

**SEEDS: The OARDC Graduate Research Enhancement Grant  
Program  
Ph.D. Application**

**Identifying Contributions of Plant Roots within Ecological  
Waste Treatment Systems**

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**Lay Abstract**

Nutrients from animal wastes are the second most common cause of water pollution leading to reductions in dissolved oxygen and contributing bacteria that are harmful to humans and fish populations. Ecological treatment systems treat wastewater in an environmentally safe and cost effective manner by relying on natural processes and renewable energy. One natural process relied upon in ecological treatment systems is the reduction in the nitrogen concentration of wastewater by bacteria living on submerged plant roots. In the current study, we will assess whether roots of two different plant functional groups, emergent herbaceous and woody, promote nitrogen removal at different rates. A preliminary study of nitrogen removal by two emergent plant species, Taro and Cyperus, was run to test the proposed methods. The results indicate that roots of species from the same functional group do not differ in rates of nitrogen removal. One of the drawbacks of ecological treatment systems is that they typically have a larger landscape footprint when compared to conventional wastewater treatment systems. If particular functional groups promote nitrogen removal at greater rates, those functional groups can be selected for, resulting in increased efficiency of ecological treatment systems. Increased efficiency will reduce the size of the system required, making ecological treatment systems more competitive with conventional wastewater treatment systems.

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### 3. Project Description

#### Introduction

In the quest to improve the sustainability of water treatment options, plant-based systems, such as wetlands and Ecological Treatment Systems, are a promising alternative. Conventional methods of wastewater treatment both for municipal and animal farming operations rely greatly on non-renewable energies and in agricultural settings lack efficacy. Alternatively, ecological treatment systems, which rely on renewable resources, have successfully treated municipal and industrial effluents with reduced costs compared to conventional methods (Austin 2000 and Todd and Josephson 1996). One of the most pressing questions concerning these systems is the interaction between vegetation and wastewater that leads to water quality improvements. While many studies prove that systems with plants out-perform similar systems without plants, the specific vegetative functions leading to increases in water quality are not understood (Kadlec 1995, Soto et al. 1999). Although the area around the submerged plant roots is thought to be critical for nitrogen transformation and removal, specific interactions between the roots and wastewater remain unknown. It is unclear how plants enhance the rates of nitrification and denitrification that lead to nitrogen removal.

This research will identify plant functional groups that better facilitate processes improving water quality and will increase the efficiency of ecological treatment systems. This will provide greater nitrogen removal per area, reducing the area needed for future ecological treatment systems.

The long-range research goal is to develop ecological treatment systems that will treat wastewater from animal production facilities. The goal of this research is to determine if different functional groups of plants (woody vs. emergent) provide a root environment that increases nitrogen removal. **The central hypothesis is that a woody species, *Hibiscus moscheutos*, will promote nitrogen removal at a greater rate than the emergent species *Cyperus spp.* and *Colocasia esculentus*.** This hypothesis is based on preliminary studies that will be detailed in later sections. The study will be carried out using roots harvested from a prototype ecological treatment system, located at the Waterman Farm Dairy Facility on The Ohio State University campus, which has been in operation for six months. The research will build on successful preliminary experiments and benefit from the expertise of the graduate committee in ecological engineering, aquatic and riparian vegetation and water chemistry analysis.

The central hypothesis will be tested by accomplishing the following specific objectives:

- **Objective 1. Determine if roots of different plant functional groups, woody or emergent herbaceous, support larger quantities of nitrifying and denitrifying bacteria.**

Hypothesis 1. A woody species, *Hibiscus moscheutos*, will support a larger population of nitrifying and denitrifying bacteria than the emergent species *Cyperus spp.* or *Colocasia esculentus*.

- **Objective 2. Quantify nitrification and denitrification rates of root-associated bacteria to determine the best plant functional groups for ecological treatment systems.**

Hypothesis 2. A woody species, *Hibiscus moscheutos*, will support greater rates of nitrification and denitrification because of a larger quantity of root-associated bacteria than the emergent species *Cyperus spp.* or *Colocasia esculentus*.

The proposed research is new and innovative because: (1) the role of plant roots in ecological treatment systems has not been fully investigated and (2) no assessment of the rates of nitrification and denitrification supported by plant roots of species commonly used in ecological treatment systems has been completed. It is expected that through the proposed research, fundamental data will be obtained, data that can be used to critically evaluate the influence of plant roots on nitrogen removal and determine whether plant species of different functional groups significantly differ in their influence on nitrification and denitrification. Collectively, my research is expected to be significant in providing data that will be used to design and operate full-scale ecological treatment systems for a variety of waste treatment applications.

### **Rationale and Significance**

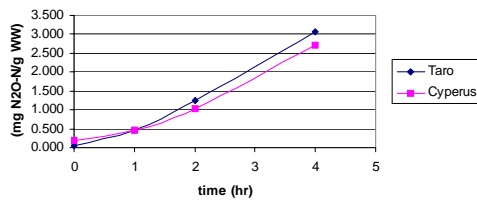
Traditionally, agricultural wastewater has been stored in lagoons, which can overflow or fail with drastic consequences to water quality (Innes 2000). Nutrients from animal wastes are the second most common cause of water pollution leading to reductions in dissolved oxygen and contributing bacteria that are harmful to humans and fish populations (USEPA 1993). Consequently, there is a great need for environmentally safe methods of wastewater treatment for animal containment facilities.

Ecological treatment systems provide an environmentally safe and cost effective method for treating wastewater from animal containment facilities. The proposed research is significant because determining what plant functional groups are best suited for ecological treatment systems, in terms of their ability to remove nitrogen, will improve the efficiency of ecological treatment systems. Greater efficiency will result in cost savings by reducing the size of the systems, which is one of the biggest drawbacks of ecological treatment systems when compared with conventional wastewater treatment systems (USEPA 2001).

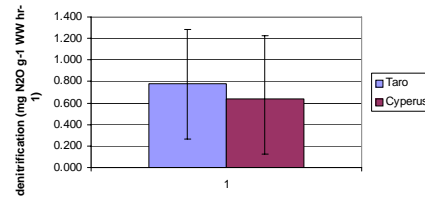
### **Preliminary Results**

A preliminary experiment was conducted in October 2004 to assess the adequacy of the proposed methods for measuring denitrification rates and bacterial population sizes. The most probable number method was used to measure denitrifier population size. *Colocasia esculentus* (Taro) roots supported a population of  $9.0 * 10^4$  MPN  $g^{-1}$  wet roots  $L^{-1}$ , while *Cyperus spp.* (Cyperus) roots supported a population of  $1.4 * 10^4$  MPN  $g^{-1}$  wet roots  $L^{-1}$ . The acetylene block method was used to measure denitrification over a ten-hour period. Rates of  $N_2O$  production were linear for the first four hours of sampling for the majority of the sample replicates (Taro  $r^2 = 0.981$  and Cyperus  $r^2 = 0.958$ ) (Figure 1). After four hours the rates fluctuated non-linearly. Consequently, only the first four sampling times (0 hr, 1 hr, 2 hr and 4 hr) were used to calculate the rates.

Denitrification rates based on wet weight did not significantly differ between Taro ( $0.775 \text{ mg N}_2\text{O-N g}^{-1} \text{ WW hr}^{-1}$ ) and Cyperus ( $0.637 \text{ mg N}_2\text{O-N g}^{-1} \text{ WW hr}^{-1}$ ) ( $p\text{-value} = 0.626$ ) (Figure 2). Rates of denitrification based on dry weight also showed no significant differences. Nitrate loss was significantly less than  $\text{N}_2\text{O}$  production during the incubation of samples ( $p\text{-value} = 0.001$  for Taro and  $0.000$  for Cyperus). Bastviken et al. (2003) reported a strong correlation between nitrate loss and  $\text{N}_2\text{O}$  production ( $r^2 = 0.76$ ). The correlation between nitrate loss and  $\text{N}_2\text{O}$  production for Taro was similarly strong ( $r^2 = 0.73$ ). This means that nitrate loss was primarily a result of denitrification, and that assimilative uptake and dissimilative reduction to ammonium were not significant sources of nitrate loss. However, there was no correlation between nitrate loss and  $\text{N}_2\text{O}$  production for Cyperus ( $r^2 = 0.18$ ).



**Fig.1** Rate of  $\text{N}_2\text{O-N}$  production over time per gram of wet weight for Taro ( $r^2 = -0.9081$ ) and Cyperus ( $r^2=0.958$ ).



**Fig. 2** Denitrification on the surfaces of Taro and Cyperus roots per gram wet weight. Error bars show standard deviation.  $P\text{-value} = 0.626$ .

Studies that have compared denitrification rates on different biotic surfaces have generally compared species of different functional groups and have found significant differences in  $\text{N}_2\text{O}$  production between the surfaces of the different functional groups (Toet et al. 2003 and Bastviken et al. 2003).

Our preliminary results indicate that species of the same functional group do not differ in rates of  $\text{N}_2\text{O}$  production. The objective of this proposal is to expand the study to include a second functional group of woody species. Woody twigs have been found to facilitate higher rates of denitrification than emergent species (Bastviken et al. 2003). The proposed research will determine whether the roots of woody species facilitate higher rates of denitrification than those of emergent species.

Low levels of nitrification can limit denitrification in treatment wetlands (Newman et al. 2000 and Toet et al. 2003). In their comparison of *P. australis* and *Elodea*, Toet et al. (2003) suggested that the lower rates of denitrification on *Elodea* shoots resulted from lower nitrate concentrations in the *Elodea* stand. As with denitrification, the differences in nitrification rate between species may result from differences in root exudates or surface characteristics (Bastviken et al. 2003). At the Solar Aquatic System in Marion, MA, nitrifying bacteria were found to be associated with the roots of *Eichhornia crassipes* only 10% of the time and with septage particulates 90% of the time (Hamersley and Howes 2003). In our next run of experiments, we will measure nitrification rate as well as denitrification, to determine whether nitrification is a limiting factor for denitrification in our ecological treatment system. By examining bacteria population sizes and nitrification and denitrification rates on plant species of different functional groups we will be able to determine which functional group supports the greatest rate of nitrogen removal.

## Literature Review

The most effective method of nitrogen removal from wastewater is the conversion of mineralized nitrogen into the gaseous compounds dinitrogen and nitrous oxide through the microbially mediated processes of nitrification and denitrification, and the subsequent loss of these gases to the atmosphere (Focht and Chang 1975). Biofilms containing heterotrophic and nitrifying bacteria form on suspended or sedimentary organic matter in aerobic water (Hamersley and Howes 2002). The consumption of organic carbon by these bacteria removes oxygen from the water faster than it can diffuse back, creating anaerobic conditions as little as 100 $\mu$ m below the organic carbon source (Hamersley and Howes 2002). One problem with wastewater treatment is that it typically involves the removal of organic matter early in the process, resulting in aerobic water with low organic carbon and a low capacity for denitrification (Hamersley and Howes 2002). Aquatic plants help to make up for this loss of organic matter in ecological treatment systems by providing accessible surface area for attachment by microorganisms (Eriksson and Weisner 1996 and 1997). The creation of anaerobic microsites within aerobic regions allows plants to simultaneously support nitrification and denitrification, making them a vital component of ecological treatment systems.

Although it is known that creation of anaerobic microsites on plants supports attached microbial communities, the significance of their role on denitrification in wastewater treatment systems is unclear and requires further investigation (Hamersley and Howes 2003). Studies by Austin (2000) and Hamersley and Howes (2003) have suggested that the role of plants as nitrifier hosts could be increased by selecting for plants with longer roots (>10-20cm), increasing plant areal densities to 20% of the water column, or increasing root concentrations. These suggestions are based on the idea that the greater the percentages of plant root volume, the greater the reduction of chemical oxygen demand. The drawbacks to increased root concentration are consequent reductions in mixing and lower hydraulic retention time that may negate any increase in nitrification (Hamersley and Howes 2003). Thus, rather than simply increasing root density, it would be useful to know whether certain functional groups support nitrification and denitrification at higher rates than others. Using plant functional groups that promote nitrogen removal would improve the efficiency of ecological treatment systems, allowing them to be smaller and more cost effective.

Studies that have compared nitrification and denitrification rates on different biotic surfaces have generally compared plant species of different functional groups and have found significant differences in nitrogen gas production between the surfaces of the different functional groups (Toet et al. 2003 and Bastviken et al. 2003). In a comparison of woody twigs, *Myriophyllum spicatum* (Eurasian watermilfoil, an herbaceous, submersed aquatic), and filamentous macroalgae (herbaceous, submersed aquatic), twigs had the highest rates of nitrification and denitrification (Bastviken et al. 2003). Toet et al. (2003) compared *Phragmites australis*, an emergent aquatic macrophyte, with *Elodea*, a submersed aquatic macrophyte, and found higher rates of denitrification on *Phragmites australis* shoots. A comparison of *Typha spp.*, *Scirpus spp.* and a mixed stand of emergents found that nitrate removal differed significantly for each of the different vegetation types and was highest in the mixed stand (Bachand and Horne 2000). These studies all examined biofilms attached to the stems or leaves of the plant.

Only one study, Hamersley and Howes (2003), has investigated the rates of nitrification supported by biofilms on the roots of a plant species (*Eichhornia crassipes*). They found that nitrification increased with increased nitrifier concentration, but cautioned that further studies are needed on the role of plant roots in wastewater treating systems. Ecological treatment systems are unique in that they lack sediment and generally do not contain submerged species; instead they contain emergent species supported on racks that allow plant roots to float in wastewater. Consequently, plant roots are a critical component of these systems, and a better understanding of the functions they perform is needed.

#### **4. Research Methods**

**Objective 1. Determine if roots of different plant functional groups, woody or emergent herbaceous, support larger quantities of nitrifying and denitrifying bacteria.**

##### **Introduction**

To determine whether plant functional groups differ in their ability to support nitrification and denitrification on their roots, data will be collected on the population sizes of denitrifiers and nitrifiers. Quantification of nitrifying and denitrifying bacteria populations will be done using the most probable number (MPN) method (APHA 1998). The data will be used to statistically evaluate the degree to which plant species from different functional groups differ in their ability to support nitrification and denitrification.

##### **Experimental Design**

Roots from two different functional groups growing in the aerobic tanks of the Waterman ecological treatment system will be harvested one day prior to the experiment. The two most prevalent species from the emergent functional group (*Colocasia esculentus* and *Cyperus sp.*) will be used. The woody functional group is represented by only one species within the aerobic tanks of the Waterman ecological treatment system, *Hibiscus mosheutos*; consequently, this will be the only woody species used in the experiment. Clippers will be used to cut the roots off the emergent stems. Upon returning to the lab, tubes used in the most probable number experiment will be inoculated with root homogenate and then incubated in order to estimate population sizes.

##### **Most Probable Number Method**

Nutrient broth supplemented with 0.5 g/L KNO<sub>3</sub> per liter will be used to culture denitrifying bacteria (Tiedje 1982), while nitrifying bacteria will be grown in nutrient broth supplemented with 0.07 g/L NH<sub>4</sub>Cl (Hamersley and Howes 2003). Ten milliliters of broth will be dispensed into 16 ml tubes with butyl rubber septa screw caps for denitrifying populations and into regular screw cap tubes for nitrifying populations. The media filled tubes will be autoclaved for 15 minutes at 21 psi and 121 °C for sterilization (Tiedje 1982).

To determine the concentration of bacteria present on the roots, we will inoculate the sterilized tubes with a mixture of roots and nanopure water rather than wastewater. Wastewater contains particulates with attached bacteria; consequently, using wastewater as the solvent would inhibit our ability to accurately determine bacterial concentrations on the roots. Ten grams of roots and

90 ml of nanopure water will be homogenized with a blender (Tiedje 1982). Serial dilutions of the homogenate [ $10^{-1}$ ,  $10^{-3}$ , and  $10^{-5}$ ] will be prepared with the homogenate, as recommended by Tiedje (1982). The media tubes will then be inoculated with 0.1 ml of the appropriate serial dilution, with five replicates each. Following inoculation, tubes for denitrification will have 1 ml of acetylene injected through a 0.22  $\mu\text{m}$  filter and syringe assembly.

After two weeks of incubation at 25°C, presumptive tests for denitrification will be performed, and at the endpoint of accumulation of  $\text{N}_2\text{O}$ , confirmatory tests will be done (Tiedje 1982). Nitrifier tubes will be incubated for three weeks at 25°C, at which point the production of active acid will be checked with diazotizing and coupling reagents. The incubations will continue for approximately 8 weeks, or until there are no changes in the number of positive tubes for two consecutive weeks. At the end point, the presence of  $\text{NO}_2^-$  will be checked for in all tubes with visible growth and in the lowest dilution with no visible growth. Nitrite negative tubes will be checked for  $\text{NO}_3^-$  with a diphenylamine spot test. The number of bacteria present in the nitrifier and denitrifier tubes will be determined using the tables and equations provided in APHA (1998).

### **Interpretation**

The data will be assessed to ensure they meet requirements of normality, and needed transformations will be performed. Differences in bacterial population size among the three species and in the nutrient content of the incubation water will be tested with one-way ANOVA. Multiple comparisons will be made using the Tukey-Kramer test. Significance will be assessed at  $\alpha = 0.05$ .

### **Means of Applying Results**

The results of the research will be used to select for, and increase the coverage of, functional groups within the Waterman ecological treatment system that support the highest level of nitrogen removal. The knowledge gained from the research will be disseminated to the public through ecological treatment system tours, conference posters and peer-reviewed articles.

### **Pitfalls and/or Limitations of the Proposed Research**

Potential problems with the MPN study include contamination of the tubes or lack of growth. Contamination will be indicated if growth occurs in the sterile control tubes. If contamination occurs the experiment will be terminated, and started anew. If no growth occurs, the nutrient media will be adjusted and the experiment repeated.

**Objective 2. Quantify nitrification and denitrification rates of root-associated bacteria to determine the best plant functional groups for ecological treatment systems.**

### **Introduction**

Data on the rates of nitrification and denitrification on the roots of each plant species will be collected in order to determine whether roots of the plant species differ in their ability to support

nitrification and denitrification. Nitrification rates will be estimated from differences in  $\text{NH}_4^+$  accumulation between nitrification inhibited microcosms and uninhibited microcosms. The acetylene block method will be used to measure the rate of denitrification on plant roots. The data will be used to statistically evaluate the degree to which plant species differ in their ability to support denitrification and nitrification.

## Experimental Design

Roots from two different functional groups growing in the aerobic tanks of the Waterman ecological treatment system will be harvested one day prior to the experiment. The two most prevalent species from the emergent functional group (*Colocasia esculentus* and *Cyperus sp.*) will be used. The woody functional group is represented by only one species within the aerobic tanks of the Waterman ecological treatment system, *Hibiscus mosheutos*; consequently, this will be the only woody species used in the experiment. Clippers will be used to cut the roots off the emergent stems. Roots will be submerged in water taken from the tanks and aerated over night at the lab. The following morning, roots will be added to denitrification microcosms and the acetylene block method performed. The next day roots that have remained submerged in the tank water will be added to the nitrification microcosms for nitrification measurement.

## Methods

**Measurement of Nitrification Rate.** The rate of N mineralization, resulting in  $\text{NH}_4^+$  accumulation, will be used to determine nitrification rates of the nitrifying bacteria colonizing root samples. A concentration of 10 wet g/L roots will be used (Hamersley and Howes 2003). Root samples will be added to 1 L Mason jars that contain 750 ml of nutrient broth. The nutrient broth will have an initial  $\text{NH}_4^+$ -N concentration of 6.65 mg/l to release nitrification from substrate limitation. Eight replicates of each species and eight controls with no roots will be used. Fifteen milligrams per liter of N-serve will be added to half of the control and root containing flasks. N-serve is a commercially available chemical used to prohibit nitrification. Rubber tubing, connected to an air pump, will be inserted into each flask to keep the broth aerated (Hamersley and Howes 2003 and Bastviken et al. 2003). The flasks will be incubated at 20°C for 12 hours, and 15 ml samples will be withdrawn at time zero and every two hours after that. Withdrawn samples will be vacuum filtered through 0.45  $\mu\text{m}$  filter and then analyzed for  $\text{NH}_4^+$  concentration by colorimetric assay on a lachat. Beginning and ending samples will also be analyzed for  $\text{NO}_3^-$ . At the end of the experiment, the nutrient broth will be sieved and the plant roots dried to constant weight at 60°C and weighed. This will allow the correlation of nitrification rates with biomass.

Rates of nitrogen mineralization will be calculated as the slope of a linear regression of the linear portion of  $\text{NH}_4^+$ -N increase in the N-serve microcosms. The mineralization rate will then be subtracted from the ammonium loss rate in the non-N-serve microcosms, giving the nitrification rate for each two-hour period (Hamersley and Howes 2003).

**Measurement Denitrification Rate.** Roots will be incubated in commercially available nutrient broth that has been enriched with 0.5 g/l  $\text{KNO}_3^-$  (Tiedje 1982), to ensure that denitrification rates are not limited by nutrient availability. Serum bottles (120 ml) will receive 100 ml of broth and then be sterilized at 21 psi, 121°C for 15 minutes. Cooled serum bottles will receive 3.5 g of roots (Hamersley and Howes 2002) and will then be degassed with He for two minutes to create

anaerobic conditions (Balderston et al. 1976). Eight replicates of each species will be run in addition to eight control bottles containing no roots (Tiedje 1982). After sealing the flasks, 20% of the gas headspace will be replaced with acetylene gas, to inhibit conversion of  $N_2O$  to  $N_2$  during denitrification (Tiedje et al. 1989).

The flasks will be kept on a shaker, at 150 rpm and 20°C (Curtis and Ingraham 1983), to maintain equilibrium between the gas and water phases (Tiedje 1982, Eriksson and Weisner 1996). The headspace gas will be sampled at hours 0, 1, 2 and every two hours after that for 12 hours. Samples will be taken with gas-tight syringes and then injected into evacuated serum bottles and stored at 4°C until analysis on a gas chromatograph can be done. The samples will be analyzed for an increase in  $N_2O$  concentration on a gas chromatograph with an electron capture detector and a Porapak Q column. The carrier gas will be 95% argon and 5% methane. At the end of the sample period, the nutrient broth will be sieved and the plant roots dried at 60°C to constant weight. This will allow the correlation of denitrification rates with biomass.

Measured concentrations of  $N_2O$  will be converted from volume/volume units to mass/volume units through application of the Ideal Gas Law. The amount of  $N_2O$  dissolved in solution will be calculated and added to the amount in the headspace. Rates of  $N_2O$  production will then be determined from the linear portion of a linear regression of  $N_2O$  concentration over time.

### **Interpretation**

The data will be assessed to ensure they meet requirements of normality, and any needed transformations performed. Differences in nitrification and denitrification rates among the three species and in the nutrient content of the incubation water will be tested with one-way ANOVA. Multiple comparisons will be made using the Tukey-Kramer test. Significance will be assessed at  $\alpha = 0.05$ .

### **Means of Applying Results**

The results of the research will be used to select for, and increase the coverage of, functional groups within the ecological treatment system that support the highest level of nitrogen removal. The knowledge gained from the research will be disseminated to the public through ecological treatment system tours, conference posters and peer-reviewed articles.

### **Pitfalls and/or Limitations of the Proposed Research**

The main problems associated with the acetylene inhibition method include failure if insufficient acetylene is added, which is particularly important when organic matter concentrations are high; the water solubility of  $N_2O$ ; and inhibition of nitrification due to acetylene addition. The last problem generally only occurs in soils with low  $NO_3^-$  concentrations (Tiedje et al. 1989). We will add sufficient  $NO_3^-$  to overcome substrate limitation by nitrification inhibition. If denitrification does not occur, the experiment will be repeated with increased acetylene. The water solubility of  $N_2O$  will be corrected for using Henry's law.

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## **6. Schedule of Activities**

The experiments will be started June 20, 2006, once vegetation has become established following winter senescence. Nitrification and denitrification experiments will be performed over two consecutive days. MPN experiments will be started at the same time and will be incubated for approximately eight weeks. Samples will then be prepared and sent to STAR Lab for analysis; it is anticipated that this will take approximately two weeks. It is expected that experiments and analysis will be completed by November 2006. Results will be analyzed and a paper written for publication; this is expected to be completed by May 1, 2006.

7. Budget

**SEEDS BUDGET FORM**

<b>Investigator Name: Jennifer A. Morgan</b>				
<b>Department: Food, Agricultural, and Environmental Engineering</b>				
	<b>Year 1 SEEDS</b>	<b>Year 2 SEEDS</b>	<b>Year 1 Match (if applicable)</b>	<b>Year 2 Match (if applicable)</b>
<b>A. Salaries and Wages</b>				
1. Research Associates / Post Doctorates				
2. Undergraduate Students				
4. Other				
<b>Total Salaries and Wages</b>	0			
<b>B. Fringe Benefits</b>				
<b>C. Total Salaries, Wages and Fringe Benefits (A plus B)</b>	0			
<b>D. Nonexpendable Equipment</b>				
<b>E. Materials and Supplies</b>			2,000	
<b>F. Travel</b>			1,200	
<b>G. Publication Costs</b>				
<b>H. Other (Describe in Budget Justification.)</b>	5,000			
<b>Total (C through H)</b>	5,000		3,200	

## 8. Budget Justification

The proposed research is intensively analytical; consequently, all of the funds requested for this proposal will be used to pay for laboratory assessment. Concentrations of  $\text{NH}_4^+$  will be assessed for approximately 700 samples taken during the nitrification experiment (700 @ \$6/each = \$4,200). Concentrations of  $\text{NO}_3^-$  will be assessed in 185 samples taken during the nitrification experiment and the denitrification experiment (185 @ \$3.50/each = \$647.50).  $\text{N}_2\text{O-N}$  concentrations will be measured in 152 samples from the denitrification experiment (152 @ \$1/each = \$152). Lab sample analysis will be conducted at Star Lab, located on the Wooster campus of OARDC.

The faculty advisor will provide \$2,000 for materials and supplies such as chemicals, plants, test tubes and racks, mason jars and volumetric flasks. In addition, the faculty advisor will cover travel to one in-state meeting, OARDC Annual Conference (which includes the cost involved for poster preparation), and one national conference, Society of Wetland Scientists, to present results.

## 9. Location, Facilities and Equipment

The study system is located at Waterman Farm on The Ohio State University campus in Columbus, Ohio. The Wetland ecological treatment system is housed in a 30 ft. x 34 ft. polyhouse and consists of four identical treatment lines, each receiving waste from a 2,000-gallon dosing tank (Fig. 3). Dairy wash-water is pumped from the wash-water reservoir to the Wetland ecological treatment system dosing daily. The wash-water is then pumped from the dosing tank to the four treatment lines.

Each treatment line is designed in the following manner: one 150 gallon anaerobic reactor, one anoxic reactor (110 gal.), one closed aerobic reactor (110 gal.), one aerobic reactor (110 gal.), one clarifier (110 gal.), one subsurface flow gravel wetland (2 ft x 4ft x 4 ft), two aerobic reactors (110 gal.), one clarifier (110 gal.) and two subsurface flow gravel wetlands (2 ft x 4 ft x 1 ft). The aerobic reactors are continually supplied with air from airlift pumps. Plastic racks attached to the sides of the aerobic reactors support vegetation on the water surface and allow the roots to be suspended in the tanks, where they provide surface area for microbial attachment. The aerobic reactors are dominated by *Cyperus papyrus* and *Colocasia esculentus* and have lower frequencies of *Iris pseudocorus*, *Hibiscus moscheutos*, *Canna spp.*, *Saururus cernuus*, and *Lemna spp.* The wetland mesocosms are vegetated with a mix of *Schoenoplectus spp.*, *Chloris spp.*, *Polygonum persicaria*, *Juncus spp.*, and *Salix spp.*

Plant roots for the experiments in this proposal will be harvested from the first and second aerobic reactors, and laboratory incubations will be conducted in facilities located in the Agricultural Engineering building. Sample analysis for  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations will be conducted at Star Lab on the Wooster campus of Ohio State University.

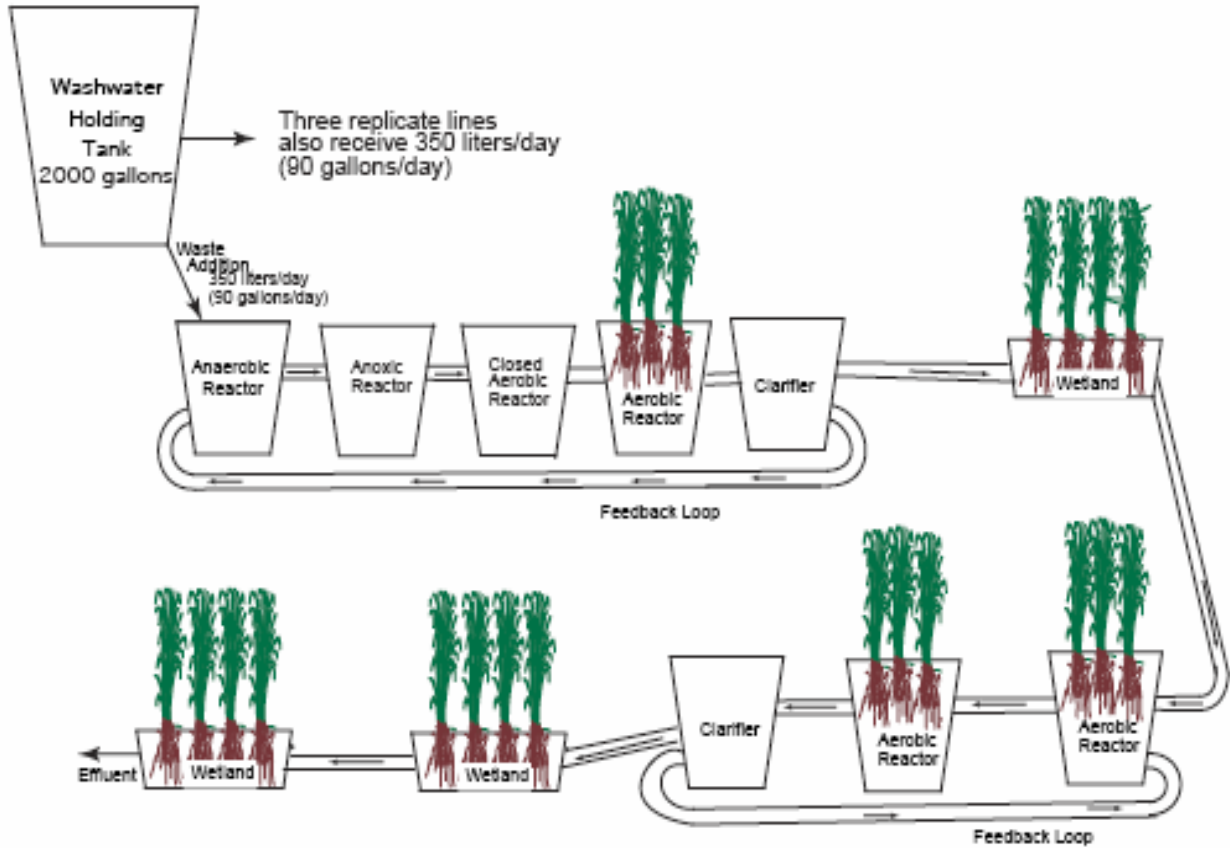


Fig. 3. Illustration of one treatment line within the Waterman ecological treatment system consisting of: one 150 gallon anaerobic reactor, one anoxic reactor (110 gal.), one closed aerobic reactor (110 gal.), one aerobic reactor (110 gal.), one clarifier (110 gal.), one subsurface flow gravel wetland (2 ft x 4ft x 4 ft), two aerobic reactors (110 gal.), one clarifier (110 gal.) and two subsurface flow gravel wetlands (2 ft x 4 ft x 1 ft). Nitrification and denitrification reactions within the first and second vegetated aerobic reactors will be investigated in this proposal.

## **10. Collaborative Arrangements**

Not applicable.

## **11. Research Risks**

Not applicable.

## 12. Curriculum Vitae

### Jennifer A. Morgan

#### Education

The Ohio State University, Columbus, OH, Master of Science, Natural Resources, June 2004

Portland State University, Portland, OR, Bachelor of Science with Honors, June 2002,  
Majors: Environmental Studies and Biology Minor: Geology

#### Employment Experience

Graduate Fellow, 2004 - present, The Ohio State University, Columbus, OH

Graduate Teaching Associate, 2002 - 2004, School of Natural Resources, The Ohio State University, Columbus, OH

Air Quality Intern, June 2001 - August 2002, Port of Portland, Portland, OR  
Biology Lab Technician, March 1998 – August 2000, Portland Community College, Portland, OR

#### Presentations

Morgan, Jennifer A. 2005. Impact of clipping *Phragmites australis* (Cav.) Trin. ex Steudel and flooding on plant species diversity and biomass in a Lake Erie wetland. The Ohio Academy of Science. Toledo, OH.

Morgan, Jennifer A. 2003. Invasive species hypotheses. Monthly meeting of E-CARP, Columbus, OH.

Morgan, Jennifer A. 2003. Impact of clipping *Phragmites australis* (Cav.) Trin. ex Steudel and flooding on plant species diversity and biomass in a Lake Erie wetland. Stone Laboratory summer seminar series, Put-in-Bay, OH.

Morgan, Jennifer A., M. Nechvatal, J. Valigore, J. Goicochea. 2003. Restoration of the Olentangy River from Dodridge Ave. to 5<sup>th</sup> Ave., following removal of the 5<sup>th</sup> Ave. dam. Symposium on the Olentangy River, Columbus, OH.

#### Honors and Awards

Environmental Science Graduate Program Fay Fellowship     September, 2004 to present  
Gamma Sigma Delta Honor Society     December, 2003  
Stone Lab Student Scholarship     June, 2003  
Barry Commoner Environmental Scholarship     May, 2001

Portland State University Transfer Student Scholarship     June, 2000  
Golden Key International Honor Society                     November, 2000

### **Academic Service**

- Ecological Engineering Society, Columbus Chapter                     May 2003 – May 2004
- Secretary: invite and coordinate guest speakers, record and post minutes, advertise meetings.  
GradRoots Seminar Committee                     September 2003 – June 2004
  - Coordinate committee members and communicate with guest lecturers to determine and fulfill audiovisual needs.  
GradRoots Minigrants Committee                     September 2002 - June 2004
  - Review grant applications for scholastic integrity, scientific merit and need, make recommendations on grant recipients.

### **Research Experience**

- Graduate Research Associate                     September 2002 - Present
- Thesis research on the aggressive wetland species *Phragmites australis*.
  - Examined the response of extant and seed bank vegetation to inundation and cutting of *P. australis*.
  - Attempted to determine the depth of inundation necessary to reduce/control *P. australis* stands.
- Nutrient Removal Research Project                     April 2003 – June 2003
- Investigated nitrogen and phosphorus removal by emergent aquatic plants in a wastewater treatment system.
  - Studied nutrient assimilation in the above and belowground biomass of the emergent aquatic plants.
- Riparian Restoration Project                     January 2003 – March 2003
- Developed restoration plans for a two-mile section of the Olentangy River riparian area following removal of a lowhead dam.
  - Designed a surface flow treatment wetland for inorganic nutrient removal.
  - Worked with landscape architecture students to increase accessibility and visibility of the river.
- Independent Field Project                     July 2000 – March 2001
- Designed and began compilation of a plant herbarium representing Portland, OR urban riparian vegetation, for Dr. Alan Yeakley.
  - Conducted vegetation surveys and collected soil samples in urban riparian areas.
- Wetland Survey Project                     June 2000 – August 2000
- Developed and implemented a habitat classification process that could be used by the public to help a local wetland park complete a habitat survey
  - Created a vegetation key and set up a grid system to identify quadrants of the wetland, both aimed at usage by a lay person.

