The Effects of Increased Nutrient Loads and Nutria Herbivory on *Panicum hemitomon* Biomass.

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Introduction

Wastewater assimilated wetlands are created in order to provide cost cutting benefits to cities that use them and to improve the quality of receiving wetlands. Discharging waste water effluent into wetlands provides a source of fresh water and nutrients, and is intended to increase the overall productivity. Numerous studies have shown environmental benefits from creating assimilation wetlands in forested wetlands (Day et al. 2004, Hesse et al. 1998, Hunter et al. 2009). However, the long term effect of discharging water that contains high nutrient loads in a marsh ecosystem is still uncertain. Concerns are that the additional source of nutrients may decrease the belowground biomass (Turner 2010); additionally, nutrient enriched vegetation may concentrate herbivores and eventually cause overgrazing and eat outs (Nyman 2014 and Day et. al 2011).

In Louisiana there are five assimilation wetlands, located in Breaux Bridge, Hammond, Mandeville, Luling, and Thibodaux; the oldest, in Breaux Bridge, has been discharging waste water effluent since the early 1950s (Hunter et al 2009). The flow rates, nutrient concentration, and size of the wetland vary among all of the sites. Depending on the location, effluent is discharged into an existing forested wetland or freshwater marsh. There is substantial evidence to suggest that the nutrient rich effluent greatly benefits forested wetlands.

Hesse et al. (1998) reported significant increase in the growth rates of baldcypress (*Taxodium distichum*) from trees that had been receiving effluent for 40 years at the Breaux Bridge site. Day et al. (2011) reported an increase in baldcypress seedling growth in the Hammond assimilation wetland; additionally the seedlings planted closest to the discharge pipes had the greatest growth, and there was an indirect relationship between the amount of growth and distance from the discharge. However, the overall benefits of increased nutrient loads provided to marsh vegetation are less understood.

Numerous field studies and experiments have demonstrated that increased nutrient loads to marsh vegetation can increase aboveground biomass (DeLaune 1986, Holm 2006, and Moerschbaecher 2008). In contrast, it is assumed that when plants receive additional nutrients, the plant responds by allocation the growth to the shoot and increasing the root to shoot ratio

(Agren and Franklin 2003). Reduced root deposition would ultimately weaken the soil structure, making the marsh more susceptible to erosion. For this reason, belowground biomass serves as a greater factor in determining marsh sustainability.

The literature about belowground biomass and nutrient loads are conflicting. Turner (2010) suggests that increased nutrient loading decreases both the root and rhizome biomass of plants in fresh, brackish, and saline coastal wetlands. Darby and Turner (2008) reported a 40% to 60% decrease in belowground biomass when adding fertilizer to a salt marsh. Similarly, Holm (2006) observed a decrease in belowground biomass in a floating freshwater marsh that received increased nutrient loads; however, there were multiple hypotheses with respect to the exact. Reduced root growth and accelerated decomposition was reported, but it was uncertain whether this was directly related to nutrient inputs, or if it was a result of the nutrients changing the species composition.

Other researchers have demonstrated positive or insignificant effects on belowground biomass. Fertilization of *Spartina patens* in the field resulted in greater belowground biomass, but the response was dependent on site salinity (Ialeggio 2014). Sites with higher salinity had the greatest increase in belowground biomass when fertilized compared to sites of lower salinity that were fertilized (Ialeggio 2014).

At the Caernarvon diversion, Moerschaecher (2008) determined that the sites receiving diversion water had greater belowground biomass than the control; the site farthest from the outfall had the greatest mean belowground biomass and the site closest to the outfall reached the greatest total belowground biomass. In a core sample study, in the Breton Sound, there was no significant difference of belowground biomass in samples that received ^{15}N treatments verses the control (White et al. 2011).

Supplying additional sources of freshwater and nutrients to wetlands may provide an overall benefit to the receiving area, but the benefits have the potential to be offset or reversed by large grazing animals, such as muskrats, waterfowl, nutria and feral hogs. These animals will graze on the aboveground vegetation and also the root rhizomes (Lynch et al. 1947, Shirley et al. 1980). It has been suggested that grazing animals will preferentially feed on plants that have been exposed to higher amounts of nutrients. Ngai and Jefferies (2004) reported that geese preferred forage species that contained higher nutrient concentrations and lower C:N and C:P ratios. Ialeggio (2014) conducted an experiment where nutria where exposed to three common occurring Louisiana coastal marsh plant species; each species was represented with fertilized and unfertilized samples. For all three species, the fertilized vegetation had an overall mean mass loss of 79.4% while the unfertilized vegetation only had a loss of 9.3% (Ialeggio 2014).

Of the grazing animals, nutria poses the greatest risk to the sustainability of Louisiana coastal marshes. Before the start of the Coastwide Nutria Control Program (CNCP), nutria damage estimates were as high as 102,585 acres in a single year (Manuel and Mouton 2014). After 13 years of intensive nutria harvests, the latest estimate is at 4,181 acres. Marsh recovery after nutria harvest has also been documented in the Hammond assimilation wetlands (Day et al 2011). Discharge of secondarily treated waste water effluent began in 2006. By 2008 the area receiving direct discharge had been converted to open water and mud flats. It was only after more than 2,000 nutria were killed in 2008 and 2009 that the marsh began to recover (Dr. Shaffer personal communication).

The purpose of this research is to test how *Panicum hemitomon* biomass responds to high nutrient loads. We are especially interested in the belowground biomass due to its roll in holding marsh soil intact. Because grazing could interact with the plant response, we evaluated the effect of exposure to tertiary treatment water with and without grazing. Results from this experiment may enhance the knowledge and potential consequences of using river diversions and assimilation wetlands as a management tool.

Methodology

The project originally began in May 2014 when *Panicum hemitomon* plants were collected from the White Lake Wetlands Conservation Area. The following day 40 plants were selected and transferred into one gallon pots filled with potting soil. The next day, the plants were brought to the Hammond assimilation wetlands. Half of the plants were placed in the outfall area receiving secondarily treated waste water and half were placed in the control. The control and outfall areas are hydrologically separated by South Slough (Figure 1). Within each of these areas, half of the plants were placed inside previously existing nutria exclosures; two exclosures were used in the outfall, and two were used in the control. The two exclosures in the outfall area had to be repaired using chicken wire.

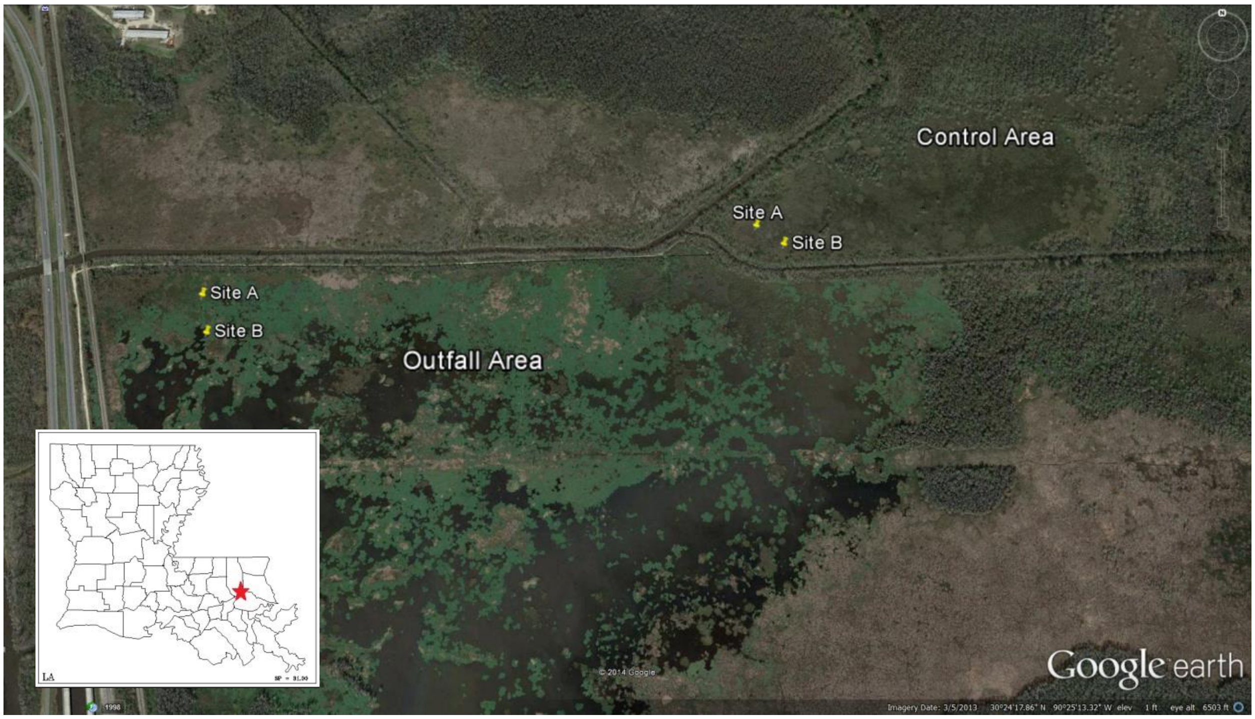

Figure 1. Study area used for the experiment.

On July 7th, the site was revisited, and all but one plant was found alive. Almost all of the plants were either found dead, over grown with floating vegetation, or the pot was lost completely. In order to ensure the survival of the plants to continue the study, future plants would have to have a longer acclimation time between collection from the marsh and transfer to the study area. Additionally, the pots would have to be designed to float in the water. More *Panicum hemitomon* plants were collected from a mesocosm at the Cade Farm, and transferred to the ULL Ecology Center. On the same day, plants were divided into single stem plantlets and put into individual one gallon pots filled with potting soil and cut up styrofoam plates. The purpose of the styrofoam was to increase the buoyancy of the pots. The plants resided under a shade house for three weeks to become acclimated to the pots. On the second week, each plant was cut six inches above the soil surface.

Twenty four of the healthiest plants were transferred to the Hammond Assimilation Wetlands on August 16th. The pots were attached to each other in groups of three. Along both sides of each group, pipe insulation was attached to allow the pots to float (Figure 2). Of the 24 plants, 12 were placed in the outfall area that received secondarily treated wastewater effluent, and 12 were placed in the control area. For both the control and outfall areas, half of the plants were placed in nutria exclosures and half were left exposed adjacent to an exclosure. Plants inside an exclosure are labeled ungrazed, and plants located outside an exclosure labeled grazed (Figure 3).

Figure 2. Pictures of floating pots and usage of nutria exlocure.

All four of the exclosures used were newly constructed using chicken wire and zip ties. Exclosures were a cylinder shaped, approximately 1 meter in diameter. Each was placed over one group of plants and staked down into the mud. The plants were located in open water areas where there was less competition for light and space, and they were also tied off to a firm object to ensure they would not float away.

The study area was revisited on September 26th to check the plants and take aboveground measurements. Nutria had turned over one exclosure in the outfall area, and consumed one plants. Repairs were made to the exclosure. Additionally, two groups of grazed plants were grazed, one in the control and one in the outfall.

On October 3, all the plants were removed from the area. Aboveground biomass was harvested, and then the belowground matter was removed from the pot and washed while at the site. Both the above and belowground biomass were placed in paper bags. The samples were transported to ULL Ecology Center and placed in 70°C drying ovens. On October 6th, all of the biomass was weighed to the nearest 0.1 g. The plant material was weighed first, followed by the empty bag that had contained the plant. The difference between the two weights provided a final biomass dry weight.

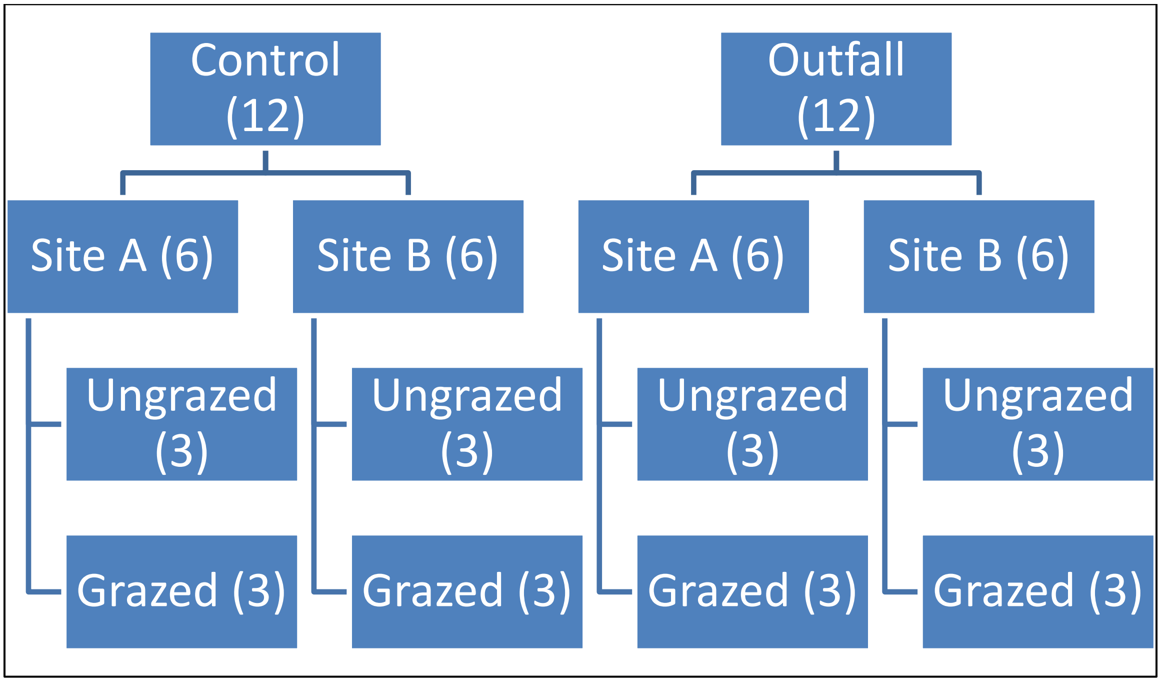

Figure 3. Layout of the experiment showing the number of plants used in each area.

Results

Herbivory was present in both the outfall and control areas. In the control, 3 of the plants without an exclosure had been eaten. Four plants were grazed in the outfall; 3 of the plants without an exclosure and 1 plant inside of an exclosure had been eaten. All herbivory was presumed to be nutria, based on scat found nearby.

The total mean aboveground biomass was .704g. The greatest biomass .966g occurred in the ungrazed outfall area, and the lowest .216g occurred in the grazed control area. Plants located in the ungrazed control had a mean aboveground biomass of .683g, and the grazed outfall was measured at .950g. The total mean belowground biomass was 1.416g with the highest being 1.9g in the ungrazed control and the lowest .533g in the grazed control. The outfall area consisted of 1.833g for grazed and 1.4g for ungrazed (Figure 4).

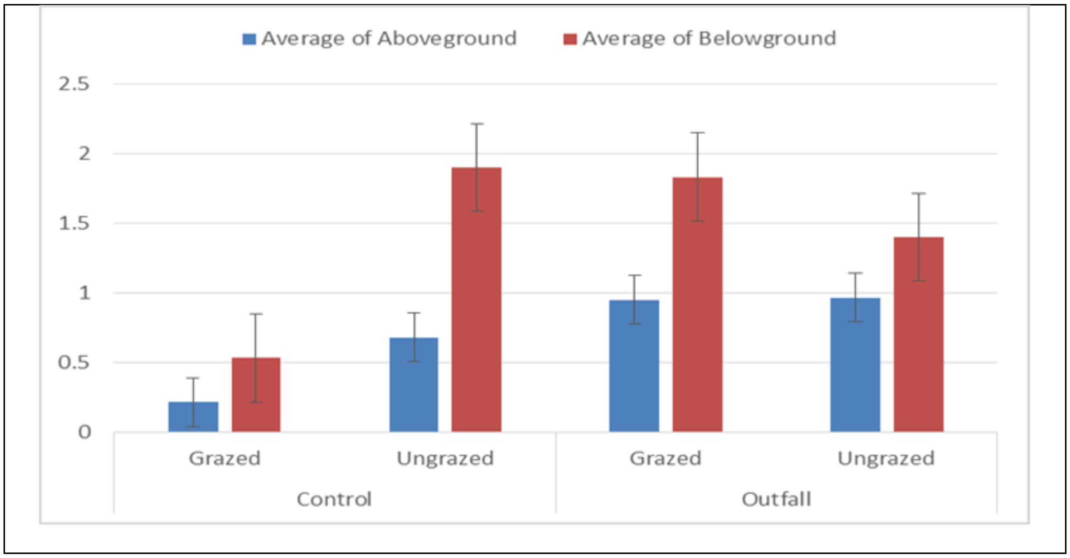

Figure 4. Comparison of Above and Belowground Biomass.

Using ANOVA, no statistical difference was found between the control and outfall areas for both above and belowground biomass, and there was no interaction found between nutrients and grazing (Table 1).

Table 1. ANOVA of aboveground biomass, belowground biomass, and interaction between nutrients and grazing.Source	Aboveground	Belowground	Total
Nutrients	0.1237	0.5641	0.3698
Grazing	0.4538	0.5016	0.4826
Nutrients*Grazing	0.4851	0.2018	0.2692

Discussion

We found no significant differences in *Panicum hemitomon* biomass when exposed to high nutrient loads and grazing. This is likely due to a combination of factors. The plants that were used in the analysis were exposed to treatments for a short period of time, 49 days. This is a short duration when compared to the growing season which lasts several months. Before the experiment began, the effect on belowground biomass was uncertain, but it was assumed there would be differences in aboveground biomass with numerous previous studies reporting a direct relationship between increased nutrient loads and aboveground biomass. (DeLaune 1986, Holm 2006, and Moerschbaecher 2008). No difference in aboveground biomass suggests the duration of the experiment was too short.

A similar study should be conducted in the future using the same study area. The area is significant because it is an existing area that is receiving high nutrient loads. In other experiments, nutrients was added to plants in the form of fertilizer (Darby and Turner 2008), which allows for human error to alter the results. The results from these experiments often suggest that adding nutrients to the marsh will decrease belowground biomass. However, in those experiments, Nyman (2014) suggests that these results can be attributed to adding too much fertilizer or adding sulfate in the fertilizer which can act as a toxin under anaerobic conditions.

Many nutrient enrichment experiments also fail to determine how other stress factors may affect the plants (Nyman 2014). Where plants receive additional sources of nutrients, they may also receive an increase in herbivory (Ialeggio 2013, Wilsey and Chabreck 1991). Our research did consider how grazing would affect biomass. Three plants in the control and four plants in the outfall were eaten by nutria. Plants that were eaten had an obviously negative affect; all of the aboveground vegetation was consumed, and with the exception of 2 plants, all of the rhizomes were consumed. What our research failed to do was demonstrate an interaction between nutrients and grazing. Given a larger sample size, then the experiment may provide results on how nutrient loads and grazing effect biomass.

In conclusion, this experiment provides the framework on how conduct a research project to study the effects of high nutrient loads and grazing. By correcting the unsuspected errors that occurred, increasing the duration of exposure to the treatments, and having a larger sample size, this experiment may be replicated to provide better results on how high nutrient loads and grazing can affect the long term sustainability a freshwater marsh.

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