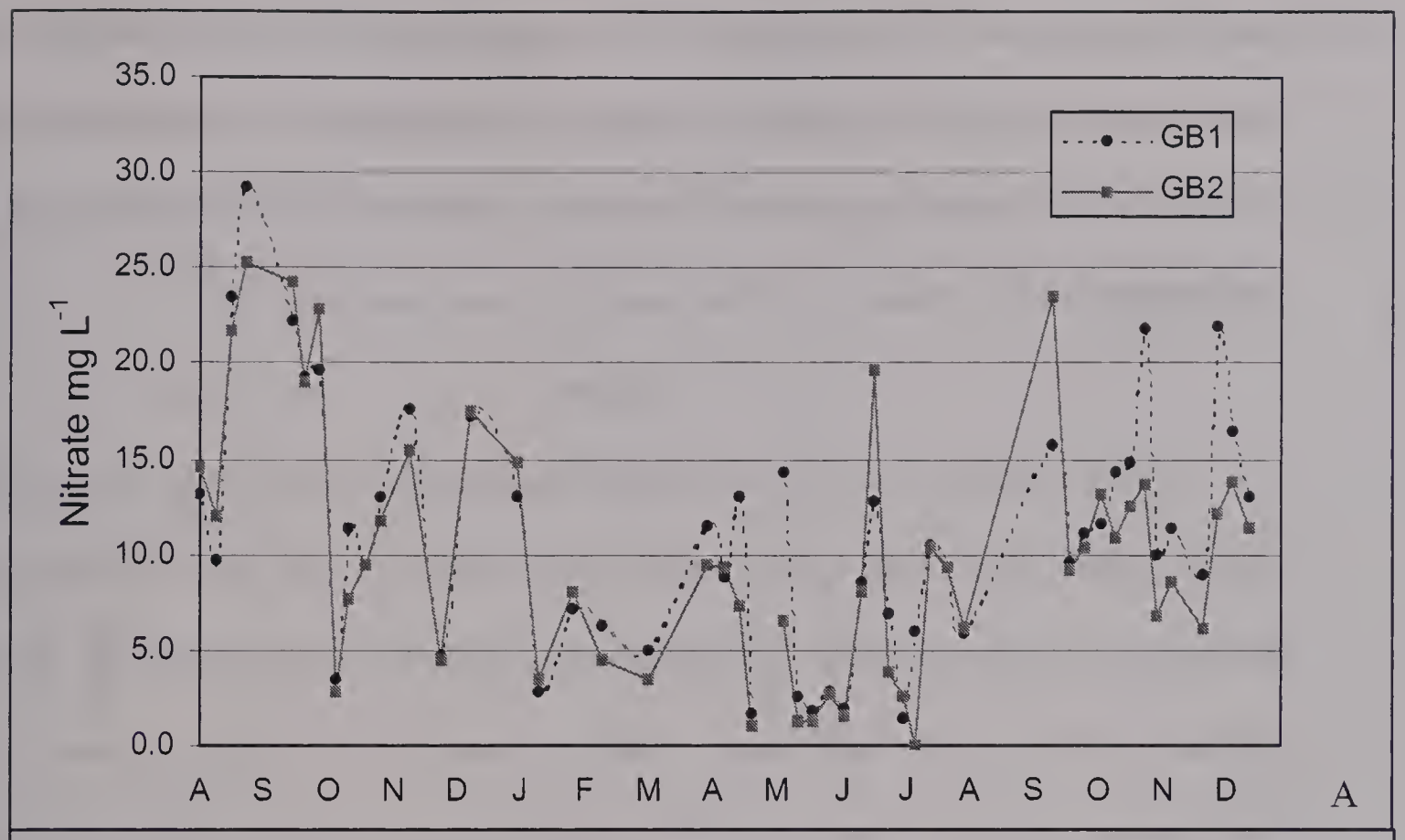
Figure 9. Mean influent and effluent ammonium concentrations for GB1 (A) and GB2 (B) from August 1, 2005 through December 14, 2006.

from the plants and evaporation from the media result in periods of "no flow", where influent volume to the system cannot keep pace with water loss due to evapotranspiration. Since daily inflow remains essentially constant, the first bed in the series (GB1) would receive normal flow. Volume loss in GB1, however, would result in reduced flow to the second bed (GB2). The lower flow to the second bed may have caused a decrease in substrate for the nitrifying organisms and resulted in reduction of performance (Kemp and George, 1997; Neralla *et al.*, 2000).

Nitrate

Effluent nitrate concentrations for all beds are shown in Figure 10. System influent nitrate concentrations (Appendix A) were low (3.1 ± 2 mg L^{-1}) as would be expected in primary effluent, but may have been skewed to the high side due to the presence of suspended solids in the samples. It was necessary to use undiluted samples to obtain enough nitrate to measure, and samples were zeroed against untreated influent. Flow to the system from the anaerobic settling tank was not constant and was influenced by both time of day and day of the week. As such, it is possible that periodically influent to the system reflected some denitrification occurring within the settling tank.

With notable exceptions (22 and 28 September, and 15 December 2005) nitrate concentrations leaving PB2 did not exceed 10 mg L^{-1} (Primary Drinking Water Standard). The highest mean nitrate concentration (25.7 mg L^{-1}) leaving PB2 during the course of the study was on October 5, 2005. The October 5th sample was the second sample collected after an absence of effluent from either PB1 or PB2 for at least 3 months. Samples were analyzed by the cadmium reduction method, which may have



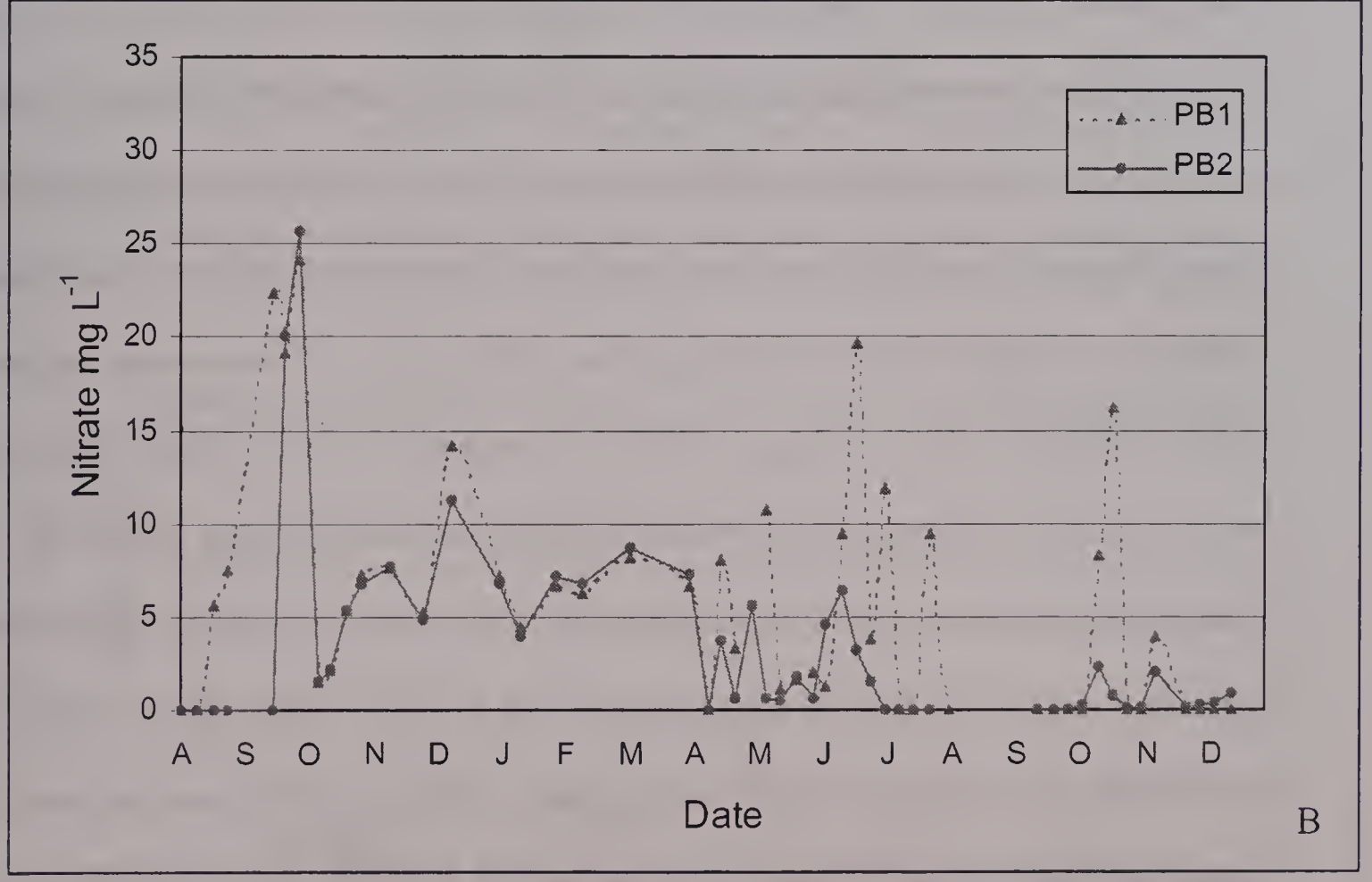


Figure 10. Mean nitrate concentrations for GB1 and GB2 (A) and PB1 and PB2 (B) from August 1, 2005 to December 14, 2006.

interferences caused by the presence of iron, copper and other metals (American Public Health Association *et al.*, 2003). Samples were analyzed for total iron (the most prevalent of the interfering metals) during May 2006 and iron was determined to be nondetectable in the effluent of all four beds (data not presented).

Nitrite

Periodic spikes in nitrite concentration occurred in GB1and PB1 during the fall of 2005 (Figure 11). These spikes were more sustained in PB1 than GB1 during this time period and did not appear to be followed by spikes in GB2 and PB2 as would be expected if the nitrite had been "flushed through" the entire system. Nitrite concentrations exceeded 1 mg L^{-1} in GB1 during most of the late winter and spring of 2006. Spikes in nitrite concentration did occur in GB2 and then PB1 following periods of heavy rain in April and May 2006 indicating that in this case nitrite probably was being "flushed" from GB1 into the other beds, but as with nitrate concentrations, effluent leaving PB2 were never higher than 0.02 mg L^{-1}. The seasonal increase in nitrite exhibited in GB1 during fall 2005 was repeated in fall of 2006. If nitrifying bacterial growth is represented by Monod kinetics, under steady-state conditions nitrite accumulation has been shown to be insignificant; thus the rate limiting step is assumed to be the oxidation of ammonium (Benefield and Randall, 1980). This would suggest that the dramatic increase in nitrite concentration exhibited in GB1 was the result of a major disturbance in the nitrite oxidizing population (i.e. *Nitrobacter*, *Nitrospira*, etc.). While this is definitely a possible explanation for this phenomenon, it seems strange that the suggested decrease in nitrite oxidation begins at the same time that there is a seasonal increase in total microbial population along with increases in both nitrification

potential and denitrifying enzyme activity (see chapter 4). An alternative explanation would be that during the summer months, the apparently temperature sensitive

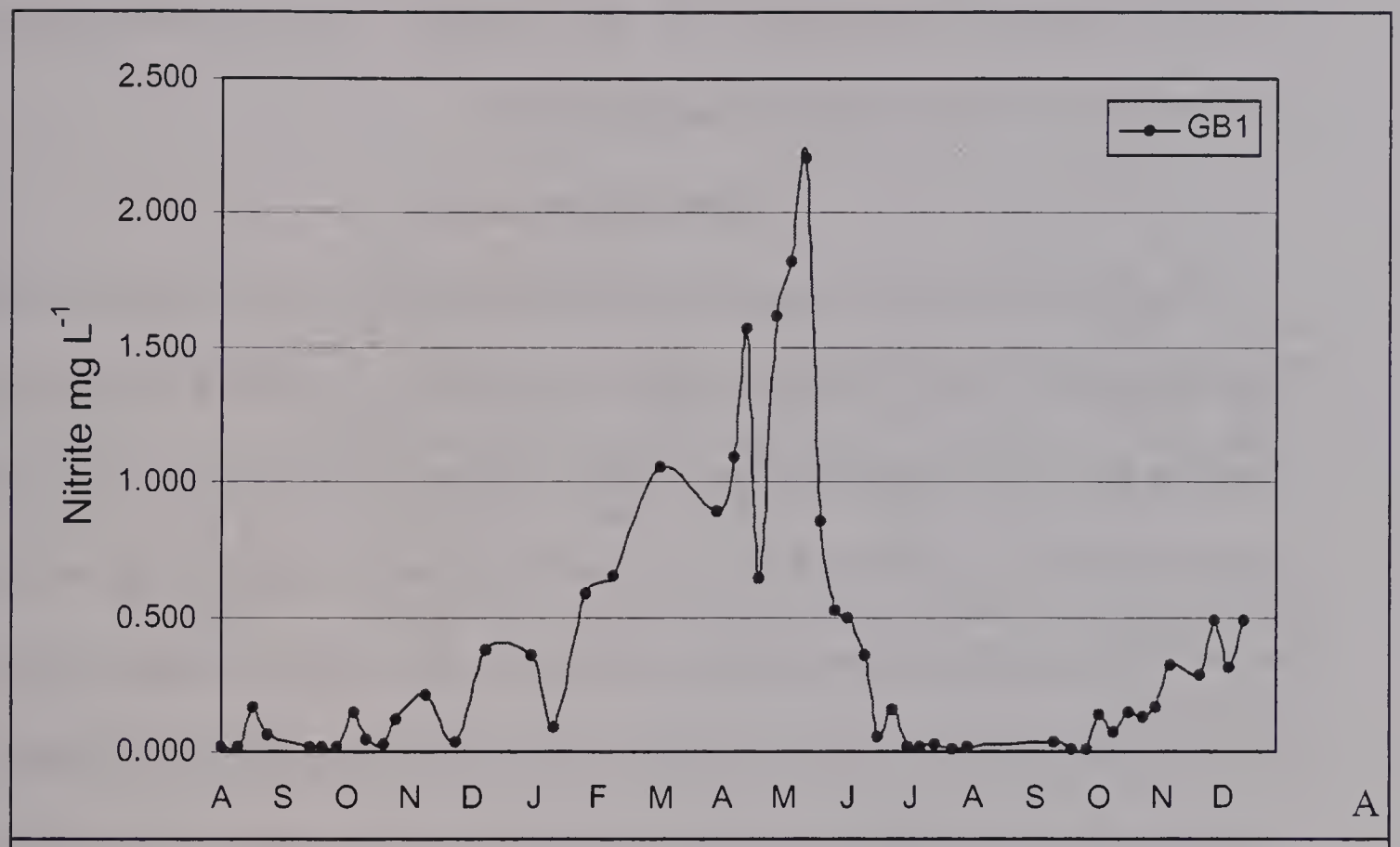


Figure 11. Mean nitrite concentrations for GB1 (A) and GB2, PB1. PB2 (B) from August 1, 2005 to December 14, 2006.

anammox reactions are occurring, consuming nitrite and ammonium in a 1.32:1 ratio (Schmidt *et al.,* 2002, Wyffels *et al*., 2003), but when temperatures begin to drop in the fall, the anammox reactions first slow, and then stop completely when temperatures drop below 6-7°C (Thamdrup and Dalsgaard, 2002).

Redox Measurements

In GB1, the corrected mean redox potential at the 15-cm depth was 440 mV, dropping below 375 mV only six or seven times during the 18-month study (Figure 12). Probes at the 15-cm depth in the other 3 beds (Figures 13, 14, and 15) were subject to the most variation in potential from one measurement to the next. This was indicative of the rapidly changing moisture content at this depth. The hydraulic regime for all of the beds kept the phreatic level at or below the 15-cm depth (controlled by the height of the overflow pipe in the control box). Evaporation from the exposed sand surface in the *Phragmites* beds and evapotranspiration from the plants in all beds would drop the phreatic level significantly during the summer. Similarly, rain events would raise the level quickly. The shallowness of the depth, and the lack of a bentonite seal around the probes also contributed to variability of readings at this depth.

The potential in GB1 was usually lower at the 30-cm depth (mean 195 ± 85 mV) than at the 60-cm depth (mean 289 ± 43 mV) indicating more microbial activity and less free oxygen at the 30-cm depth. Potential at both depths was consistently below the threshold levels for nitrate reduction. A similar trend was seen in both GB2 and PB2 during the calendar year 2006. For PB2, the readings at the 30- and 45-cm depth during the late summer and fall of 2005 were virtually indistinguishable from each other. In GB2, however, the potential was higher at both the 30 and 45-cm depths during 2005

than at these depths during the summer of 2006. This change in redox potential mirrored a noticeable lack of vigor, and browning of the leaf blades in GB2 during summer 2006.

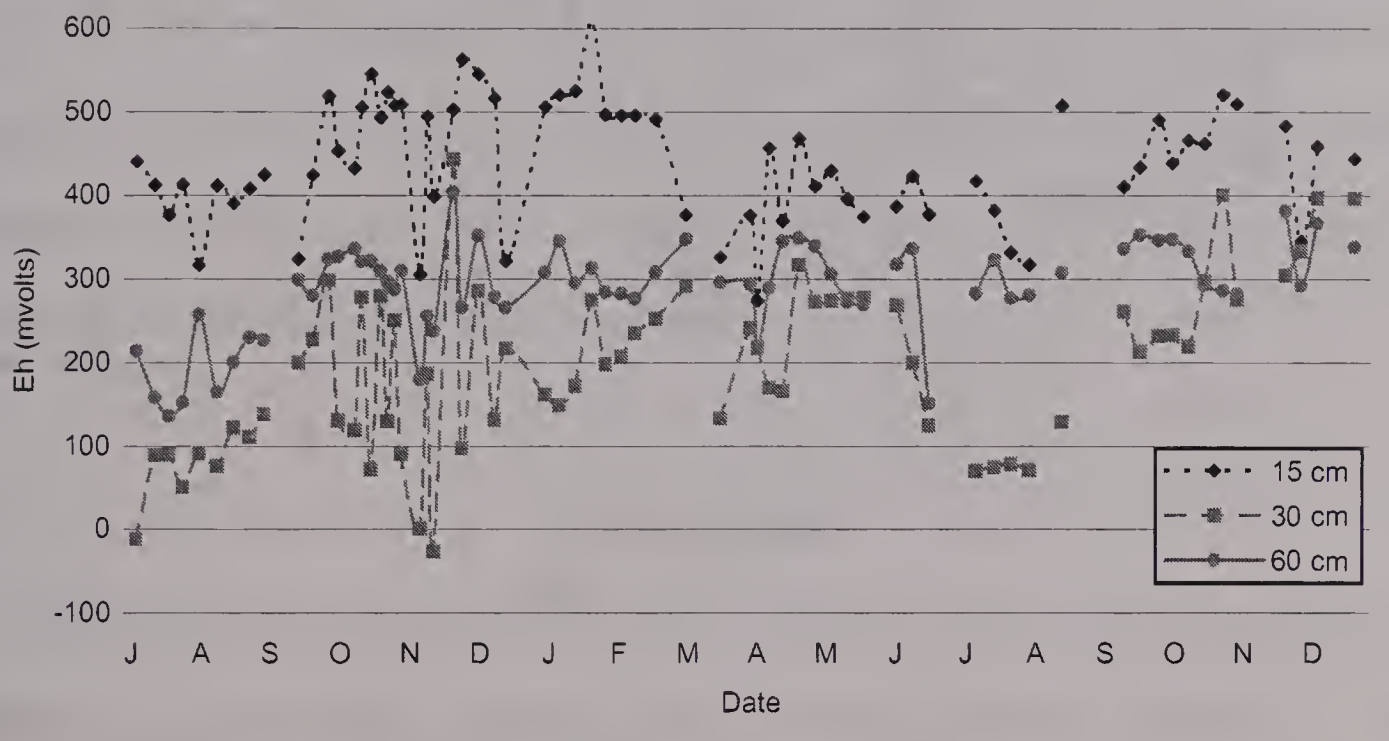


Figure 12. Redox potential in GB1 from July 1, 2005 to December 22, 2006.

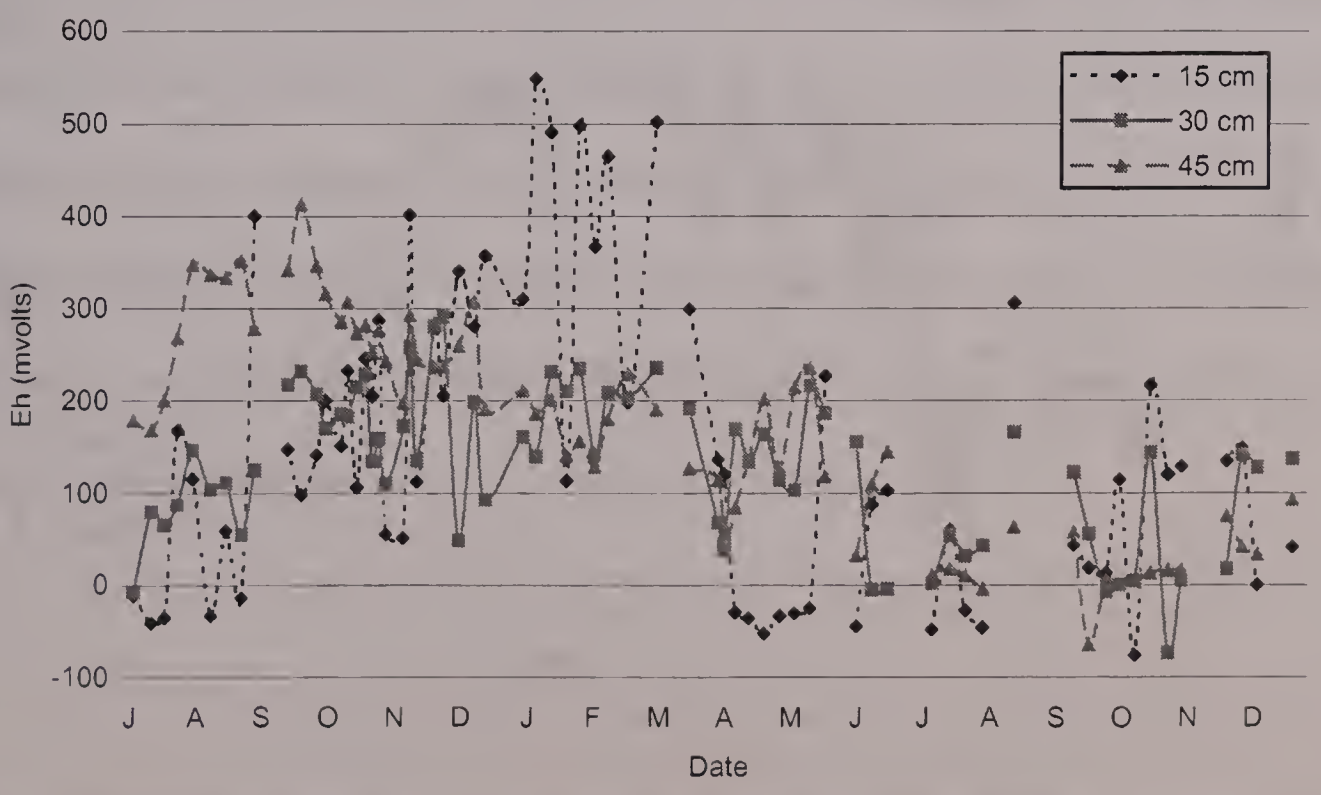


Figure 13. Redox potential in GB2 from July 1, 2005 to December 22, 2006.

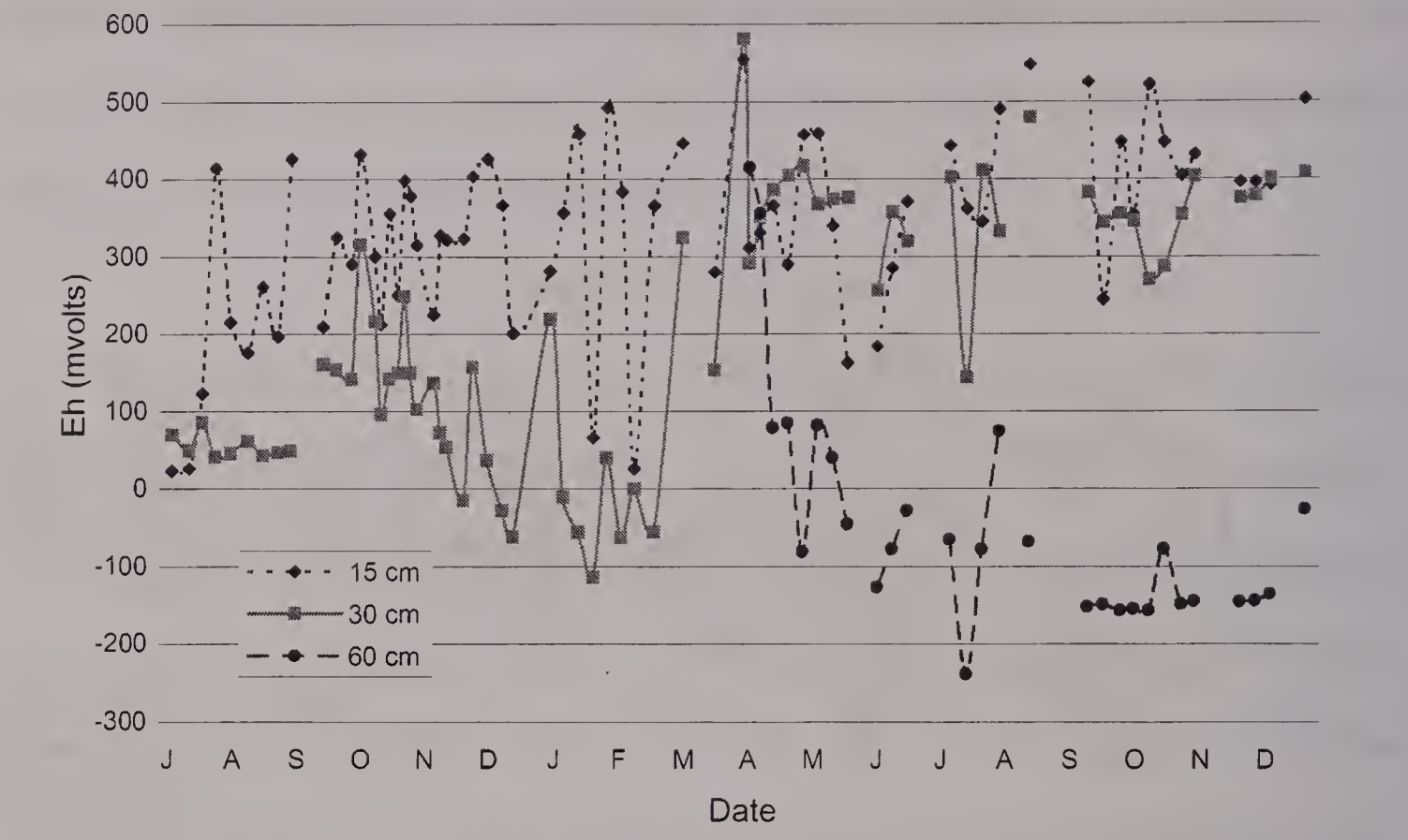


Figure 14. Redox potential in PB1 from July 1, 2005 to December 22, 2006.

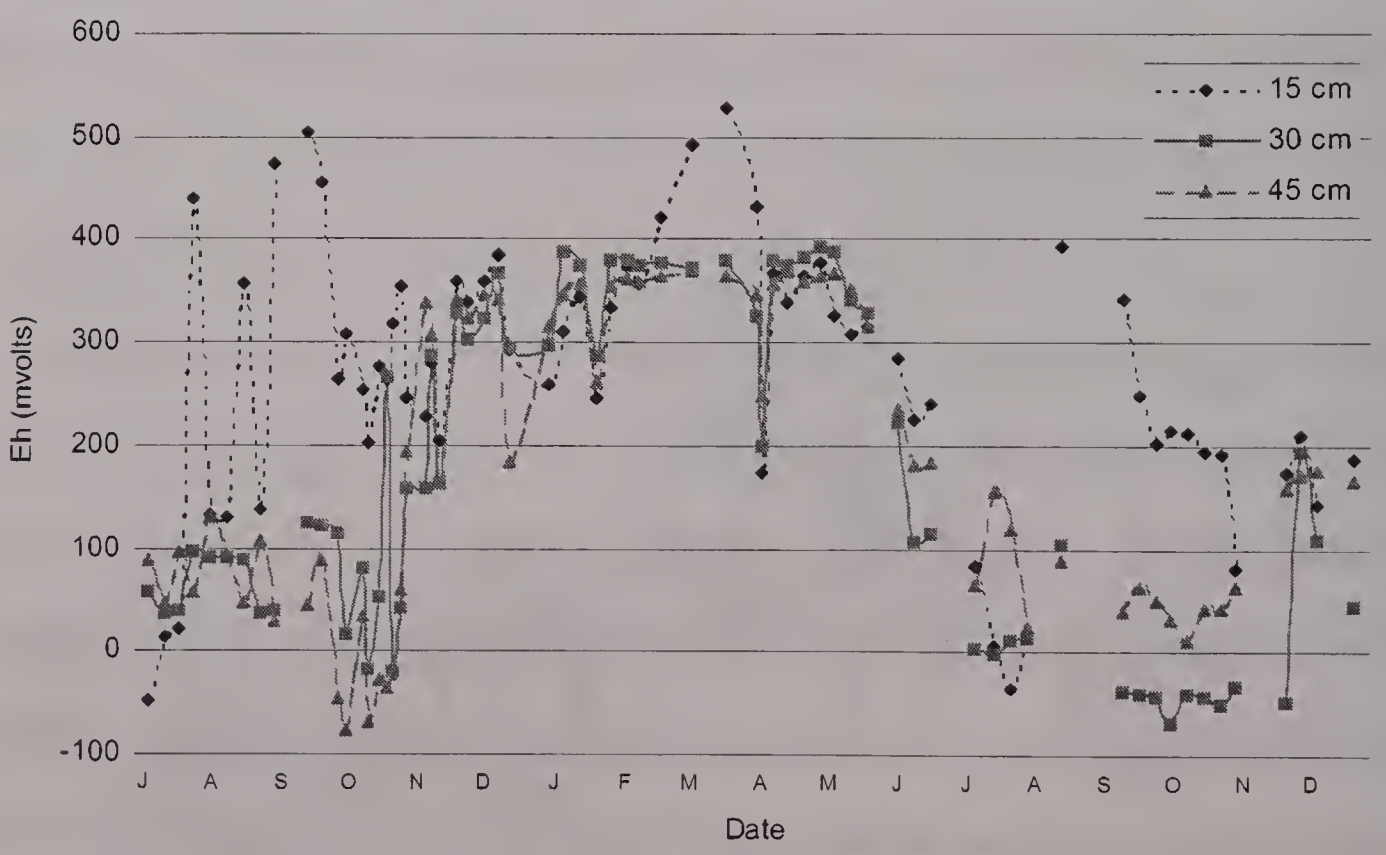


Figure 15. Redox potential in PB2 from July 1, 2005 to December 22, 2006.

PB1 did not have deep redox probes during 2005. In April 2006 all probes in PB1 were moved to a new location where it was possible to install probes at the 60-cm depth. In the new location less variability was seen in the replicate readings, than was exhibited during 2005. The probes at the 60-cm depth took approximately 2 weeks to stabilize at less than 100 mV, dropped to -50 mV by May where they remained until early September when they dropped to -150 mV. There was very little variation in reading at this depth once the rains started in September, while the shallow probes in this bed continued to indicate lack of moisture (400 mV).

Conclusions

As indicated by most published evaluations, treating $CBOD_5$ to permit levels was not difficult to accomplish, although lack of carbon source ($CBOD_5$) for other microorganisms in GB2 may be a problem. Ammonium oxidation in GB1 was greater than in GB2 (based on percent reduction) and this trend was not influenced by lack of plant vigor during 2006. Nitrite accumulation in GB1 appeared to be seasonal, and not directly related to nitrite oxidation. Neither nitrite nor nitrate leave the system at levels that might be considered environmentally significant (i.e. both are below primary drinking water standards). Although redox measurements appear to be indicative of microbial activity within the beds, it is unclear if the costs of the probes (both time and money) are justified.

CHAPTER 4

MICROBIAL ANALYSIS

Introduction

Discussion of microbial processes in the artificial wetland literature generally falls into two categories, pathogen removal efficiencies (Gersberg *et al.*, 1989a; Ulrich et al., 2005; Song *et al.*,2006) and discussion of microbially mediated pollutant removal (typically nitrogen and BOD). Pollutant removal is the parameter measured; results and discussion are focused on removal efficiencies (i.e. influent ammonium concentration, effluent ammonium concentration, % removal etc.) (Gersberg *et al.*, 1989b; Kemp and George, 1997; Kantawanichkul *et al.*, 2001); and there is no discussion of the microorganisms responsible for the reductions. Few studies have undertaken significant analyses of the actual microbial communities, or measurement of the microbially mediated process themselves. One published study attributed lower microbial populations, which were actually quantified; to reduced rooting of *Phalaris arundinacea* (20-30-cm) as compared to *Phragmites* sp. where roots were found to a depth of 60-80-cm (Ottová *et al.*,1997). The lack of rooting depth in the *Phalaris* bed in Chmelná wetland may have been an artifact peculiar to that system (i.e., media type or other environmental factor). At the Staff Wetland in Highland, NY two beds are planted with *Phalaris arundinacea* and they have typically out performed the *Phragmites* beds. This would indicate if not a more extensive root system, then a greater ability to provide oxygen to the microsites.

The current study was undertaken to elucidate some of the factors responsible for the range and fluctuations of nitrification and denitrification within a top loading

vertical flow submerged bed treatment system (TLVFSPTS). Heterotrophic potential, nitrification potential, denitrifying enzyme activity, and total microbial biomass were evaluated throughout the growing season to determine environmental factors influencing the processes but not to determine which specific organisms might be responsible. The Zumtobel Staff Wetland is located in the town of Highland, NY on the western bank of the Hudson River approximately 90 miles north of New York City. The Staff system began receiving wastewater in June 2001. This system was constructed using TLVFSBTS technology, details of which are to be found in chapter 3.

Methods and Materials

Sampling

Samples for each of the microbial analyses were collected with a Dutch auger at a depth of 15 cm. Only the portion of the sample at the very tip of the auger was used. Samples were collected from random locations within each wetland cell, and no sample was collected from the same place twice. Five samples were collected on each sampling date. Analyses were repeated on a monthly basis.

Heterotrophic Potential

Heterotrophic potential measurements were modified from those of Wright and Hobbie (1966), Behera and Wagner (1974), and Scow *et al.* (1986), and are summarized below. Media samples were collected from 15-cm depth at 5 random locations within each wetland cell and transported to the lab on ice. Ten-gram sub-samples were placed into 125-mL Erlenmeyer flasks and allowed to acclimate to incubation temperature for 12 hours prior to initiation of each experiment. Two and a half mL of 1M NaOH solution were placed in a 7-mL trap within each flask. Samples received 1 mL glucose

solution (2 mg mL^{-1}) amended with 1-^{14}C-D-Glucose- (46.6 mCi $mmol^{-1}$; Sigma Chemical, St. Louis, MO) to provide 1.8 x 10^5 dpm mL^{-1}. Microbial metabolism was stopped by the addition of 1 mL 0.5 M sulfuric acid injected through the rubber turnover septum stopper. Samples were incubated for 20, 20, 30, 60, 90, and 180 min at 27, 20, 16, 12, 8, and 4°C, respectively. A 2-mL aliquot from each trap was added to 18 mL of Scintiverse BD scintillation cocktail (Fisher Scientific) and placed in the dark, at room temperature, overnight, to reduce chemiluminescence. Samples were counted using a multi-purpose scintillation counter (Model LS 6500; Beckman-Coultur, Fullerton, CA).

Potential for glucose assimilation and respiration was expressed as V_{MAX} for total uptake in micrograms per gram of wetland media per hour (Wyndham and Costerton, 1981). Preliminary experiments were conducted with media from a single bed at each incubation temperature to determine incubation duration. Incubation times were adjusted so that no more than 3% of the substrate was metabolized during the course of the experiment. To determine if differences in heterotrophic potential existed, the experiment was repeated using media from each of the four beds.

Microbial Biomass

Microbial biomass (carbon:nitrogen ratio) was calculated from ninhydrin reactive nitrogen values (Joergenson and Brooks, 1990) obtained following simultaneous fumigation and soil extraction (Fierer, 2003). Five media samples (A-E) from each wetland cell (GB1, GB2, PB1, PB2) were isolated, split in half, and 5-grams of each isolate weighed into 20-ml scintillation vials. Samples were extracted with 15 mL of 0.5 M potassium sulfate (Doyle *et al.*, 2004). One set of the isolated samples

received 0.5 mL of ethanol-free chloroform prior to placement on either a reciprocal shaker (75 strokes per minute) or an orbital shaker (50 revolutions per minute) at ambient temperature for 4 hours. Samples were then filtered through Whatman #1 filter paper and the filtrate stored at 4°C for analysis. Extracting solution with and without chloroform addition was also filtered to determine the background concentration of nitrogen in the extracting solution and on the filter paper. Following filtration a 0.75 mL aliquot of each sample was combined with 1.75 mL of citric acid buffer (pH 5) and 1.25 mL ninhydrin reagent (0.11 M in 3:1 DMSO to lithium acetate buffer {4 M, pH 5.2}). Samples were then placed in a "vigorously boiling" water bath for 25 minutes; cooled to room temperature; diluted with 4.5 mL of ethanol-water (50:50); mixed; and had absorbance read at 570 nm on a Hitachi U-2000 UV-VIS Double Beam Spectrophotometer (Hitachi Instruments, Schaumburg, Illinois).

Ninhydrin reactive nitrogen was calculated using Eq.[5]; where NRN = ninhydrin reactive nitrogen (mg kg^{-1}), r = regression coefficient, A = sample absorbance, V = sample volume (ml) and W = sample weight (g).

$$NRN = (rA \cdot V) \cdot W^{-1}) \qquad [5]$$

Background absorbance values were subtracted from sample absorbance when needed (i.e., when extracting solution/filter paper absorbance ≠ 0). The value calculated for a nonfumigated sub-sample was subtracted from the value calculated for the fumigated sub-sample (i.e., NRN for GB1A non fumigated would be subtracted from NRN for GB1A fumigated). The mean of the difference was calculated and then multiplied by 5.0 to convert to total microbial nitrogen. Total microbial carbon was calculated by multiplying the NRN value by 20.6 (Joergenson and Brooks. 1990).

Nitrification Potential

Five replicate samples (15 g moist media) were weighed into individual 125-mL Erlenmeyer flasks to which 45 mL 0.5 M phosphate buffer (pH between 7.0 and 7.2) and 0.1 mL of 0.25 M ammonium sulfate solution were added. Flasks were placed on either a reciprocating shaker (75 strokes per minute) or an orbital shaker (50 revolutions per minute) and 0.5 mL of 1 M chlorate solution was added per flask to inhibit conversion of nitrite to nitrate (Schmidt and Belser, 1994). A 5-mL aliquot was removed at ten minutes, and then hourly for 6 to 8 hours. Reactions were stopped by the addition of 0.06 ml merthiolate (1% weight:volume) and allowed to settle overnight prior to nitrite analysis by the modified Griesse-Ilsovay method (Mulvaney, 1996). Sample concentrations were adjusted to reflect the reduced volume but constant solid mass remaining within the flask following each aliquot withdrawal. Nitrification potential rates (NPR) were calculated with Eq. [6].

$$NPR(\mu g\ kg^{-1}\ h^{-1}) = (A_t - A_0)\ r\ V_t \cdot W^{-1} \cdot 1000 \cdot t_i^{-1} \qquad [6]$$

Where A_0 = absorbance at 10 min
V_0 = volume at 10 minutes
i = 1,2,3… counts sampling times
A_t = absorbance at time t_i
r = regression coefficient
V_t = volume at t_i (mL) = $V_0 - (v \cdot i)$
v = volume of sample aliquot (5 mL)
W = sample weight (g)
t_i = time of the i^{th} sample (hrs)

Denitrifying Enzyme Activity

Prior to denitrifying enzyme activity (DEA) analysis 125-ml Erlenmeyer flasks were permanently numbered and the volume of each determined by taring the balance with the empty flask on the balance, filling the flask with water, inserting the rubber turnover septa stopper with a needle through the septa to allow the excess water to be

ejected as the stopper was inserted, drying the flask, and weighing the full flask. These flasks were used for all DEA experiments. For DEA analysis media samples (25 g) were placed into premeasured flasks along with 10 mL of "feed" solution (1 mM glucose as carbon source, 5 mM potassium nitrate as nitrate source, 0.25 g L^{-1} chloramphenicol to inhibit microbial division). Flasks were capped with gas-tight stoppers and made anaerobic by flushing with N_2 gas for 4 minutes. Acetylene was then injected into each flask to achieve a final concentration of 10%. Flasks were placed on a reciprocal shaker (75 strokes per minute) to incubate for 2 hours. Headspace was sampled by gas tight syringe every 30 minutes and stored in 4-ml glass shell vials fitted with rubber turnover septa stoppers which, had been evacuated for 2 minutes at 8 kPa (600± mm Hg). Nitrous oxide was analyzed using a Shimadzu GC 17A gas chromatograph equipped with a ^{63}Ni electron capture detector (Shimadzu Corporation, Columbia, MD), and a 2-mm x 2-m Poropak Q stainless steel column (Supelco, Bellefonte, PA). The carrier gas was 95% argon with 5% methane. Flow rate was 40 mL min^{-1}, column temperature 90°C and detector temperature 240°C. Nitrous oxide produced was calculated using Eq. [7] through Eq.[15]

where: V_a = volume of C_2H_2 injected into flask (0.015 L)
V_h = volume of flask headspace (≈ 0.135 L)
V_1 = volume of primary (flask sampling) syringe (0.002 L)
V_w = volume of water in the flask (water added + water content of sample)
m_i = µmol of N_2O injected for the i^{th} sample = (peak area · regression coefficient)
i = 1,2,3,4 counts sampling time
k_H^{cc} = unitless Henry's constant for N_2O (µmol L_{water}^{-1}) · (µmol L_{gas}^{-1})$^{-1}$

F_1 = fraction of headspace removed during i^{th} sampling = $V_1 \cdot (V_h + V_1)^{-1}$ [7]

P_0 = Initial pressure in the flask (atm.) = $(V_a + V_h) \cdot V_h^{-1}$ [8]

P_i = Pressure in flask after the i^{th} sample is withdrawn = $P_{i-1} V_h \cdot (V_h + V_1)^{-1}$ [9]

Φ_i = Fraction of N_2O withdrawn from flask during i^{th} sampling that remains in the primary syringe after depressurization = $1 \cdot P_i^{-1}$ [10]

f_i = fraction of headspace moles injected into vial during the i^{th} sampling = $F_1 \cdot \Phi_1$ [11]

H_1 = μmol of N_2O actually in the headspace immediately before the i^{th} sample = $5\ m_i\ (F_1 \cdot \Phi_1)^{-1}$ [12]

Q_i = μmol of N_2O dissolved in water in the flask just before the i^{th} sample = $H_i \cdot (V_w \cdot V_h^{-1}) \cdot k_H^{cc}$ [13]

M_i = μmol of N_2O that would have been present in the headspace at the time of the i^{th} sample if no prior samples had been taken
$M_1 = H_1$ for $i > 1$, $M_i = H_i + F_{i-1} M_{i-1}$ [14]

T_1 = Total μmol of N_2O created within the flask by the time of the i^{th} sample = $M_i + Q_i$ [15]

To obtain a DEA rate, the total number of μmol of N_2O was converted to mg N_2O produced per hour per kilogram of wetland media.

Results and Discussion

Heterotrophic Potential

Heterotrophic potential experiments for all temperatures could not all be run simultaneously due to lack of incubator space. Preliminary experiments to determine incubation times and initial incubations at 4, 20, and 27°C were run during October 2005. The complete experiment was run during June 2006. Samples were run at 16 and 8°C on June 1, 2006, at 12°C on June 7, 2006 and at 4, 20 and 27°C on June 15, 2006. Preliminary experiments had indicated differences between the four-wetland beds, but when samples were run in June 2006 there were no significant differences between

wetland cells. A final experiment was run in late September 2006, again with no differences between beds (data not presented). Combined data from all beds (4 beds, 5 reps per bed) and all temperatures (6) were plotted (Figure 16) and a nonlinear regression curve [Eq.16] was fit to the data using Sigma Plot 8.03 (Systat Software, Inc., San Jose, CA).

$$V_{MAX} = 1.04 + \frac{6.75}{1 + e^{-\left(\frac{x-13.07}{1.70}\right)}} \qquad [16]$$

R^2 for the above equation 0.72 at P=.0001.

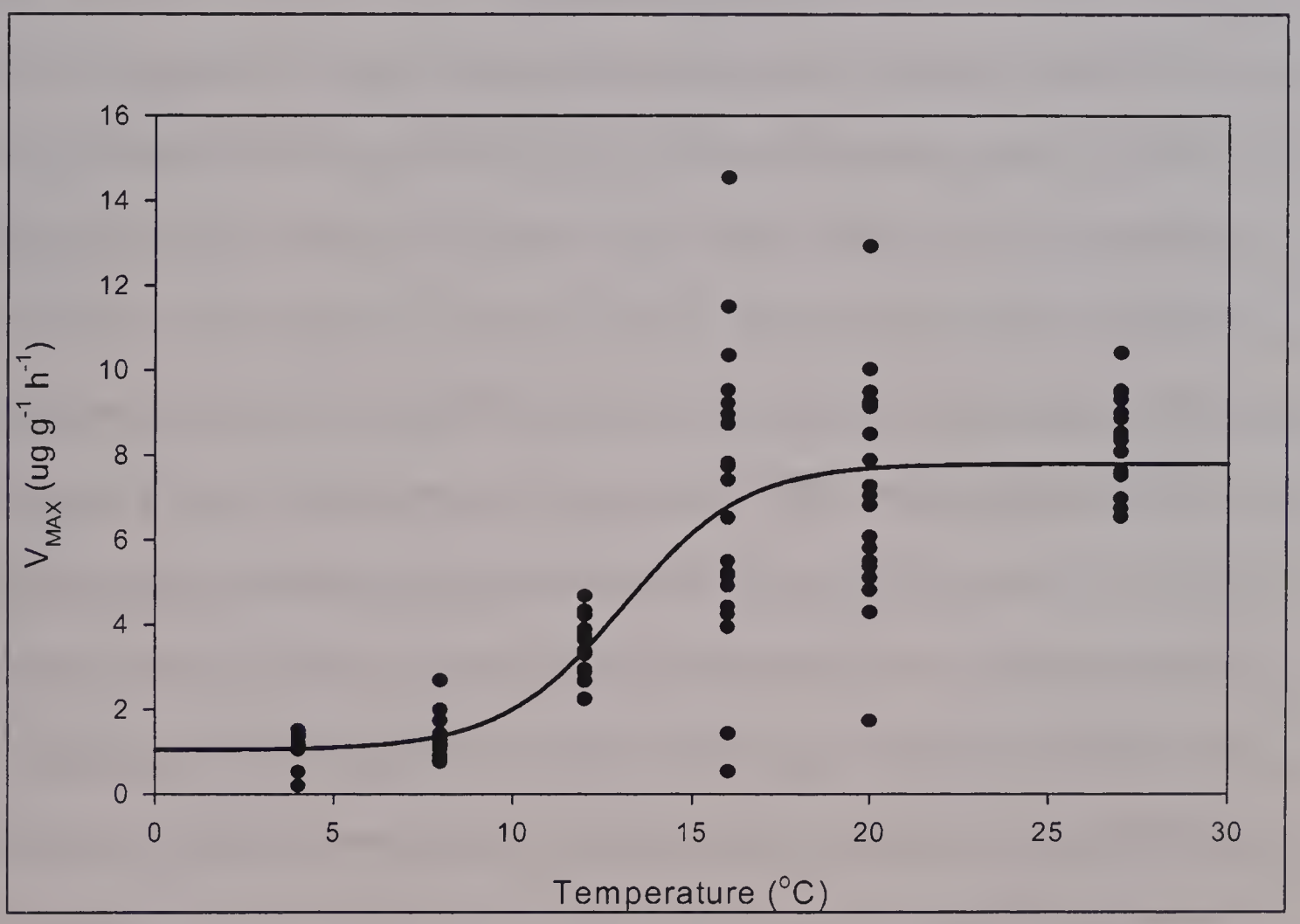


Figure 16. Heterotrophic potential measurements for all samples taken during June 2006 from the Staff Wetland. The curve shows a nonlinear regression to the data of a sigmoidal model of the form $y = y_o + a/(1+e^{-((X-Xo)/b)})$.

It is unclear why there were differences in V_{MAX} at both 20 and 27°C between the beds during the fall 2005, and not at other times. Two different sets of samples were analyzed, with each set run through the scintillation counter twice with similar results, indicating that there was significantly lower heterotrophic potential in GB1 than in the other three-wetland cells (Table 5). The decreased population of heterotrophs that would be indicated by the lower potential was not associated with a loss of performance (measured nitrate concentration) and is difficult to explain. Seventeen cm of rain fell on the system on October 8 and a total of 38 cm fell within a week (October 7 – 15). It seems unlikely, however, that if heterotrophs were being "flushed" from the sand media by the heavy rain that it would occur in only one of 4 beds. Similarly, it does not appear that the increased heterotrophic activity in the other three cells was enhanced by "extra" heterotrophs, as the range of potential measured for these beds during June and September 2006 was equivalent to that measured in October 2005. The decreased activity could also be explained by a sampling difference (i.e., samples were taken from a shallower/deeper depth, less active microsites were sampled, etc.). This possibility, however, seems less likely, as the difference occurred in two consecutive samples. The third explanation is that the measured difference was the result of a "disturbance" which had occurred previous to sampling and was reflected in the October 2005 measurements. Regardless of the reason, the data represent the range of heterotrophic potentials possible.

Table 5. Heterotrophic potential of samples taken during October 2005. Values are the mean of 20 samples ± 1 standard deviation. Values within a column followed by the same letter are not significantly different from each other according to Duncan's New Multiple Range Test.

Sample Location	Incubation temperature (°C)		
	27	20	4
	---------- V_{MAX} ($\mu g\ g^{-1}\ hr^{-1}$) ----------		
GB1	4.81 ±1.5 b**	3.16 ±1.0 b*	0.57 ±.06 ns
GB2	11.09 ±0.5 a**	6.02 ±2.3 a*	0.95 ±.04 ns
PB1	11.01 ±1.7 a**	8.16 ±1.5 a*	0.96 ±.04 ns
PB2	12.16 ±3.7 a**	8.08 ±2.7 a*	0.83 ±.04 ns

*, **, ns Significant at the P = 0.05, 0.01, and not significant, respectively.

Microbial Biomass

Microbial biomass, analyzed as ninhydrin reactive nitrogen, exhibited a bimodal curve when plotted across a calendar year. One local maximum occurred in late May/early June and the other in October. GB1 had significantly more measured biomass (July 26 and November 8, 2006) than the other three beds, and so a curve was fit to the data from GB1 and a second curve fit to the mean data from the other three beds (n = 5 and 15 for each date, respectively). Both 6th order polynomial curves and double bell curves were applied to the data. While the polynomial curves had greater R^2 values (0.97 and 0.92, respectively), the double bell curves were considered to be more appropriate, as the biomass values return to a single baseline value after each local maximum (Figure 17). The double bell model was algebraically expressed as shown in Eq. [17].

$$y = A\, e^{-\alpha(t-t_1)^2} + Be^{-\beta(t-t_2)^2} + C \qquad [17]$$

where: A = the height of the first maxima
α = the width of the first maxima
B = the height of the second maxima
β = the width of the second maxima
C = the baseline value

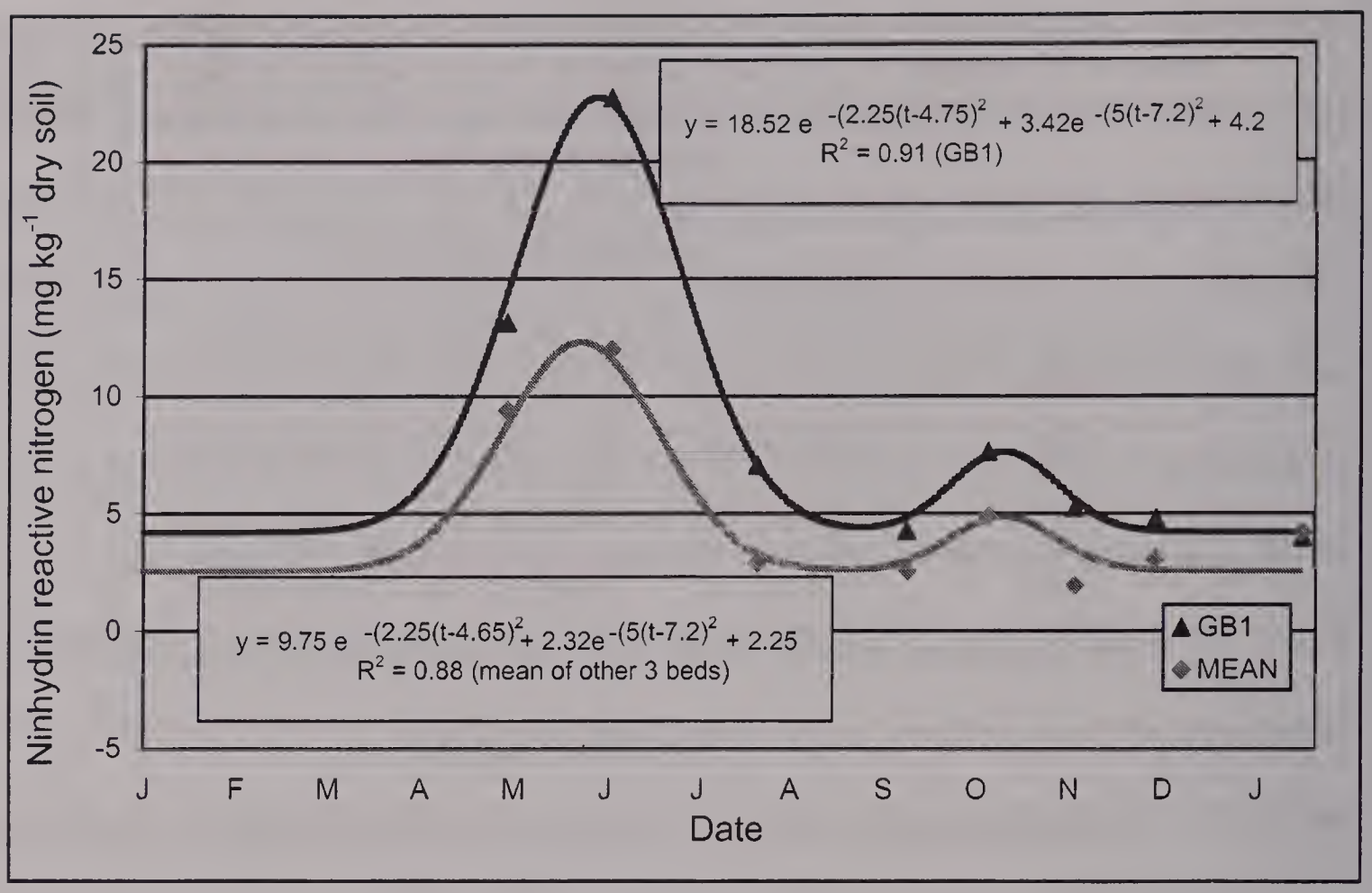


Figure 17. Bimodal distribution curves fitted to ninhydrin reactive nitrogen mean values for GB1 (diamonds) and the other three beds combined (circles). Equations and R^2 values for each are given.

The seasonal trend in the location of the first local maximum corresponded to the warming of the sand media and resumption of active plant growth. The second maximum occurred in early fall, corresponding to the arrival of cooler temperatures and fall rains. While the measured heterotrophic potential reached its maximum value in the laboratory at temperatures above 20°C, in the natural environment temperatures above 20°C lead to increased evapotranspirational cooling by the plants with more energy expended on plant maintenance than on active growth. The combined results at a microbial level were decreased oxygen, less moisture (lowered phreatic level as evidenced by no measurable outflow) and/or more competition for both. It is expected that the location and extent of the microbial growth maxima may vary slightly from

year to year with fluctuations in actual local weather conditions but that the bimodal trend will continue to be expressed.

The measured ninhydrin reactive nitrogen was converted to total microbial carbon and nitrogen (Joergenson and Brooks, 1990) to allow comparison to published values. Mean total microbial N values calculated for GB1 (56.7 mg N kg^{-1} dry soil) are in keeping with those reported by Groffman *et al.* (2001) for tropical riparian forest soil in Puerto Viejo, Costa Rica (40-50 mg N kg^{-1}). Maximum values from GB1 (130 mg N kg^{-1} dry soil) were also similar to total microbial N in an intact forest in Gandoca, Costa Rica (114 and 120 mg N kg^{-1}, respectively). In another study of natural wetlands in Dutchess County, NY Groffman *et al.* (1996) report microbial biomass nitrogen at values as high as 300 mg N kg^{-1} soil in red maple swamps and woodland pools. The difference in the range of values can be explained by the measurement technique. Groffman *et al.* (1996, 2001) used a chloroform fumigation-incubation method followed by analysis of the CO_2 flushes and extractable mineral N (NH_4^+, NO_3^-). That analysis was likely measuring microorganisms in the log phase of growth after they had been reintroduced to the fumigated soil. Limitations of the analytical methods used in these experiments may also be the source of the discrepancies in biomass estimates. Ninhydrin reactive nitrogen analysis specifically measures amino-N compounds and was chosen for use because it would not include measurements of ammonium or nitrate, both of which would both be present in the wetland solution. It should also be noted that biomass measurements in GB1 are an underestimation of actual numbers since the color intensity of the solutions were often beyond the linear portion of the standard curve.

Neither dilution of samples following extraction nor extraction of a reduced soil volume reduced the color intensity of the final solution.

Wastewater engineers use stoichiometry to estimate biomass yield. $C_5H_7O_2N$ is typically used to represent the molecular formula of bacterial cells (Hoover and Porges, 1952 as cited by Metcalf and Eddy, 2003). This represents 0.105 g N g cell^{-1} (14 g N 133g cell^{-1}). The calculated microbial total N values were used to calculate the potential number of bacteria per gram of dry soil in the wetland cells.

$$\left(\frac{\text{g N g soil}^{-1}}{0.105 \text{ g N g cell}^{-1}} \right) \text{ x number cells g cell}^{-1} = \text{cells g soil}^{-1}$$

The average, maximum and minimum numbers of bacterial cells possible per gram soil are listed in Table 6. For these calculations it was assumed that all microorganisms present were bacteria. While not a valid assumption in the "real world", it does allow for calculation of a theoretical microorganism population that can be used for comparison to other wetland and soil microcosms. Ottová *et al.* (1997) report denitrifying and ammonifying bacteria present on the roots and rhizomes of plants from the Doksy wetland (Poland) in the range of $10^6 - 10^8$ per gram of dry material. This range was 3 orders of magnitude higher than the reported nitrifying bacteria ($10^3 - 10^5$ per gram of dry matter).

Table 6. Possible number of bacterial cells present in wetland media as calculated from ninhydrin reactive nitrogen measurements.

		Multiplier used for calculations	
		5.2×10^{11} cells g^{-1}†	1.28×10^{13} cells g^{-1}‡
		Bacterial cells g $soil^{-1}$	
GB1	Average	2.1×10^{8}	5.2×10^{9}
	Maximum	5.6×10^{8}	13.8×10^{9}
	Minimum	1.6×10^{8}	3.9×10^{9}
Other three beds combined	Average	9.8×10^{7}	2.4×10^{8}
	Maximum	3.6×10^{8}	8.8×10^{9}
	Minimum	8.3×10^{7}	2.0×10^{8}

† Whipps and Lynch, 1983
‡ Schnüner *et al.*, 1986

Nitrification Potential

Nitrification potential measurements exhibited the expected seasonal trend, with greater potential exhibited between August and November (Fig. 18) than between November and August. GB1 and PB1 had significantly greater nitrification potential as would be expected due to the inflow of primary effluent with a high ammonium concentration. The single maximum coincides with the secondary maximum (October) exhibited in total microbial biomass. The possibility exists that the nitrification trend should also be bimodal, and that the sensitivity of nitrifying organisms to disturbance as well as some methodology problems during early analyses were responsible for the low potentials measured during May and June.

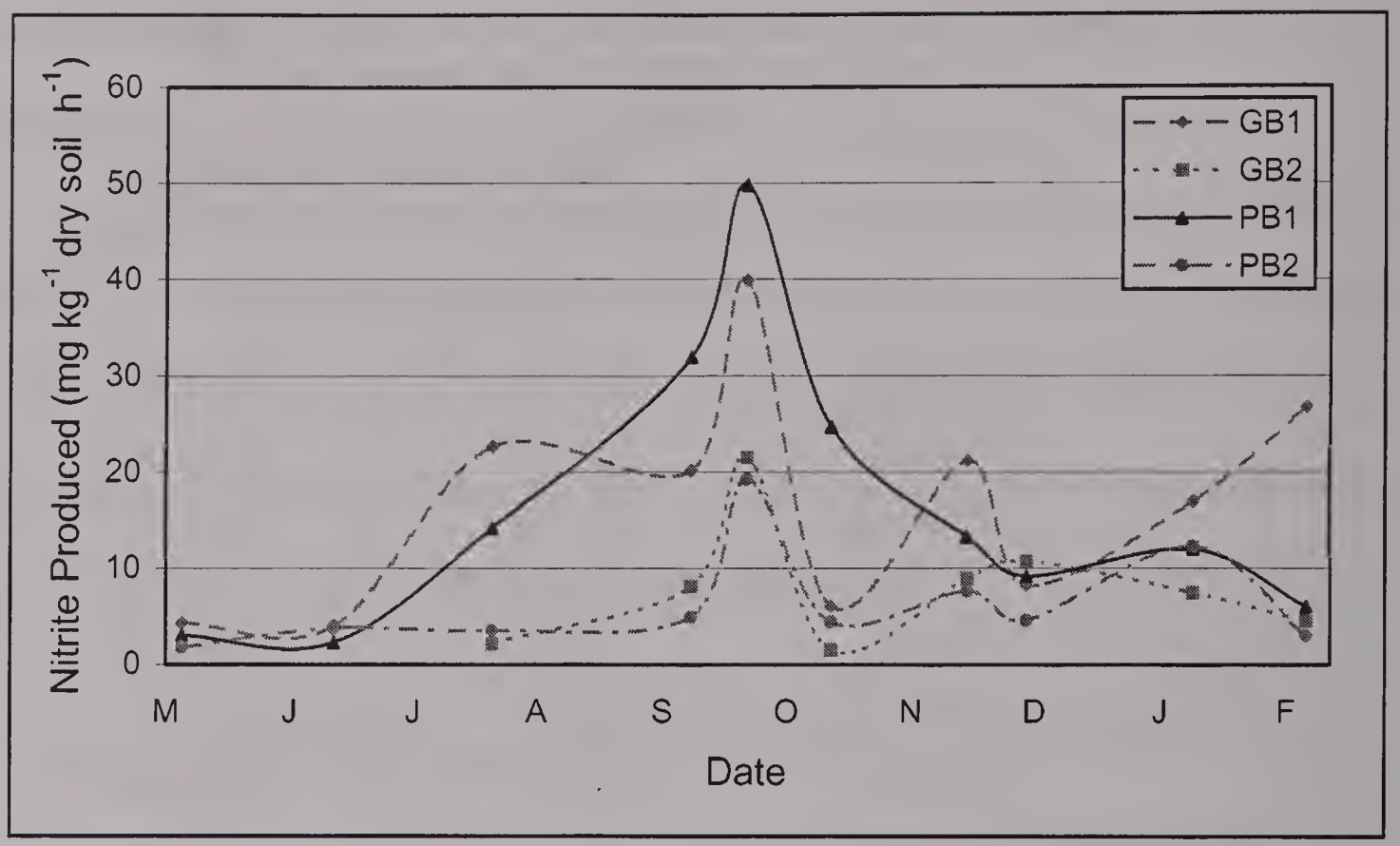


Figure 18. Nitrification potential measured every 3 to 4 weeks throughout the year.

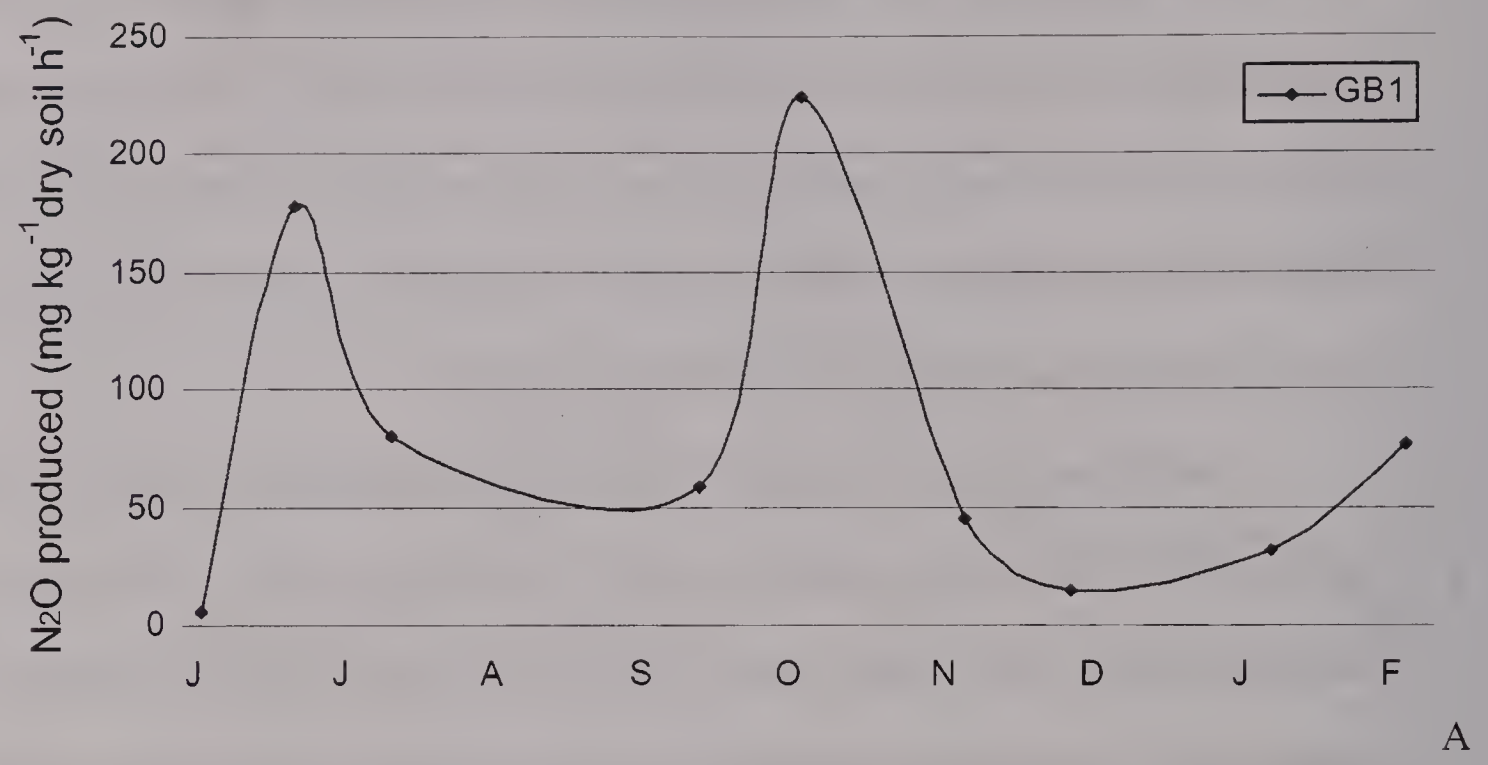
The average nitrification rate measured during this study (all 4 beds, all measurements) was 0.402 mg N L^{-1} h^{-1} with the maximum rate (2.66 mg N L^{-1} h^{-1}) in PB1 and the minimum rate (0.01 mg N L^{-1} h^{-1}) in GB2. These values lie within the range of most published values (D'Angelo and Reddy, 1993; Bowden, 1986; Reddy *et al.*, 1980; Chen *et al.*, 1972). Kemp *et al.* (1990) reported a maximum nitrification potential of 1.44 mg L^{-1} h^{-1} in marine sediments, noting that the rate was limited by the penetration of oxygen into the sediments. Groffman *et al.* (1996, 2001) report nitrification rates ranging from 0.2 – 5.3 mg N kg^{-1} d^{-1} in tropical soils and 0 – 0.9 mg N kg^{-1} d^{-1} in natural wetlands. These low rates can in part be explained by the method of analysis (measurement of NO_3^- accumulated during a 10-day incubation period). The method as described does not account for nitrate that may have been denitrified, or ammonium which may only have been oxidized to nitrite. Maximum nitrification rates measured in both GB1 and PB1 (40 and 50 mg N kg^{-1} h^{-1}, respectively) during the

course of these experiments exceeded all of those found in the literature. The ability of the hydrophytic vegetation to supply oxygen below the surface, the constant supply of ammonium, and the larger pores of the sand medium (allowing movement within the medium) all contribute to a nitrifier population acclimated to the specific environment and thus the increased nitrification potential measured.

Nitrification potential was somewhat elevated beginning in January 2007 when a variable speed rotary shaker became available. With the new shaker, motion during the incubation period was a "gentle swirl" rather than an abrupt agitation. February 2007 samples were removed from beneath snow and ice except in GB1 where warm influent entering the bed had prevented ice formation. All samples were incubated in a cardboard box at 20°C overnight to allow microorganisms to warm slowly and prevent cell lysing. It is possible that a combination of: 1) warmer temperatures in GB1, 2) nitrifier populations at a somewhat higher level than would be typical winter levels (due to unseasonably warm temperatures in December and early January), and 3) a gradual increase from field temperature to 20°C were responsible for the high nitrification rate measured in GB1 during February.

Denitrifying Enzyme Activity

Denitrifying enzyme activity exhibited a bimodal curve (Fig. 19) similar to that for total microbial biomass. All four beds followed the same pattern, with GB1 having the greatest activity (an order of magnitude larger), followed by GB2, PB1 and PB2. This ranking of the denitrification rate in the four-wetland cells follows the current flow pattern (GB1 → GB2 → PB1 → PB2). While reducing conditions (Eh < 375 mv) existed at the 30-cm depth and below in all four wetland cells during most of the



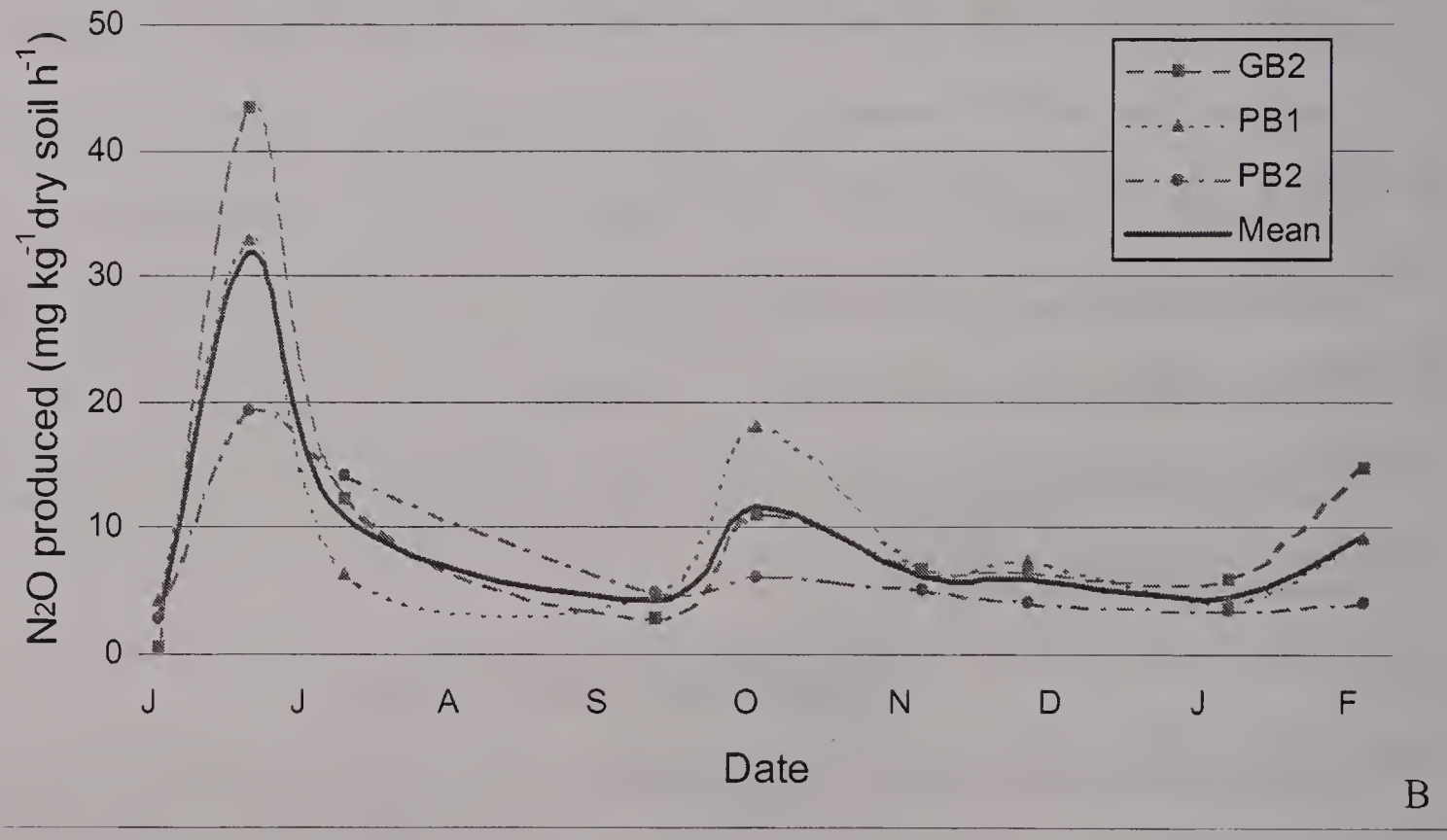


Figure 19. Denitrifying enzyme activity for GB1 (A), GB2, PB1, PB2 and mean of all 4 beds (B).

experimental period (Fig. 12-15, Chapter 3), the amount of available carbon (as measured by $CBOD_5$) was significantly different within the beds. The primary effluent entering the system was carbon rich (259 mg L^{-1} mean $CBOD_5$), but the majority of the

$CBOD_5$ was consumed in GB1 and very little left GB2. Phragmites Bed 1 received some carbon inflow during times of influent bypass, but this input was not constant and was only a small portion of the total inflow to PB1. The decline in measured denitrification rate through the system was most likely due to the lack of carbon source for the heterotrophic denitrifiers.

Denitrification potentials measured during this experiment 0.20 mg N L^{-1} h^{-1} (December) to 1.9 mg N L^{-1} h^{-1} (June) are much larger than those published in the literature. D'Angelo and Reddy (1993) reported DEA rates of 0.108 mg L^{-1} h^{-1} for lake sediments amended with 100 mg L^{-1} of nitrate prior to measurement. When expressed as the rate of N_2O produced per kilogram soil per hour, rate from these experiments (monthly mean of 83.8 mg N_2O kg^{-1} h^{-1} for GB1) greatly exceed published rates. The monthly mean of the other beds (12.4, 11.0, and 7.5 mg N_2O kg^{-1} h^{-1} for GB2, PB1, and PB2 respectively) was more in line with published rates. Groffman *et al.* reported denitrification rates ranging from 2.5 – 4.5 mg N kg^{-1} h^{-1} in both natural wetlands in NY state (1996) and tropical soils in Costa Rica (2001). Ellis *et al.* (1998) reported 8 mg kg^{-1} over a 6-hr incubation period and 23 mg kg^{-1} over a 12-hour period in grass land soils in the U.K. These correspond to 1.3 and 1.9 mg kg^{-1} h^{-1}, respectively. Lowrance and Smittle (1988) reported the highest rate of their experiments (1.1 mg N_2O kg^{-1} dry soil d^{-1}) when Bonifay sand cores were incubated in a N_2-H_2-CO_2 atmosphere in an anaerobic chamber for 3 hours prior to treatment with added nitrate and glucose. The overall low denitrification rates reported by these researchers were presumably due to a fairly small microbial population in the Bonifay sand in combination with the infrequent fertilizer applications of the study. Both Lowrance and Smittle (1988) and D'Angelo

and Reddy (1993) reported the highest rates following addition of either fertilizer or fertilizer and glucose. The higher rates of denitrification activity measured for this study reflect the nature of the treatment wetland system. In GB1, there is a constant source of both ammonium to nitrify (and thus nitrate to denitrify) and ample carbon to sustain the denitrifier populations.

Correlations between microbial biomass, nitrification potential, and denitrification rate were examined for each month and for each bed but were considered to be inappropriate, in that measurements were not all made on the same samples or during the same week. When all data for the study were grouped (n = 116, 95, and 128, for biomass, nitrification potential and denitrification rate, respectively) and correlations run, denitrifying enzyme activity was significantly correlated to microbial biomass (P=0.0005), with Pearson's correlation coefficient = 0.32. This suggests that total biomass is a good predictor of denitrification activity, but not of nitrification potential. This lack of correlation between nitrification potential and biomass as well as the lack of significant differences in both analyses (biomass and nitrification potential) on some dates was likely due to the various problems associated with the measurements themselves. Nitrification potential measurements exhibited the most variability and were the most sensitive to mechanical and/or methodology changes. Measurements in July were all negative, presumably due to failure to adjust the pH of the phosphate buffer. May and June measurements failed to detect any nitrification occurring in GB2, and all measurements were low in September, due to problems with the speed of the shaker. Biomass measurements also proved problematic from time to time. July biomass measurements were not used because fumigated cells had less biomass than

non-fumigated cells in two of the beds (including GB1). It was determined that sample handling at the time of sample collection was the most likely cause. Variations within replicates of DEA measurements were the least problematic in that regardless of the amount of variation between replications, the means were still significantly different from each other.

Conclusions

It is interesting to note that the standard heterotrophic potential measurement (Wright and Hobbie, 1965, 1966; Hobbie and Wright, 1968; Hobbie and Crawford, 1969; Wyndham and Costerton, 1981; Scow *et al.*, 1986) used for these experiments failed to predict the differences in microbial biomass exhibited by the different wetland cells. Since the majority of the heterotrophs within the wetland cells should be bacteria, and the only heterotrophs measured by the glucose addition method were glucose consuming bacteria, the lack of differences in heterotrophic potential between wetland cells would suggest microbial biomass would also exhibit no differences. Total microbial biomass in GB1, however, was always at least double that of the other beds, and yet heterotrophic potential in GB1 was either not different from or was less than the other three cells.

Both nitrification potential and denitrification enzyme activity in the wetland system, especially GB1, exceeded published values for natural wetlands, tropical soils and for both marine and freshwater sediments. These findings demonstrate the ability of top loading vertical flow submerged bed treatment wetlands to remove the various nitrogen constituents once the microbial population becomes acclimated to the influent wastewater.

CHAPTER 5

MODEL EVALUATION

Introduction

Early design models for artificial wetlands were based on first order kinetic decay models with plug flow hydraulics and a single design parameter (Reed, 1995; Kadlec and Knight, 1996). Originally the typical design parameter was biochemical oxygen demand or total suspended solids. As use of constructed wetlands expanded nitrogen removal often became the design parameter. When the constructed wetlands failed to meet the predicted effluent concentrations, temperature or other environmental factors were added to the equations to lower predicted effluent concentrations. As the desire for use of artificial wetland treatment systems has grown, fueled by their low cost and sustainability, the need for better predictive models has been the focus of many research studies (Gidley, 1995; Kadlec, 2000; Wynn and Liehr, 2001). As models have evolved, they have become increasingly complicated, including multiple sub-models and feedback loops, and yet still fail to adequately predict measured effluent concentrations (Marsili-Libelli and Checchi, 2005).

From the modeling perspective, treatment wetlands are considerably more complex than conventional wastewater treatment plants (Marsili-Libelli and Checchi, 2005). Natural environmental dynamics (i.e., temperature, precipitation, aeration, etc.) are often not predictable and definitely not controllable. Hydraulic flow dynamics in free water surface wetland systems are much different than in submerged bed systems and horizontal flow is different from vertical flow. None of the various hydraulic

regimes are adequately modeled by plug-flow hydraulics (Kadlec, 2000) or sequencing batch reactor hydraulics (Wynn and Liehr, 2001).

The current study evaluated a mechanistic, compartmental simulation model (Gidley, 1995; Wynn and Liehr, 2001) to determine if it was an appropriate model for top loading vertical flow submerged bed treatment systems (TLVFSBTS). To this end, wetland system performance data (Chapter 3), microbial process data (Chapter 4) and environmental data were collected over a period of 17 months. The treatment wetland system studied during this research is located in the town of Highland, NY on the western bank of the Hudson River approximately 90 miles north of New York City. The system began receiving wastewater in June 2001. The system was constructed using TLVFSBTS technology, details of which are to be found in chapter 3.

The original data from the Mayo Wetland (Ann Arundel, MD), as well as the model input and output data were obtained (Gidley, 1995) in order to correctly recreate the model. All model inputs were duplicated, and the model was run using the original input data. Output data obtained did not replicate the published output, and many mistakes in model values and statements were discovered. Initial attempts to debug the complete model failed. The individual model compartments (water budget, oxygen budget, heterotroph dynamics, etc.) were then run separately, again, using the original input data (Gidley, 1995). The water budget compartment failed to produce the reported output (evapotranspiration was not correctly computed), and heterotroph and autotroph dynamics failed to produce realistic values. Further attempts to debug the existing model were not made. A new simpler model has been designed, but is only partially successful. The current progress is, however, notable and offers several insights into the

type of dynamics and interactions occurring in the wetland system as well as the direction future modeling efforts should take.

Model Development

STELLA 8.1 (isee systems, inc) simulation software is based on J.W. Forrester's dynamic systems models (Gidley, 1995) and utilizes several methods for numerically solving systems of differential equations (Euler's, 2nd order and 4th order Runge-Kutta numerical methods (isee systems, inc.)). The user constructs a diagram of the processes to be modeled, and the software develops the mathematical equations. The modeler simulates conditions with the use of "stocks" (rectangles), "flows" (valves), "converters" (circles), and "action connectors" (arrows). Stocks can be reservoirs, conveyors, queues, or ovens, depending on the processes occurring within. Flows can be used as inputs to or as outputs from stocks. Converters allow the modeler to modify an input variable, while action connectors are used to direct the converter to the proper location, thus defining the specific relationship. Converters may be either mathematical functions or graphs. For example, in the simple model below (Fig. 20.) "Water Volume" is a reservoir (stock), with precipitation as input , and evapotranspiration (ET)

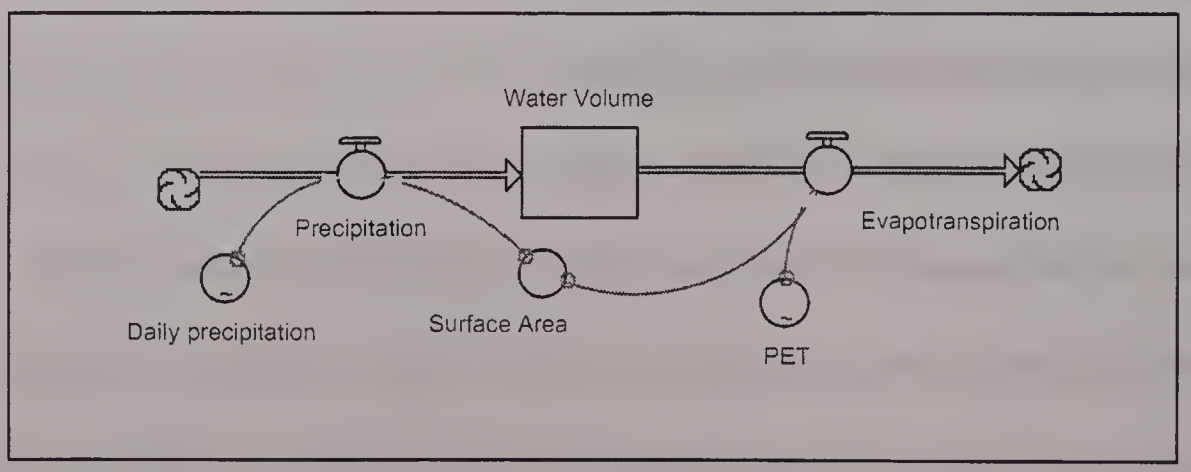


Figure 20. Simple model showing use of stocks, flows, converters, and action connectors. Arrows indicate direction of movement. Squiggles (~) within a converter indicate that the input is graphical.

as output (flows). Daily precipitation, surface area, and potential evapotranspiration (PET) are converters used to convert the input values (cm of either precipitation or PET) to volumes. Bed area is the area (m^2) on which the precipitation is falling or from which ET is lost. The statements on the equation level are listed below.

Water_Volume(t) = Water_Volume(t - dt) + (Precipitation - Evapotranspiration) * dt
INIT Water_Volume = 26.75 m^3

INFLOWS:
Precipitation = (Daily_precipitation {cm}/100)*Surface_Area {m^2}

OUTFLOWS:
Evapotranspiration = (PET{cm}/100)*Surface_Area {m^2}

Surface_Area = Bed_width*Bed_length = 15.24 m x 15.24 m

Daily_precipitation = GRAPH (Time)
(0, 0.00), (1, 0.00), (2, 0.00), (3, 0.00), (4, 1.98), (5, 0.03), (6, 0.00), (7., 0.00), (8, 0.00), (9, 0.00), (10, 0.00), (11, 1.19), (12, 0.00), …

PET = GRAPH (Time)
(0, 0.33), (1, 0.483), (2, 0.533), (3, 0.559), (4, 0.432), (5, 0.432), (6, 0.406), (7, 0.305), (8, 0.356), (9, 0.508), (10, 0.432), (11, 0.508), (12, 0), …

Water Budget

The first compartment of the new model to be created was the Water Budget (Fig. 21). This segment began as a simple model for the first wetland cell only. Inputs were measured inflow and measured precipitation, both graphed against time (i.e., date, precipitation or inflow). Outflows calculated by the model were evapotranspiration and effluent volume. Initial water volume was set as the volume of the bed below the phreatic level times the porosity of the sand (0.3). Bed capacity was established as the volume of water required within the bed in order for outflow to occur (i.e., bed volume with the phreatic level at the level of the outflow pipe). Therefore, water volume within

the bed at any time was current inflow + precipitation (adjusted for bed area) minus ET adjusted for bed area, minus outflow (bed volume – bed capacity). Once this portion of the water budget was running, the remaining wetland cells were added to the compartment to simulate flow from one bed to another (the current flow pattern). Thus, outflow from GB1 became the inflow to GB2 etc. Bed capacity for each bed was set to reflect current phreatic levels. Initial simulation output was determined to be inaccurate, as the model did not adequately predict times when there would be no outflow from the system.

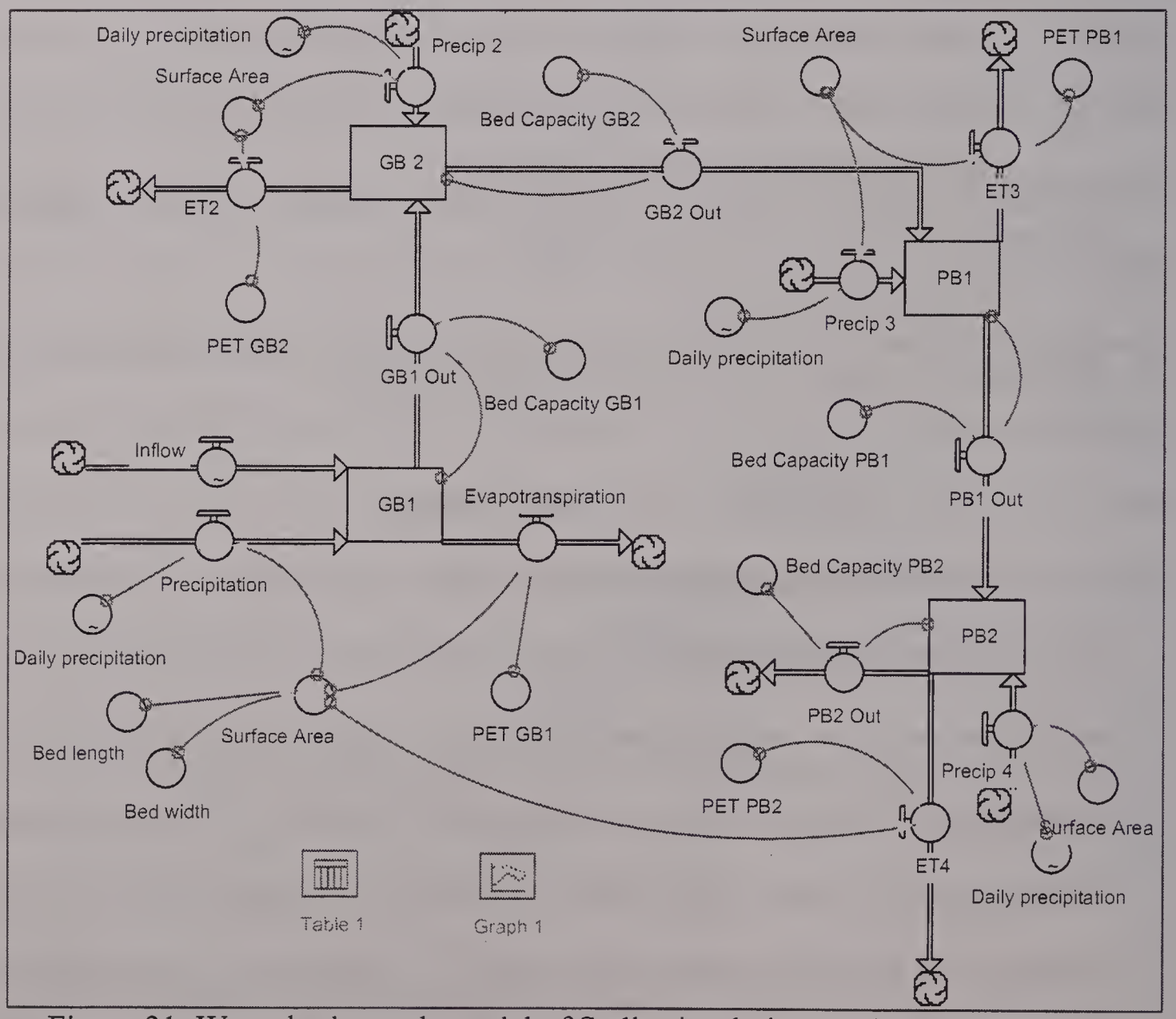


Figure 21. Water budget sub-model of Stella simulation model developed for a TLVFSBTS.

Evapotranspiration rates used in the original model runs were considered to be underestimates of actual ET. Calculated PET values were used rather than calculated ET as PET values are for a short green crop of uniform height that completely shades the soil surface and adequate (i.e., unlimited) water, whereas ET is calculated as if the surface were a single plane. The PET values originally used were calculated for Stewart Airport approximately 20 miles south of the research site. The vegetation in both grass beds was dense enough to shade the sand surface, but the grass was between 2 and 3-feet tall. In the *Phragmites* beds, the sand surface was exposed between plants, allowing direct evaporation. The plants, however, were 8 to 10-feet tall, increasing the transpirational surface area considerably. Probable ET rates for GB1 were calculated as the difference between measured inflow (water meter) and measured outflow (tipping bucket with counter). Probable ET was plotted against time, fitted to a cosine curve, and compared to the cosine curve of the calculated PET. During non-growth periods (October – March) probable ET and calculated PET were equivalent, while during the growing season probable ET was determined to be higher, with the largest differences occurring in July and August. Probable ET curves were then used on subsequent model runs (Fig.22), and the model simulation better matched days when there was no measurable outflow from the system; 48 of 53 and 40 of 46 days in 2005 and 2006, respectively.

It should be noted, that the STELLA (isee systems, inc.) "smooths" the flow in the model. This effect is dependent on which numerical method is chosen, and what the integration interval is set at. Fourth order Runge-Kutta gives the best output, but also requires the most computational "space", so it was often necessary to set simulation

runs time of 100 days, rather than the full 500 day study period. The shorter simulation duration allowed for shorter integration intervals. Figure 23 illustrates the difference between the output for a 100-day simulation run, with time interval set to 1 day, or to 3 hours (0.125 d). The "smoothing effect" shifts the curve, but otherwise has no effect.

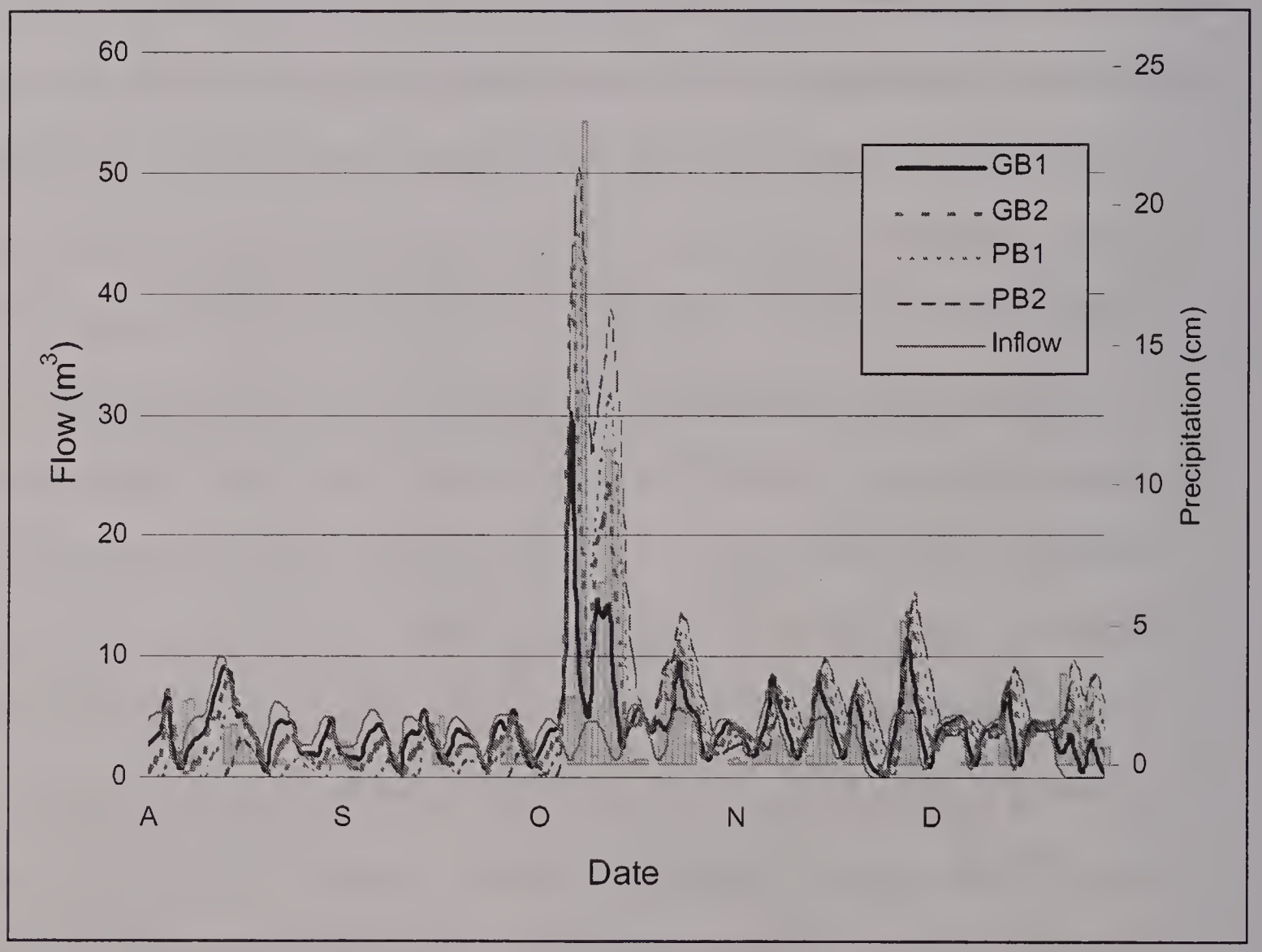


Figure 22. Sample output from the model simulation of outflow from the 4 individual wetland cells during the fall of 2005. Precipitation is plotted as bars.

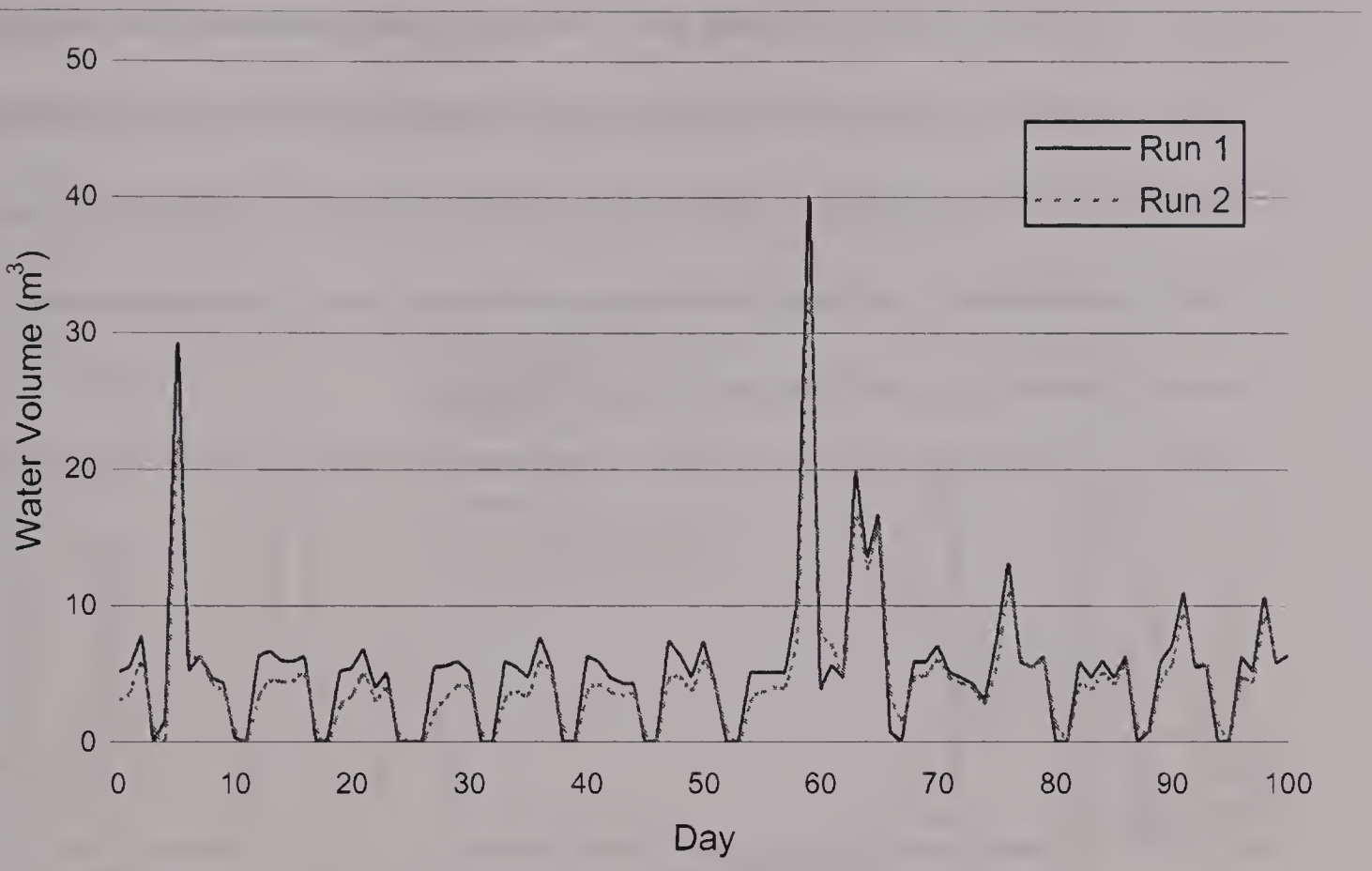


Figure 23. STELLA 8.1 simulation output for total flow into the wetland system over a 100-day simulation period using a 2nd order Runge-Kutta numerical method. Run 1 was set for a 3-hr time interval, while run 2 was set for a 1-day time interval.

Nitrogen Cycle

The Gidley (1995) model contained a sub-model 'autotroph dynamics' to simulate the nitrification process. Similarly 'heterotroph dynamics' was the sub-model for denitrification processes. The number of autotrophs and heterotrophs, autotroph and heterotroph growth and death rates, and half saturation constants for both autotrophs and heterotrophs were obtained from published data and various textbooks (Gidley, 1995). The sub-model outputs were then used as input to the 'Nitrogen Cycle' sub-model. When model simulations did not adequately approximate the collected data, these original model values were adjusted as needed (orders of magnitude adjustments). While this is a valid method for modeling, it was decided that a more realistic approach would be to measure the dynamics of the processes, and use the measured rates for future simulation models.

In the TLVFSBTS model, the 'Nitrogen Cycle' sub-model is fairly simple (Fig. 24). It consists of an ammonium reservoir, a biomass N reservoir, a nitrite reservoir, and a nitrate reservoir. Inflows to the various reservoirs were manipulated by converters for flow, precipitation, and nitrification/denitrification rate. Nitrification was a two-step process, ammonium oxidation and nitrite oxidation.

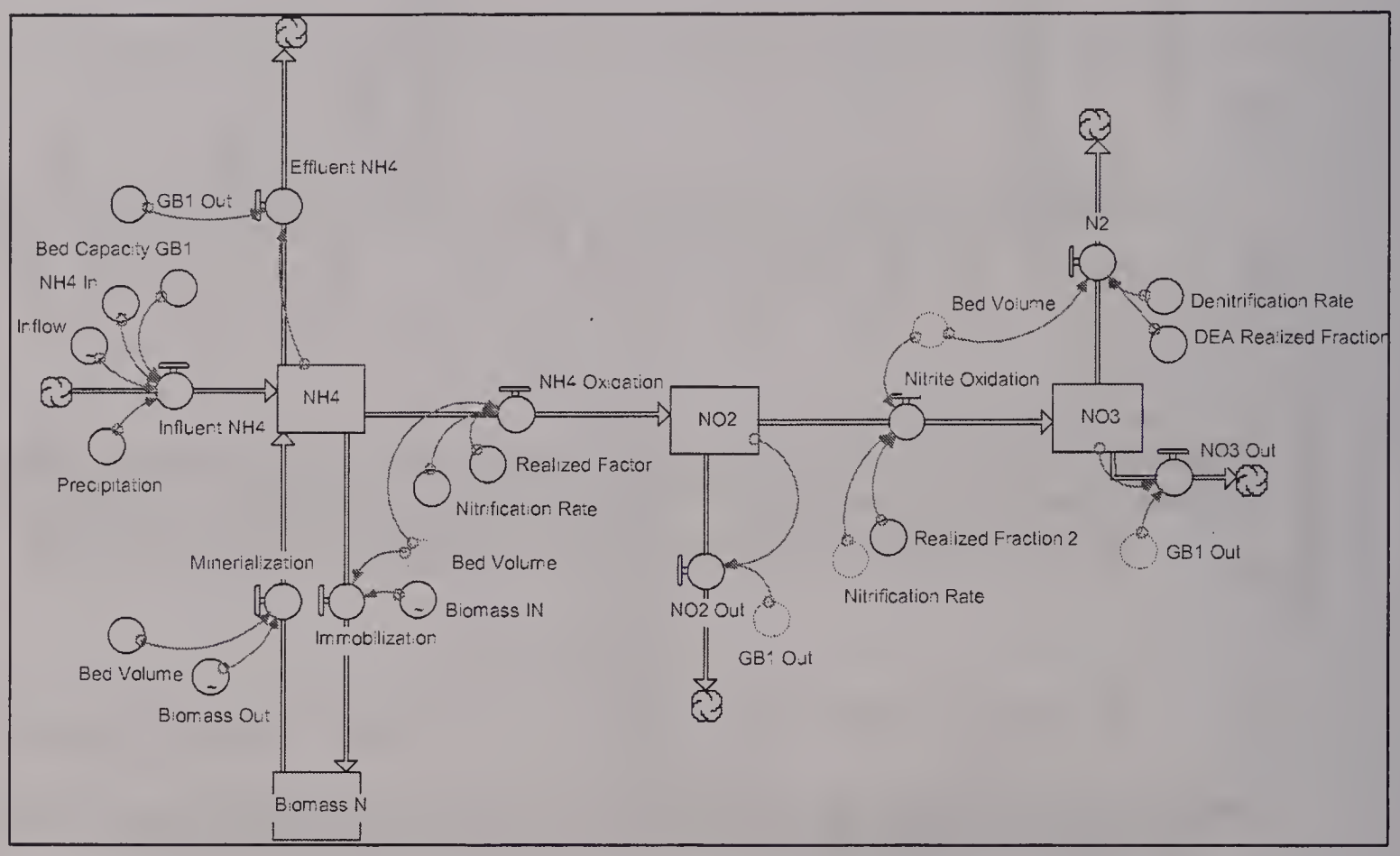


Figure 24. Nitrogen Cycle sub-model of Stella simulation model developed for a TLVFSBTS.

Nitrification Rate

Nitrification potential (chlorate blockage method) was measured monthly during the growing season (Chapter 4). Rates were obtained for the potential amount of ammonium oxidized to nitrite per gram of dry soil per hour. Using the bulk density of the sand media, the rates were converted to grams of nitrite produced per cubic meter of bed volume per day. Data were then plotted against time to determine seasonal trends.

A double bell curve was then fit to the data (Fig. 25), and the curve extrapolated to include the entire 500-day evaluation period.

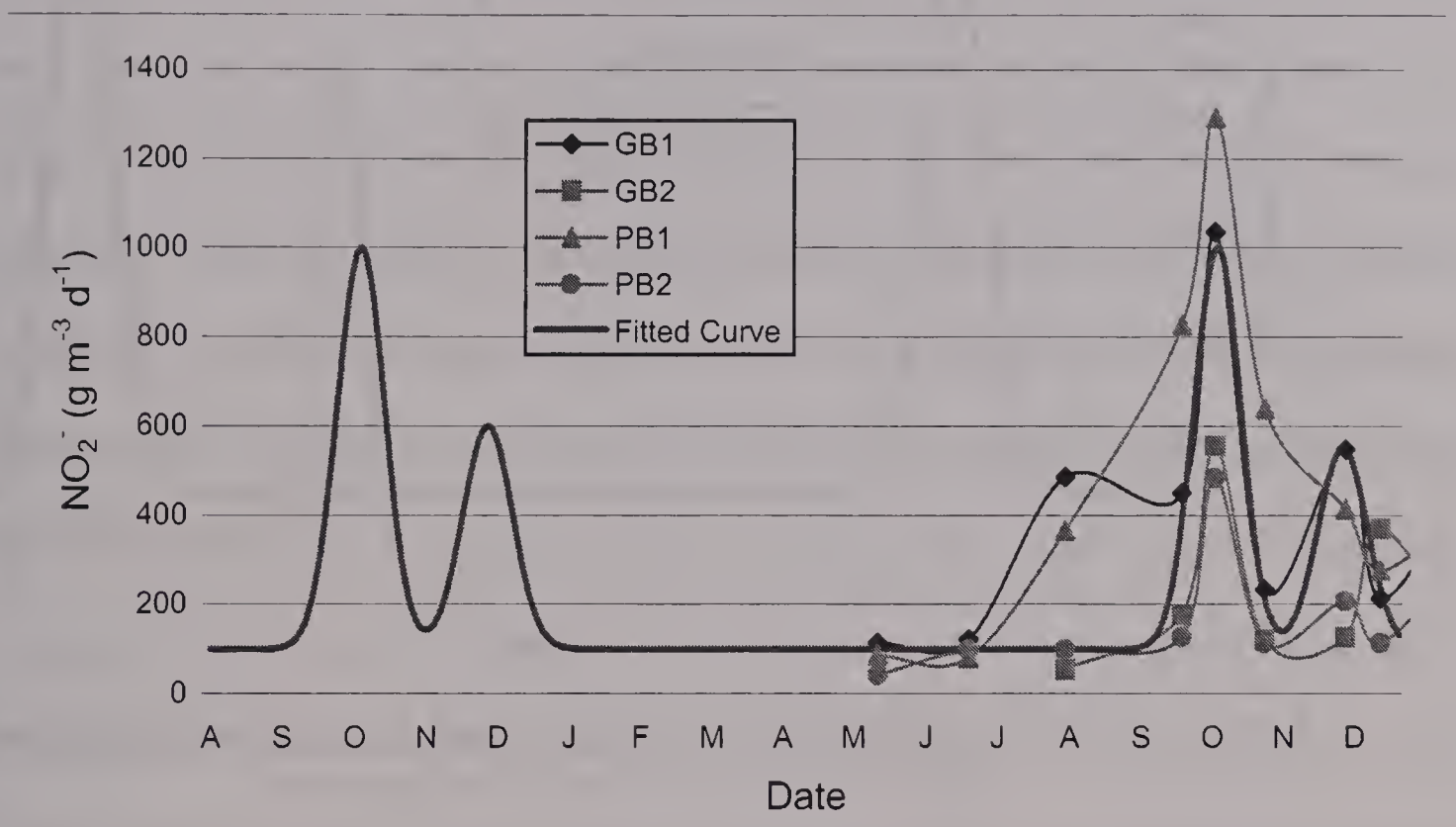


Figure 25. Nitrification rate curve fit to measured data and then extrapolated to include the entire 500-day evaluation period.

Denitrification Rate

Denitrifying enzyme activity (acetylene blockage method) was also measured throughout the growing season. Analysis was conducted immediately after return from the field so that the measurements reflected actual field conditions as closely as possible. Denitrification rates were calculated for each wetland cell as grams of nitrous oxide produced per kilogram dry media per hour. Denitrification rates were then converted to grams nitrous oxide produced per cubic meter of wetland cell per day. A double bell curve was fit to the data (Fig. 26) and the curve extrapolated to cover the full evaluation period.

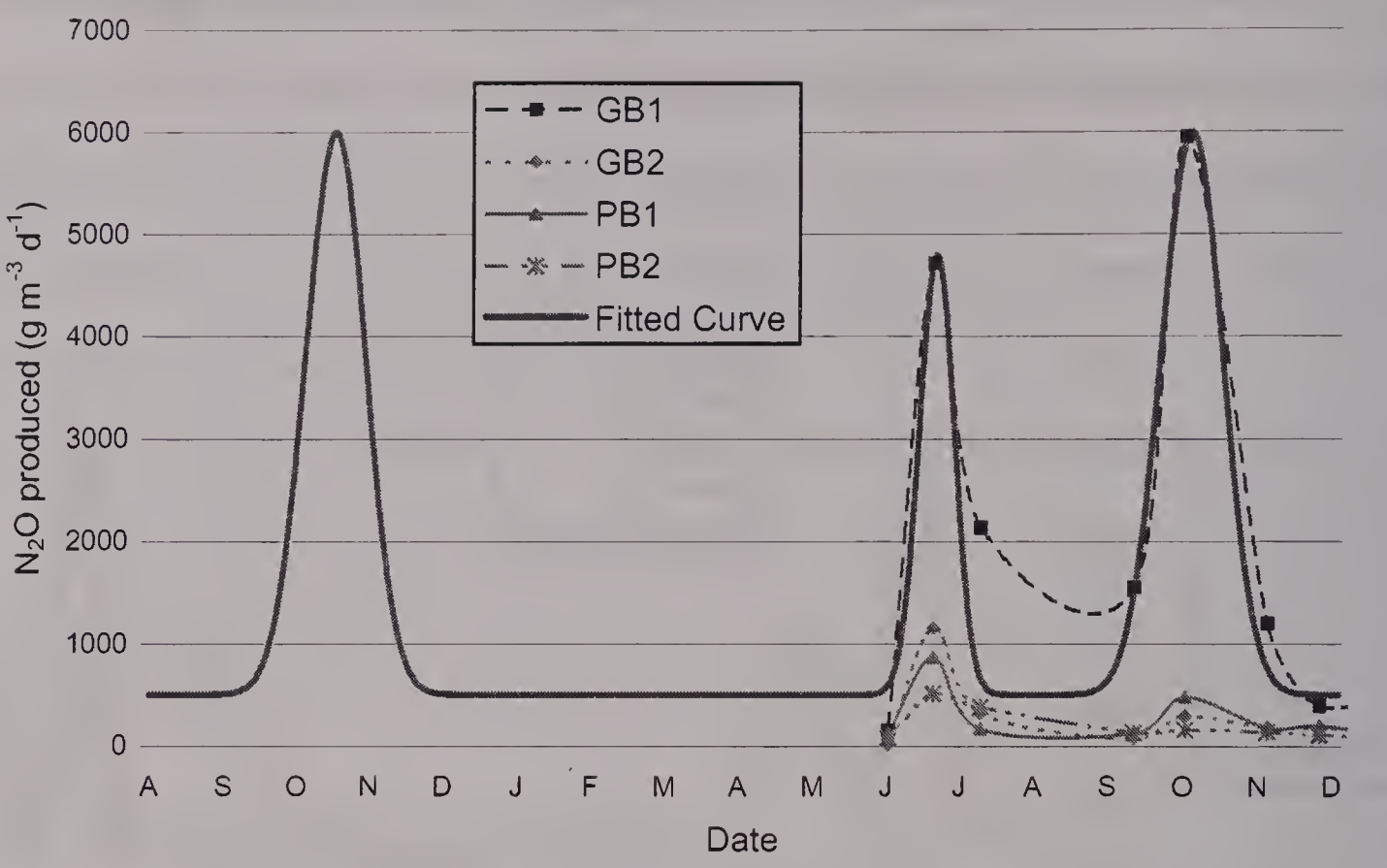


Figure 26. Denitrification rate curve fit to measured data and then extrapolated to include the entire 500-day evaluation period.

Mineralization and Immobilization

For modeling purposes, immobilization of incoming ammonium and mineralization of organic nitrogen were estimated by the amount of microbial nitrogen measured within each bed (Chapter 4 Fig. 17). The difference between microbial nitrogen on day t and the amount of microbial nitrogen on day t-1 was calculated on a daily basis for the evaluation period. The daily difference was input into the new model as converters to flows into and out of the biomass N reservoir.

Although plant growth and death are another source of immobilization and mineralization, the Nitrogen Cycle of the TLVFSBTS model does not currently contain a separate plant biomass component. Plant uptake was not measured, but total plant biomass was measured in October of 2005, and typical C:N ratios for the two species could be obtained from the literature to add this component in the future. The Staff

Wetland is never nitrogen deficient, at least not in grass bed 1 (GB1) and thus it was determined that the nitrification and denitrification rates would be sufficient for estimation of the nitrification and denitrification occurring within GB1. It was anticipated that only a portion of the measured nitrification/denitrification rates would actually be realized within the wetland system, so a "realized fraction" was used as a converter within the model. The realized fraction addresses the differences between laboratory- measured potential and the actual rates occurring within the treatment wetland cells. Mass balance analysis performed on the weekly nitrogen totals indicates that if only 10% of the measured nitrification potential were realized, there would be no measurable ammonium leaving the system. Measured nitrification potentials, however, are reflective of the more ideal conditions of the laboratory. The analysis requires a pH range between 7 and 7.2, as nitrifying bacteria are extremely pH sensitive (Paul and Clark, 1996; Sylvia *et al.*, 1999) This was clearly demonstrated when one set of samples (July 17, 2006) were inadvertently run without adjusting the pH of the incubation solution and no readable results were obtained, even after 6 hours of incubation.

Wetland pH has been measured by the Highland Sewer Department three times per week since January 2004, but measurements were for influent and effluent only. Beginning in May 2006 effluent pH values were considerably higher than previously reported (data not presented). This raised questions as to the value of the reported influent and effluent measurements. Therefore, system influent and effluent from each of the individual wetland cells was analyzed for pH on site from June 21 through December 14, 2006 (data not presented). Average influent pH during this time period was 7.35. The pH dropped in GB1 to an average value of 6.63 and returned to close to

neutral (6.87) before leaving PB2. The drop in pH within GB1 was the result of the known acidification accompanying nitrification. This pH drop, however, also functions as a type of "feed back inhibition" decreasing nitrification potential in the less well-buffered wetland microsites.

It may be surmised that only a fraction of the denitrification potential would be realized by the enzymes nitrate reductase, nitrite reductase and nitric oxide reductase, which are inhibited by oxygen (Paul and Clark, 1996; Sylvia *et al.*, 1999). Thus, denitrification will occur only in the anoxic microsites, regardless of the location (oxic or anoxic microsite) of the denitrifying organisms. During the laboratory experiments to measure DEA, the incubation flasks were purged with N_2 gas for 4 minutes to insure anoxic conditions. Although the measured redox potential (chapter 3) indicated that the wetland cells were within the anoxic range, $-100 < Eh < +300$ mV (Headley *et al.*, 2005), the heterogeneity of the wetland environment and the measured nitrification within the wetland units indicated that not all microsites were anoxic.

TLVFSBTS Model Evaluation

As previously mentioned, simulation runs of the 'water budget' sub-model realistically predicted flow rates from each bed, and from the system as a whole. Input and output data from this simulation may be found in Appendix D. Graphical output as seen in Fig. 22 clearly showed precipitation events moving from one wetland cell to another as the additive effect of the additional water volume moved through the system. The simulation output also adequately predicted times when no outflow occurred.

Simulation of the 'nitrogen cycle', however, was not successful. The simplest version of the nitrogen cycle merely added ammonium concentration as a converter to

the inflow component from the "water budget'. Ammonium was treated as 'inert' (i.e. non reactive) to test the inflow, outflow, and dilution effect (precipitation). This portion of the model accurately predicted outflow as being diluted by precipitation, and reduced to outflow volume by evapotranspiration.

The next component added to the nitrogen cycle was the first step of nitrification, ammonium oxidation to nitrite. This outflow was defined as the nitrification rate (g m^{-3} d^{-1}) multiplied by the bed volume (m^3) and then multiplied by the realized fraction of the nitrification rate (unitless). If the realized fraction was set equal to 0 (i.e., no nitrification occurring) the model correctly simulated all nitrogen leaving the wetland cell as ammonium. By varying the 'realized fraction' between 0 and 0.1 simulated nitrogen output changed from all ammonium to all nitrite.

Since the simulation software assigns priority to reservoir outflows based on the order in which they are created, the sub-model was recreated with the first outflow going to Biomass N (immobilization), the second outflow to NO2 (NH4 oxidation), and the third outflow to ammonium in the wetland effluent (Effluent NH4). With the realized fraction of nitrification rate set equal to 0, the simulation model realistically modeled immobilization and minerialization as well as Biomass N (Fig. 27).

The remaining portions of the nitrogen cycle model, nitrite oxidation and denitrification, were added to the model after the Biomass N portion. With 'realized fraction 2' and 'DEA realized fraction' both set equal to 0, ammonium N should have gone to Biomass N first, then to NO2 via NH4 Oxidation, and the remainder to Effluent NH4. The problem with the TLVFSBTS model occurs when the 'realized fraction' of the nitrification rate during ammonium oxidation is set to a value greater than 0.01. At

a realized fraction at or below 0.01, the output resembles the curve fit to the measured nitrification potential. When the realized fraction is set to a larger value, the program is unable to correctly calculate the nitrification rate, and erroneous data are generated (Fig. 28). The wide fluctuations in the nitrification rate curve when the realized fraction is set to a value greater than 0.01, cause equally wide fluctuations in the Effluent NH4 component of the model. Efforts to overcome this problem have failed.

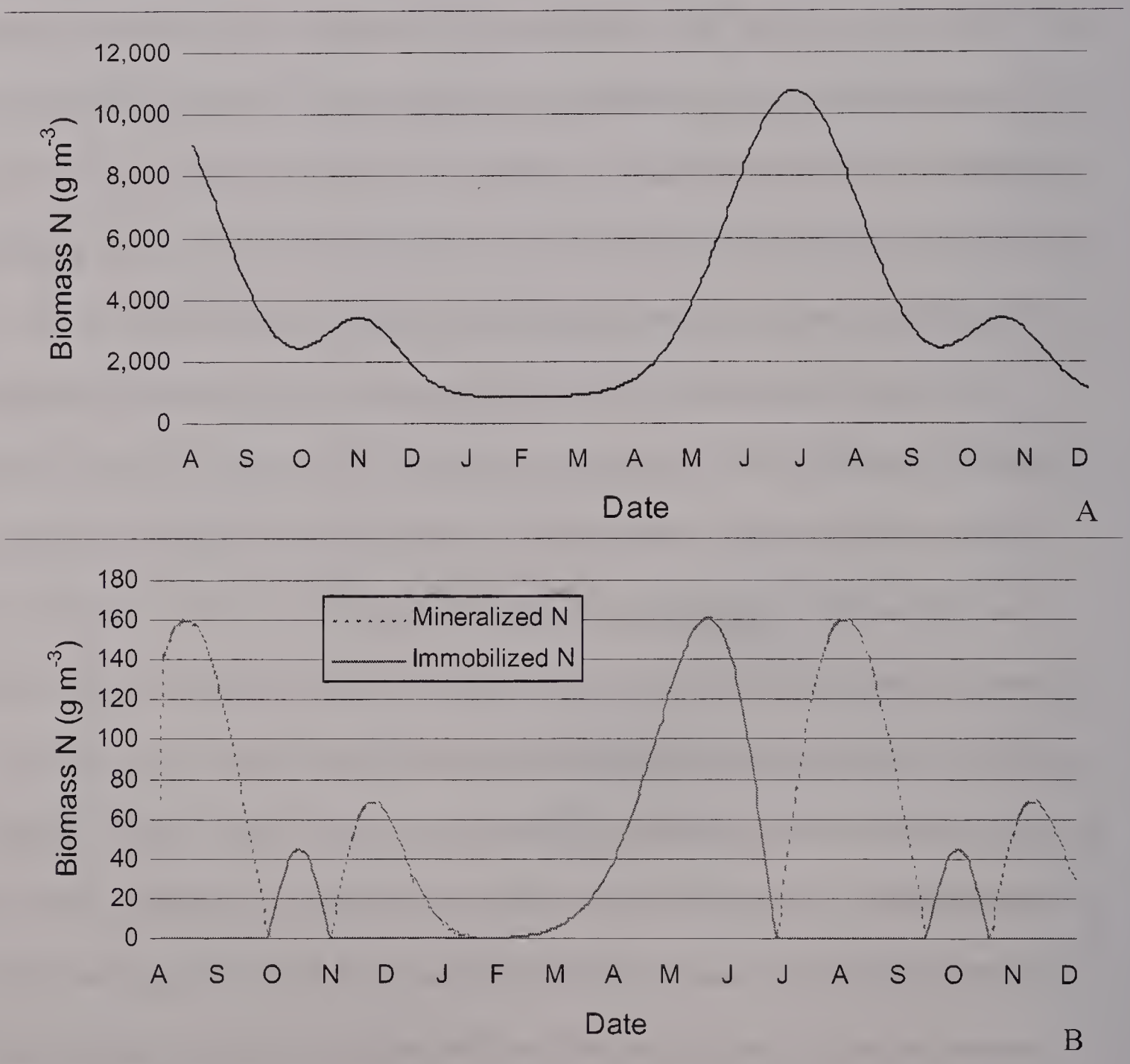


Figure 27. Simulation output from TLVFSBTS Model output for Biomass N (A) and immobilization/minerialization of nitrogen (B).

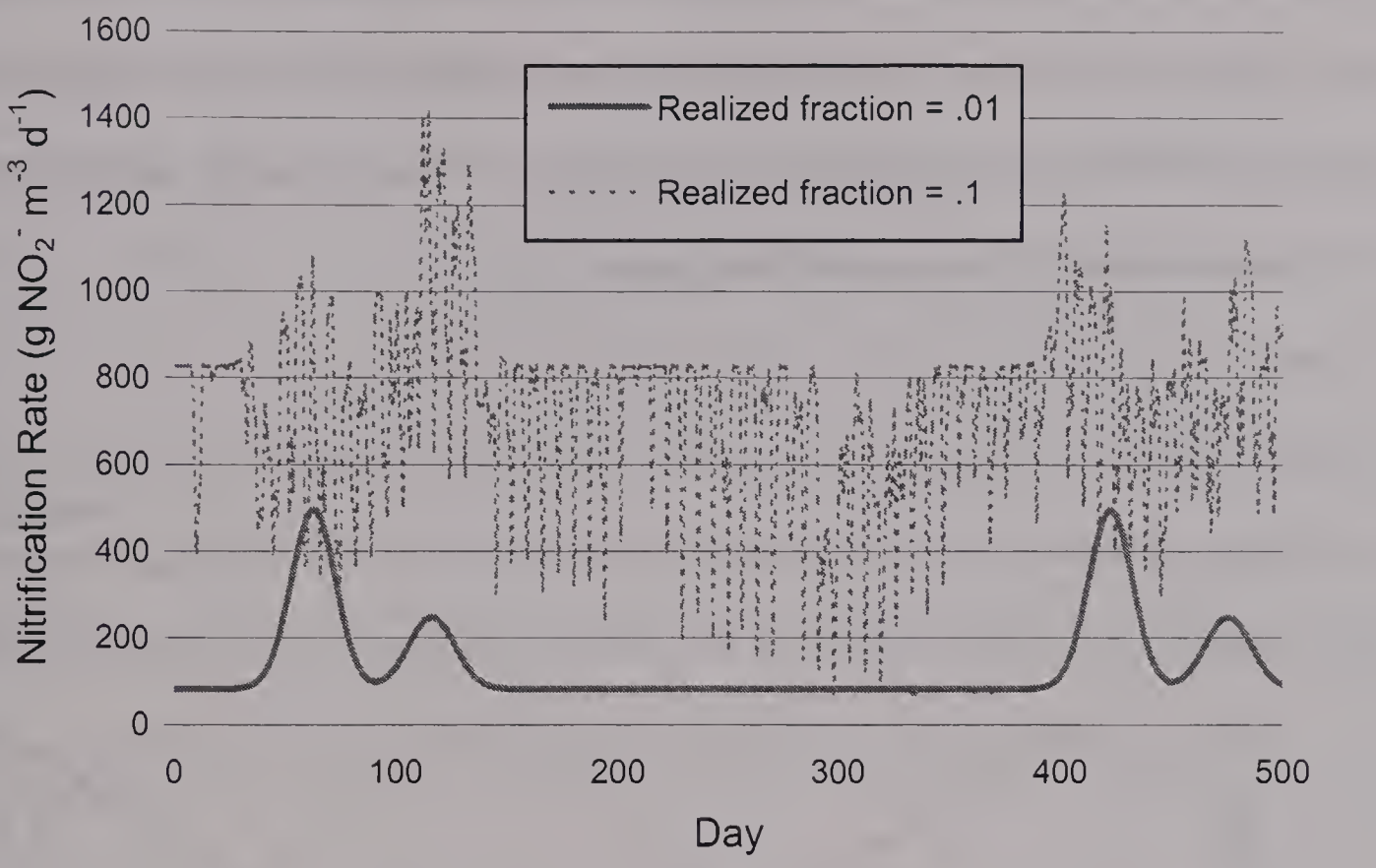


Figure 28. Simulation output for NH4 Oxidation portion of the TLVFSBTS model showing the nitrification rate curve shape when realized fraction = 0.01 and the erroneous data when realized fraction = 0.1.

When the difference between influent ammonium concentration and GB1 effluent ammonium concentration were plotted, a distinct seasonal trend existed. A cosine curve was fit to the data (Fig. 29) and would have been used as the 'seasonal factor' modifying the 'realized potential' or as the new 'realized potential' for both nitrification and denitrification rates in the TLVFSBTS model if the calculation errors could have been overcome. It should be noted that the curve fit to the difference between the influent and effluent ammonium concentrations in GB1 is nearly a mirror image of the PET curve, indicating that when probable evapotranspiration is greatest, the difference between influent and effluent concentrations is the smallest. The calculated PET values as the basis for the probable ET curve take into account environmental factors, which are difficult to measure accurately within the canopy of a

treatment wetland (solar radiation, wind velocity, humidity, etc.). It should also be noted that the use of a seasonally varying realized potential makes a great deal of sense in that it allows incorporation of the majority of "environmental" factors influencing the performance of a living treatment system.

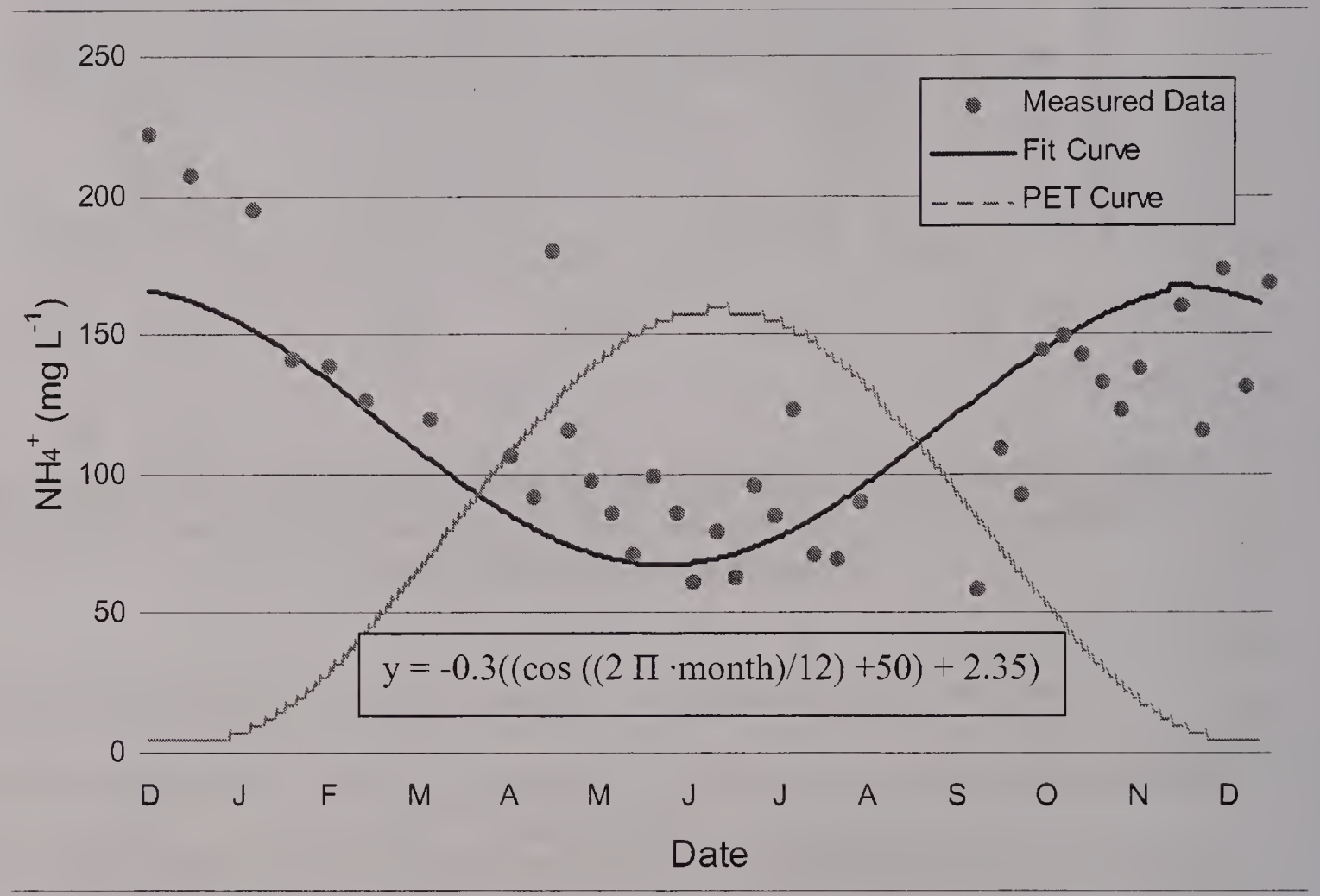


Figure 29. Difference between influent and effluent ammonium concentration in GB1 of the treatment wetland. Circles represent measured date, the solid line represents fitted curve for the equation in the figure, and the dashed line represents the PET curve.

Conclusions

The original simulation model evaluated was that of Gidley (1995). This model was determined to be exceedingly complicated, and there were numerous differences between the original (Gidley, 1995) and the published version (Wynn and Liehr, 2001). Due to cumulative effects of erroneous values, or misstatements, it was impossible to

resolve the differences between the two versions or to debug the model statements. A new, simpler TLVFSBTS model was developed, parts of which worked quite well. There appears, however, to be a computational problem in the simulation software, which is interfering with the simulation output. This makes an accurate assessment of the new, simple model impossible. Future work will continue to pursue development of the current model, perhaps with different software. A great deal was learned about the interactions between the microbial systems studied and the environmental factors influencing the wetland treatment system, all of which reinforce the notion that a simpler model will ultimately be the best predictor of system performance.

CHAPTER 6

CONCLUSIONS AND FUTURE WORK

System Performance and Microbial Evaluation

Artificial or constructed treatment wetlands such as the top loading vertical flow submerged bed treatment system (TLVFSBTS) studied during this research represent, or should represent, the future of wastewater treatment. These systems mimic nature, and are low technology, energy efficient, and when properly built and maintained, are capable of out performing conventional concrete and steel technology. Since Global Climate Change is one of the leading issues for the political and scientific leaders of the world, it only seems appropriate to look at all measures that could reduce global energy consumption and greenhouse gas production. Conventional wastewater treatment plants are tremendous "energy hogs". Aeration requires electricity, which in turn often requires consumption of petroleum products. Biosolids reduction usually requires the use of synthetic chemical polymers, which must be produced (consuming more petroleum products and electricity) and transported to the treatment plant, again consuming petroleum products. The "treated" biosolids, must then be removed from the treatment plant (in liquid or 'dry' form) again consuming energy.

The "treated" effluent from conventional wastewater treatment plants is often discharged only partially treated (as evidenced by NPDES Permit Limits of 30 mg L^{-1} for both BOD_5 and TSS). When stricter discharge limits are required, as is mandated in parts of upstate New York the cost of treatment becomes incredible. Grahamsville, NY a small rural community in the Catskill region built a $30,000,000 sewer treatment plant in the early 1990's to treat 180,000 GPD. The plant requires 11 fulltime operators. In

comparison, Highland, NY has a treatment plant treating 1.75 MGD, which can be operated by only three fulltime employees. The difference is that the Highland plant discharges into the Hudson River, while the Grahamsville plant discharges into a tributary to one of New York City's drinking water reservoirs. As evidenced by the system performance evaluation of this study, the TLVFSBTS wetland in Highland is capable of excellent ammonium removal while meeting the drinking water nitrate MCL (maximum contaminant level) of 10 ppm. If microorganisms within the wetland are capable of carrying out the ANAMOX process, as has been suggested by this research, then the ammonium removal (ammonium → nitrite → nitrogen gas) is producing N_2 directly, thereby eliminating the possibility of producing greenhouse gases (NO_x). The combination of producing a near drinking water quality effluent (from a nutrient standpoint); with non-detectable suspended solids (allowing easy UV disinfection); while not consuming electricity or petroleum products; and not discharging greenhouse gases would seem to be an obvious choice.

The next question, therefore, would be why are such systems not being used? The short answer is regulators. In order to feel comfortable approving and permitting wetland systems, regulators subject these systems to more stringent discharge levels than most existing conventional treatment plants; thus the permit for the Staff wetland requires meeting NY State DEC Intermittent Stream Standards (< 5, <10, <1 and <2.2 mg L^{-1} for $CBOD_5$, TSS, PO_4^{2-}, and NH_4^+, respectively). Not all artificial wetland systems can meet these strict limits. Free water surface systems grow algae, thus increasing effluent BOD_5 and TSS. End-loading horizontal flow submerged bed systems produce excessive nitrate, or do not reduce ammonium, depending

predominantly on the size and type of media used within the system. Both nitrification potential and denitrification enzyme activity as measured in the wetland system, especially GB1, demonstrate the ability of top loading vertical flow submerged bed treatment wetlands to remove the various nitrogen constituents once the microbial population has acclimated to the influent wastewater. It would appear from the results of this study and a survey of the literature, that top-loading vertical flow through medium sand is the right combination for the desired level of treatment.

Future research investigations of this particular treatment wetland system should focus on identification of the microorganisms present within the wetland cells. It would be most productive to determine if ANAMOX bacteria are present. If it could be clearly demonstrated that TLVFSBTS wetlands are capable of a level of treatment (ANAMOX) difficult to achieve or sustain in conventional wastewater treatment except at exorbitant costs, regulators would have a greater 'comfort factor' in approving the use of these systems. Similarly, if natural systems (i.e., non concrete and steel), and especially constructed wastewater treatment wetlands, are capable of removing pharmaceutical compounds from wastewater, as early research in the mid-west is indicating, this too would be a boon for the permitting and use of constructed wastewater treatment wetland systems. The Staff wetland would be an ideal candidate for such a study precisely because it is essentially a "black water" system (i.e., bathroom waste only). The lack of dilution (from laundry, showers, dish washers, etc) which result in the extremely high influent BOD_5 and NH_4^+ concentrations, would also keep the concentration of pharmaceutical compounds (synthetic hormones, antibiotics, and other prescription compounds) from being diluted to levels that might make them difficult to detect.

Additionally, since the "users" of the system are a small, known population, determining exactly which compounds might be in the waste stream may be easier than in a system with a large input.

During the course of this study, the heterotrophic potential measurements failed to predict the large differences in total microbial biomass between the wetland cells. It would be interesting to repeat the heterotrophic potential experiment using both the labeled glucose and a labeled amino acid. Perhaps this would better predict the differences in total microbial biomass and help to differentiate between the glucose-consuming bacteria and the more diverse heterotrophs.

From the soil scientist viewpoint, it would be of great interest to determine if the use of processed shale sand, rather than quartz sand, is a factor in treatment. Black shale has been shown to be high in both iron and manganese. It would appear that reduced iron and/or manganese weathering from the sand may be a contributing factor to continued phosphorous removal within the wetland system. The lack of phosphorous 'flushes' from the system would also point to sorption or precipitation of stable phosphorous compounds, which do not 'desorb' or otherwise flush out of the sand. The possibility that manganese is reduced in the first wetland cell and then oxidized in the second wetland cell should also be investigated. Manganese toxicity in plants is not common, but would be more apt to occur when reduced manganese is retained within the system. Should manganese toxicity prove to be a problem in the second wetland cell, it would suggest that over time it might become a problem in cells three and four, leading to the possibility of reduced performance. Use of manganese-tolerant plant species would overcome this obstacle, should it prove to be a problem.

Model Evaluation and Future Model Development

The existence of programs such as STELLA 8.1 (isee systems, inc.) has led to the ability of people other than computer programmers and mathematicians to develop simulation models. This advancement is beneficial to the extent that future simulation models may be developed by scientists knowledgeable of the living systems being modeled. These programs also have drawbacks, however, in that such models may not be mathematically valid. This would be particularly true if care is not taken to insure that the mathematics are correct with respect to "units" (i.e., 24 g m^{-3} d^{-1} x 200 g m^{-3} d^{-1} gives an answer 4800, but with meaningless units g^2 m^{-6} d^{-2}). Conversely, simulation models may be developed by those who understand the mathematics, but not the living systems being modeled. This was the case with the original simulation model evaluated for this study (Gidley,1995; Wynn and Liehr, 2001). This model was determined to be exceedingly complicated, leading to an inability to debug the model statements when the simulation runs produced meaningless output. For complex artificial wetland treatment systems, however, the simple empirical models originally used also failed to adequately predict system performance. It seems clear that a new, simpler TLVFSBTS model, such as the one partially developed using research and data from this study, are needed. It may be that Stella 8.1 (isee systems, inc.) software is not robust enough for the interactions required to adequately model TLVFSBTS wetlands.

A great deal was learned about the interactions between the microbial systems studied and the environmental factors influencing the wetland treatment system. There appear to be distinct, seasonal trends influencing all systems. The concept that there might be "constants" within the system was quickly disabused. The results of this study,

especially those from the modeling phase, reinforces the notion there are "patterns" in nature, that these patterns can be simulated with fairly simple mathematical equations, and that a simpler model will ultimately be the best predictor of system performance.

APPENDIX A

STAFF WETLAND PERFORMANCE DATA

Table 7. Mean nitrate concentration from all samples. Numbers within a row followed by the same letter are not statistically different from each other according to Duncan's New Multiple Range Test. Dates when no sample was available are indicated by nf.

Date	Influent	GB1	GB2	PB1	PB2
	---------- mg L^{-1} ----------				
2005					
10-Aug	11.2 c***	13.2 b***	14.5 b***	nf	nf
18-Aug	8.0 c***	9.7 b***	12.0 a***	nf	nf
25-Aug	4.8 c***	23.5a***	21.7 b***	5.7 c***	nf
1-Sep	7.2 c***	29.2 a***	25.3 b***	7.5 c***	nf
22-Sep	2.8 c***	22.2 b***	24.3 a***	22.3 b***	nf
28-Sep	2.3 b***	19.3 a***	19.0 a***	19.2 a***	20.0 a***
5-Oct	4.2 d***	19.7 c***	22.8 b***	24.2 ab***	25.7 a***
13-Oct	2.2 c***	3.5 a***	2.8 b***	1.5 d***	1.5 d***
19-Oct	2.2 c***	11.3 a***	7.7 b***	2.2 c***	2.2 c***
27-Oct	4.0 c***	9.5 a***	9.5 a***	4.3 b***	4.3 b***
2-Nov	4.0 e***	13.1 a***	11.7 b***	7.3 c***	6.8 d***
16-Nov	6.3 b***	17.7 a***	15.5 a***	7.7 b***	7.7 b***
1-Dec	3.0 c***	4.7 b***	4.5 b***	5.2 a***	4.8 ab***
15-Dec	4.7 c***	17.3 a***	17.5 a***	14.2 ab***	11.3 b***
2006					
6-Jan	5.2 d***	13.0 b***	14.8 a***	7.2 c***	6.8 c***
19-Jan	4.8 a***	2.8 e***	3.5 d***	4.5 b***	4.0 c***
1-Feb	5.0 c***	7.2 b***	8.2 a***	6.7 b***	7.2 b***
14-Feb	4.5 c***	6.3 b***	4.5 c***	6.2 b***	6.8 a***
8-Mar	0 e***	5.0 b***	3.5 c***	8.2 b***	8.7 a***
5-Apr	4.7 d***	11.5 a***	9.5 b***	6.7 c***	7.3 c***
13-Apr	4.8 b***	8.8 a***	9.3 a***	nf	nf
19-Apr	3.5 d***	13.0 a***	7.3 c***	8.0 b***	3.7 d***
25-Apr	0.4 d**	1.2 a**	1.0 ab**	0.9bc**	0.7 cd**
3-May					
10-May	6.0 e***	14.3 a***	6.5 c***	10.7 b***	5.7 d***
17-May	0.5 c***	2.6 a***	1.3 b***	1.1 b***	0.7 c***
24-May	0.4 c***	1.8 a***	1.3 b***	1.2 b***	0.5 c***
1-Jun	3.2 a*	2.9 ab*	2.6 abc*	2.1 bc*	1.8 c*
7-Jun	0.4 e***	1.9 a***	1.6 b***	1.3 c***	0.6 d***
15-Jun	1.4 d***	8.6 b***	8.0 b***	9.4 a***	4.6 c***
21-Jun	6.0 c***	9.7 b***	9.7 b***	24.5 a***	9.5 b***
28-Jun	1.2 d***	6.9 a***	3.8 b***	3.8 b***	3.2 c***
5-Jul	1.7 c***	1.4 d***	2.5 b***	11.8 a***	1.5 d***

Date	Influent	GB1	GB2	PB1	PB2
	---------- mg L^{-1} ----------				
11-Jul	1.3 c***	6.1 b***	9.7 a***	nf	0.9 c***
18-Jul	1.9 b***	10.4 a***	10.3 a***	nf	nf
26-Jul	2.1 b***	9.3 a***	9.3 a***	9.5 a***	0.5 c***
3-Aug	1.5 b***	5.8 a***	6.2 a***	nf	nf
13-Sep	1.6 c***	15.7 b***	23.5 a***	nf	nf
28-Sep	1.2 c***	11.2 a***	10.3 b***	nf	nf
5-Oct	1.2 c***	11.7 b***	13.2 a***	nf	0.3 d***
12-Oct	1.2 e***	14.3 a***	10.8 b***	8.3 c***	2.3 d***
19-Oct	1.4 d***	14.8 b***	12.5 c***	16.2 a***	0.8 e***
26-Oct	1.5 c***	21.8 a***	13.7 b***	nf	0.1 d***
1-Nov	1.5 c***	10.0 a***	6.8 b***	nf	0.1 c***
8-Nov	1.3 e***	11.3 a***	8.5 b***	3.9 c***	2.1 d***
22-Nov	1.4 c***	9.0 a***	6.2 b***	nf	0.1 d***
29-Nov	1.4 c***	22.0 a***	12.2 b***	nf	0.3 c***
6-Dec	1.5 c***	16.5 a***	13.8 b***	nf	0.3 d***
14-Dec	1.4 c***	13.0 a***	11.8 b***	nf	0.9 d***

*, **, *** Significant at P=0.05, 0.01 and 0.001, respectively.

Table 8. Mean ammonium concentration from all samples. Numbers within a row followed by the same letter are not statistically different from each other according to Duncan's New Multiple Range Test. Dates when no sample was available are indicated by nf.

Date	Influent	GB1	GB2	PB1	PB2
	---------- mg L^{-1} ----------				
2005					
10-Aug	152.3 a***	44.3 b***	34.7 c***	nf	nf
18-Aug	94.0 a***	21.7 b***	15.7 c***	nf	nf
25-Aug	231.7 a***	33.0 b***	26.0 c***	22.1 d***	nf
1-Sep	160.0 a***	32.7 b***	23.7 b***	9.0 b***	nf
22-Sep	104.0 a***	40.3 b***	32.7 c***	19.7 d***	nf
28-Sep	177.3 a***	47.0 b***	31.3 c***	19.0 d***	10.0 e***
5-Oct	216.0 a***	41.3 b***	29.3 bc***	13.0 cd***	7.3 d***
13-Oct	156.7 a***	13.0 b***	8.0 bc***	4.0 cd***	0.7 d***
19-Oct	147.3 a***	25.3 b***	13.3 c***	4.0 d***	0.0 e***
27-Oct	160.0 a***	21.0 b***	14.3 c***	4.0 d***	1.0 d***
2-Nov	178.7 a***	29.0 b***	18.3 c***	0.7 d***	0.0 d***
16-Nov	250.0 a***	27.7 b***	21.0 b***	0 b***	0 b***
1-Dec	230.0 a***	22.7 b***	12.0 c***	0 d***	0 d***
15-Dec	227.3 a***	33.0 b***	20.7 c***	0 d***	0 d***
2006					
6-Jan	174.0 a***	33.0 b***	20.7 c***	3.7 d***	4.0 d***
19-Jan	163.7 a***	25.3 b***	16.3 b***	0.0 c***	3.3 c***
1-Feb	146.0 a***	40.0 b***	23.0 c***	1.7 d***	0.0 d***
14-Feb	179.2 a***	59.7 b***	23.7 c***	2.3 d***	0.0 d***
8-Mar	158.0 a***	51.7 b***	25.3 c***	0.7 d***	1.3 d***
5-Apr	150.0 a***	58.3 b***	33.0 c***	1.0 d***	0.0 d***
13-Apr	265.0 a***	85.0 b***	38.3 c***	nf	nf
19-Apr	202.3 a***	86.7 b***	44.0 c***	0.0 d***	0.0 d***
26-Apr	152.7 a***	55.0 b***	25.3 c***	11.7 d***	3.3 e***
3-May	175.3 a***	89.3 b***	36.0 c***	18.3 d***	4.3 e***
10-May	155.0 a***	84.0 b***	32.7 c***	19.7 d***	5.0 e***
17-May	176.0 a***	77.0 b***	41.3 c***	14.7 d***	4.3 e***
24-May	159.3 a***	93.3 b***	46.7 c***	17.0 d***	4.0 e***
1-Jun	144.7 a***	83.3 b***	47.7 c***	19.7 d***	6.7 e***
7-Jun	164.7 a***	85.7 b***	58.0 c***	22.3 d***	4.7 e***
15-Jun	108.7 a***	46.3 b***	35.3 c***	17.3 d***	3.7 e***
21-Jun	168.7 a***	73.3 b***	50.3 c***	34.7 d***	0.0 e***
28-Jun	116.7 a***	31.7 b***	27.7 b***	7.0 c***	4.0 c***
5-Jul	174.0 a***	51.3 b***	26.7 c***	16.7 d***	1.0 e***
11-Jul	151.3 a***	80.7 b***	39.3 c***	nf	0.0 d***
18-Jul	144.0 a***	75.0 b***	48.7 c***	nf	nf
26-Jul	162.7 a***	73.0 b***	44.0 c***	15.3 d***	4.7 e***
3-Aug	119.3 a***	60.7 b***	40.7 c***	nf	nf

Date	Influent	GB1	GB2	PB1	PB2
	---------- mg L^{-1} ----------				
13-Sep	159.3 a***	50.7 b***	42.3 c***	nf	nf
21-Sep	143.3 a***	50.7 b***	39.0 b***	nf	nf
28-Sep	202.0 a***	57.7 b***	43.0 c***	nf	nf
5-Oct	194.7 a***	45.7 b***	35.3 c***	nf	0.0 d***
12-Oct	182.0 a***	39.7 b***	32.3 c***	6.3 d***	2.3 e***
19-Oct	175.3 a***	42.7 b***	31.7 c***	5.0 d***	0.0 e***
26-Oct	166.0 a***	43.0 b***	30.0 c***	nf	0.3 d***
1-Nov	171.3 a***	33.7 b***	22.0 c***	nf	0.0 d***
8-Nov	197.3 a***	37.0 b***	22.3 c***	4.0 d***	0.3 d***
22-Nov	152.7 a***	37.3 b***	16.7 c***	nf	0.0 d***
29-Nov	218.0 a***	44.7 b***	23.0 c***	nf	1.0 d***
6-Dec	174.0 a***	42.7 b***	25.7 c***	nf	0.0 d***
14-Dec	207.3 a***	38.7 b***	29.3 c***	nf	0.0 d***

*** Significant at P=0.001.

Table 9. Mean nitrite concentration from all samples. Numbers within a row followed by the same letter are not statistically different from each other according to Duncan's New Multiple Range Test. Dates when no sample was available are indicated by nf.

Date	Influent	GB1	GB2	PB1	PB2
	---------- mg L^{-1} ----------				
2005					
10-Aug	0.015 a***	0.019 a***	0.006 b***	nf	nf
18-Aug	0.008 b***	0.017 a***	0.006 b***	nf	nf
25-Aug	0.005 c***	0.168 a***	0.008 c***	0.019 b***	nf
1-Sep	0.000 c***	0.065 a***	0.013 b***	0.011 b***	nf
22-Sep	0.012 bc***	0.022 a***	0.009 c***	0.013 b***	nf
28-Sep	0.017 ab***	0.017 ab***	0.009 c***	0.014 b***	0.019 a
5-Oct	0.007 b***	0.020 a***	0.020 a***	0.006 b***	0.007 b***
13-Oct	0.007 b***	0.151 a***	0.008 b***	0.005 b***	0.002 b***
19-Oct	0.011 b***	0.048 a***	0.006 b***	0.008 b***	0.004 b***
27-Oct	0.005 c***	0.026 a***	0.002 d***	0.008 b***	0.000 e***
2-Nov	0.011 bc***	0.119 a***	0.005 c***	0.049 ab***	0.001 c***
16-Nov	0.019 c***	0.215 a***	0.006 d***	0.037b***	0.002 d***
1-Dec	0.013 c***	0.038 a***	0.006 d***	0.032 b***	0.002 e***
15-Dec	0.010 c***	0.373 a***	0.009 c***	0.053 b***	0.001 d***
2006					
6-Jan	0.018 c***	0.362 a***	0.009 d***	0.028 b***	0.001 e***
19-Jan	0.020 c***	0.090 a***	0.020 c***	0.030 b***	0.001 d***
1-Feb	0.017 bc***	0.584 a***	0.029 b***	0.020 bc***	0.001 c***
14-Feb	0.022 b***	0.655 a***	0.007 bc***	0.008 bc***	0.001 c***
8-Mar	0.010 c***	1.058 a***	0.090 b***	0.009 c***	0.001 c***
5-Apr	0.018 bc***	0.894 a***	0.038 b***	0.002 c***	0.001 c***
13-Apr	0.018 c***	1.094 a***	0.161 b***	nf	nf
19-Apr	0.019 d***	1.570 a***	0.038 c***	0.135 b***	0.000 e***
26-Apr	0.018 b***	0.641 a***	0.005 b***	0.011 b***	0.000 b***
3-May	0.102 b***	1.618 a***	0.002 c***	0.003 c***	0.000 c***
10-May	0.022 b***	1.820 a***	0.006 b***	0.008 b***	0.002 b***
17-May	0.013 c***	2.204 a***	0.065 b***	0.005 c***	0.001 c***
24-May	0.010 b***	0.858 a***	0.007 c***	0.004 c***	0.001 d***
1-Jun	0.000 d***	0.522 a***	0.191 b***	0.011 c***	0.001 d***
7-Jun	0.003 c***	0.493 a***	0.005 c***	0.044 b***	0.001 c***
15-Jun	0.017 b***	0.361 a***	0.008 cd***	0.013 bc***	0.003 d***
21-Jun	0.014 c***	0.052 a***	0.107 b***	0.009 d***	0.005 e***
28-Jun	0.020 b***	0.159 a***	0.010 c***	0.008 c***	0.003 d***
5-Jul	0.014 d***	0.017 c***	0.078 a***	0.024 b***	0.001 e***
11-Jul	0.026 b***	0.019 c***	0.052 a***	nf	0.002 d***
18-Jul	0.024 a***	0.023 a***	0.008 b***	nf	nf
26-Jul	0.019 b***	0.011 c***	0.005 d***	0.041 a***	0.000 e***
3-Aug	0.024 a***	0.014 b***	0.009 c***	nf	nf

Date	Influent	GB1	GB2	PB1	PB2
	---------- mg L^{-1} ----------				
13-Sep	0.018 b***	0.038 a***	0.011 c***	nf	nf
21-Sep	0.013 a***	0.010 b***	0.008 c***	nf	nf
28-Sep	0.010 a***	0.012 a***	0.006 b***	nf	nf
5-Oct	0.001 c***	0.138 a***	0.007 b***	nf	0.002 bc***
12-Oct	0.006 c***	0.070 a***	0.005 c***	0.034 b***	0.004 c***
19-Oct	0.017 b***	0.150 a***	0.007 c***	0.007 c***	0.002 c***
26-Oct	0.016 b***	0.127 a***	0.017 b***	nf	0.002 c***
1-Nov	0.012 b***	0.162 a***	0.007 c***	nf	0.001 d***
8-Nov	0.009 b***	0.320 a***	0.006 b***	0.005 b***	0.002 b***
22-Nov	0.003 b***	0.288 a***	0.005 b***	nf	0.001 b***
29-Nov	0.006 b***	0.488 a***	0.004 b***	nf	0.001 b***
6-Dec	0.011 b***	0.310 a***	0.004 bc***	nf	0.001 c***
14-Dec	0.005 b***	0.485 a***	0.005 b***	nf	0.001 b***

*** Significant at P=0.001.

Table 10. Mean $CBOD_5$ concentration from influent and GB1 effluent and GB2 effluent. Numbers within a row followed by the same letter are not statistically different from each other according to Duncan's New Multiple Range Test.

Date	Influent	GB1	GB2
	---------- mg L^{-1} ----------		
2005			
14-Jul	295 a***	1.60 b***	0.20 b***
22-Jul	286 a***	3.70 b***	1.80 b***
28-Jul	253 a***	3.40 b***	2.60 b***
3-Aug	199 a***	1.20 b***	0.80 b***
10-Aug	232 a***	3.20 b***	2.00 b***
17-Aug	204 a***	2.40 b***	0.40 b***
25-Aug	262 a***	2.90 b***	0.60 b***
1-Sep	199 a***	1.20 b***	0.80 b***
22-Sep	235 a***	3.30 b***	1.30 b***
28-Sep	164 a***	0.84 b***	0.61 b***
5-Oct	227 a***	3.30 b***	2.70 b***
13-Oct	201 a***	4.70 b***	1.00 b***
19-Oct	130 a***	3.70 b***	0.50 b***
27-Oct	179 a***	2.80 b***	1.40 b***
2-Nov	103 a***	3.30 b***	0.90 b***
16-Nov	265 a***	5.60 b***	1.20 b***
16-Dec	285 a***	7.95 b***	b***
2006			
6-Jan	290 a***	9.10 b***	1.90 b***
19-Jan	220 a***	8.50 b***	1.70 b***
1-Feb	277 a***	12.60 b***	1.80 b***
14-Feb	327 a***	11.60 b***	1.33 b***
5-Apr	292 a***	17.60 b***	2.20 b***
13-Apr	314 a***	11.30 b***	2.10 b***
19-Apr	311 a***	15.00 b***	2.30 b***
26-Apr	332 a***	15.60 b***	2.20 b***
3-May	287 a***	11.60 b***	2.00 b***
10-May	307 a***	17.90 b***	1.10 b***
17-May	323 a***	14.10 b***	1.30 b***
24-May	318 a***	6.08 b***	2.30 b***
15-Jun	312 a***	9.66 b***	2.54 b***
21-Jun	281 a***	5.36 b***	2.74 b***
28-Jun	289 a***	4.68 b***	3.30 b***
5-Jul	260 a***	3.43 b***	2.33 b***
12-Jul	332 a***	3.73 b***	3.79 b***
19-Jul	326 a***	8.07 b***	2.83 b***
26-Jul	421 a***	7.32 b***	3.76 b***
13-Sep	291 a***	1.90 b***	2.65 b***

Date	Influent	GB1	GB2
	---------- mg L^{-1} ----------		
21-Sep	283 a***	4.19 b***	1.44 b***
28-Sep	253 a***	4.73 b***	0.99 b***
5-Oct	276 a***	2.69 b***	1.37 b***
12-Oct	173 a***	3.08 b***	1.11 b***
19-Oct	244 a***	2.43 b***	0.92 b***
26-Oct	225 a***	3.00 b***	0.91 b***
1-Nov	233 a***	2.27 b***	4.06 b***
22-Nov	221 a***	4.15 b***	1.40 b***

*** Significant at P= 0.001.

APPENDIX B

MICROBIAL ANALYSIS DATA

Table 11. Nitrification potential expressed at mg nitrite produced per gram dry sand per hour. Values within a row followed by the same letter are not significantly different according to Duncan's New Multiple Range Test.

Date	GB1	GB2	PB1	PB2
	---------- mg kg^{-1} dry soil h^{-1} ----------			
May 17	4.3 ns		3.0 ns	1.8 ns
Jun 24	3.9 ns		2.3 ns	3.7 ns
Aug 3	18.8 a*	1.3 b*	14.1 ab*	3.6 b*
Oct 5	39.9 ns	21.4 ns	49.8 ns	19.2 ns
Oct 26	6.0 b***	1.5 b***	24.6 a***	4.4 b***
Nov 29	21.2 a**	4.7 c**	15.9 ab**	7.8 bc**
Dec 14	8.2 ns	10.7 ns	9.2 ns	4.6 ns
Jan 24	16.9 a***	3.1 c***	14.8 ab***	10.7 b***
Feb 21	25.7	4.5	6.0	3.0

* ** *** Significant at P = 0.05, 0.01, 0.001, and not significant, respectively.

Table 12. Denitrifying enzyme activity expressed at mg nitrite produced per gram dry sand per hour. Values within a row followed by the same letter are not significantly different according to Duncan's New Multiple Range Test.

Date	GB1	GB2	PB1	PB2
	---------- mg kg^{-1} dry soil h^{-1} ----------			
Jun1	5.77 a*	0.62 b*	4.37 a*	2.29 ab*
Jul 16	177.7 a***	43.50 b***	32.85 b***	19.35 b***
Jul 26	80.4 a***	12.27 b***	14.25 b***	6.35 b***
Sep 28	58.4 a***	2.87 b***	4.70 b***	5.03 b***
Oct 19	224.1 a***	11.11 b***	18.04 b***	6.08 b***
Nov 22	45.0 a***	6.75 b***	6.83 b***	5.09 b***
Dec 14	14.8 a***	6.67 b***	7.39 b***	4.12 b***
Jan 24	31.2 a***	5.94 b***	4.06 b***	3.44 b***
Feb 21	102.4 a***	18.11 b***	11.51 b***	6.10 b***

* *** Significant at P = 0.05, 0.001, respectively.

APPENDIX C

STELLA EQUATIONS

Water Budget

GB1(t) = GB1(t - dt) + (Inflow + Precipitation - Evapotranspiration - GB1_Out) * dt

INIT GB1 = 26.75

INFLOWS:
Inflow = GRAPH (TIME)
(0.00, 4.92), (1.00, 4.54), (2.00, 4.92), (3.01, 5.30), (4.01, 5.30), (5.01, 1.33), (6.01, 1.33), (7.01, 2.65), (8.02, 4.54), (9.02, 4.92), (10.0, 5.30), (11.0, 4.92), (12.0, 9.84), (13.0, 9.84), (14.0, 9.84), (15.0, 4.92), (16.0, 6.06), (17.0, 4.54), (18.0, 4.16), (19.0, 1.51), (20.0, 1.51), (21.0, 6.06), (22.0, 6.43), (23.0, 5.68), (24.0, 5.68), (25.0, 2.74), (26.1, 2.74), (27.1, 2.74), (28.1, 2.74), (29.1, 4.92), (30.1, 5.30), (31.1, 3.79), (32.1, 2.46), (33.1, 2.46), (34.1, 2.65),

Precipitation = (Daily_precipitation/100)*Surface_Area

OUTFLOWS:
Evapotranspiration = (PET_GB1/100)*Surface_Area
GB1_Out = GB1-Bed_Capacity_GB1

GB_2(t) = GB_2(t - dt) + (GB1_Out + Precip_2 - GB2_Out - ET2) * dt

INIT GB_2 = 26.75

INFLOWS:
GB1_Out = GB1-Bed_Capacity_GB1
Precip_2 = (Daily_precipitation/100)*Surface_Area

OUTFLOWS:
GB2_Out = GB_2-Bed_Capacity_GB2
ET2 = (PET_GB2/100)*Surface_Area

PB1(t) = PB1(t - dt) + (GB2_Out + Precip_3 - PB1_Out - ET3) * dt

INIT PB1 = 26.75

INFLOWS:
GB2_Out = GB_2-Bed_Capacity_GB2
Precip_3 = (Daily_precipitation/100)*Surface_Area
OUTFLOWS:
PB1_Out = PB1-Bed_Capacity_PB1
ET3 = (PET_PB1/100)*Surface_Area

PB2(t) = PB2(t - dt) + (PB1_Out + Precip_4 - PB2_Out - ET4) * dt
INIT PB2 = 26.75

INFLOWS:
PB1_Out = PB1-Bed_Capacity_PB1
Precip_4 = (Daily_precipitation/100)*Surface_Area

OUTFLOWS:
PB2_Out = PB2-Bed_Capacity_PB2
ET4 = (PET_PB2/100)*Surface_Area

Bed_Capacity_GB1 = 24.4
Bed_Capacity_GB2 = 26.5
Bed_Capacity_PB1 = 31.8
Bed_Capacity_PB2 = 31.8
Bed_length = 15.24
Bed_width = 15.24
PET_GB1 = .31*(cos(2*PI*(TIME+30)/365)+1.05)
PET_GB2 = .31*(cos(2*PI*(TIME+30)/365)+1.05)
PET_PB1 = .375*(cos(2*PI*(TIME+30)/365)+1.05)
PET_PB2 = .375*(cos(2*PI*(TIME+30)/365)+1.05)
Surface_Area = Bed_width*Bed_length

Daily_precipitation = GRAPH(TIME)
(0.00, 0.00), (1.00, 0.00), (2.00, 0.00), (3.01, 0.00), (4.01, 1.98), (5.01, 0.03), (6.01, 0.00), (7.01, 0.00), (8.02, 0.00), (9.02, 0.00), (10.0, 0.00), (11.0, 1.19), (12.0, 0.00), (13.0, 0.66), (14.0, 0.03), (15.0, 0.05), (16.0, 0.00), (17.0, 0.00), (18.0, 0.00), (19.0, 0.13), (20.0, 0.00), (21.0, 0.00), (22.0, 0.00), (23.0, 0.03), (24.0, 0.00), (25.0, 0.00), (26.1, 0.03), (27.1, 0.08), (28.1, 0.03), (29.1, 0.13), (30.1, 0.58), (31.1, 0.00), (32.1, 0.00), (33.1, 0.00), (34.1, 0.00), (35.1, 0.00), (36.1, 0.00), (37.1, 0.03), (38.1, 0.00), (39.1, 0.00), (40.1, 0.00), (41.1, 0.00),

Nitrogen Cycle

DEA Equation

alpha_dea = 9
A_dea = 5500*EXP(e1_dea)
beta_dea = 3
B_dea = 4300*EXP(e2_dea)
C_dea = 5500*EXP(e3_dea)
DEA_Potential = A_dea+B_dea+C_dea+D_dea
D_dea = 500
e1_dea = -beta_dea*((date_3-t1_dea)^2)
e2_dea = -alpha_dea*((date_3-t2_dea)^2)
e3_dea = -beta_dea*((date_3-t3_dea)^2)
t1_dea = 2.5
t2_dea = 10.6
t3_dea = 14
date_3 = GRAPH(TIME)
(0.00, 0.00), (31.3, 1.00), (62.5, 2.00), (93.8, 3.00), (125, 4.00), (156, 5.00), (188, 6.00), (219, 7.00), (250, 8.00), (281, 9.00), (313, 10.0), (344, 11.0), (375, 12.0), (406, 13.0), (438, 14.0), (469, 15.0), (500, 16.0)

Flow

GB1(t) = GB1(t - dt) + (Staff_Inflow + Precipitation - Evapotranspiration - GB1_Out) * dt
INIT GB1 = 26.75

INFLOWS:
Staff_Inflow = Inflow
Precipitation = (Daily_precipitation/100)*Surface_Area

OUTFLOWS:
Evapotranspiration = (PET_GB1/100)*Surface_Area
GB1_Out = GB1-Bed_Capacity_GB1

Bed_Capacity_GB1 = 24.4
Bed_length = 15.24
Bed_width = 15.24
PET_GB1 = .31*(cos(2*PI*(TIME+30)/365)+1.05)
Surface_Area = Bed_width*Bed_length
Daily_precipitation = GRAPH(TIME)
(0.00, 0.00), (1.00, 0.00), (2.00, 1.19), (3.01, 0.00), (4.01, 0.66), (5.01, 0.03), (6.01, 0.05), (7.01, 0.00), (8.02, 0.00), (9.02, 0.00), (10.0, 0.13), (11.0, 0.00), (12.0, 0.00),

Inflow = GRAPH(TIME)
(0.00, 5.12), (1.00, 5.52), (2.00, 5.12), (3.01, 5.00), (4.01, 5.00), (5.01, 5.00), (6.01, 5.12), (7.01, 6.30), (8.02, 4.73), (9.02, 4.33), (10.0, 0.00), (11.0, 0.00), (12.0, 6.30), (13.0, 6.70), (14.0, 5.91), (15.0, 5.91), (16.0, 6.30), (17.0, 0.00), (18.0, 0.00), (19.0, 5.12), (20.0, 5.12),

Nitrification Equation

alpha_nt = 5
A_nt = 500*EXP(e1_nt)
beta_nt = 4.5
B_nt = 200*EXP(e2_nt)
C_nt = 500*EXP(e3_nt)
D_nt = 200*EXP(e4_nt)
e1_nt = -alpha_nt*((date_2-t1_nt)^2)
e2_nt = -beta_nt*((date_2-t2_nt)^2)
e3_nt = -alpha_nt*((date_2-t3_nt)^2)
e4_nt = -beta_nt*((date_2-t4_nt)^2)
E_nt = 100
Nitrification_Potential = A_nt+B_nt+C_nt+D_nt+E_nt
t1_nt = 2
t2_nt = 3.7
t3_nt = 13.5
t4_nt = 15.2
date_2 = GRAPH(TIME)
(0.00, 0.00), (31.3, 1.00), (62.5, 2.00), (93.8, 3.00), (125, 4.00), (156, 5.00), (188, 6.00), (219, 7.00), (250, 8.00), (281, 9.00), (313, 10.0), (344, 11.0), (375, 12.0), (406, 13.0), (438, 14.0), (469, 15.0), (500, 16.0)

Biomass_N(t) = Biomass_N(t - dt) + (Immobilization - Mineralization) * dt

INIT Biomass_N = 9000

INFLOWS:
Immobilization = Biomass_IN*Bed_Volume

OUTFLOWS:
Mineralization = Biomass_Out*Bed_Volume

NH4(t) = NH4(t - dt) + (Influent_NH4 + Mineralization - Immobilization – NH4_Oxidation - Effluent_NH4) * dt

INIT NH4 = 1500

INFLOWS:
Influent_NH4 = IF Inflow=0 THEN

NH4_In*(Inflow+4)*(Bed_Capacity_GB1/(Bed_Capacity_GB1+Precipitation))
ELSE NH4_In*Inflow*(Bed_Capacity_GB1/(Bed_Capacity_GB1+Precipitation))
Mineralization = Biomass_Out*Bed_Volume

OUTFLOWS:
Immobilization = Biomass_IN*Bed_Volume
NH4_Oxidation = Nitrification_Potential*Realized_Factor*Bed_Volume
Effluent_NH4 = IF GB1_Out=0 THEN 0 ELSE NH4/GB1_Out

NO2(t) = NO2(t - dt) + (NH4_Oxidation - Nitrification_Step_2 - NO2_Out) * dt

INIT NO2 = .24

INFLOWS:
NH4_Oxidation = Nitrification_Potential*Realized_Factor*Bed_Volume

OUTFLOWS:
Nitrification_Step_2 =
Nitrification_Potential*Realized_Fraction_2*Bed_Volume
NO2_Out = IF GB1_Out=0 THEN 0 ELSE NO2/GB1_Out

NO3(t) = NO3(t - dt) + (Nitrification_Step_2 - NO3_Out - N2) * dt
INIT NO3 = .24

INFLOWS:
Nitrification_Step_2 =
Nitrification_Potential*Realized_Fraction_2*Bed_Volume

OUTFLOWS:
NO3_Out = IF GB1_Out=0 THEN 0 ELSE (NO3/GB1_Out)
N2 = DEA_Potential*DEA_Realized_Fraction*Bed_Volume

Bed_Volume = 82.6
DEA_Realized_Fraction = .05
Realized_Factor = .0001
Realized_Fraction_2 = 0

Biomass_IN = GRAPH(TIME)
(0.00, 0.00), (1.00, 0.00), (2.00, 0.00), (3.00, 0.00), (4.00, 0.00), (5.00, 0.00), (6.00, 0.00), (7.00, 0.00), (8.00, 0.00), (9.00, 0.00), (10.0, 0.00), (11.0, 0.00), (12.0, 0.00), (13.0, 0.00), (14.0, 0.00), (15.0, 0.00), (16.0, 0.00), (17.0, 0.00), (18.0, 0.00), (19.0, 0.00), (20.0, 0.00), (21.0, 0.00), (22.0, 0.00), (23.0, 0.00), (24.0, 0.00), (25.0, 0.00), (26.0, 0.00), (27.0, 0.00), (28.0, 0.00), (29.0, 0.00), (30.0, 0.00), (31.0, 0.00), (32.0, 0.00), (33.0, 0.00), (34.0, 0.00), (35.0, 0.00), (36.0, 0.00), (37.0, 0.00), (38.0, 0.00), (39.0, 0.00), (40.0, 0.00), (41.0, 0.00), (42.0, 0.00), (43.0, 0.00), (44.0, 0.00), (45.0, 0.00), (46.0, 0.00), (47.0, 0.00), (48.0, 0.00),

Biomass_Out = GRAPH(TIME)
(0.00, 0.00), (1.00, 1.68), (2.00, 1.71), (3.00, 1.75), (4.00, 1.79), (5.00, 1.81), (6.00, 1.85), (7.00, 1.86), (8.00, 1.89), (9.00, 1.90), (10.0, 1.92), (11.0, 1.92), (12.0, 1.94), (13.0, 1.94), (14.0, 1.94), (15.0, 1.94), (16.0, 1.94), (17.0, 1.93), (18.0, 1.91), (19.0, 1.91), (20.0, 1.89), (21.0, 1.88), (22.0, 1.85), (23.0, 1.83), (24.0, 1.80), (25.0, 1.78), (26.0, 1.75), (27.0, 1.72), (28.0, 1.68), (29.0, 1.65), (30.0, 1.61), (31.0, 1.57), (32.0, 1.53), (33.0, 1.49), (34.0, 1.44), (35.0, 1.40), (36.0, 1.34), (37.0, 1.30), (38.0, 1.25), (39.0, 1.20), (40.0, 1.15), (41.0, 1.09), (42.0, 1.03), (43.0, 0.98), (44.0, 0.93), (45.0, 0.86), (46.0, 0.81), (47.0, 0.75), (48.0, 0.68),

NH4_In = GRAPH(TIME)
(0.00, 152), (10.2, 94.0), (20.4, 232), (30.6, 160), (40.8, 104), (51.0, 177), (61.2, 216), (71.4, 157), (81.6, 147), (91.8, 160), (102, 179), (112, 250), (122, 230), (133, 227), (143, 174), (153, 164), (163, 146), (173, 179), (184, 158), (194, 150), (204, 265), (214, 202), (224, 153), (235, 175), (245, 155), (255, 176), (265, 159), (276, 145), (286, 165), (296, 109), (306, 169), (316, 117), (327, 174), (337, 151), (347, 144), (357, 163), (367, 119), (378, 159), (388, 143), (398, 202), (408, 195), (418, 182), (429, 175), (439, 166), (449, 171), (459, 197), (469, 153), (480, 218), (490, 174), (500, 207)

Seasonal_Factor = GRAPH(TIME)
(0.00, 0.014), (1.00, 0.014), (2.00, 0.014), (3.00, 0.014), (4.00, 0.014), (5.00, 0.013), (6.00, 0.013), (7.00, 0.013), (8.00, 0.013), (9.00, 0.013), (10.0, 0.013), (11.0, 0.013), (12.0, 0.013), (13.0, 0.013), (14.0, 0.013), (15.0, 0.013), (16.0, 0.013), (17.0, 0.012), (18.0, 0.012), (19.0, 0.012), (20.0, 0.012), (21.0, 0.012), (22.0, 0.012), (23.0, 0.012), (24.0, 0.012), (25.0, 0.012), (26.0, 0.012), (27.0, 0.011), (28.0, 0.011), (29.0, 0.011), (30.0, 0.011), (31.0, 0.011), (32.0, 0.011), (33.0, 0.011), (34.0, 0.011), (35.0, 0.011),

APPENDIX D

MODEL INPUT AND PREDICTIONS

Table 13. TLVFSBTS model Water Budget input and output values. Measured and Precip. are measured inputs, STELLA and all output values were calculated by the software.

	Input			Output				
	Measured	STELLA	Precip.	PET	GB1	GB2	PB1	PB2
Date	---------- m^3 ------		cm	---------- m^3 ----------				
10-Aug	5.12	5.32	0	1.37	3.24	0.61	0	0
11-Aug	5.52	2.70	0	1.37	3.41	1.60	0	0
12-Aug	5.12	0.14	2.77	1.36	0.49	0.98	0	0
13-Aug	0	14.00	0	1.35	2.38	3.57	0.39	0
14-Aug	0	17.93	1.53	1.35	20.47	7.55	0.01	0
15-Aug	30.73	7.00	0.06	1.34	8.42	11.40	8.76	0
16-Aug	5.12	5.55	0.12	1.33	6.20	7.87	7.11	1.84
17-Aug	6.30	4.67	0	1.32	4.61	5.38	5.19	3.25
18-Aug	4.73	2.58	0	1.32	3.71	3.71	3.44	2.72
19-Aug	4.33	0.38	0	1.31	0.95	1.83	2.13	1.63
20-Aug	0	2.48	0.29	1.30	1.39	2.51	2.80	2.86
21-Aug	0	5.79	0	1.29	3.14	1.26	0.88	1.18
22-Aug	6.30	6.36	0	1.28	5.75	3.73	1.93	1.16
23-Aug	6.70	6.01	0	1.27	4.87	3.87	2.54	1.01
24-Aug	5.91	6.05	0.06	1.27	4.77	3.79	2.42	1.02
25-Aug	5.91	4.05	0	1.26	4.90	3.66	2.20	0.88
26-Aug	6.30	0.95	0	1.25	1.70	2.60	2.17	0.75
27-Aug	0	1.70	0.06	1.24	0.38	0.78	0.56	0.39
28-Aug	0	4.26	0.18	1.23	1.72	0.05	0	0
29-Aug	5.12	5.25	0.06	1.22	3.18	0.94	0	0
30-Aug	5.12	4.96	0.29	1.21	3.73	1.88	0	0
31-Aug	5.52	4.60	1.36	1.20	3.34	2.21	0	0
1-Sep	3.94	3.41	0	1.19	3.46	2.28	0.10	0
2-Sep	5.12	1.09	0	1.18	1.60	1.66	0.48	0
3-Sep	0	0.00	0	1.17	0.26	0.53	0	0
4-Sep	0	1.44	0	1.16	0.00	0.00	0	0
5-Sep	0	4.20	0	1.15	0.71	0.00	0	0
6-Sep	5.52	5.61	0	1.14	3.21	0.00	0	0

	Input			Output				
	Measured	STELLA	Precip.	PET	GB1	GB2	PB1	PB2
Date	---------- m^3 ------		cm	---------- m^3 ----------				
7-Sep	5.52	5.62	0.06	1.13	4.14	1.56	0	0
8-Sep	5.91	4.18	0	1.12	5.13	3.75	0	0
9-Sep	5.12	1.41	0	1.11	2.48	2.93	0.89	0
10-Sep	0	1.23	0	1.10	0.52	1.10	0.46	0
11-Sep	0	4.10	0	1.09	1.00	0.26	0	0
12-Sep	5.91	5.48	0	1.08	3.67	0.88	0	0
13-Sep	5.52	4.97	0	1.06	3.77	2.11	0	0
14-Sep	4.73	4.80	0	1.05	3.60	2.48	0	0
15-Sep	4.73	4.16	3.01	1.04	3.79	2.66	1	0
16-Sep	5.12	1.72	0.41	1.03	2.74	2.37	1.09	0
17-Sep	0	0.92	0	1.02	0.51	1.03	0.78	0
18-Sep	0	4.02	0	1.01	0.62	0.26	0	0
19-Sep	6.30	5.90	0	1.00	4.00	0.83	0	0
20-Sep	5.91	5.12	0	0.98	4.16	2.38	0	0
21-Sep	4.73	4.48	0	0.97	3.67	2.77	1	0
22-Sep	4.33	3.89	0	0.96	3.45	2.66	1.08	0
23-Sep	4.33	1.73	0	0.95	4.82	5.07	3.96	0
24-Sep	0	0.46	0	0.94	1.02	3.11	4.00	0
25-Sep	0	3.28	0.06	0.92	0.46	0.89	1.53	0
26-Sep	5.52	5.87	1.94	0.91	3.76	1.17	0.31	0
27-Sep	6.30	5.48	0.06	0.90	4.59	2.61	0.70	0
28-Sep	4.73	5.31	0	0.89	3.98	3.15	1.66	0
29-Sep	5.91	4.88	1.53	0.88	4.65	3.35	1.84	0
30-Sep	4.33	2.00	0	0.86	3.45	3.24	2.29	0
1-Oct	0	0.11	0	0.85	0.73	1.76	1.92	0
2-Oct	0	2.67	0	0.84	0.00	0.52	0.39	0
3-Oct	5.12	5.12	0	0.83	3.10	0.40	0	0
4-Oct	5.12	5.12	0	0.81	5.00	3.68	1.73	0
5-Oct	5.12	5.12	0	0.80	4.43	3.87	2.75	0
6-Oct	5.12	4.93	0	0.79	4.32	3.71	2.72	0
7-Oct	4.73	2.52	5.48	0.78	5.06	4.89	4.01	0
8-Oct	0	0.15	41.96	0.77	1.13	3.12	3.87	0
9-Oct	0	2.49	3.83	0.75	0.30	0.95	1.66	0
10-Oct	5.52	4.90	0.12	0.74	3.13	0.89	0.36	0

	Input			Output				
	Measured	STELLA	Precip.	PET	GB1	GB2	PB1	PB2
Date	---------- m^3 ------		cm	---------- m^3 ----------				
11-Oct	4.73	4.94	0	0.73	3.57	2.16	0.73	0
12-Oct	5.12	4.93	15.09	0.72	4.03	2.83	1.36	0
13-Oct	4.73	4.59	8.66	0.70	3.98	3.17	1.91	0
14-Oct	4.33	2.61	12.49	0.69	3.75	3.18	2.18	0.15
15-Oct	0	0.41	0.77	0.68	4.61	6.97	6.97	5.6
16-Oct	0	2.30	0	0.67	28.41	40.12	42.15	42.57
17-Oct	5.91	5.10	0	0.65	14.81	29.56	43.57	49.56
18-Oct	5.52	6.15	0.35	0.64	8.36	17.31	29.10	39.41
19-Oct	7.09	6.18	0	0.63	7.33	11.11	18.05	27.61
20-Oct	5.12	5.25	0	0.62	15.65	20.98	24.96	31.56
21-Oct	4.73	3.16	0	0.61	13.69	22.09	29.22	35.12
22-Oct	0	0.74	4.24	0.59	13.00	23.96	32.73	39.82
23-Oct	0	1.80	3.01	0.58	4.73	14.26	24.71	33.68
24-Oct	5.52	4.94	2.06	0.57	4.25	7.57	14.20	23.39
25-Oct	6.70	6.12	6.72	0.56	5.61	5.69	8.60	14.8
26-Oct	5.52	5.74	0.35	0.55	5.66	5.65	6.79	9.87
27-Oct	5.52	5.75	0	0.53	5.18	5.21	5.69	7.16
28-Oct	6.30	4.36	0	0.52	5.42	4.95	4.85	5.55
29-Oct	0	1.37	0	0.51	3.02	4.14	4.56	4.62
30-Oct	0	1.56	0	0.50	3.25	5.81	6.80	7.34
31-Oct	5.91	4.22	0	0.49	5.25	6.42	7.70	9.2
1-Nov	4.73	5.21	0	0.48	6.45	7.06	8.17	9.45
2-Nov	5.52	5.13	0.47	0.46	9.41	11.62	12.74	13.64
3-Nov	4.73	5.30	0	0.45	6.64	9.35	12.00	13.51
4-Nov	6.30	4.49	0	0.44	5.59	6.93	8.89	10.94
5-Nov	0	1.76	0	0.43	3.85	5.27	6.76	8.41
6-Nov	0	1.20	0.77	0.42	1.04	3.09	4.83	6.33
7-Nov	5.91	4.38	0	0.41	1.90	1.73	2.48	4.21
8-Nov	7.09	7.09	0	0.40	4.93	2.65	1.75	2.48
9-Nov	9.06	7.82	2.06	0.39	6.54	4.48	2.91	2.09
10-Nov	5.52	6.53	0	0.38	7.27	6.15	4.68	3.21
11-Nov	4.73	4.24	0.88	0.37	5.41	6.05	5.65	4.31
12-Nov	0	1.61	0	0.36	3.61	4.79	5.13	4.75
13-Nov	0	0.89	0.06	0.35	1.00	2.85	3.99	4.34

	Input			Output				
	Measured	STELLA	Precip.	PET	GB1	GB2	PB1	PB2
Date	---------- m^3 ------		cm	---------- m^3 ----------				
14-Nov	6.30	3.78	0	0.34	1.95	2.15	2.74	3.81
15-Nov	4.33	5.16	0.82	0.33	4.67	2.78	2.13	2.73
16-Nov	5.12	4.82	5.77	0.32	4.09	3.65	3.04	2.37
17-Nov	5.12	5.25	0.65	0.31	5.85	5.59	4.88	4.29
18-Nov	6.3	5.23	0	0.30	5.42	5.53	5.44	4.99
19-Nov	0	2.54	0	0.29	5.46	5.79	5.81	5.61
20-Nov	0	0.47	0	0.28	1.33	4.06	5.51	5.54
21-Nov	5.91	3.34	0.24	0.27	1.17	2.05	3.17	4.53
22-Nov	4.73	5.21	4.95	0.26	4.53	2.44	1.90	2.98
23-Nov	4.33	4.28	0	0.25	4.71	4.24	3.60	3.07
24-Nov	0	1.94	0	0.24	7.36	8.75	8.62	8.09
25-Nov	0	0.00	0	0.23	2.72	6.85	9.47	9.88
26-Nov	0	0.00	0	0.22	0.96	3.34	5.98	8.23
27-Nov	0	0.10	0	0.22	0.40	1.45	3.23	5.52
28-Nov	5.91	3.05	0	0.21	0.14	0.56	1.58	3.23
29-Nov	5.91	5.91	2.18	0.20	4.48	1.78	0.82	1.83
30-Nov	5.52	5.72	10.61	0.19	7.98	7.55	6.33	5.47
1-Dec	5.52	5.52	0	0.18	6.42	7.55	8.06	7.39
2-Dec	5.52	5.52	0	0.18	5.60	6.36	7.04	7.25
3-Dec	0	2.96	0	0.17	5.47	5.76	6.13	6.6
4-Dec	0	0.20	0	0.16	1.58	4.19	5.62	5.98
5-Dec	5.52	2.46	0	0.16	0.69	2.04	3.42	4.89
6-Dec	4.73	4.87	0.06	0.15	3.88	2.10	1.89	3.28
7-Dec	5.12	4.95	0	0.14	5.51	5.01	4.39	4.18
8-Dec	5.52	5.26	0	0.14	11.32	13.44	13.40	13.03
9-Dec	5.12	5.32	0	0.13	8.18	11.77	14.76	15.63
10-Dec	0	3.10	0.12	0.12	5.92	8.42	11.33	13.54
11-Dec	0	0.50	0.77	0.12	2.36	5.64	8.49	10.83
12-Dec	5.52	2.12	0.53	0.11	0.93	2.96	5.30	8.1
13-Dec	3.94	4.28	0	0.11	3.53	2.43	2.99	5.39
14-Dec	5.12	4.57	0	0.10	3.95	3.30	3.20	3.84
15-Dec	4.73	4.82	0	0.10	4.45	3.86	3.48	3.47
16-Dec	5.52	5.06	4.77	0.09	4.60	4.24	3.86	3.55
17-Dec	0	3.52	0	0.09	5.00	4.56	4.13	3.79

	Input			Output				
	Measured	STELLA	Precip.	PET	GB1	GB2	PB1	PB2
Date	---------- m^3 ------		cm	---------- m^3 ----------				
18-Dec	0	0.88	0	0.08	2.53	4.04	4.56	4.16
19-Dec	5.91	1.90	0	0.08	1.21	2.98	4.06	4.6
20-Dec	5.12	4.61	0	0.07	3.72	2.96	3.15	4.29
21-Dec	4.73	5.15	0	0.07	4.89	3.85	3.49	3.68
22-Dec	4.73	4.81	0	0.07	4.69	4.43	4.12	3.69
23-Dec	4.73	4.73	0	0.06	4.64	4.55	4.33	3.98
24-Dec	0	3.42	0	0.06	7.27	8.07	7.91	7.69
25-Dec	0	1.05	3.83	0.06	4.10	6.99	8.83	9.11
26-Dec	0	0.00	3.83	0.05	1.20	3.96	6.40	7.98
27-Dec	0	0.00	0	0.05	0.57	1.93	3.75	5.91
28-Dec	0	0.00	0	0.05	0.26	0.91	2.10	3.86
29-Dec	0	0.00	5.42	0.05	0.11	0.41	1.13	2.33
30-Dec	0	0.00	0.24	0.04	0.03	0.16	0.57	1.31
31-Dec	0	0.00	0	0.04	0.00	0.07	0.25	0.68
1-Jan	0	0.00	0.06	0.04	0.00	0.01	0.09	0.32
2-Jan	3.94	1	1.77	0.04	1.98	2.65	2.72	2.85
3-Jan	5.52	3	0	0.04	4.33	5.51	6.14	6.53
4-Jan	5.91	5	0	0.04	5.04	5.35	6.31	7.09
5-Jan	5.52	6	3.71	0.04	5.33	5.19	5.63	6.18
6-Jan	4.73	6	0.88	0.04	8.44	9.25	9.27	9.47
7-Jan	0	4	0	0.04	7.08	9.12	10.59	11.09
8-Jan	0	2	0	0.04	4.27	6.73	8.86	10.13
9-Jan	5.91	0.80	0	0.04	1.32	4.20	6.58	8.44
10-Jan	5.12	3.66	0	0.04	2.77	3.71	5.16	7.52
11-Jan	5.52	5.46	2.36	0.04	5.34	4.19	4.46	6.11
12-Jan	5.12	5.32	0	0.04	5.00	4.79	4.86	5.11
13-Jan	5.52	5.32	0	0.04	7.18	7.58	7.46	7.47
14-Jan	0	4.85	4.42	0.04	6.79	8.17	9.04	9.24
15-Jan	0	2.25	0	0.04	5.22	6.75	8.15	8.94
16-Jan	7.88	0.58	0	0.04	1.41	4.46	6.64	7.79
17-Jan	5.12	4.34	0	0.04	1.54	2.48	3.98	6.07
18-Jan	5.52	6.34	7.37	0.05	6.10	3.36	2.71	4.22
19-Jan	6.30	5.38	0	0.05	6.22	6.42	5.96	5.34
20-Jan	5.12	5.90	0	0.05	6.16	6.61	6.80	6.58

	Input			Output				
	Measured	STELLA	Precip.	PET	GB1	GB2	PB1	PB2
Date	---------- m^3 ------		cm	---------- m^3 ----------				
21-Jan	0	5.52	0	0.05	6.12	6.20	6.42	6.53
22-Jan	0	2.41	0	0.06	7.33	8.79	9.20	9.29
23-Jan	5.91	0.07	1.24	0.06	2.78	7.00	9.66	10.43
24-Jan	5.12	3.02	0.12	0.06	1.10	3.47	6.15	8.64
25-Jan	5.52	5.52	0.00	0.07	4.91	3.18	3.55	5.98
26-Jan	5.52	5.32	0	0.07	8.48	9.20	9.07	9.41
27-Jan	5.12	5.51	0	0.07	7.68	9.67	11.18	11.7
28-Jan	0	5.33	0	0.08	5.94	7.51	9.35	10.6
29-Jan	0	2.79	1.71	0.08	5.41	6.34	7.56	8.96
30-Jan	6.30	0.21	0.06	0.09	1.69	4.48	6.39	7.55
31-Jan	5.52	2.78	1.53	0.09	1.30	3.06	4.75	6.71
1-Feb	5.52	5.59	0	0.10	4.95	3.33	3.42	5.28
2-Feb	5.52	5.57	0	0.10	5.15	4.40	4.12	4.22
3-Feb	5.52	5.52	4.07	0.11	5.24	4.88	4.52	4.19
4-Feb	0	5.52	2.53	0.11	5.32	5.09	4.76	4.4
5-Feb	0	3.33	1.59	0.12	5.36	5.19	4.92	4.61
6-Feb	5.12	0.57	0	0.12	2.92	5.24	6.11	5.86
7-Feb	5.12	1.94	0	0.13	1.33	3.44	5.04	6.06
8-Feb	5.52	4.50	0	0.14	4.10	3.56	4.04	5.59
9-Feb	5.52	5.26	0	0.14	5.01	4.45	4.47	4.97
10-Feb	7.49	5.46	0	0.15	5.07	4.71	4.58	4.53
11-Feb	0	6.19	0	0.16	7.17	7.51	7.23	7.01
12-Feb	0	4.67	0	0.16	8.96	9.79	10.12	10.16
13-Feb	5.91	1.24	1	0.17	5.22	9.01	11.30	11.72
14-Feb	4.73	1.87	0	0.18	1.86	5.55	8.66	10.75
15-Feb	5.52	4.46	0	0.18	3.50	3.53	5.06	7.93
16-Feb	4.73	5.19	0	0.19	4.60	3.81	4.07	5.5
17-Feb	4.73	5.13	0.77	0.20	4.81	4.30	4.12	4.42
18-Feb	0	4.89	0	0.21	4.79	4.51	4.24	4.11
19-Feb	0	3.44	0	0.22	4.57	4.49	4.28	4.06
20-Feb	5.52	1.07	0	0.22	2.60	3.74	4.18	4.01
21-Feb	5.52	1.41	0	0.23	1.05	2.72	3.68	4.14
22-Feb	5.12	4.16	0	0.24	2.64	2.17	2.45	3.49
23-Feb	4.73	5.42	0	0.25	4.54	2.92	2.27	2.53

	Input			Output				
	Measured	STELLA	Precip.	PET	GB1	GB2	PB1	PB2
Date	---------- m^3 ------		cm	---------- m^3 ----------				
24-Feb	5.12	5.14	0	0.26	4.76	3.92	3.12	2.42
25-Feb	0	4.92	0	0.27	4.97	4.73	4.11	3.31
26-Feb	0	3.93	0	0.28	4.90	4.86	4.54	3.99
27-Feb	5.52	1.48	0	0.29	3.23	4.11	4.41	4.08
28-Feb	5.12	1.06	0	0.30	0.87	2.51	3.50	3.75
1-Mar	5.12	3.75	0	0.31	1.73	1.48	1.85	2.76
2-Mar	5.12	5.25	0	0.32	4.20	2.31	1.45	1.75
3-Mar	5.12	5.12	0.18	0.33	4.44	3.46	2.47	1.64
4-Mar	0	5.12	0.41	0.34	4.61	3.96	3.09	2.15
5-Mar	0	4.36	0	0.35	4.69	4.20	3.47	2.64
6-Mar	5.91	1.80	0	0.36	3.59	3.93	3.70	3
7-Mar	4.73	0.77	0	0.37	0.89	2.50	3.24	3.03
8-Mar	5.12	3.58	0	0.38	1.24	1.27	1.66	2.32
9-Mar	4.73	5.22	0	0.39	4.25	2.04	1.03	1.33
10-Mar	4.73	4.93	0	0.40	4.14	3.24	2.21	1.21
11-Mar	0	4.88	0	0.41	4.52	3.79	2.83	1.85
12-Mar	0	4.32	0	0.42	4.55	4.24	3.54	2.64
13-Mar	6.30	1.95	2.47	0.43	3.86	4.03	3.75	3.1
14-Mar	5.12	0.43	0.12	0.44	0.91	2.56	3.32	3.04
15-Mar	5.12	3.52	0	0.45	0.66	1.05	1.62	2.26
16-Mar	5.52	5.64	0	0.46	4.50	1.82	0.63	1.1
17-Mar	6.30	5.14	0	0.48	4.31	3.26	2.05	0.89
18-Mar	0	5.36	0	0.49	4.50	3.72	2.65	1.5
19-Mar	0	5.78	0	0.50	4.89	4.05	3.03	2.01
20-Mar	7.09	3.00	0	0.51	5.29	4.41	3.40	2.42
21-Mar	5.52	0.05	0	0.52	1.93	4.58	5.17	4.18
22-Mar	5.12	3.58	0	0.53	1.30	2.95	4.13	4.91
23-Mar	5.12	6.31	0	0.55	5.40	2.78	2.17	3.33
24-Mar	5.12	5.34	0.35	0.56	4.80	3.99	3.17	2.39
25-Mar	0	5.13	0.00	0.57	4.58	4.10	3.37	2.51
26-Mar	0	5.12	0.41	0.58	4.56	4.04	3.34	2.61
27-Mar	5.12	2.80	0	0.59	4.54	3.99	3.30	2.62
28-Mar	5.52	0.24	0	0.61	1.11	2.80	3.26	2.58
29-Mar	5.52	2.23	0	0.62	0.37	0.91	1.51	1.95

	Input			Output				
	Measured	STELLA	Precip.	PET	GB1	GB2	PB1	PB2
Date	---------- m^3 ------		cm	---------- m^3 ----------				
30-Mar	5.52	4.96	0	0.63	2.91	0.95	0.36	0.69
31-Mar	5.91	5.49	0	0.64	4.11	2.32	0.82	0.14
1-Apr	0	5.52	0.77	0.65	4.64	3.44	1.94	0.52
2-Apr	0	5.67	0	0.67	4.82	3.98	2.72	1.35
3-Apr	5.91	3.56	2.89	0.68	5.19	4.38	3.25	2.07
4-Apr	5.52	0.64	3.01	0.69	1.84	3.48	3.66	2.59
5-Apr	5.12	2.21	0.06	0.70	0.55	1.36	1.98	2.19
6-Apr	6.30	5.02	0	0.72	2.88	1.08	0.51	0.92
7-Apr	5.12	5.43	0.65	0.73	4.19	2.33	0.87	0.2
8-Apr	0	5.59	0.82	0.74	4.25	3.14	1.72	0.3
9-Apr	0	5.72	0	0.75	5.19	4.02	2.56	1.22
10-Apr	6.30	3.62	0	0.77	5.03	4.48	3.40	2.07
11-Apr	5.12	0.87	0	0.78	3.09	4.77	4.86	3.83
12-Apr	4.73	1.97	0	0.79	2.55	4.99	6.06	6.31
13-Apr	4.73	4.76	0	0.80	4.22	4.19	4.93	6.19
14-Apr	0	5.24	2.00	0.81	4.56	3.70	3.71	4.24
15-Apr	0	4.81	0	0.83	4.38	3.98	3.56	3.31
16-Apr	0	3.42	0	0.84	4.57	4.31	3.79	3.38
17-Apr	5.91	1.06	0	0.85	2.38	3.43	3.66	3.23
18-Apr	6.70	0.00	0	0.86	0.56	1.39	2.07	2.29
19-Apr	5.52	1.48	0	0.88	0.00	0.41	0.47	0.85
20-Apr	4.73	4.62	0	0.89	1.18	0.00	0	0.13
21-Apr	5.52	6.22	0	0.90	4.12	0.89	0	0
22-Apr	0	5.66	4.54	0.91	5.39	3.64	0.79	0
23-Apr	0	5	9.43	0.92	5.08	4.74	3.09	0.41
24-Apr	6.30	4.16	1.12	0.94	4.33	4.00	3.05	1.45
25-Apr	5.52	1.62	0.59	0.95	2.86	3.13	2.65	1.58
26-Apr	4.73	1.19	0	0.96	0.61	1.42	2	1.2
27-Apr	5.52	4.20	0	0.97	1.12	0.40	0	0.46
28-Apr	5.12	5.63	0	0.98	3.99	1.26	0	0
29-Apr	0	5.12	0	1.00	3.93	2.46	0.33	0
30-Apr	0	5.19	0	1.01	5.52	4.97	3.21	0.56
1-May	6.30	4.52	0	1.02	9.67	10.95	10.13	8.43
2-May	5.12	1.82	0	1.03	8.09	12.18	14.24	13.92

	Input			Output				
	Measured	STELLA	Precip.	PET	GB1	GB2	PB1	PB2
Date	---------- m^3 ------		cm	---------- m^3 ----------				
3-May	5.12	0.79	0.24	1.04	1.98	6.83	10.88	12.66
4-May	5.91	3.81	0	1.05	1.58	3.07	5.58	8.78
5-May	6.30	5.57	0	1.06	4.30	2.38	2.41	4.64
6-May	0	5.20	0	1.08	3.95	2.91	2.17	2.22
7-May	0	5.63	0	1.09	4.11	2.97	1.87	1.25
8-May	5.91	5.63	0	1.10	4.67	3.21	1.78	0.79
9-May	11.03	2.64	0	1.11	4.24	3.31	2.01	0.67
10-May	7.49	0.38	0	1.12	0.80	1.86	2	0.64
11-May	6.30	3.62	0	1.13	0.06	0.55	0	0.34
12-May	7.88	8.59	6.66	1.14	4.06	0.62	0	0
13-May	0	9.05	0	1.15	7.83	3.46	0	0
14-May	0	6.90	0.06	1.16	6.21	5.10	2.37	0
15-May	5.12	6.98	1.83	1.17	5.46	4.83	3.00	0
16-May	6.7	3.78	2.65	1.18	6.22	4.73	3	0.24
17-May	5.12	0.01	0	1.19	1.22	3.07	3	1.05
18-May	5.52	2.54	0.71	1.20	0.02	0.93	1	0.62
19-May	6.70	5.85	2.71	1.21	2.60	0.00	0	0.04
20-May	0	5.92	0	1.22	6.62	4.59	2.03	0.76
21-May	0	5.34	0.94	1.23	7.00	7.77	6.71	4.93
22-May	6.70	6.04	0	1.24	4.79	5.35	5.52	4.89
23-May	5.52	3.64	0	1.25	5.97	5.29	4.62	4.45
24-May	7.88	0.34	0	1.26	2.65	5.40	6.09	5.54
25-May	5.12	2.89	0	1.27	1.10	3.12	4.48	5.45
26-May	7.09	5.74	2.24	1.27	4.09	2.03	1.69	3.05
27-May	0	6.59	0	1.28	5.33	4.16	3.04	2.55
28-May	0	6.56	0	1.29	6.98	5.65	4.32	3.36
29-May	0	6.16	0	1.30	5.12	5.21	4.62	3.34
30-May	6.30	4.14	2.12	1.31	5.82	4.94	3.98	3.25
31-May	5.12	0.80	0	1.32	1.65	3.23	3.45	2.51
1-Jun	5.12	0.00	2	1.32	0.42	0.94	1.09	1.27
2-Jun	4.73	2.27	0	1.33	0.00	0.02	0.07	0.22
3-Jun	0	5.01	3.42	1.34	2.32	0.00	0	0
4-Jun	0	5.30	0	1.35	4.22	2.11	0	0
5-Jun	6.30	5	0	1.35	3.57	2.36	0.33	0

	Input			Output				
	Measured	STELLA	Precip.	PET	GB1	GB2	PB1	PB2
Date	---------- m^3 ------		cm	---------- m^3 ----------				
6-Jun	5.91	3.26	0	1.36	3.47	2.32	0.45	0
7-Jun	5.91	0.83	6.25	1.37	1.72	2.28	1	0
8-Jun	5.52	1.94	0.47	1.37	0.55	1.45	1	0
9-Jun	5.91	4.97	0.35	1.38	2.64	1.01	0.26	0
10-Jun	0	5.99	0.41	1.39	4.81	2.64	0.77	0
11-Jun	0	5.80	0	1.39	5.39	4.28	2.61	0
12-Jun	6.30	5.71	0	1.40	5.99	5.71	4.52	1.61
13-Jun	5.91	4.27	0	1.40	4.65	4.31	3.71	1.97
14-Jun	5.52	1.40	1.12	1.41	2.29	2.88	2.63	1.44
15-Jun	5.91	1.54	0.29	1.41	1.57	3.17	3.34	2.89
16-Jun	5.52	4.60	0.06	1.42	4.79	4.95	4.89	5.32
17-Jun	0	5.92	0.06	1.42	4.81	3.77	3.69	3.84
18-Jun	0	5.71	0	1.43	4.64	3.80	3.05	2.46
19-Jun	9.06	5.72	0	1.43	4.54	3.58	2.52	1.66
20-Jun	5.52	4.52	1.94	1.44	4.37	3.22	1.95	0.89
21-Jun	5.12	1.65	0	1.44	2.51	2.43	2	0.32
22-Jun	5.52	1.66	0.82	1.44	0.53	1.02	1	0.18
23-Jun	5.52	5.57	0.18	1.45	2.02	0.70	0.04	0
24-Jun	0	6.61	0.94	1.45	5.29	1.90	0	0
25-Jun	0	5.32	11.73	1.45	4.00	2.87	0.63	0
26-Jun	5.91	5.38	1.30	1.46	3.87	2.68	0.75	0
27-Jun	5.12	4.75	0.12	1.46	4.02	2.57	1	0
28-Jun	5.12	1.99	9.49	1.46	3.42	2.91	1	0
29-Jun	4.33	0.72	0.77	1.46	0.85	2.23	2.07	0
30-Jun	5.52	3.58	0	1.47	0.57	0.46	0.42	0
1-Jul	0	5.43	0	1.47	3.87	1.01	0	0
2-Jul	0	5.05	0	1.47	3.80	2.21	0	0
3-Jul	0	4.75	4.42	1.47	7.08	6.93	5.01	0.05
4-Jul	0	4.60	0.77	1.47	9.81	12.42	12.47	9.76
5-Jul	5.12	2.33	0	1.47	5.19	7.93	10.40	10.2
6-Jul	4.73	0	0.06	1.47	3.01	7.04	9.55	10.66
7-Jul	5.91	0	0	1.48	4.77	8.52	11.12	13.54
8-Jul	0	0.21	0	1.48	0.89	3.29	6.82	9.75
9-Jul	0	2.76	0	1.48	0.00	1.07	2.23	4.73

	Input			Output				
	Measured	STELLA	Precip.	PET	GB1	GB2	PB1	PB2
Date	---------- m^3 ------		cm	---------- m^3 ----------				
10-Jul	5.91	4.94	0	1.48	2.39	0.00	0.58	1.43
11-Jul	5.12	5.26	0	1.48	3.90	2.14	0.26	0.49
12-Jul	4.73	2.87	7.72	1.48	6.34	5.42	3.65	2.36
13-Jul	5.91	0	0.06	1.47	1.06	3.18	3.88	2.62
14-Jul	4.33	2.89	0	1.47	0.00	0.94	1	1.21
15-Jul	0	5.46	2.59	1.47	2.82	0.00	0	0.16
16-Jul	0	4.95	0	1.47	3.09	0.95	0	0
17-Jul	5.91	5.29	0	1.47	3.08	1.48	0	0
18-Jul	5.12	5.15	0	1.47	3.98	1.91	0	0
19-Jul	5.12	2.48	0	1.47	3.17	2.06	0	0
20-Jul	5.52	0.24	0	1.46	1.96	3.29	1	0
21-Jul	5.52	2.52	0.35	1.46	3.47	5.59	5.30	1.15
22-Jul	0	5.14	8.25	1.46	3.50	2.64	2.82	1.65
23-Jul	0	5.19	0	1.46	4.30	3.26	2.37	1.2
24-Jul	6.70	5.28	0	1.45	5.09	4.55	3.51	2.26
25-Jul	4.73	5.48	0	1.45	4.17	3.46	2.70	1.65
26-Jul	7.09	3.41	0	1.45	4.14	3.02	1.83	0.8
27-Jul	5.12	0.65	1.83	1.44	1.02	1.77	1.39	0.25
28-Jul	5.12	2.44	1.00	1.44	0.11	0.38	0	0
29-Jul	0	5.09	0	1.44	2.36	0.00	0	0
30-Jul	0	5.83	0.06	1.43	5.24	3.31	0.46	0
31-Jul	5.52	6.06	0.06	1.43	9.02	8.94	7.06	4.46
1-Aug	5.52	5.44	0	1.42	5.08	6.08	6.47	4.97
2-Aug	5.52	3.48	0	1.42	4.23	4.34	4.20	3.81
3-Aug	5.12	0.92	0.06	1.41	1.51	2.50	2.65	2.36
4-Aug	4.73	1.67	0	1.41	0.43	0.84	1.06	1.26
5-Aug	0	4.42	0	1.40	2.64	1.43	0.79	0.94
6-Aug	0	5.52	0	1.40	4.06	2.24	0.88	0.27
7-Aug	5.91	5.41	0.71	1.39	3.93	2.50	0.95	0
8-Aug	4.73	5.11	0	1.39	3.91	2.64	0.97	0
9-Aug	5.12	3.60	0	1.38	3.65	2.54	0.97	0
10-Aug	4.73	1.14	0	1.37	1.61	1.69	0.79	0
11-Aug	5.52	1.42	0	1.37	0.24	0.50	0	0
12-Aug	0	4.10	0	1.36	0.93	0.00	0	0

	Input			Output				
	Measured	STELLA	Precip.	PET	GB1	GB2	PB1	PB2
Date	---------- m^3 ------		cm	---------- m^3 ----------				
13-Aug	0	5.13	0	1.35	3.09	0.00	0	0
14-Aug	5.52	4.93	0	1.35	3.21	1.13	0	0
15-Aug	5.12	5.00	2.00	1.34	3.56	1.87	0	0
16-Aug	4.73	4.20	0	1.33	4.00	2.61	0	0
17-Aug	5.91	1.68	0	1.32	2.47	2.08	0	0
18-Aug	5.12	0.98	0	1.32	0.40	0.82	0	0
19-Aug	0	3.67	5.07	1.31	0.36	0.00	0	0
20-Aug	0	5.19	0.35	1.30	3.07	0.00	0	0
21-Aug	5.91	5.04	0	1.29	3.29	1.16	0	0
22-Aug	5.12	5.39	0	1.28	3.57	1.81	0	0
23-Aug	4.73	4.72	0	1.27	4.52	2.83	0	0
24-Aug	6.30	1.88	0	1.27	4.01	4.00	0	0
25-Aug	4.73	0.69	1.59	1.26	0.70	1.67	0	0
26-Aug	0	3.56	1.41	1.25	0.12	0.49	0	0
27-Aug	0	5.39	7.37	1.24	4.34	1.46	0	0
28-Aug	5.91	5.03	0.06	1.23	6.53	6.11	3.86	0
29-Aug	5.52	5.53	7.37	1.22	4.37	4.60	4.10	0
30-Aug	4.73	5.05	0.53	1.21	4.80	3.85	2.89	0
31-Aug	4.73	2.02	0	1.20	3.32	3.24	2.51	0
1-Sep	5.12	0.32	0	1.19	0.65	1.44	1.60	0
2-Sep	0	3.26	1.53	1.18	0.00	0.49	0.23	0
3-Sep	0	5.67	1.18	1.17	4.63	1.83	0.33	0
4-Sep	0	5.09	0	1.16	6.07	5.38	3.99	0
5-Sep	5.91	4.74	0.59	1.15	8.23	9.79	9.46	2.68
6-Sep	5.52	4.88	0	1.14	5.78	7.84	9.31	6.58
7-Sep	5.91	2.51	0	1.13	8.86	11.08	12.14	11.69
8-Sep	5.52	0	0	1.12	1.82	6.08	9.67	10.44
9-Sep	0	0	1.18	1.11	0.45	2.03	4.52	6.91
10-Sep	0	2.80	0	1.10	0.00	0.77	1.60	3.47
11-Sep	6.30	5.58	0	1.09	4.04	1.52	1.05	2.26
12-Sep	5.12	5.71	0	1.08	4.57	3.40	2.28	1.51
13-Sep	7.09	5.72	0.06	1.06	4.64	3.61	2.37	1.28
14-Sep	6.30	3.12	6.07	1.05	4.80	4.06	2.79	1.62
15-Sep	5.12	0.34	7.48	1.04	1.07	2.54	2.64	1.57

	Input			Output				
	Measured	STELLA	Precip.	PET	GB1	GB2	PB1	PB2
Date	---------- m^3 ------		cm	---------- m^3 ----------				
16-Sep	0	2.66	0.06	1.03	0.18	0.74	0.71	0.83
17-Sep	0	5.32	0	1.02	3.23	0.58	0.05	0.21
18-Sep	5.12	6.02	0.06	1.01	4.43	2.88	0.97	0
19-Sep	5.12	6.59	0.24	1.00	5.18	3.45	1.59	0
20-Sep	4.33	5.93	0	0.98	5.27	4.03	2.29	0.42
21-Sep	5.52	3.33	0	0.97	4.59	4.02	2.72	1.05
22-Sep	5.12	0.63	0	0.96	2.34	3.95	3.83	2.61
23-Sep	0	1.84	0.18	0.95	4.84	7.69	8.39	8.48
24-Sep	0	4.40	0.94	0.94	7.68	9.91	11.65	13.03
25-Sep	5.91	4.85	0	0.92	4.82	6.28	8.70	10.4
26-Sep	4.73	4.85	0	0.91	4.11	4.66	5.76	7.24
27-Sep	5.12	5.19	0	0.90	4.37	3.95	4.03	4.95
28-Sep	5.12	3.58	0	0.89	4.52	3.87	3.40	3.56
29-Sep	4.73	0.95	4.12	0.88	1.88	2.84	2.99	2.64
30-Sep	0	1.76	0.24	0.86	0.47	1.02	1.47	1.69
1-Oct	0	4.37	3.59	0.85	2.03	0.57	0.27	0.58
2-Oct	7.88	5.09	0	0.84	3.87	1.94	0.33	0
3-Oct	5.52	5.04	0	0.83	4.44	3.47	1.97	0.39
4-Oct	4.73	5.02	0	0.81	4.22	3.43	2.30	0.94
5-Oct	6.30	3.63	0	0.80	4.11	3.42	2.36	1.21
6-Oct	4.73	1.17	0	0.79	2.19	2.72	2.36	1.35
7-Oct	0	1.85	0	0.78	0.68	1.85	2.09	1.77
8-Oct	0	5.26	0	0.77	5.14	4.58	4.03	4.37
9-Oct	5.12	5.99	0	0.75	6.08	4.93	4.52	4.33
10-Oct	5.12	5.28	0	0.74	6.92	7.74	7.50	6.76
11-Oct	4.33	5.53	6.25	0.73	5.09	5.78	6.40	6.36
12-Oct	4.33	4.30	0.29	0.72	5.10	4.93	4.95	5.19
13-Oct	5.12	1.46	0	0.70	2.97	3.91	4.24	4.2
14-Oct	0	0.89	0	0.69	0.76	1.99	2.83	3.21
15-Oct	0	3.45	0	0.68	1.02	0.75	1.04	1.77
16-Oct	5.91	5.00	0	0.67	3.58	1.43	0.29	0.57
17-Oct	4.73	4.61	3.48	0.65	3.84	2.55	1.20	0.08
18-Oct	4.73	4.44	0.12	0.64	3.63	2.93	1.81	0.53
19-Oct	4.73	4.18	0	0.63	4.49	3.94	2.90	1.85

	Input			Output				
	Measured	STELLA	Precip.	PET	GB1	GB2	PB1	PB2
Date	---------- m^3 ------		cm	---------- m^3 ----------				
20-Oct	4.73	1.90	2.95	0.62	7.49	8.85	8.43	7.55
21-Oct	0	0.66	0	0.61	1.51	5.02	7.54	7.72
22-Oct	0	3.49	0	0.59	1.20	2.24	3.87	5.65
23-Oct	6.70	5.20	0	0.58	4.23	2.28	1.89	3.23
24-Oct	4.33	4.73	0	0.57	3.96	3.16	2.43	2.06
25-Oct	4.33	4.73	0	0.56	4.41	3.84	3.02	2.38
26-Oct	5.12	4.41	0	0.55	6.47	6.78	6.16	5.5
27-Oct	4.73	2.05	0.77	0.53	4.17	5.16	5.82	5.59
28-Oct	0	0.33	12.61	0.52	1.13	3.44	4.78	5.1
29-Oct	0	3.58	0	0.51	2.57	3.96	4.98	6.24
30-Oct	4.73	5.42	0	0.50	5.11	3.27	3.22	4.49
31-Oct	4.73	4.35	0	0.49	3.93	3.85	3.62	3.28
1-Nov	4.73	4.74	0.06	0.48	3.92	3.62	3.25	2.98
2-Nov	4.73	4.90	2.42	0.46	4.47	3.71	3.07	2.73
3-Nov	5.12	2.36	0	0.45	4.24	3.85	3.26	2.67
4-Nov	0	0.02	0	0.44	0.98	2.74	3.34	2.8
5-Nov	0	2.26	0	0.43	1.62	2.99	3.73	4.29
6-Nov	5.52	4.63	0	0.42	11.95	13.49	13.50	14.42
7-Nov	5.12	4.73	0	0.41	5.36	8.65	12.27	13.46
8-Nov	5.12	4.73	13.44	0.40	4.85	6.28	8.37	10.56
9-Nov	4.73	4.90	0.06	0.39	4.60	5.12	6.18	7.96
10-Nov	4.33	2.88	0	0.38	4.96	4.93	5.27	6.38
11-Nov	0	0.34	0	0.37	3.10	5.71	6.89	7.39
12-Nov	0	2.30	0	0.36	0.84	2.67	4.59	6.11
13-Nov	4.73	4.89	0.12	0.35	3.51	2.13	2.33	3.97
14-Nov	4.73	5.16	2.24	0.34	4.34	3.17	2.54	2.64
15-Nov	4.33	4.97	0	0.33	4.55	3.83	3.08	2.5
16-Nov	5.12	4.62	3.48	0.32	5.32	5.25	4.62	3.95
17-Nov	4.73	2.77	0.06	0.31	13.57	16.94	16.94	16.54
18-Nov	0	0.55	0	0.30	3.33	9.62	14.99	16.44
19-Nov	0	1.68	0	0.29	1.37	4.79	8.84	12.67
20-Nov	4.33	4.04	0	0.28	2.92	2.95	4.80	8.39
21-Nov	4.73	4.60	0	0.27	4.04	3.23	3.56	5.35
22-Nov	4.33	4.66	0	0.26	4.26	3.93	3.79	4.25

	Input			Output				
	Measured	STELLA	Precip.	PET	GB1	GB2	PB1	PB2
Date	---------- m^3 ------		cm	---------- m^3 ----------				
23-Nov	0	4.86	0	0.25	6.02	6.13	5.83	5.87
24-Nov	0	3.33	0	0.24	5.00	5.49	5.90	5.95
25-Nov	0	0.90	0	0.23	4.89	7.43	8.44	8.68
26-Nov	0	1.27	0	0.22	1.20	4.00	6.47	7.84
27-Nov	5.91	3.55	0	0.22	2.29	2.41	3.59	5.65
28-Nov	3.94	4.53	0	0.21	3.83	2.77	2.71	3.78
29-Nov	4.73	3.27	2.77	0.20	4.06	3.45	3.04	3.02
30-Nov	5.12	1.02	0	0.19	2.33	3.16	3.33	3
1-Dec	4.33	0	5.24	0.18	0.66	1.90	2.62	2.85
2-Dec	0	0	0	0.18	0.24	0.77	1.44	2.16
3-Dec	0	1.36	0	0.17	0.03	0.27	0.65	1.25
4-Dec	5.12	3.88	0	0.16	1.89	0.59	0.19	0.56
5-Dec	5.12	4.66	0	0.16	3.96	2.08	0.83	0.38
6-Dec	5.12	4.58	0	0.15	3.85	3.21	2.23	1.05
7-Dec	5.12	4.85	0	0.14	4.55	3.88	3.01	2.08
8-Dec	4.73	3.77	0	0.14	6.55	6.87	6.27	5.45
9-Dec	0	1.36	0	0.13	3.55	5.40	6.48	6.22
10-Dec	0	0.86	0	0.12	4.76	8.30	9.91	10.7
11-Dec	4.73	3.42	0	0.12	2.35	4.46	7.20	9.35
12-Dec	4.33	5.12	1.36	0.11	4.53	3.83	4.65	6.77
13-Dec	4.73	5.12	0	0.11	4.77	4.36	4.47	5.16
14-Dec	3.94	5.07	0	0.10	4.90	4.64	4.51	4.64
15-Dec	5.12	4.29	0	0.10	4.88	4.76	4.59	4.52
16-Dec	0	1.78	0	0.09	3.81	4.43	4.63	4.52
17-Dec	0	0.50	0	0.09	1.02	3.01	4.12	4.37
18-Dec	4.73	2.83	0	0.08	1.18	1.67	2.48	3.59
19-Dec	4.73	4.53	0	0.08	3.79	2.17	1.74	2.53
20-Dec	3.94	4.50	0	0.07	4.02	3.30	2.70	2.29
21-Dec	4.73	4.35	0	0.07	5.31	5.15	4.60	4.03
22-Dec	4.33	4.30	0	0.07	4.22	4.69	4.88	4.51
23-Dec	0	2.24	0	0.06	4.42	4.43	4.48	4.52
24-Dec	0	0.21	0	0.06	1.09	3.27	4.38	4.4
25-Dec	0	2.57	0	0.06	0.80	1.68	2.67	3.77
26-Dec	0	4.71	0	0.05	3.81	2.07	1.67	2.66

	Input			Output				
	Measured	STELLA	Precip.	PET	GB1	GB2	PB1	PB2
Date	---------- m^3 -----		cm	---------- m^3 ----------				
27-Dec	0	4.33	0	0.05	4.21	3.34	2.69	2.3
28-Dec	0	4.34	0	0.05	3.89	3.78	3.39	2.76
29-Dec	0	4.53	0	0.05	4.47	4.02	3.59	3.21

LITERATURE CITED

American Public Health Association, Amer. Water Works Assoc. and Water Poll. Cont. Fed. 2003. Standard Methods for the Examination of Water and Wastewater 20th Ed. L.S. Clesceri, A.D. Eaton, E.W. Rice and A.E. Greenberg (eds). Am. Pub. Health Assoc. Washington, DC.

Armstrong, J., W. Armstrong, and P.M. Beckett. 1990. Measurement and modeling of oxygen release from roots of *Phragmites australis*. p 41-52 *In*: P.F. Cooper and B.C. Findlater (eds) Constructed Wetlands in Water Pollution Control. Pergamon Press, Oxford.

Bates, R.G. 1973. Determination of pH, 2nd ed. John Wiley and Sons, NY.

Behera, B. and G.H. Wagner. 1974. Microbial growth rate in glucose-amended soil. Soil Sci. Soc. Am. Proc. 38:591-594.

Benefield, L.D. and C.W. Randall. 1980. Biological Process Design for Wastewater Treatment. Prentice Hall, Englewood Cliffs, NJ. 526 pps.

Blazejewski, R. and S. Murat-Blazejewski. 1997. Soil clogging phenomena in constructed wetlands with subsurface flow. Water Sci. Tech. 35(5):183-188.

Bowden, W.B. 1986. Nitrification, nitrate reduction, and nitrogen immobilization in tidal freshwater marsh sediment. Ecology 67:88-99.

Breen, P.F. and A.J. Chick. 1995. Rootzone dynamics on constructed wetlands receiving wastewater: A comparison of vertical and horizontal flow systems. Water Sci. Tech. 32:281-290.

Brix, H. 1993. Wastewater treatment in constructed wetlands: System design, removal processes and treatment performance. p. 9-22. *In* G.A. Moshiri (ed.) Constructed Wetlands for Water Quality Improvement. CRC Press, Inc. Boca Raton, FL.

Brix, H. B.K. Sorrell, H.H. Schierup. 1996. Gas fluxes achieved by *in situ* convective flow in *Phragmites australis*. Aquat. Bot. 54:151-163.

Carter, J.P. Y.H. Hsiao, S. Spirp, and D.J. Richardson. 1995. Soil and sediment bacteria capable of aerobic nitrate respiration. Appl. Environ. Microbiol. 61(8):2852-2858.

Castignetti, D. and T.C. Hollocher. 1984. Heterotrophic nitrification among denitrifiers. Appl. Environ. Microbiol. 47:620-623.

Castignetti, D. R. Yanong, and R. Gramzinski. 1985. Substrate diversity of an active heterotrophic nitrifier, an *Alcaligenes sp*. Can. J. Microbiol. 31:441-445.

Chen, Y.K., D.R. Keeney, D.A. Graetz, and A.Holding. 1972. Nitrification in lake sediments. J. Environ. Qual. 1: 151-154.

Clean Water Act. 1972. U.S. Code, Public Law 92-240, Section 2.

Cooper, P. 1999. A review of the design and performance of vertical flow and hybrid reedbed treatment systems. Water Sci. Tech. 40(3):1-9.

Crites, R. W. and G. Tchobanoglous. 1998. Small and Decentralized Wastewater Management Systems. McGraw Hill, Boston, MA.

Dalsgaard, T. D.E. Canfield, J. Petersen, B. Thamdrup, and J. Acuña-González. 2003. N_2 production by anammox reaction in the anoxic water column of Golfo Dulce, Costa Rica. Nature 422(10):606-608.

D'Angelo, E.M. and K.R. Reddy. 1993. Ammonium oxidation and nitrate reduction in sediments of a hypereutrophic lake. Soil Sci. Soc. Am. J. 57:1156-1163.

Davis, M.L. and S.J. Masten. 2004. Principles of Environmental Engineering and Science. McGraw Hill, Boston. 704 pps.

Doyle, A., M. N. Weintraub, and J. P. Schimel. 2004. Persulfate digestion and simultaneous colorimetric analysis of carbon and nitrogen in soil extracts. Soil Sci. Soc. Am. J. 68:669-676.

Drozd, J.W., A. Godley, and M.I. Baley. 1978. Ammonia oxidation by methane oxidizing bacteria. Soc. Gen. Microbiol. Proc. 5:66-67.

Ellis, S. M.T. Howe, K.W.T. Goulding, M.A. Mugglestone and L. Dendooven. 1998. Carbon and nitrogen dynamics in a grassland soil with varying pH: Effect of pH on the denitrification potential and dynamics of the reduction enzymes. Soil Biol Biochem. 30:359-367.

Environmental Protection Agency. 1975. Process Design Manual for Nitrogen Control. Office Technol. Transfer, Washington, DC.

Environmental Protection Agency. 1993. Subsurface flow constructed wetlands for wastewater treatment: A technology review. EPA 832-R-93-008. EPA office of Research and Development, Washington, DC

Fierer, N. 2003. Stress ecology and the dynamics of microbial communities and processes in soil. Ph.D. Thesis. University of California, 226 pages. (http://fiesta.bren.ucsb.edu/~fierer/microbialbiomassdetermination.htm)

Gersberg, R.M., B.V. Elkins, and C.R. Goldman. 1986. Role of aquatic plants in wastewater treatment by artificial wetlands. Water Res. 20:363-368.

Gersberg, R.M., R.A. Gearheart, and M. Ives. 1989a. Pathogen removal in constructed wetlands. p.431-445. *In* D.A. Hammer (ed.) Constructed Wetlands for Wastewater Treatment. Lewis Publishers, Chelsea, MI.

Gersberg, R.M.,S.R. Lyon, R. Brenner and B.V. Elkin. 1989b.Integrated wastewater treatment using artificial wetlands: a gravel marsh case study. p.145-152. *In* D.A. Hammer (Ed.) Constructed Wetlands for Wastewater Treatment. Lewis Publishers, Chelsea, MI.

Gidley, T.M. 1995. Development of a constructed subsurface flow wetland simulation model. M.S. Thesis, N.C. State Univ. Raleigh, NC.

Goreau, T.J., W.A. Kaplan, S.C. Wofsy. M.B. McElroy. F.W. Valois, and S.W. Watson. 1980. Production of nitrite and N_2O by nitrifying bacteria at reduced concentrations of oxygen. Appl. Environ. Microbiol. 40:526-532.

Green, M., E. Friedler, Y. Ruskol and I, Safrai. 1997. Investigation of alternative method for nitrification in constructed wetlands. Water Sci. Tech. 35(5):63-70.

Gries, C., L. Kappen, and R. Lösch. 1990. Mechanisms of flood tolerance in reed, *Phragmites australis* (Cav.) Trin. Ex Stuedel. New Phytol. 114:589-593.

Groffman, P.M., W.H. McDowell, J.C. Meyers and J.L. Merriam. 2001. Soil microbial biomass and activity in tropical riparian forests. Soil Biol. and Biochem. 33:1339-1348.

Groffman, P.M., G.C. Hanson, E. Kiviat, and G. Stevens. 1996. Variation in Microbial biomass and activity in four different wetland types. Soil Sci. Soc. Am. J. 60:622-629.

Good, B.J and W.H. Patrick, Jr. 1987. Root-water-sediment interface processes. p 359-371. *In* K.R. Reddy and W.H. Smith (Eds.) Aquatic Plants for Water Treatment and Resource Recovery. Magnolia Publishing, Orlando FL.

Guang, J. T. Kelly, M. Freeman, and M. Callahan. 2002. Removal of N, P, BOD_5 in pilot-scale constructed wetland systems. Inter. J. Phytoremediation 4(2):127-141.

Hammersley, M.R. and B.L. Howes. 2002. Control of denitrification in a septage-treating artificial wetland: the dual role of particulate organic carbon. Water Res. 36:4415-4427.

Hammersley, M.R., B.L. Howes, and D.S. White. 2003. Particulates, not plants, dominate nitrogen processing in a septage-treating aerated pond system. J. Environ. Qual. 32:1895-1904.

Headley, T.R., E. Herity, and L. Davidson. 2005. Treatment at different depths and vertical mixing within a 1-m deep horizontal subsurface-flow wetland. Ecol. Eng. 25: 567-582.

Hobbie, J.E. and C.C. Crawford. 1969. Respiration corrections for bacterial uptake of dissolved organic compounds in natural waters. Limnology and Oceanography 14:528-532.

Hobbie, J.E. and R.T. Wright. 1968. A new method for the study of bacteriain lakes: description and results. Milteilungen Internationale Vereinigung fur Theoretisch und Argewandte Limnologie 14:64-71

Hoover, S.R. and N. Porges. 1952. Assimilation of dairy wastes by activated sludge, II: The equation of synthesis and oxygen utilization. Sewage and Industrial Wastes, Vol 24.

Joergensen, R.G. and P.C. Brookes. 1990. Ninhydrin-reactive nitrogen measurements of microbial biomass in 0.5 m K_2SO_4 soil extracts. Soil Biol. Biochem. 22(8):1023-1027.

Kadlec, R.H. 2000. The inadequacy of first-order treatment wetland models. Ecol. Eng. 15:105-119.

Kadlec, R.H. 2003. Pond and wetland treatment. Water Sci. Technol. 48(5):1-8.

Kadlec, R.H. and R.L. Knight. 1996. Treatment Wetlands. Lewis, Boca Raton, FL.

Kantawanichkul, P., P. Neamkam and R.B.E. Shutes. 2001. Nitrogen removal in a combined system: vertical vegetated bed over horizontal flow sand bed. Water Sci. Tech. 44(11):137-142.

Kemp, M.C. and D.B. George. 1997. Subsurface flow constructed wetlands treating municipal wastewater for nitrogen transformation and removal. Water Env. Res. 6(7):1254-1262.

Kemp, W.M., P. Sampou, J. Caffrey, M. Mayer, K. Henriksen, and W.R. Boynton. 1990. Ammonium recycling verses denitrification in Chesapeake Bay sediments. Limnol. Oceanogr. 35:1545-1563.

Knight, R.L., R.W. Ruble, R.H. Kadlec, and S. Reed. 1993. Wetlands for wastewater treatment: Performance database. p. 35-58 *In* G.A. Moshiri (ed.) Constructed Wetlands for Water Quality Improvement. CRC Press, Inc. Boca Raton, FL.

Kuypers, M.M.M., A.O. Sliekers, G. Lavik, M. Schmid, B.B. Joergensen, J.G. Kuenen, J.S.S. Damste, M. Strous, and M. S.M. Jetten. 2003. Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. Nature 422(10):608-611.

Lavigne, R.L. and J. Jankiewicz. 2000. Artificial wetland treatment technology and its use in the Amazon rain forests of Ecuador. p 813-821. *In* Proceedings of the 7th International Conference on Wetland Systems for Water Pollution Control, Nov. 11-16, 2000. Orlando, FL.

Lowrance, R. and D. Smittle. 1988. Nitrogen cycling in a multiple-crop vegetable production system. J. Environ. Qual. 17:158-162.

Marsili-Libelli, S. and N. Checchi. 2005. Identification of dynamic models for horizontal subsurface constructed wetlands. Ecol. Modelling 187:201-218.

Luederitz, V., E. Eckert, M. Lange-Weber, A. Lange, R.M. Gersberg. 2001. Nutrient removal efficiency and resource economics of vertical flow and horizontal flow constructed wetlands. Ecological Engineering 18:157-171.

Metcalf and Eddy, 2003. Wastewater Engineering: Treatment and Reuse. 4th ed. McGraw- Hill, Boston, MA.

Mitsch, W.J. and J.G. Gosselink. 2000. Wetlands, 3rd Ed. John Whiley and Sons., New York, NY

Morris, M. and R. Herbert. 1997. The design and performance of a vertical flow reed bed for the treatment of high ammonia, low suspended solids organic effluents. Water Sci. Tech. 35(5):197-204.

Mulvaney, R.L. 1996. Nitrogen – Inorganic forms. p 1123-1184. *In* D.L. Sparks *et al.* (eds) Methods of Soil Analysis, Part 3.Chemical Methods. Soil Sci. Soc. Am., Madison, WI.

Neralla, S., R.W. Weaver, B.J. Lesikar, and R.A. Persyn. 2000. Improvement of domestic wastewater quality by subsurface flow constructed wetlands. Biosource Technology 75:19-25.

Nielson, M. L.H. Larsen, M.S.M. Jetten, and N.P. Revsbech. 2004. Bacterium-based NO_2^- biosensor for environmental applications. Appl. Environ. Microbiol.70(11) 6551-6558.

Nielson, M. A, Bollman, A.O. Sliekers, M. Jetten, M. Schmid, M. Strous, I. Schmidt, L. H. Larsen, L.P. Nielson, and N.P. Revsbech. 2005. Kinetics, diffusional limitation and microscale distribution of chemistry and organisms in a CANON reactor. FEMS Microbiology Ecology 51:247-256.

Ottová, V., J. Balcarová, and J. Vymazal. 1997. Microbial characteristics of constructed wetlands. Water Sci. Tech. 35(5):117-123.

Paul, E.A. and F.E. Clark.1996. Soil Microbiology and Biochemistry. 2nd Ed. Academic Press. San Diego, CA.

Platzer, C. 1998. Entwicklung eines Bemesssungsansatzes zur stickstoffelinination in pflanzenkläranlagen. Diss. TU Berlin.

Pynaert, K. B.F. Smets, S. Wyffels, D. Beheydt., S.D. Siciliano, and W. Verstraete. 2003. Characterization of an autotrophic nitrogen-removing biofilm from a highly loaded lab-scale rotating biological contactor. Appl. Environ. Microbiol. 69:3626-3635.

Ralt, D., R.F. Gomez, and S.R. Tannenbaum. 1981. Conversion of acetyhydroxamate and hydroxylamine to nitrite by intestinal microorganisms. Eur. J. of Microbiol. Biotechnol. 12:226-230.

Reddy, K.R., W.H. Patrick, Jr., and R.E. Phillips. 1980. Evaluation of selected processes controlling nitrogen loss in flooded soil. Soil Sci. Soc. Am. J. 44:1241-1246.

Reddy, K.R. and W.H. Patrick 1983. Nitrogen transformations and loss in flooded soils and sediments. CRC Crit. Rev. Envirn. Cont. 13(4):273-309.

Reed, S.C. 1995. Design of Subsurface flow constructed wetlands for wastewater treatment. p. XX. *In* S.C. Reed, E.J. Middlebrooks, and R.W. Crites (Eds) Natural Systems for Waste Management and Treatment, 2nd edn. McGraw-Hill, New York, NY.

Reed, S.C. , E.J. Middlebrooks, and R.W. Crites 1995. Natural Systems for Waste Management and Treatment, 2nd edn. McGraw-Hill, New York, NY.

Robertson, L.A., E.W.J. van Niel, R.A.M. Torremans, and J.G. Kuenen. 1988. Simultaneous nitrification and denitrification in aerobic chemostat cultures of *Thiosphaera pantotropha*. Appl. Environ. Microbiol. 54:2814-2818.

Schmidt, E.L. and L.W. Belser.1994. Autotrophic nitrifying bacteria. p 159-178. *In* R.W. Weaver *et al*. ed. Methods of Soil Analysis. Part 2. Microbial and Biochemical Properties. Soil Sci. Soc. Am., Madison, WI.

Schmidt, I., O Sliekers, M. Schmid, I. Cirpus, M. Strous, E. Bock, J.G. Kuenen, and M.S.M. Jetten. 2002. Aerobic and anaerobic ammonia oxidizing bacteria – competitors or natural partners? FEMS Microbiology Ecology 39:175-181.

Schmidt, I., P.J.M. Steenbakkers, H.J.M. op den Camp, K. Schmidt, and M.S.M. Jetten. 2004. Physiologic and proteomic evidence for a role of nitric oxide in biofilm formation by *Nitrosomonas europaea* and other ammonia oxidizers. J. Bacteriol. 186(9):2781-2788.

Schnürer, J., M. Clarholm, T Rosswall. 1986. Fungi, bacteria and protozoa in soil from four arable cropping systems. Biology and Fertility of Soils 2(3):119-126.

Scow, K.M., S. Simkins, and M. Alexander 1986. Kinetics of minerialization of organic compounds at low concentrations in soil. Appl Environ Microbiol. 51(5): 1028-1035.

Seidel, K. 1970. Macrophytes and water purification. p 109-121. *In* J. Tourbier and R.W. Pierson (Eds) Biological Control of Water Pollution. Univ. of Penn. Press, Philadelphia, PA.

Shannon, R. D., O.P. Flite III, and M. Hunter. 2000. Subsurface flow constructed wetland performance at a Pennsylvania campground and conference center. J. Environ. Qual. 29:2029-2036.

Sikora, F.J., T. Zhu, L.L. Behrends, S.L. Steinberg, and H.S. Coonrod.1995. Ammonia removal in constructed wetlands with recirculating subsurface flow: removal rates and mechanism. Water Sci. Tech. 32(3):193-202.

Sliekers, A.O., S.C.M. Haaijer, M.H. Stafsnes, J.G. Kuenen, M.S.M. Jetten. 2005. Competition and coexistence of aerobic ammonium- and nitrite-oxidizing bacteria at low oxygen concentrations. Appl. Microbiol. Biotechnol. 68:808-817

Song, Z. Z. Zheng, J. Li, X. Sun, X Han, W. Wang, and M. Xu. 2006. Seasonal and annual performance of a full-scale constructed wetland system for sewage treatment in China. Ecol. Eng. 26:272-282.

Sorrell, B.K. and H. Brix. 2003. Effects of water vapor pressure deficit and stomatal conductance on photosysthesis, internal pressurization and conductive flow in three emergent wetland plants. Plant & Soil 253:71-79.

Sylvia, D.M. J.F. Fuhrmann, P.G. Hartel, and D.A. Zuberer. 1999. Principles and Applications of Soil Microbiology. Prentice Hall, Saddle River, NJ.

Szogi, A.A., P.G. Hunt, and F.J. Humenik. 2003. Nitrogen distribution in soils of constructed wetlands treating lagoon wastewater. Soil Sci. Soc. Am. J. 67:1943-1951.

Tanner, C. 2001. Plants as ecosystem engineers in subsurface-flow treatment wetlands. Water Sci. Technol. 44: 9-17.

Tanner, C., J.P.S. Sukais, and M.P. Upsdell. 1998. Relationship between loading rates and pollutant removal during maturation of gravel-bed constructed wetlands. J. Environ. Qual. 27:448-454.

Tanner. C. C. and J.P.S. Sukais. 1995. Accumulation of organic solids in gravel-bed constructed wetlands. Water Sci. Technol. 32:229-240.

Teal, J.M. and J.W. Kanwisher. 1966. Gas transport in the marsh grass *Spartina alternaflora*. J. Expt. Bot. 17(51):355-361.

Thamdrup, B. and T. Dalsgaard. 2002. Production of N_2 through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. Appl. Environ. Microbiol.68:1312-1318.

Ulrich, H., K. Diehl, I. Feuerpfiel, A. Hummel, J. Lopez-Pila, and R. Szewzyk. 2005. Microbial investigations for sanitary assessment of wastewater treated in constructed wetlands. Water Research 39:4849-4858.

van Niel, et.al. 1987. Proc. 4th Eur. Congr. Biotechnol. 3: 363.

Veneman, P.L.M. and E.W. Pickering. 1983. Salt bridge for field redox potential measurements. Commun. Soil Sci. Plant Anal. 14:669-677.

Vepraskas, M.J. and J. Bouma. 1976. Model experiments on mottle formation simulating field conditions. Geoderma 15:217-230.

Verstraete, W. and M. Alexander. 1972. Hetrotrophic nitrification by *Arthrobacter sp.*J. Bacteriol. 110:955-961.

von Felde, K. and S. Kunst. 1997. N-and COD- removal in vertical flow systems. Water Sci. Tech. 35(5):79-85.

Weedon, C.M. 2003. Compact vertical flow constructed wetland systems - first 2 years performance. Water Sci. Tech. 48(5):15-23.

Weiner, S.E.B. and W. Granéli. 1989. Influence of substrate conditions on the growth of *Phragmites austral is* after a reduction in oxygen transport to below-ground parts. Aquatic Botany 35:71-80. Weisner, SE.B. and W. Granéli. 1989. Influence of substrate conditions on the growth of *Phragmites australis* after a reduction in oxygen transport to below-ground parts. Aquat. Bot. 35:71-80.

Whipps, J.M. and J.M. Lynch. 1983. Substrate flow and utilization in the rhizosphere of cereals. New Phytol. 95:605-623.

White, K. 1995. Enhancement of nitrogen removal in subsurface flow constructed wetlands employing a 2-stage configuration, an unsaturated zone and recirculation. Water Sci. Tech. 32(3):59-67.

Wiesner, A., P. Kuschk, and U. Stottmeister. 2002. Abilities of helophyte species to release oxygen into rhizospheres with varying redox conditions in laboratory-scale hydroponic systems. Inter. J. Phytoremediation 4:1-15.

Winter, K.J. and D. Goetz. 2003. The impact of sewage composition on the soil clogging phenomena of vertical flow constructed wetlands. Water Sci. Tech. 48(5):9-14.

Wright, R.T. and J.E. Hobbie. 1965. The uptake of organic solutes in lake water. Limnology & Oceanography 10:22-28.

Wright, R.T. and J.E. Hobbie. 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology 47(3):447-464.

Wyndham, R.C. and J.W. Costerton, 1981 Heterotrophic potentials and hydrocarbon biodegradation potentials of sediment microorganisms within the Athabasca Oil Sands deposit. Appl. Environ. Microbiol. 41(3): 783-790.

Wyffels, S. P. Boeck, K. Pynaert, W. Verstraete, and O. Van Cleemput. 2003. Sustained nitrite accumulation in a membrane-assisted bioreactor (MBR) for the treatment of ammonium-rich wastewater. J. Chem. Technol. Biotechnol. 78:412-419.

Wynn, T.M. and S.K. Liehr. 2001. Development of a constructed subsurface-flow wetland simulation model. Ecol. Eng. 16:519-536.

Zhu, T. and F.J. Sikora. 1995. Ammonia and nitrate removal in vegetated and unvegetated gravel bed microcosm wetlands. Water Sci. Tech. 32(3):219-228.