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A Biofuel-Capable Wetland With Optimal Nitrate Uptake from Chesapeake Bay Waters Affected by Agricultural Runoff

Gemstone Team SWAMP (Superior Wetlands Against Malicious Pollutants)

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We pledge on our honor that we have not given or received any unauthorized assistance on this assignment or examination.

Arsh Agarwal
Allie Bradford
Kerry Cheng
Ramita Dewan
Enrique Disla
Addison Goodley
Nathan Lim
Lisa Liu
Lucas Place
Raevathi Ramadorai
Jaishri Shankar
Michael Wellen
Diane Ye
Edward Yu

Mentor: Dr. Dave Tilley - Yes
Librarian: Robert Kackley - No

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Abstract

Harmful algal blooms caused by nitrates and phosphates negatively affect estuarine ecosystems, such as the Chesapeake Bay. These blooms release toxins and block sunlight needed for submerged aquatic vegetation, creating hypoxic areas of the Bay. Artificial wetlands have been utilized to reduce the amount of nitrate pollution. This project will test the *Typha latifolia* (cattail), *Panicum virgatum* (switchgrass), and *Schoenoplectus validus* (soft-stem bulrush) for denitrification potential. In order to amplify the differences between the plants, we will use a carbon-based denitrification factor to be found through testing. We plan to use the ANOVA test in order to determine the significance of our findings. Based on our data, future environmental groups can better choose the species they will plant in artificial wetlands.

Introduction

Adverse effects of agricultural runoff into the Chesapeake Bay directly affect surrounding aquatic, terrestrial, and industrial life, as well as residents of the Chesapeake Bay Watershed. This results in a poor quality of life for plants and animals alike, leaving many residents who depend on the Bay for their livelihood without the necessary resources to sustain their businesses and their families.

The Problem: Effects of Pollutants from Agricultural Runoff

Nitrates and phosphates from agricultural areas run off into the Chesapeake Bay Watershed. These chemicals cause harmful algal blooms that lead to massive dead zones as nutrients vital to aquatic wildlife are depleted (Carpenter et al., 1998). A dead zone is an area that has been overtaken by harmful algal blooms. These algal blooms deplete oxygen from the surrounding waters resulting in areas that have little to no wildlife or nutrients necessary for organism growth. Algal blooms also decrease water clarity and quality. In addition, they inhibit aquatic wildlife from thriving, leading to the loss of various aquatic species (Anderson, Glibert, & Burkholder, 2002). Reducing runoff into the bay is vital to the success of the fishing industry, the health of seafood consumers, and the biodiversity of the Chesapeake. Furthermore, environmental groups concerned with the health of the bay are also invested in reducing nitrate pollution.

Our team aims to mitigate the effects of these pollutants, caused by agricultural runoff, by identifying plant species that are efficient at absorbing nitrates and show potential as biofuel crops (Brisson & Chazarenc, 2008). By utilizing water-purifying plants that can also act as biofuels, we hope to select a combination of plants that can both maximize denitrification in a

wetland environment located in the Bay Watershed and be utilized as an environmentally-friendly alternative energy source.

The Research Question

We will conduct our experiment based on the question, "What combination of plants with the potential to be used as biofuels most efficiently removes nitrates, the result of agricultural runoff, from the Chesapeake Bay Watershed in a wetland environment?" Efficiency will be defined as the percentage of nitrate uptake over a specified period of time. Nitrates will remain the focus of this study, as phosphate removal in a wetland environment has been shown to require extensive resources that extend beyond our scope (Vymazal, 2007). Because the Chesapeake Bay is such a large body of water, our team has chosen to focus on a smaller, more accessible river that is part of the watershed. After reviewing literature, we opted to emulate the conditions of the Choptank River, a major tributary of the Chesapeake Bay that has been adversely affected by agricultural runoff (U.S. Geological Survey Virginia Water Science Center, 2005). Sixty percent of the land surrounding the Choptank River is used for agricultural purposes, so the majority of runoff is theoretically composed of nitrates and other agricultural pollutants. For the sake of accessibility and convenience while collecting hydrology samples, we chose the Tuckahoe Creek, a representative branch of the Choptank River (Whitall et al., 2010).

Research Hypotheses

Our study will be guided by several statistical hypotheses. As our current research design includes two separate phases, we have separate statistical hypotheses for each phase. For the first phase, which includes testing which denitrification factors are most efficient at magnifying the difference in nitrogen uptake, the null hypothesis is: there is no difference in the nitrogen uptake of plants when denitrification factors A/B/C are added to the system. The alternative hypothesis

is: there is a significant difference in the nitrogen uptake of plants when denitrification factors A/B/C (sawdust, wheat straw, glucose) are added to the system.

The second phase of the study tests different combinations of plants to find an optimal combination for denitrification efficiency. The null hypothesis for this phase is: there is no significant difference in nitrogen uptake between different plant combinations. The alternative hypothesis is: there is a significant difference in nitrogen uptake between different plant combinations (Hien, 2010).

In the contents of this paper, we will begin by discussing the basis of our research through a literature review. We will then describe the specifics of our proposed methodology, starting with a general overview of our experimental design followed by our experimental setup and protocol. An overview of our data analysis and anticipated results will follow. We will conclude by providing a timeline and budget for the next 3 years.

Literature Review

Agricultural Runoff

Agricultural runoff is one of the most significant sources of pollution to the Chesapeake Bay Watershed. The main sources of nutrients from agricultural runoff are fertilizer and manure, which have high concentrations of nitrates and phosphates (Carpenter et al., 1998). Plants only absorb 18 percent of the nitrogen input from the fertilizer, and up to 35 percent of the nitrogen from the fertilizer runs off into coastal waters and surrounding bodies of water (Carpenter et al., 1998; Zedler, 2003). This nitrate and phosphate rich agricultural runoff causes a steep increase in the nutrient concentration of the neighboring bodies of water. This process, known as eutrophication, can cause harmful algal blooms that reduce water quality and lead to massive dead zones since nutrients essential to aquatic wildlife are depleted by the algae

(Carpenter, et al., 1998) As these algal blooms decompose, oxygen is depleted from the surrounding waters, resulting in dead zones. Furthermore, algal blooms inhibit aquatic wildlife from thriving, leading to the loss of various aquatic species (Anderson, 2002).

Constructed wetlands are one of many methods that mitigate the problems created by agricultural runoff. Past research has shown that strategically placed wetlands can remove up to 80 percent of inflowing nitrates (Crumpton & Baker, 1993). Because they are so effective, constructed wetlands are especially applicable to the Chesapeake Bay, which being subjected to heavy loads of agricultural runoff (McConnell et al., 2007). Nitrates will remain the focus of this study, as phosphate removal in a wetland environment has been shown to require extensive resources that reach beyond our scope (Vymazal, 2007). Thus, Team SWAMP will study the effects of constructed wetlands on denitrification in bodies of water running into and surrounding the Chesapeake Bay.

River Selection

In order to make the results generalizable, we will need to emulate the conditions of a particular area of the Chesapeake Bay Watershed. The Choptank River is the largest eastern tributary of the Chesapeake Bay (Staver, Staver, & Stevenson, 1996). Seventy percent of the total nitrogen input in the Choptank River Basin comes from agricultural sources (Karrh, Romano, Raves-Golden, & Tango, 2007). Specifically, from mid-February to mid-June, large amounts of nutrients flow into the river from grain and corn industries (Whitall et al., 2010).

Around the 1980s, the relationship between high nutrient concentrations and declining amounts of submerged aquatic vegetation was discovered. Many studies were performed and models were implemented in order to decrease the effect of the nutrients (Twilley, Kemp, Staver, Stevenson, & Boynton, 1985). Since then, the Choptank River has been able to cut down

millions of pounds of nitrogen input per year. Although it now contributes less than one percent of the total nitrogen load to the Chesapeake Bay, the river still contains high levels of nutrients that support environmentally harmful algal blooms (Karrh et al., 2007). In addition, different species of algal blooms have been found, most likely resulting from excessive nutrient loading along different tributaries of the Chesapeake Bay, including the Choptank, from 1997-1999 (Glibert et al., 2001). In 1995, a Tributary Strategy Team was formed to address the problems in the Chesapeake Bay and its subwatersheds. As of 2005, the nutrient levels were still exceeding Tributary Strategy goals by 1.55 million pounds per year (Karrh et al., 2007). Until the nutrient loading to the Bay is decreased, the Bay ecosystem will continue to be threatened.

Because the Choptank River is such a large part of the Chesapeake Bay Watershed, we chose to emulate its conditions in the lab and greenhouse. However, for the sake of accessibility and convenience, we have chosen to focus on the Tuckahoe Creek, a tributary of the Choptank River on the Eastern Shore of Maryland. The Tuckahoe Creek sub-basin represents 34 percent of the Choptank Watershed, so by emulating the conditions of the Tuckahoe Creek, we hope to make our results generalizable to a large part of the Choptank River Watershed as well (United States Department of Agriculture, 2009).

Plant Selection

Denitrification, the chemical transformation from nitrate to nitrogen (N_2) gas, accounts for most nitrate removal and is primarily carried out by bacteria. However, it has been observed that these microfauna are affected by the plants in their environment and that macrophyte selection can have a significant impact on efficiency of denitrification (Brisson, 2008). Therefore, three plants will be tested based on their potential for aiding nitrate removal, their

potential as biofuel crops, and their native presence near the Chesapeake Bay. Many of these plants have been tested before, but not concurrently under these experimental conditions.

The first plant will be used is switchgrass, which was selected because of its effectiveness in reducing nitrate levels. A study found that switchgrass had the greatest amount of nitrate reduction as compared to three other plants known to take up nitrates in wetlands (Larson, n.d.). Another reason switchgrass is an ideal plant to use is its native presence in the Chesapeake Bay Watershed, its ability to thrive with little fertilization or irrigation, and its resistance to drought (Larson, n.d.).

The second plant that will be used is the soft-stem bulrush. The soft-stem bulrush is a wetland plant that has proven to be promising in several studies. One study tested four plant species for their effectiveness in reducing pollution levels in subsurface wetland microcosms. It was found that *Schoenoplectus validus* was more effective than *Carex lacustris*, *Phalaris arundinacea*, and *Typha latifolia*, a cattail plant (Fraser, Carty, & Steer, 2004). Another study measuring the effectiveness of *Schoenoplectus* at absorbing nitrates showed that the plant was responsible for 90% of the nitrogen removal in all experimental treatments (Rogers, Breen, & Chick, 1991).

The third plant that will be used is the cattail. Cattails are the most frequently researched as a potential plant for wetlands. One study found that it was the most effective at reducing nitrogen at high nitrate concentrations (Fraser, 2004). Another study investigated nitrate removal from runoff from dairy pastures and found cattails were very effective at reducing nitrate concentration (Matheson, 2010). Cattails also have a strong potential as biofuel crops. In one biofuel research method, the cellulose in cattails was transformed into glucose that could potentially be fermented into ethanol for fuel (Zhang, 2010).

Although more than three quarters of nitrogen is removed by denitrifying bacteria rather than plants, the environment created by different plants affects the denitrification rates of the bacteria (Matheson & Sukias, 2010). It has been found that macrophyte selection has a significant impact on nitrate absorption (Brisson, 2008). This means that plants do aid the denitrification process, but indirectly, and the plants that we have chosen best assist the bacteria in optimizing denitrification. However, in order to be able to quantify differences in nitrate uptake, we need to ensure that the differences are not insignificant. As such, we found literature that outlined certain factors that affect denitrification among plants and bacteria. Since the factors would affect the microbial ecosystem, rather than the addition of these factors could improve the denitrification in all of the plant samples equally, thereby magnifying the differences in nitrate uptake.

Biofuel-Capable Plants

In order to potentially accommodate changing energy and environmental needs, our constructed wetland will contain mostly biofuel-capable plants. In particular, many species of cattails have been shown to have high biofuel potential. One particular study analyzed a potential means for harvesting cattails as a source of ethanol using a hot-water pretreatment process using a Dionex accelerated solvent extractor. The team varied temperature and the duration of heating in order to obtain the maximum product of cellulose. The pretreatment at 190 degrees Celsius for 10 minutes effectively dissolved the Xylanase. This harvested cellulose can then be turned into glucose at a 77.6 percent yield (Zhang, Shahbazi, Wang, Diallo, & Whitmore, 2010). Cattails' promise as a biofuel source provided our team with the idea to include biofuel potential as a secondary data analysis element of the plant selection and screening process.

After determining that cattails were a highly viable biofuel crop, we further researched biofuel-capable plants and cross-referenced with a list of Maryland-native, Bay area plants. Switchgrass was a particularly popular plant studied for its biofuel capabilities and its ability to filter agricultural runoff from Chesapeake Bay waters. A Virginia Tech study of switchgrass and its biomass yields found that in 1989, a single hectare plot of switchgrass yielded 16.2 dry milligrams of biomass. The study compared the switchgrass to other biofuel-capable plants, including sorghum-sudangrass, birdsfoot trefoil, and flatpea. Out of all of the plants in the study, switchgrass consistently yielded the highest amount of dry biomass per hectare. Many of the plants completely or partially failed while switchgrass almost always yielded results (Wright & Turhollow, 2010).

In addition, a third plant seemed to appear extensively on a list of biofuel-capable plants and a list of Maryland-native, Bay area plants: the soft-stem bulrush. One study found that out of twenty wetland species, soft-stem bulrush ranked second in energy output per unit mass. The average energy content was 20.5 kilojoules per gram (kJ/g), only surpassed by cattail with an energy content of 21.5 kJ/g. In addition, soft-stem bulrush was found to have a high biomass yield per unit area. It was found to range from 18 to 42 metric tons per hectare (Fedler, Hammond, Chennupati & Ranjan, 2007).

Denitrification Factors

In order to maximize variability in quantitative data, we chose to include certain factors that affect denitrification efficiency among plants. Based on previous research, three factors for denitrification will be used: glucose, sawdust, and wheat straw, which are all primarily carbon-based. Glucose has been chosen as the first factor due to its ability to greatly increase denitrification rates in artificial wetlands (Weisner, Eriksson, Graneli, & Leonardson, 1994). In

another study, glucose was analyzed in comparison to sawdust, and was found to be more effective than sawdust on the scale of a few days in increasing denitrification rates. However, as time progressed to eight days the sawdust aided in denitrification on a comparable level to the glucose. As a result, sawdust was chosen as the second denitrification factor to be included in the experimental setup and design (Hien, 2010).

The third denitrification factor is wheat straw, which has been found to increase denitrification rates for approximately a week, and then gradually decrease in effectiveness (Ines, Soares, & Abeliovich, 1998). Even though the wheat straw denitrification effectiveness decreased after a week, it still has potential to be used as a factor in our study because our base testing time for each factor is 7 days.

Methodology

Experimental Design and Setup

This project will primarily consist of experimental lab research, but it will also involve data collection in the field. This high constraint approach is necessary because we want to avoid confounding variables that would result from field research. In order to apply our results to the Tuckahoe Creek, we first need to test various microbial factors in a lab environment, controlling for as many variables as possible. In order to save time, we will buy fully grown plants rather than seeds. If fully grown plants are not feasible, we will buy young plants and grow them for a period of time determined through literature review, so that they can reach maturity by the time we begin testing (Brisson, 2008). The plants will be grown in the wetland microcosms, which are artificially created ecosystem. After the plants mature, we will change the water to match the nitrate concentrations of the samples from Tuckahoe Creek (Rice, Szogi, Broome, Humenik, & Hunt, 1998).

Specific soil and hydraulic variables for the microcosm will also be determined by comparison with a sampling point on the Tuckahoe tributary and kept constant in the microcosm. In emulating our river environment in the constructed wetland, we will focus on three particular aspects of the river: the nitrate concentration, the flow rate, and the temperature. In order to determine the appropriate nitrate concentration, flow rate, and temperature, we will take nine total water samples (three samples each during three separate visits in spring, summer, and fall) from one access point along the Tuckahoe Creek. We will analyze these samples for the appropriate characteristics, and take the highest values to apply in our wetland. Feasibly, these are three aspects that we can control in a lab or greenhouse environment. Other specifications for the construction of the microcosms have not yet been decided, but will probably include a pump for water circulation (Rice et al., 1998).

In order to ensure external validity, we want our microcosms to emulate our chosen wetland environment. One of the most important aspects of the river is the flow rate over the wetlands. A certain volume of water crosses a certain area of wetland in a given amount of time. We will measure rate of water flow in the wetland sections of the river and multiply that by the width and depth of the area being measured. To emulate this in the microcosms, we will first match the depth of the water. Then, the inflow and outflow rates will both be set to be proportional to the rate of the river. After the water passes through the wetland, it will be sent to a separate outflow area where we will take samples to test for nitrate levels. Additional types of wetland construction, such as horizontal or vertical flow, are also being considered (Vymazal, 2007).

In the first part of the experiment, we will use a single plant species, either switchgrass or cattail, to test the denitrification factors (Larson, n.d.). The purpose of these factors is to increase

variation between microcosms for statistical significance. The factors will be carbon sources designed to stimulate the microbial ecosystem built around the plants. The factors we have chosen are sawdust, glucose, and straw. The plant species will be grown in separate microcosms, to which a single denitrification factor or different combinations of factors will be added. These different factors will be tested in order to determine which combination per unit mass is most effective at increasing the efficiency of nitrate uptake. Nitrate levels of the effluent will be measured once a day for a period of eight days per trial and will be tested in a laboratory using standard methods for water analysis (Hien, 2010).

In the second stage of the project, we will choose combinations of the predetermined three species to plant in the microcosms. The plants chosen will be based on literature review and certain criteria. First, the plants must be native to the Chesapeake Bay Watershed and non-invasive. Second, we will choose plants that foster microbial denitrification so that the microcosms will be more effective. Finally, the selected plants must have the potential to be harvested as high-yield biofuel crops. At this time, it seems likely that switchgrass, cattail, and soft-stem bulrush will be the three plants we choose (Wright & Turhollow, 2010; Zhang, 2010).

The plants will be evenly distributed through each of the microcosms for the second phase of the experiment. Each microcosm will have a different combination of plants. We will design several different microcosms: some containing only one species and others containing combinations of species we have chosen. A possible design is a one square meter microcosm, lined with PVC film, filled with potting soil inoculated with wetland from the Tuckahoe Creek (Rice et al., 1998). We will add water with a constant concentration of nitrates to the microcosm. Then, we will allow the water to flow through for eight days, collecting daily water samples to determine percentage of nitrate uptake over time. Samples and effluent will be tested by either

sending the water to an outside lab or by using a combination of nitrate test kits (Grumbles, 2008).

Once these preliminary second-phase trials have been completed, we will move our experimentation to a larger scale environment, incorporating flow rate into our constructed wetland. We will place the most efficient combinations of plants into a larger microcosm, containing the same inoculated soil combination as before. The plants will be given a ten-day acclimation period after which we will begin our trials. In these larger scale trials, water will flow into the microcosms at a rate similar to that of the Tuckahoe Creek. Once again, the outflow will be measured to determine the final nitrate concentration, and based on these results, we will be able to confirm which plant or combination of plants will most efficiently remove nitrates from the Tuckahoe Creek and its surrounding environment.

We decided to measure nitrate uptake through reduction of nitrate levels in the water for several reasons. There are several different ways a wetland environment eliminates nitrates: absorption by plants, breakdown by algae, and transformation to gaseous nitrogen by bacteria (Kadlec & Wallace, 2010). Measuring nitrate levels in plants and microbial communities is difficult, while the measurement of nitrate levels entering and exiting is easy and replicable in experiments outside of the greenhouse.

The experiment requires two primary kinds of laboratory space. The group needs a greenhouse to establish the wetland microcosms. This will minimize the effects of confounding variables on the experiment. The group also requires a small amount of lab space in order to test nitrate concentrations of the microcosm water.

Data Collection

Our field data will be used to set up the microcosms. We will test at a to-be-determined location on the Tuckahoe Creek in order to measure salinity, flow rates, and other hydrological, soil, and water quality data. This will produce data integral to creating a generalizable environment for growth and analysis in a greenhouse setting.

Nitrate concentration reduction and factor data will be collected daily during lab and greenhouse testing. We will measure the efficiency of the plants' nitrogen uptake by tracking the change in nitrate concentration in the water, measured in milligrams per liter. A control microcosm without any denitrification factor will be needed to identify a baseline with which to compare the factor results. The addition of the factors should ideally increase the denitrification rates as compared to the control uptake rate.

Data Analysis

Each microcosm contains a unique combination of three possible plant species, totaling seven possible experimental microcosms and one control microcosm without plants. Variables related to salinity, flow rate, hydrology, soil conditions, and water quality will be kept constant so that the inflow and outflow nitrate concentration data will best represent the effect of different plant species combinations. Constraining these variables is vital to the validity of our experiment. The combination with the greatest difference that is statistically significant from the control indicates the one with highest ability to remove nitrate.

There is one independent variable for each phase of the project; it is a between-subjects factor, and there are multiple levels based on the number of factors tested and number of plant species tested. There are two research hypotheses, one for each phase of the project.

Because we are testing the variance in nitrate uptake between microcosms in each phase of the project, we will employ ANOVA tests to determine if the reductions in nitrate

concentration are statistically significant. Statistical Analysis Software (SAS) is a software package that can be used to run these analysis of variance tests. The exact conditions and details of the statistical analysis are yet to be determined. As we collect our data, we will have to decide which types of tests to use for data analysis.

Anticipated Results

For the first part of our experiment, we expect to find a certain set of denitrification factors that will best stimulate the denitrification process in the wetland plants. For example, organic materials such as sawdust, hay, and straw help create oxygen-deficient environments for processes like denitrification (Davis, 1995). Studies have found that adding varied carbon sources or other materials will affect denitrification rates differently (Weisner, 1994). Therefore, for the first part of our experiment, we would expect to find one or more denitrification factors that can increase nitrate uptake in the wetland microcosms.

A study found that denitrification rates differed in swamps that contained different combinations of wetland plants (Gray & Serivedhin, 2006). Similarly, we expect to find a difference in nitrate removal between different combinations of wetland plants. We also expect the differences to be greater due to the denitrification factors. From this second phase, we expect to find the combination of plants that is most effective at removing nitrates from our microcosm.

Limitations

We have several extraneous variables that we need to address. The most significant confounding variable is environmental conditions. We need to take into account hydrology, temperature, humidity and light exposure. These conditions can only be simulated to a certain degree in a laboratory setting, and they are known to affect plant growth. Attrition is a

confounding variable that we will need to be wary of, as plants that die will no longer reduce nitrate concentrations. Another confounding variable is the age of the plants. The age of a plant affects the denitrification potential, so we need to control the age of the plants that we will use by beginning our experimental tests once plants reach maturity (Von Rheinbaben & Trolldenier, 2007). One other extraneous variable is the base composition of the microcosms. We are aware that there is some association between plant species and certain microbes (Glick, 2010), so the microbial composition of the soil we use is important. However, there is no way to identify every species in the soil, so it is probable that we will have to use soil taken from the sampling site on the Tuckahoe Creek. In addition, the soil inherently contains a certain concentration of nitrates that will be very difficult to control for. Thus, to determine plant uptake of only the concentration of nitrogen we put into the microcosm through water, we could either compare our experimental groups with a control to eliminate the nitrogen increase from the soil, or even exchange soil for gravel that will need to be inoculated with necessary plant nutrients.

Previously, we established how we can interpret our data, percent decrease in nitrate concentration, to find the most effective combination of plants for our study. However, how can this result be applied beyond the research setting to answer our research question? Since it is not possible for the time frame of our project to test all the possible wetland plant species native to Maryland, our choice of the three plant species in phase two must be either extremely viable or effective to our location based on field observation and literature review. Similarly, the controlled conditions of the microcosms also need to agree with our Tuckahoe Creek location. The conditions we try to duplicate in the lab are not representative of all locations of the Chesapeake bay, and as such our proposed plants may not be optimal when hydrologic conditions, flow rate, salinity, and nitrate concentrations, and other factors are changed. We will

also have to assume that other conditions, including pH, salinity, and other compounds in the water, cannot be realistically controlled, emulated, and taken into consideration with the goals of our project.

Conclusion

Due to agricultural nitrate runoff, algal blooms and the resultant dead zones form in the Chesapeake Bay. To deal with such pollution, artificial wetlands are often constructed for denitrification. This project will experiment with combinations of the following three plants native to the Chesapeake Bay for denitrification potential: cattail, switchgrass, and soft-stem bulrush. In order to amplify the differences between the denitrification rates of the experimental groups, we will use a combination of carbon-based factors. The possible factors that will be tested are glucose, sawdust, and straw. We plan to use the ANOVA test in order to determine the significance of our findings. Based on our data, future investigators will have a better foundation for testing different plant species and denitrification factors. Furthermore, future environmental, government, and business groups will be able to better choose plant species for artificial wetlands.

Appendices

Budget

Wetland Expenses:

- Greenhouse: ($30/table/month x 12 months) - $1800
- Potting soil - $0*
- Plants - $180
  - Seedling cattails ($10/plant x 60) - $600
  - Seedling softstem bulrush ($10/plant x 60) - $600
  - Seedling switchgrass ($10/plant x 60) - $600
- Factors - $80
  - Glucose (5kg) - $80
  - Straw - $0*
  - Sawdust - $0*
  - Chicken litter - $10

Data Analysis Expenses:

- Pipettes (200 9 inch eye droppers) - $25
- 15mL conical tubes (1000) - $250
- Sample Analysis (by outside lab) - $2400
  - 600 samples x $4

Miscellaneous:

- Transportation to river - $200

Grand total: $6,745 (*to be obtained from on-campus greenhouse, woodshop, and farm)

Glossary

Algal bloom: A rapid increase in the numbers of algae, usually caused by a change in the flow, light, temperature or nutrient levels of the water in which it lives and deprives the water of oxygen.

ANOVA: Analysis of variance (ANOVA) is a group of models and methods which associate variance in a single variable with different sources of variation

Biofuels: A form of renewable fuel that's derived from biomass, which includes organic materials produced by plants, animals or microorganisms

Constructed wetland: Constructed wetland treatment systems are engineered systems that have been designed and constructed to utilize the natural processes involving wetland vegetation, soils, and their associated microbial assemblages to assist in treating wastewater. They are designed to take advantage of many of the processes that occur in natural wetlands, but do so within a more controlled environment.

Dead zones: areas of low-oxygen water in the aquatic environment, often caused by decomposition of vast algal blooms.

Denitrification: The microbially facilitated process by which nitrate is reduced that may eventually produce molecular nitrogen.

Denitrification Factors: A substance or substrate that aids the process of denitrification

Effluent: Outflow of water or gas from a source

Eutrophication: Overflow of nutrients into a body of water which can cause loss of oxygen and extreme population growth or loss

Fossil fuels: any fuel derived from hydrocarbon deposits such as coal, petroleum, natural gas and, to some extent, peat; these fuels are irreplaceable, and their burning generates the

greenhouse gas carbon dioxide

Greenhouse gases: is a gas that traps heat into the atmosphere. The gas works in the same way as the glass in a greenhouse. Heat energy enters the atmosphere, in a short wavelength form, however when the energy reflects off the earth it is in long wave form and so is trapped in the earths atmosphere.

Hectare: a unit of area, 10,000 square meters, used in the measurement of land

Hydrology: Movement, sources, amount, and properties of water in an environment

Macrophyte: A large, multicellular, land based organism belonging to the plant kingdom.

Microcosm: Artificial ecosystems used to simulate natural conditions for the purpose of experimentation

Microfauna: small microscopic animals, but also including fungi and bacteria

Nitrates: The nitrate ion is a polyatomic ion with the molecular formula $NO^{-}3$. It is the conjugate base of nitric acid, consisting of one central nitrogen atom surrounded by three identical oxygen atoms in a trigonal planar arrangement.

Nitrification: The conversion of ammonia to nitrate through oxygen addition

Phosphates: are natural minerals containing phosphorus and are important to the maintenance of all life. They are used in laundry and dishwasher detergents and fertilizers. Their residues can cause growth of algal bloom in freshwater lakes and streams.

PVC film: polyvinyl chloride (PVC) is a synthetically produced polymer plastic that is present in many different forms; PVC film is a clear malleable and waterproof plastic

Runoff: water flow from saturated soil that may contain man-made contaminants.

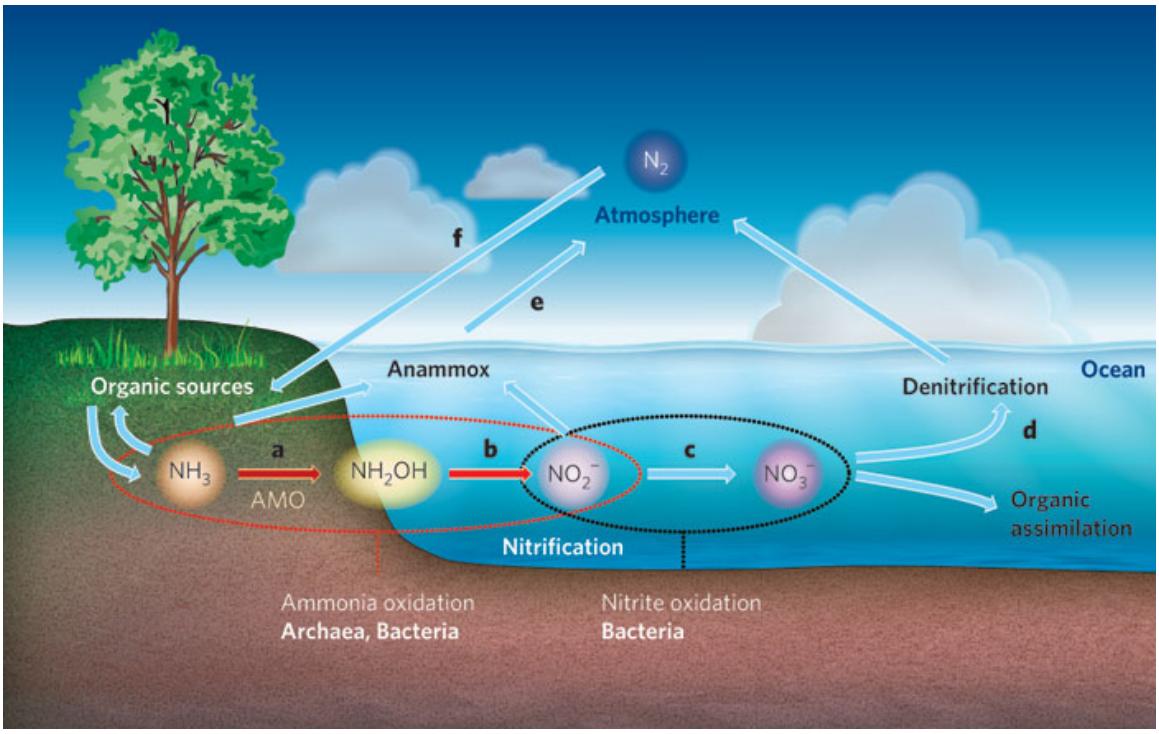
Salinity: the level of different salts in a body of water or soil usually reported in mg/L or parts per million.

Soil Inoculation: The process of mixing soil with a desired microbial community into a larger sample of soil in order to give the original microbial community to the larger sample.

Spectrophotometer: A spectrophotometer is a light intensity-measuring device that can measure intensity as a function of light source wavelength. It is useful in measuring absorption and therefore concentration differences because the spectrophotometer detects more light passing through the sample when more substance is absorbed.

Xylanase: a class of enzymes that degrade hemicellulose, a major component of plant cell walls

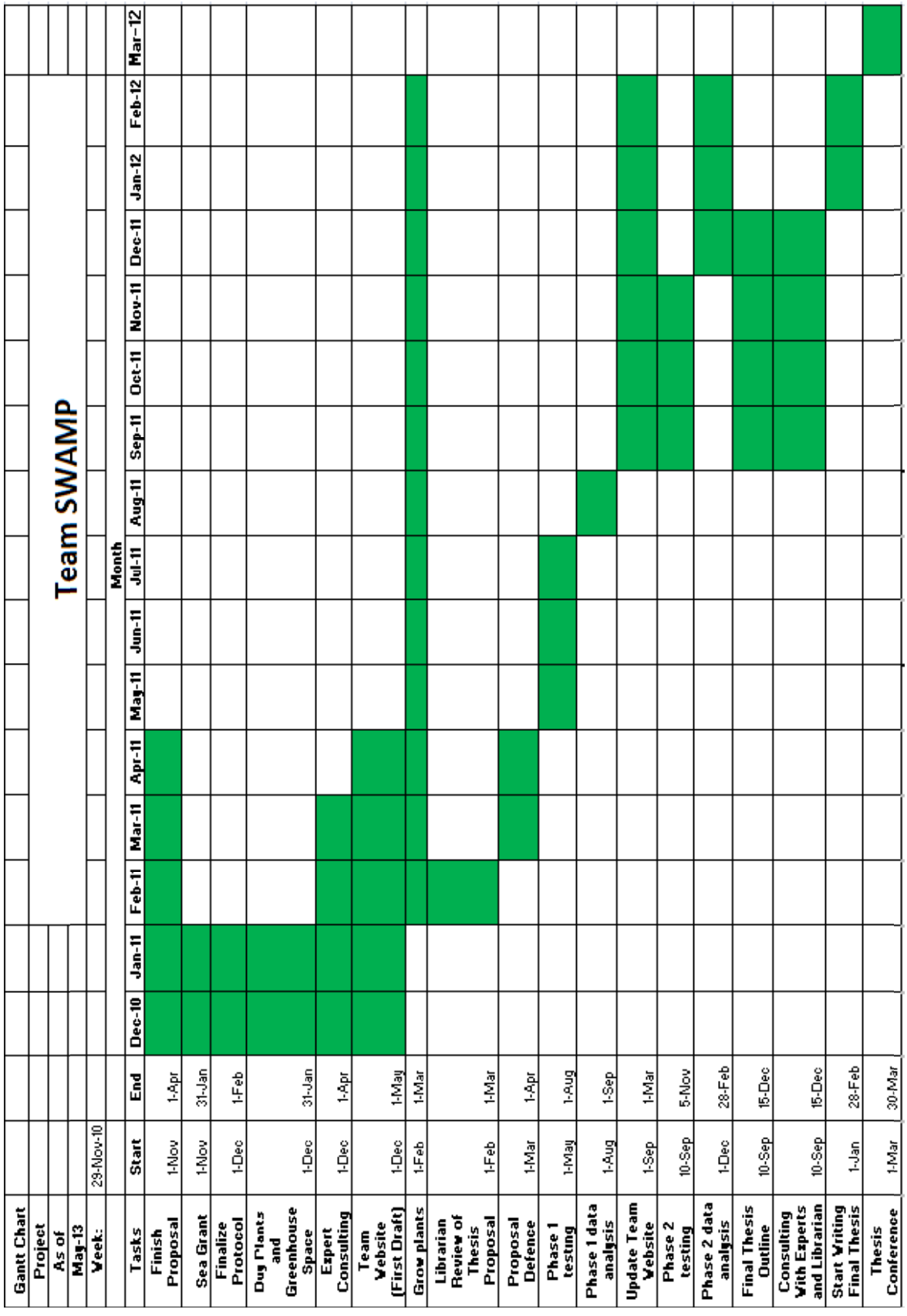
The Nitrogen Cycle



Schleper, C. (2008). Microbial ecology: Metabolism of the deep. *Nature, 456*(7223), 712-714

During nitrogen fixation, plant bacteria use nitrogen, which becomes ammonia and ammonium. Ammonium and ammonia from organic sources goes through nitrification and is converted to nitrates. Afterwards, nitrates go through denitrification to become nitrogen gas and entered into the atmosphere.

Timeline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Project
As of
May-13
Week:
29-Nov-10
Team SWAMP
Month
Tasks
Start
End
Dec-10
Jan-11
Feb-11
Mar-11
Apr-11
May-11
Jun-11
Jul-11
Aug-11
Sep-11
Oct-11
Nov-11
Dec-11
Jan-12
Feb-12
Mar-12
Finish Proposal
1-Nov
1-Apr
Sea Grant
1-Nov
31-Jan
Finalize Protocol
1-Dec
1-Feb
Dug Plants and Greenhouse Space
1-Dec
31-Jan
Expert Consulting
1-Dec
1-Apr
Team Website (First Draft)
1-Dec
1-May
Grow plants
1-Feb
1-Mar
Librarian Review of Thesis Proposal
1-Feb
1-Mar
Proposal Defence
1-Mar
1-Apr
Phase 1 testing
1-May
1-Aug
Phase 1 data analysis
1-Aug
1-Sep
Update Team Website
1-Sep
1-Mar
Phase 2 testing
10-Sep
5-Nov
Phase 2 data analysis
1-Dec
28-Feb
Final Thesis Outline
10-Sep
15-Dec
Consulting With Experts and Librarian
10-Sep
15-Dec
Start Writing Final Thesis
1-Jan
28-Feb
Thesis Conference
1-Mar
30-Mar

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