Resource competition between aquatic primary producers and emergent macrophytes before and after fertilization in restored experimental wetlands of varying biodiversity

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Comment [JP1]: David B. thinks the title is causally wrong. I disagree with him, I think it makes reasonable sense.

Abstract:

The replacement of wetland ecosystems with agricultural systems has degraded Ohio's watersheds. Wetlands restoration strategies that employ ecological theory to create wetlands that harbor biodiversity and filter nutrient high agricultural runoff are needed. We used six hydrologically isolated experimental wetland ecosystems to understand the relationship between restoration planting regime, plant biodiversity, measures of primary productivity, and measures of nutrient removal before and after agricultural runoff simulation : addition of Nitrogen (N) and Phosphorous (P). We hypothesized that nutrient and light competition between emergent aquatic primary producers would play a major role in the relationship between biodiversity and aquatic primary productivity and aboveground macrophyte biomass. Before fertilization, the oligotrophic systems were less productive within the water column in higher diversity wetlands. Results support the hypothesis that high diversity wetlands select more emergent macrophytes which outshade the water column limiting light to aquatic primary producers. Fertilized wetlands removed [N] and [P] to below 10% of initial concentration in 32 and 5 days respectively. After fertilization, aquatic productivity decreased in fertilized wetlands. Results were inconclusive to provide a mechanism for lowered rates of aquatic productivity or to elucidate relationships between biodiversity and ecosystem function. Future nutrient pulses at this study site should be much larger to observe conclusive results above the noise created by other ecological stressors.

Comment [JP2]: Degraded is a bit value laden and ambiguous

Comment [JP3]: Think about using "low nutrient input"

Introduction:

Three parallel trends in basic and applied ecological research provided the basis for this experimental study: 1) interest in ecosystem services such as wetlands that remove nutrients and sediments from agricultural runoff, 2) interest in restoring degraded ecosystems including wetlands that both harbor biodiversity and provide ecosystem services, and 3) interest in the relationship between biodiversity and ecosystem function, specifically nutrient removal and primary productivity. This study used six hydrologically isolated constructed experimental wetlands to assess how macrophyte biodiversity affects both nutrient uptake and two different measures of primary productivity: above ground macrophyte biomass and aquatic primary productivity via dissolved oxygen dynamics.

Harboring biodiversity, removing nutrients, and filtering sediments are well documented economically valuable ecosystem services that characterize wetland ecosystems (Costanza and Daly 1992; Kadlec and Knight 1996; Mitsch and Gosselink 2007; Zedler 2003,). Wetland ecosystem services are now threatened since wetlands have been destroyed and degraded; 90% of wetlands in Ohio have been drained or drastically altered over the past 200 years (Mitsch and Day 2005). Although recent wetland loss is due to suburban and commercial development, initial and vast majority of wetland loss in Northern Ohio and other regions was due to agricultural 'tiling' that lowered the water table (Mitsch and Gosselink 2007).

Large scale agriculture practices produce high nutrient and sediment runoff that have negative downstream ecological impacts (Zedler 2003). Transition from wetlands to conventional agriculture also decreases support for biodiversity by creating large monocultures that highly fragment landscapes (Henle et al 1996). So the double impact of agricultural transition is to replace ecological systems that remove nutrients and harbor biodiversity with systems that generate nutrients and minimize biodiversity. Given this negative effect, it is advantageous to restore wetlands in order to support biodiversity and to address critical socioeconomic services including nutrient removal and sediment filtering (Zedler 2003). Indeed, several local, state, and federal programs have recognized and developed incentive programs that encourage conversion of marginal farmland and farmland in riparian zones to wetlands (U.S. Department of the Interior 2004).

Comment [JP4]: Good

Arguments presented in preceding paragraphs suggest that, in the context of agriculturally dominated Northern Ohio, wetland restoration efforts should focus on the parallel goals of creating biodiverse ecosystems that provide the necessary functions of nutrient removal and sediment filtering. Bradshaw (1987), Jordan et al. (1987), and Palmer et al (2006) argue that restoration projects should use ecological theory to best design experimental studies that are explicitly designed to advance theory as well as accomplishing management goals. One area of great interest and relevance in ecological theory is the effect of biodiversity on ecosystem function, and specifically the effect of plant species diversity on overall primary productivity and nutrient uptake. Biodiversity and ecosystem functioning (BEF) has a long history of interest (Darwin 1859; Odum 1953; Elton 1958; Tilman and Downing 1994; Kinzig and Pacala 2001; Tilman and Lehman 2002). Experimental findings on this topic are not uniform (Huston 1994), but some studies have found that nutrient uptake and primary productivity are positively related to plant species richness (Tilman and Lehman 2002). Although a great deal of attention has been given to the functional role of wetlands, not much research has been done to compare BEF in wetlands ecosystems (Callaway et al. 2003; Kucharik and Zedler 2011; Thiere 2010).

Comment [JP5]: Not so clear from context whether these are studies that HAVE examined this issue or whether these are studies that have documented that lack of such studies.

BEF studies in wetlands have primarily focused on relationships between macrophyte biodiversity and measurements of macrophyte biomass (Callaway et al. 2003;Kucharik and Zedler 2011) or macrophyte biodiversity and overall rates of nutrient uptake (Thiere 2010). ~~In some cases, it may be useful to~~These studies have not, however [?] draw a distinction between emergent plants and submerged aquatic primary producers. Although coexisting within the same or overlapping physical space, these two different groups of primary producers occupy distinct environments with different access to key limiting resources including nutrients and light. To some extent, these communities can be thought of as competitors for light and nutrient resources; for example, upland plants may have first access to nutrient runoff and emergent plants can out shade aquatic primary producers in competition for light. On the other hand, under some circumstances, submerged aquatic producers, particularly plankton, have the capacity for rapid growth. Consequently, the functional effects of macrophyte biodiversity may be distinct between these two communities. Very few studies have measured the impact of total plant biodiversity on water column primary productivity in wetlands. This study attempts to do this.

Comment [JP6]: Very nice introduction!

The site of this research, the Jones Wetlands, is located in Oberlin, OH. The experimental system consists of six hydrologically isolated wetlands. In 2003 0.18 acre perched wetlands were constructed to be replicates of each other oriented in a west to east row. The ~~overall~~ two primary goals of the project are to compare the long-term effects of different wetland restoration practices on plant biodiversity and to assess the effects of plant species diversity on system function (Petersen 2002). With these goals in mind, three different planting regimes were employed each with two replicates: unplanted self-organizing wetlands, singly planted wetlands, and repetitively planted wetlands.

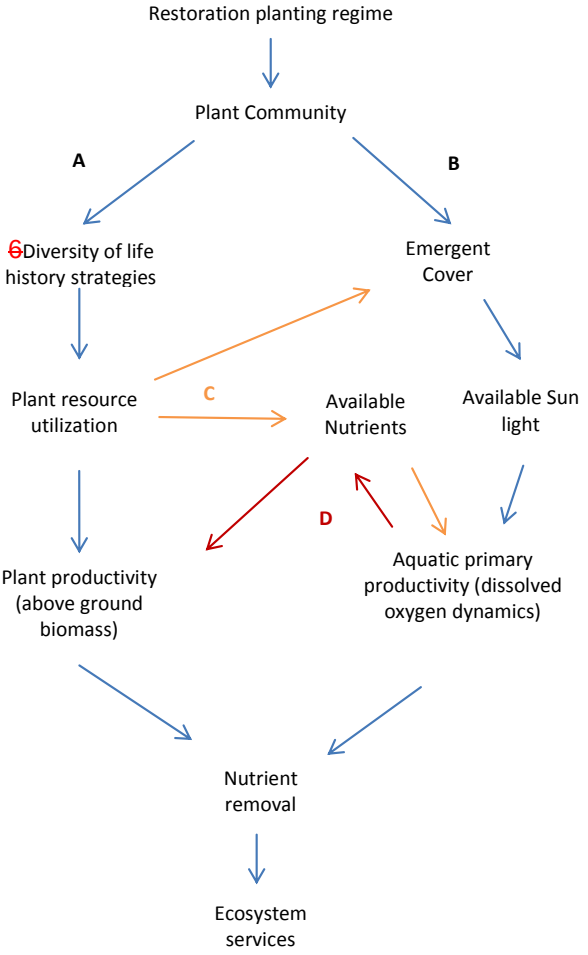
Ongoing research in this system has been used to assess the effect of planting strategy on macrophyte biodiversity and consequential effects of biodiversity on function. Four years after wetland construction, differences in planting regimes have, indeed, shown differences in several different measures of biodiversity (Grossman 2008). Grossman (2008) found that both of the planted treatments resulted in higher plant diversity than that of the unplanted control; but there was no difference between singly planted cells and repetitively planted cells.

Prior work in this experimental system has not revealed differences in ecological function among the wetlands with high and low macrophyte biodiversity. Yet ~~these~~ the hydrologically isolated wetlands that comprise this experimental system have minimal watersheds ~~and are~~ have minimal nutrient input. ~~consequently~~ The fact that they have been ~~highly~~ oligotrophic ~~systems that do not confer~~has minimized the opportunity for ~~the resolution for~~ measuring differences in nutrient dynamics that might reveal differences in ~~ecosystem~~ the functional capacity of the different diversity systems. Grossman found no differences in nutrient dynamics among planted and unplanted cells and generally found that concentrations of dissolved inorganic nitrogen and phosphate were consistently low and often below detection limits (Grossman 2008). Prior to this study no mechanism had been developed to measure primary productivity in the water column and changes in emergent plant biomass had not been assessed. So, while the different biodiversity wetlands may possess different capacities for functional response to nutrients, the initial phase of the project, which focused on creating and documenting different levels of macrophyte biodiversity, was not well suited for ~~there has been no mechanism for~~ assessing ~~these~~ differences in function resulting from this biodiversity. Controlled fertilization of one of each planting regime provides a means of 1) simulating wetland ecosystems that receive direct runoff from upstream

agricultural activity and 2) providing the resolution to assess the effects of planting regime on plant community and ecosystem productivity.

~~Where~~ ~~H~~harvesting above and/or belowground plant biomass ~~may have proved~~is a common ~~a sufficient~~ measure of completely annual primary productivity in ~~some ecosystems~~communities composed of vascular herbaceous plants such as grassland ecosystems [insert reference~~,~~]. A full accounting of primary productivity in wetland ecosystems ~~should include~~must consider production by ~~all~~ submerged aquatic primary producers as well as emergent plants and the opportunities and techniques available for monitoring primary productivity of the submerged and emergent communities differ. In this study, we measured primary productivity in two different ways: 1) changes in macrophyte aboveground biomass was assessed as a measure of annual primary productivity of this community, and 2) patterns of diel change in dissolved oxygen concentration were used to assess primary productivity of the submerged aquatic community ~~productivity via dissolved oxygen dynamics~~. Interestingly, macrophyte biodiversity

Comment [JP7]: We need to be careful about terminology. To me, "aquatic" alone is too vague as it easily covers both emergent and submerged. So I think the appropriate designation for primary productivity is "submerged aquatic" and "emergent".



may affect these two measures of primary productivity via different causal paths. Figure 1 shows the hypothesized causal model of how this study frames the connection between restoration planting regime, macrophyte biodiversity, primary productivity and nutrient removal.

Figure 1: Conceptual model of ~~causality~~relationship between planting, competition between plant communities, primary productivity and nutrient dynamics: Planting regime is an independent variable that drives differences in macrophyte biodiversity (labeled plant community). This diversity in turn affects both macrophyte productivity and submerged aquatic productivity, through two different mechanisms, shown with letters A and B. Mechanism A follows the traditional BEF argument that high diversity means a diversity of life history strategies that utilize resources more efficiently than low diversity systems (see text). Mechanism B suggests that high diversity systems are more probable to have more emergent macrophytes, which alter the extent to which the water surface is exposed to sunlight. An aquatic community limited by sunlight will have lower rates of aquatic primary productivity (see text). Mechanism B and C show potential resource competition between the aquatic community and the macrophyte community (see text). Overall rates of primary productivity are expected to play a major role in nutrient removal –a valuable ecosystem service in agriculturally dominated landscapes like Northern Ohio.

Comment [JP8]: Seems to me that you need a feedback between emergent plant cover and available nutrients just like you have this feedback for aquatic primary productivity. I suggest converting this to a genuine causal loop diagram with arrows and loops labeled + and – as appropriate (not all).

The conceptual model described in Figure 1 shows two mechanisms by which diversity may affect primary productivity and ultimately nutrient removal. Mechanism A describes the relationship between our measures of diversity and macrophyte biomass. High diversity systems are predicted to have a larger variety of life history strategies. A community that has a diversity of ways in which organisms utilize resources is predicted to have more efficient resource utilization overall; the efficiency of resource utilization is equated to higher rates of ecosystem productivity (Tilman and Lehman 2002). Because our measures of macrophyte biomass and macrophyte biodiversity are of the same community, the aforementioned trends in biodiversity and primary productivity were predicted for our experimental system. As these wetlands are nutrient limited, macrophyte biomass is predicted to increase as a result of fertilization. Drawing on the research of Tilman and Downing (1994), increases in macrophyte biomass in fertilized cells were predicted to be positively related to the diversity in these systems.

Comment [JP9]: Life history covers a lot of ground.

Comment [JP10]: Hmm, my sense is that Tilman is mostly saying the diversity causes productivity rather than productivity causes biodiversity

Mechanism B in Figure 1 describes the relationship between our measure of biodiversity and aquatic primary productivity. Like mechanism A, high diversity ecosystems are predicted to have a larger variety of life history strategies. Among these life history strategies are emergent plants that grow in standing water but receive sunlight and exchange carbon and oxygen gases above the water column instead of within it. Simply by probability, higher diversity ecosystems are predicted to have more emergent macrophytes than low diversity ecosystems. We hypothesized that spatial pattern of emergent macrophytes —how patches of plants are located in space and the gaps around them– will play a major role in the rates of aquatic primary productivity. We predict this because the location in which energy is being captured is different between ecosystems with densely populated versus scarcely populated emergent macrophyte communities. In an ecosystem where emergent macrophytes dominate, energy is being captured above the water by emergent leaf cover. On the other hand, in an ecosystem where

Comment [JP11]: There is a literature that distinguishes biodiversity effects between "sampling effects" (the mechanism you are implicitly invoking here) and "complementarity" effects (whole is greater than sum of parts). You should at least address these in passing.

emergent macrophytes are scarce, energy is being captured by phytoplankton and submerged macrophytes below the surface of the water.

When energy is captured above the water gas exchange through photosynthesis (CO2 and O2) is occurring with the atmosphere. When energy is captured below the surface of the water, gas exchange is occurring with the water column. This distinction between the submerged aquatic community and the emergent macrophyte community are characteristics that are important for understanding where the ecosystem is exchanging carbon and oxygen, and how ecosystem function is measured in this study. Indeed, several studies have identified the importance of emergent versus submergent aquatic communities on dissolved oxygen dynamics (Caraco and Cole 2002; Goodwin et al 2008). Both Caraco and Cole (2002) and Goodwin et al (2008) found that dense emergent plant communities meant a higher frequency of anoxia than in sparsely populated emergent plant communities due to light limitation. Because our measure of aquatic productivity is calculated from dissolved oxygen dynamics, emergent plants were predicted to out compete the submerged community for light, therefore limiting rates of productivity in the submerged community.

Mechanisms C and D in Figure 1 describe the relationship of nutrient competition between aquatic primary producers and macrophytes. The abundance and diversity of macrophytes potentially alters the entrance of nutrients and nutrient removal from the water column (mechanism C in Figure 1). Similar to mechanism A, an efficiency of resource utilization via differences in life history strategies, may alter the availability of nutrients that enter the water column. High diversity systems may therefore have the potential to substantially alter the rates of primary productivity within the water column. Importantly, if the macrophyte community responds quickly to a nutrient pulse, then the amount of emergent cover may increase. This increase will limit light availability in the water column, which in turn limits rates of aquatic primary productivity in fertilized cells.

Conversely, phytoplankton in the aquatic community are potentially quicker to respond to a nutrient pulse(mechanism D in Figure 1). Phytoplankton are small primary producers that contribute substantially to aquatic primary productivity in many wetland ecosystems. Phytoplankton are characterized by a large surface to area ratio and a fast turnover rate enabling these organism to quickly uptake nutrients and proliferate. Phytoplankton potentially may indeed outcompete macrophytes for available nutrients. Consequently, macrophyte biomass in fertilized may not change due to nutrient competition with phytoplankton.

We predicted that high biodiversity ecosystems would have lower rates of submerged aquatic primary productivity for two potential reasons: 1) because the water column is more shaded and therefore receives less solar energy to fuel aquatic productivity (see figure 1) and 2) because high plant diversity systems presumably take up nutrients that might otherwise fuel aquatic primary productivity. Depending on whether the aquatic or macrophyte community responded -more quickly to utilize the pulse of nutrients, fertilization could have a positive or negative effect on aquatic productivity. On one hand, rapid utilization of nutrients by the aquatic community would logically produce higher rates of aquatic productivity in fertilized cells. On the other hand, rapid utilization of nutrients by the emergent community would limit sunlight to the aquatic community which would reduce rates of aquatic metabolism in fertilized cells. Consistent macrophyte utilization of nutrients, some studies have found

Comment [JP12]: I have mentioned this before, but we need to consider the effects of decomposition of biomass of emergent plants that takes place within the water column and perhaps also respiration within roots below the water – both of these affect water column oxygen and are part of what we measure. Even if there was NO water column productivity, you might expect anoxic water to result from large carbon input from emergent plants. So net primary productivity is a good measure of in-situ photosynthesis, but the respiration values include the effects of input from the emergent and root respiration/

Comment [JP13]: This is good.

lower rates of aquatic productivity with increased macrophyte cover as one moves from oligotrophic to eutrophic systems (Hagerthy et al. 2010; Grimshaw et al. 1997).

In summary, our study comprised of an experimental system of six hydrologically isolated constructed wetland cells. Four years after initial treatments of different planting regimes has shown differences in macrophyte biodiversity between these treatments, but a lack of nutrients provided little resolution for observing differences in ecosystem function (Grossman 2008). In this study we measured ecosystem structure and ecosystem function before and after simulating agricultural runoff with a single Nitrogen (N) and Phosphorous (P) pulse in the spring of 2010. We measured ecosystem structure in two different ways: 1) macrophyte biodiversity and 2) percent open water. Ecosystem function is measured in three different ways in this experiment: 1) macrophyte biomass, 2) aquatic primary productivity and respiration within the water column, and 3) rate of nutrient removal from water column. We hypothesized that a complex relationship of resource competition between aquatic primary producers and emergent macrohpytes will predict differences in aquatic primary productivity, aboveground macrophyte biomass, and nutrient removal (see figure 1).

Comment [JP14]: Nice summary

Experimental System and Methods:

Experimental System:

The experimental system is comprised of constructed wetlands located in North East Ohio that are a part of the George Jones Memorial Farm at 44270 Oberlin-Elyria Road, New Russia Township, Lorain County, Ohio. Average annual precipitation between 1970 and 2000 was 92.02 cm; the distribution of precipitation was fairly even throughout the year (Grossman Petersen Benzing 2008 needs bibliography citation and NCDC 2004 also needs bibliography). Between 1970 and 2000 mean temperature in January was -4.6*C, mean temperature in July was 22.1*C, and total mean temperature was 9.6*C (Grossman Petersen Benzing 2008). In 2003 six 30m (east west) by 60m (north south) basins (.18ha) were dug with earth movers creating six hydrologically isolated "wetland cells" situated side by side. The cells are numbered 1 through 6 from west to east. Each cell has a permanent rebar grid that divides the wetland into 36 five by ten meter "quadrates"; each rebar has a coordinate signature A-G (east to west) and 1-7 (north to south) used for referencing spatial information. Each cell has a control box with an adjustable weir that can be used to alter the maximum water level in of each cell. At the north end of each cell is a deeper basin approximately 1.5m deep designed to be submerged year round. From the deep end of each cell, the basin inclines steep at first then gradual stretching the 60m length ending in a seasonally wet meadow. This shape is designed to provide habitat for a wide diversity of obligate and facultative wetland plants. The morphology of each marsh was constructed with as little variability as possible both within and among marshes. A study by Brodnare et al. (2003) was conducted prior to planting and showed a slight east to west gradient of SOM, Silt, and Clay measured immediately after construction. The very small watershed area that drains into each wetlands is between .5 and 1.0 ha. The watershed is dominated by annual grasses, is unfertilized, and is mowed once per year.

Comment [JP15]: Get

Immediately following the construction of the Jones Farm wetlands, three planting regimes were implemented: unplanted self-organizing wetlands (Cells 1 and 4), singly planted wetlands (Cells 3 and 6), and ~~repetitively~~ repeatedly planted wetlands (Cells 2 and 5). Figure 2 shows the experimental system and the treatment regime. All cells have been periodically weeded for *Typha latifolia* and *Phaelaris arundinacea*; muskrats have periodically been trapped in all cells to control damage. Four years after

construction, two distinct levels of diversity existed: low diversity in the unplanted cells and high diversity in all planted cells (Grossman 2008). Grossman (2008) also found that nitrate and nitrite concentrations were depleted in all cells by 2007.

Figure 2: Experimental system from above. Six wetlands cells orders one through six from west to east. Original planting regimes are shown in shades of orange. Resulting measures of biodiversity are shown in shade of green. Fertilized wetlands are shown with diversity background color, but with red stripes if fertilized.

Measurements of Ecosystem Structure –Biodiversity and Spatial Pattern:

Biodiversity is measured ~~at the level of all~~for all macrophytes, including submerged aquatic macrophytes but not including other aquatic primary producers such as phytoplankton and benthic algae. Five indices of macrophyte diversity as described by Grossman (2008) were used to assess changes and difference in biodiversity between 2004 and 2008. Although biodiversity data were collected for 2009 and 2010, these data have yet to be analyzed. Grossman (2008) found that wetland biodiversity was stable from 2006 to 2008. For the purpose of this study we assumed that biodiversity continued to remain stable through 2010. Therefore, Shannon Weaver biodiversity used in this study is from 2008.

Spatial pattern was assessed by identifying patches of plants and open water form aerial photographs. Aerial photographs of the Jones Wetlands were taken from a camera mounted on a balloon in the growing seasons of 2008, 2009 and 2010. Photographs from 2008 were imported into GIS, georeferenced and rubbersheeted to lay directly on top of GPS measurements taken at each grid rebar. Polygons (areas characterized by a defined cover type) were assigned to four different cover types: *Nymphae*, *Juncus*, other emergent macrophytes (not including *Nymphae* and *Juncus*), and open water. *Nymphae* was planted in the high diversity cells and only exists in these cells while the dominant *Juncus* species in all cells, *Juncus effuses*, self-recruited in all cells. With polygons assigned to all cover types, each grid cell of the wetland landscape was assigned a numerical value based on their respective cover type for computing spatial statistics.

Polygons were first assigned manually in ARC GIS (see Figure 3). In order to standardize the delineation of macrophyte patches, the area of each patch was delineated by hand to a degree of fine detail. Following the hand delineation, the patches were aggregated and then simplified by standardized normalization parameters at a relaxed degree of precision. As long as hand delineation was at a degree of precision higher than the precision of the normalization, the result of the normalization would not be biased by hand delineation. The values for the normalization process are reported in Table 1. FRAGSTATS was then used to measure several indices of spatial heterogeneity.

Aggregate Polygons Parameters

Aggregation distance (m)	Minimum Area (m2)	Minimum Hole (m2)
0.25	0.5	0.25

Simplify Polygon Parameters

Simplification Algorithm	Maximum Allowable Offset	minimal Area	Keep Collapsed Points
point_remove	0.1	0	no

Table 1: Parameters in GIS used to standardize polygon shapes.

Comment [JP16]: Not so much info in here that you really need a table and formatting of table is confusing; I suggest moving this content into the text description

Although several measures of spatial pattern were analyzed in FRAGSTATS, the amount of open water proved to be the most important result to report in the context of this study. From these data, percent open water is the percent of the entire wetland area that contains standing water that is not shaded by upright or floating emergent plants; open water is water that receives direct overhead sunlight.

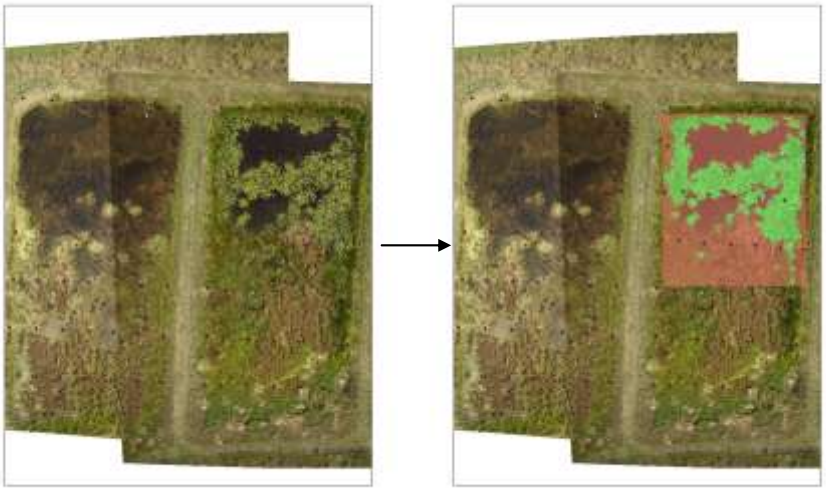


Figure 3: Example of process of using aerial photographs to assign polygons to spatial characteristics.

Although aerial photographs ~~in~~ were taken in years 2009 and 2010, these data have yet to be processed and analyzed. In order to compare recent measured of ecosystem function to measures of spatial pattern, we analyzed potentially proxies for 2010 percent open water –our best measure of sun exposed water – including emergent cover and re-use of 2008 percent open water. Figure 4 shows the spatial patterns determined from aerial photographs in 2008.

Comment [JP17]: Need to include dates on which photographs were taken.

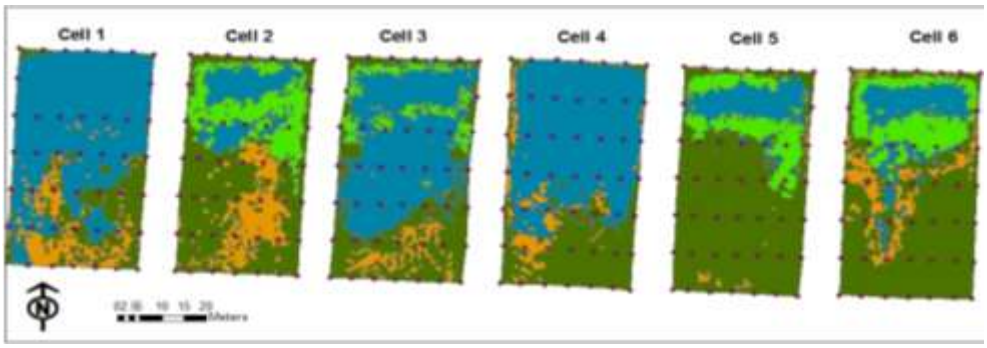


Figure 4: Spatial pattern of wetland cells in 2008. Note that high diversity cells, 2,3,5, and 6, have less open water (blue) than low diversity cells (1 and 4). Cells 2,3, and 4 were fertilized.

Choice of Experimental Units for Fertilization:

Cells were selected for fertilization treatment by first grouping cells into pairs based on similarity in levels of macrophyte diversity and ~~with similar~~similarity in percent open water. One of each pair was then randomly selected for fertilization. Biodiversity indices for the Jones wetlands taken in 2008 (Grossman 2008) were used to pair wetlands by similar biodiversity indices (see Table 2). Shannon Weaver diversity of wetland plants was chosen as an appropriate index as it accounts for richness and evenness, but does not weigh rare or specialist species more heavily. Measurements of spatial pattern of the Jones wetlands from aerial photographs taken in [insert specific date] of 2008 data was used to pair wetlands by similar percent open water (see Table 2). Based on this assessment, fertilizer was added to cells 2, 3, and 4. This then allowed wetland primary productivity and nutrient uptake to be compared between fertilized treatments an unfertilized control of both similar diversity and similar amount open water

Table 2: Choice of units for fertilization

Cell	Diversity 2008	Open water (ha) 2008
1	2.1	0.12
2	2.4	0.02
3	2.3	0.09
4	2	0.11

5	2.4	0.02
6	2.5	0.03

Table 2: Yellow highlights on cell numbers indicate the wetlands that were fertilized. Closest diversity values are paired (orange, blue, green). One of each of these pairs was fertilized (yellow). Closest hectares of open water values are paired (dark green, dark blue, red). One of each of these pairs was fertilized (yellow).

Choice of Fertilizer Load to Simulate Agricultural Runoff:

Based on a wide variety of load values of N and P in agricultural runoff (see Tables 3 and 4), we simulated runoff from a watershed dominated by conventional corn and soy rotation agriculture. Based on literature values of wetland area:watershed area ratios (see Table 3 in Apendix), each 0.18ha wetland would 'treat' a 4.5ha watershed. Proposed nutrient loads were calculated by multiplying the average agricultural runoff load (Tables 4 and 5 in appendix) by the wetland:watershed treatment ratio(Table 3 in appendix): 8.685kg elemental-P and 59.4 kg elemental-N to each of the fertilized cells (Table 6). After converting these loads to the equivalent weight in commercial fertilizers –84.1 lbs monoammonium phosphate ($NH_2H_2PO_4$) and 264.57 lbs Urea ($(NH_2)_2CO$) per fertilized cell– we decided to apply half of this calculation to make fertilization logistically feasible; this equates to a loading of nutrient runoff from 2.25 ha watershed drainage basin that is entirely in agricultural production. A summary of the fertilizer loads is provided in Table 6. Although most agricultural runoff N is in the form of nitrate that has nitrified from the previous year's application (McCartney, personal communication), we assumed that the urea we added in our experiment was quickly decomposed into ammonium, which is subject to rapid conversion to nitrate via nitrification.

Comment [JP18]: We need to specify what these ratios from the literature represent – whether these are normal ratios found in watersheds or whether these are ratios assumed in other experiments. What are we assuming in terms of fertilization per Ha of agriculture?

Comment [JP19]: This table is actually not very long – I suggest moving it back into the body of the text.

Comment [JP20]: Be specific, what is the wetland area:watershed area that this represents and how /why did we select this particular ratio. For example, is it in the middle of the range?

Table 6: Fertilization summary

Parameter	Value
Wetlands Cell Area (ha)	.18 ha
Simulated treatment watershed area (ha)*	4.5 ha
Proposed load of elemental-P per cell (kg)**	8.685 kg
Proposed load of elemental-N per cell (kg)**	59.4 kg
Applied load of elemental-P per cell ***	4.325
Applied load of elemental-N per cell ***	29.7 kg
Commercial Monoammonium Phosphate applied (lbs) ****	42.06
Commercial Urea applied (lbs) ****	132.29

Table 6: Summary of results and calculations for choice of fertilizer loads. *Simulated treatment watershed area was calculated by dividing the area of each cell by the average wetland to watershed treatment area listed in Table 4. **kg loads per cell were determined by multiplying the simulated

treatment watershed area by the average loading value for each nutrient listed in tables 2 and 3; this is an annual value. *** Applied load is ½ proposed load for logistics. ****Commercial loads were calculated using the fraction of elemental mass to commercial fertilizer mass, and then converted into lbs.

Comment [JP21]: Need to remove asterisks

Application of Fertilizer:

We altered the hydrology of each wetland cell to ensure that a heavy rainfall following fertilization could not raise the water in the wetlands above the effluent weir level allowing fertilizer to be lost. Two days before the fertilization, we lowered the adjustable weirs one stop to lower the water level well below the height of the weir when it was replaced to the original height.

Comment [JP22]: This is not going to mean anything to anyone but you and me. Need to estimate inches or volume.

Both of the fertilizers we added break down to release nitrogen that is initially in the form of ammonium (NH4). This is potentially problematic because at high concentrations and at moderate to high pH ammonium ~~and~~ will be converted to ammonia which can then leave the system as a gas. To prevent this, the day before fertilization, a commercial agricultural product called Agrotain™ was mixed with urea according to directions on the container: 5 quarts of Agrotain™ per ton of urea. Agrotain™ is an agrochemical designed to block ammonia volatilization.

Each manipulated cell was fertilized within 24 hours of each other on June 18th and 19th except for 30lbs of MAP for cell 3 that was initially over looked and not spread until June 29th. We spread fertilizer evenly across each 30m by 60m basin by walking a zig-zag pattern at a constant speed using a handheld grass seed spreader to distribute the chemicals.

Nutrient and Water Quality Parameters Data:

Rate of nutrient removal was measured from the water column; therefore, nutrient removal was measured in two ways. First, the difference was calculated between total nitrogen and phosphorus added to the wetlands as fertilizer and the amount that actually appeared in the water column. Next, rates of depletion within the water column were calculated. In this second case, the measures of water column depletion include removal by all communities that have access to nutrients through the water column: phytoplankton, benthic and macro algae, submerged macrophytes, emergent macrophytes, and heterotrophic decomposers.

Water samples were collected from each wetland on a weekly basis for inorganic nutrient analysis during the growing season. Immediately before and after nutrient addition water samples were taken from all wetlands twice a day for two days. For nearly a month following the nutrient addition, samples were taken daily (see Figure 5 in appendix). Weekly water samples resumed following the month of daily sampling. The process of analyzing each water sample was that 40 ml of sample was filtered through 47mm glass microfiber filters and frozen in two 20ml scintillation vials for later analysis. Upon analysis, anion concentration of Cl^-, NO_2^{2-}, PO_4^{3-}, NO_3^-, and SO_4^{2-} was determined using a Dionex™ ion chromatograph; NH_4^+ concentration was analyzed with an Orion ammonia probe; procedures for ion chromatography and NH_4^+ are described in Grossman (2008).

Nutrient depletion rates (r) for each experimental wetland were calculated by fitting the decline in nutrient concentration in that wetland to an exponential decay function (Concentration $C(t)=C_oe^{-rt}$) based on an initial concentration measured on the date of the peak concentration in each wetlands following nutrient edition. Exponential decay curves were fit by minimizing the sum of the squares of the residuals between

the modeled data and the actual data by changing the value for the decay constant, r. The absolute value of the decay constant, r, was reported for ease of comparison between fertilized cells.

The volume of water in each wetland had to be estimated in order to convert concentrations into numbers for absolute nutrients present in the water. The volume of water in each wetland was calculated using ~~the~~ bathymetry data collected in 2005 by Stenger (unpublished data). Relative depth measurements from the bathymetry data were converted into water depth measurements by zeroing the relative depth measurements to water extent data taken on the day before fertilization. Then, water depth measurements were numerically integrated in Excel per column first and then across columns. Multiplying the volume calculations by the peak concentration of nutrients measured after fertilization provided an estimate of the peak amount of fertilizer present in the water column. The difference between the actual load applied and the calculated peak water column concentration is as measure of fertilizer rapidly removed in the dry land portion of the cell and the wetted portion.

Comment [JP23]: This needs to be further explained for reader – they won't understand

At each nutrient sampling event, pH, redox potential, total dissolved solids, conductivity, dissolved oxygen, and water temperature were measured and recorded with a YSI model xxx multi probe (Yellow Springs Instruments). Figure 5 (Appendix), graphically displays sampling dates, dates with good data, and other key events within the experimental timeline.

Comment [JP24]: Insert info

Aquatic System Metabolism:

The metabolic activity occurring within the water column and sediments was assessed using in situ dissolved oxygen probes; measured changes in oxygen were used to calculate primary productivity and respiration of the entire aquatic community including: phytoplankton, benthic algae, submerged macrophytes, and benthic and planktonic heterotrophs. In situ dissolved oxygen probes were deployed in each of the wetland cells on June 14 2010. Dataloggers were programmed take readings every 15 minutes for the duration of the summer. Figure 5 (Appendix) shows for what periods of time data was deemed reliable from this data set. Probes in each pair of adjacent wetland shared a battery charging and data logging station ~~to share~~. Dissolved oxygen probes were tethered to flotation buoys approximately 5m from either the east or west berm and 10m from the northern berm. The one foot tether placed the probes approximately one foot below the water surface in the deepest section of each wetland cell. Oxygen probes were cleaned and calibrated ~~about~~ approximately ~~every~~ once per week until retrieval on September 4, 2010.

Photosynthesis and respiration in the water column and benthos were assessed by tracing diurnal fluctuations in dissolved oxygen in the water column. Change in dissolved oxygen over the night was attributed to respiration (R) as no photosynthesis is occurring without sunlight. Change in dissolved oxygen during the day was attributed to a net of the processes of photosynthesis and respiration that both occur during the day –Net Primary Productivity (NPP). A literature value for the minimum amount of light needed for photosynthesis (Ryther 1956). was used to calculate when change in dissolved oxygen dictated NPP; when PAR ≥1.46, change in dissolved oxygen was deemed NPP, when PAR < 1.46, change in dissolved oxygen ~~meant~~ was interpreted as R. Respiration was calculated for every 15 minute interval until change in dissolved oxygen was no longer linear due to hypoxic conditions: below 2mg/L. NPP was calculated for every 15minute interval during the day. Because the data monitoring system produced outlying data, Median R and NPP rates were used to represent the central tendency for each day. The absolute amount of photosynthesis before any amount of carbon is respired, or Gross Primary Productivty (GPP), was calculated by adding NPP to an average of the median rate of respiration from the night

Comment [JP25]: Not clear if this is minimum for net photosynthesis (integrated over the water column) or any photosynthesis.

Comment [JP26]: What light units are these? uEins/m2/sec?

before and the night following that day. After calculating GPP for each 15-minute NPP measurement, the median was used to find a central tendency for each days GPP data.

The accumulation of fixed carbon from day to day, or Net Ecosystem Productivity, was calculated by subtracting the peak dissolved oxygen concentration in day 2 from the peak concentration of dissolved oxygen in day 1. Positive NEP was ~~attributed~~ interpreted as ~~to~~ net accumulation of fixed carbon within the water column, while a negative NEP was ~~attributed~~ interpreted as~~to~~ a net loss of fixed carbon.

For the purpose of analyzing the response of the system to nutrient addition, metabolic data were averaged over various intervals ~~so~~ and then compared among treatments. After calculating daily metabolic dynamics, each of these measures were averaged over a set of time intervals (time bins) created based on 1) the rate at which nutrients became nominal in the fertilized cells and 2) when nutrient samples were available for comparison. Figure 5 shows the time periods ~~where~~ over which data ~~is~~ are binned.

The strength of the relationship between rates of NPP and rates of R provides a measure of the importance of heterotrophic respiration within the aquatic community. The strength of the NPP:R ratio was calculated by determining the square of the Pearson moment correlation coefficient (r^2) of daily NPP and R measurements regressed over the same time bins as other dissolved oxygen dynamics.

Comment [JP27]: This statement really requires the discussion of macrophyte contributions to water carbon that I suggest for the introduction.

In conjunction with dissolved oxygen data, temperature, light, and humidity sensors were deployed on the berm in between cell 3 and 4. These data loggers were programed to collected data every 5 minutes on the hour for the rest of the duration of the summer. After averaging the light data over fifteen minute intervals, light data was used as a conditional to calculate either NPP or R.

Comment [JP28]: Need to say something about conversion of light data

Biomass:

In this experiment, macrophyte biomass is an aggregate measure of all macrophyte -tissue that is above-ground, including submerged macrophytes. Macrophyte biomass was sampled in all cells as baseline data in mid-August of 2009 and post fertilization data in late August 2010. $1m^2$ plots were harvested at the corners of rebars b3, f3, b5, f5, b7, and f7. Opposite corners were selected for harvest in the two years [?]. Figure Y shows the harvested areas. All samples were placed into plastic bags and then dried. After drying for 24hrs at 105°C in a drying oven, plant material grouped by sampling plot was massed. Change in biomass per cell from 2009 to 2010 was calculated in order to measure the response to fertilization.

Comment [JP29]: ?

***Statistical** Analysis:*

Analysis of variance (ANOVA) was used measure differences between treatments: fertilized vs. unfertilized and high diversity versus low diversity. This analysis was done for: 2009 aquatic GPP, 2009 above ground biomass, 2010 aquatic GPP, change in turbidity, change in above ground biomass and change in emergent cover. Pearson moment correlation coefficients were used to determine significance of X:Y regresions: percent open water vs. diversity, aquatic GPP vs. percent open water, percent emergent cover vs. percent open water, aquatic GPP vs, and percent emergent cover. Table 7 reports the Pearson r values needed to meet a 0.05 significance based on the number of samples.

Comment [JP30]: Change over what time period?

With such a small degree of replication it is difficult to discern if the data meets the assumptions needed ANOVA to determine significance between groups or the Pearson product moment correlation coefficient (r) to calculating the correlation between dependent and independent variables. In this study I have appealed to the literature to make the case that in general these types of data sets do indeed meet the assumptions needed for these statistical techniques. Furthermore, in place of an ANOVA, randomization

tests can be used without the assumption of normal distribution. Like other statistical techniques the Randomization test loses power when the sample size is low; each randomization test is reported with the best possible p value based on sample size and the p value of the result.

Sample size (n)	Pearson's r	r^2
3	0.997	0.994
4	0.950	0.903
5	0.878	0.771
6	0.811	0.658

Table 7: Two tail critical point, $p<.05$, for Pearson correlation coefficient and r^2 values based on number of samples in the regression. In order for a regression to be significant to 95% confidence, the Pearson r value must be above the reported value. Table adapted from (Currell and Dowman 2005).

Results:

Baseline data six years after construction:

After measuring five days of diurnal dissolved oxygen dynamics with a handheld probe in July 2009, unplanted low diversity cells were found to have higher aquatic GPP than planted high diversity cells (n=2,4; p = 0.027) (Figure 6). Percent open water in 2008 was found to have a significant negative correlation to macrophyte biodiversity measured in 2008 (See Figure 7); this correlation is significant (n = 6; Pearson r = -0.989, see Table 7). We found that aquatic GPP was significantly positively correlated to percent open water (Figure 8) (n= 6; Pearson r =). We found there to be no difference in macrophyte biomass between treatments (Figure 9) (n=2,4; p = 0.573).

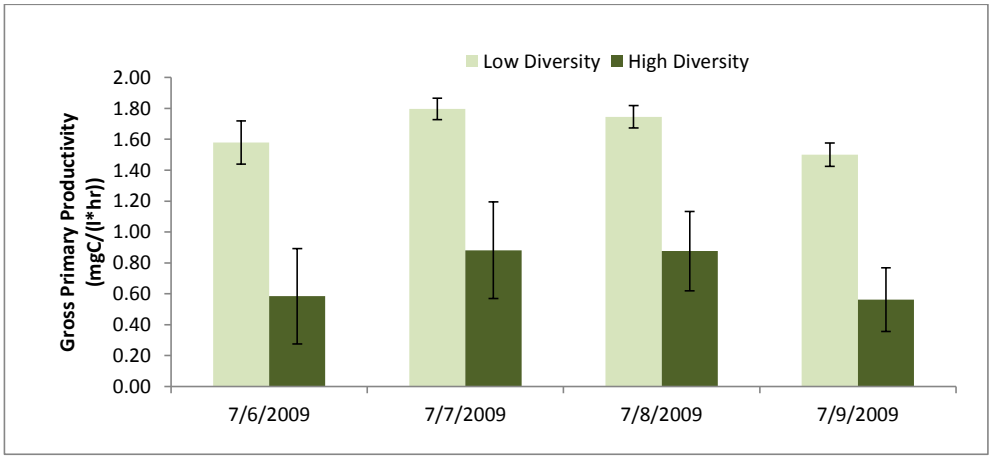


Figure 6: Four days of aquatic GPP the summer before fertilization averaged by diversity groups. Error bars for low diversity show range as there are only two replicates; error bars for high diversity are standard deviation: three replicates.

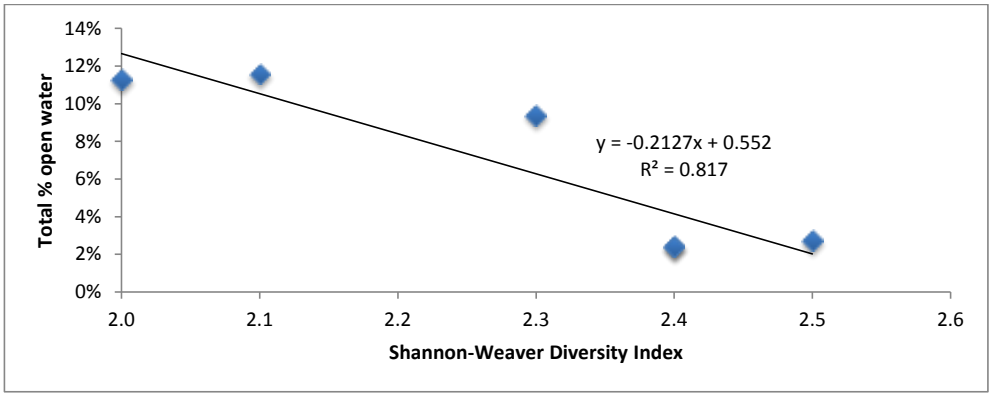


Figure 7: Percent open water measured via 2008 aerial photographs as a function of diversity measured in 2008

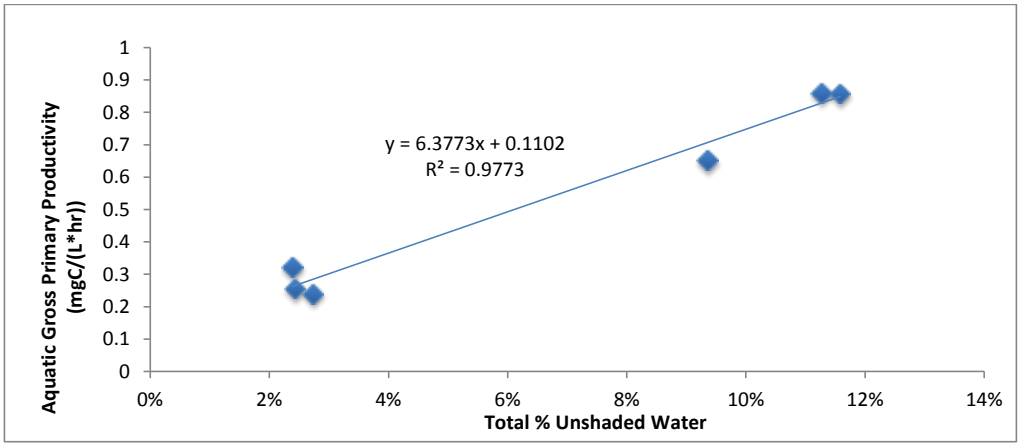


Figure 8: 2009 gross primary productivty as afunction of 2008 percent open water.

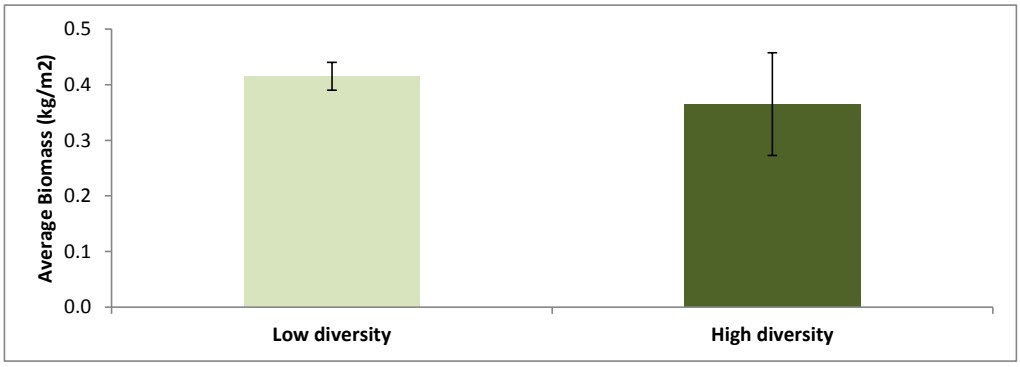


Figure 9: Summer before fertilization, 2009, aboveground macrophyte biomass averaged by diversity groups. Error bars in low diversity group represent range as there are only two replicates; error bars in high diversity are standard deviation as there are three replicates.

Finding proxies for post fertilization measures of ecosystem structure:

Grossman (2008) found that measures of biodiversity from years 2005 to 2008 have remained stable (Figure 10). Because percent emergent plant cover is measured through observation in select sampling quadrates, this measure does not exactly equate to 1 – percent open water. The best measure of sun exposure to the aqautic community in 2008, percent open water, was found to be negatively correlated to an alternative measure of percent emergent plant cover in 2008 (n= 6; Pearson r = -0.977; see Table 7) (Figure 11).

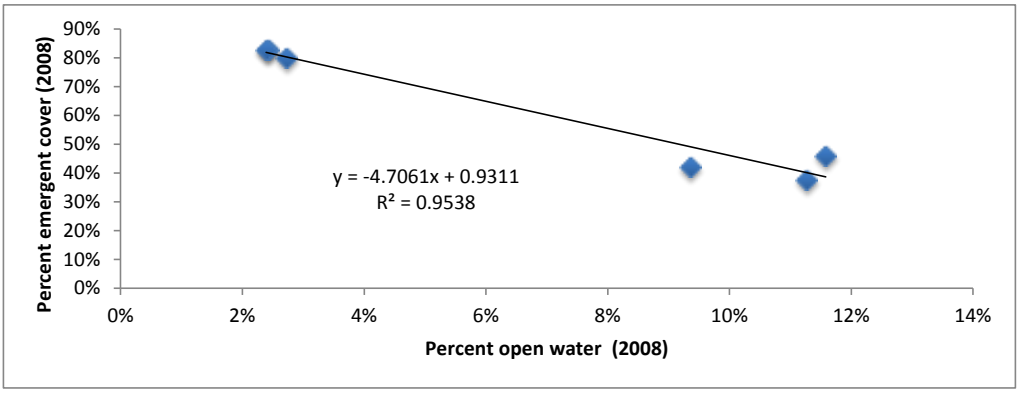


Figure 11: Observed percent emergent cover as a function of measured percent open water. Both measuresments were taken in 2008.

Comment [JP31]: Probably need to explain "observed" again.

Structure and aqautic GPP after fertilization (2010):

Before fertilization, we found no difference in GPP between the "to be fertilized cells" and the "to be control cells" (n = 3,2; p = 0.615). In general, all fertilized cells observed a significant decrease in GPP, NPP, and R after fertilization (Figure 12)(n= 3,2; p = 0.037).

To test the hypothesis that aquatic GPP is limited by the amount of sunlight that reaches the aquatic community, we compared rates of aquatic productivity to different measures of emergent cover. We found no correlation between percent emergent cover and aquatic GPP after fertilization (Figure 13). In addition, when comparing fertilized cells to unfertilized cells, unfertilized cells ~~lay~~ had much higher GPP than fertilized cells regardless of differences in percent emergent cover (Figure 13). Relative abundance of phytoplankton can often be observed thorugh measures of turbidity. We found that the fertilized cells were significantly more turbid than unfertilized cells (n=3.3; p = 0.002) (Figure 14).

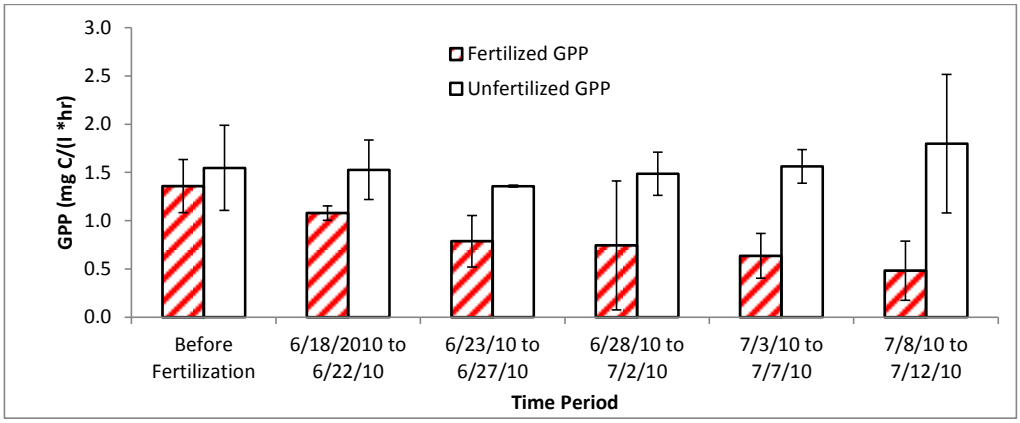


Figure 12: Aquatic GPP immediately before and after fertilization. Time binned averages are shown. Fertilized error bars are standard deviation: three replicates; unfertilized error bars show the range: two replicates.

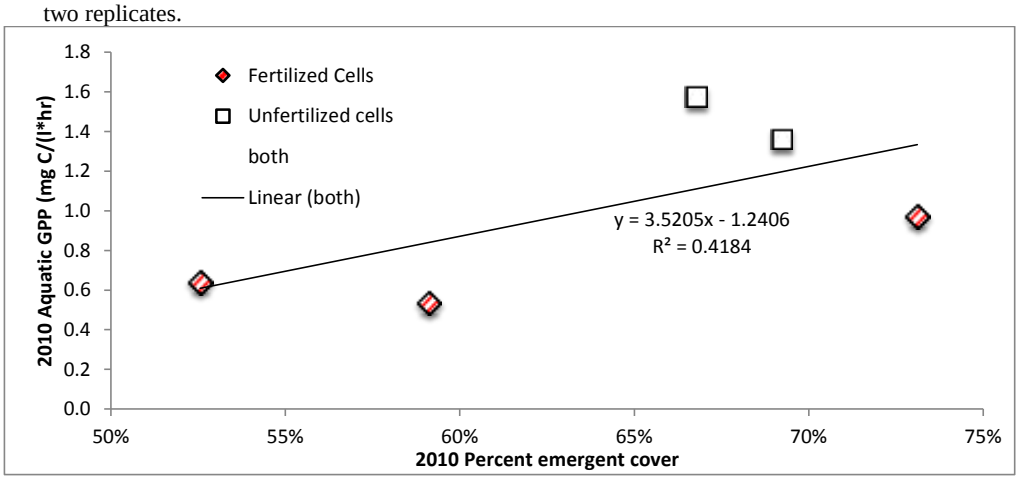


Figure 13: Aquatic GPP as a function of percent emergent cover after fertilization, 2010. Regression is made with both fertilized and unfertilized cells.

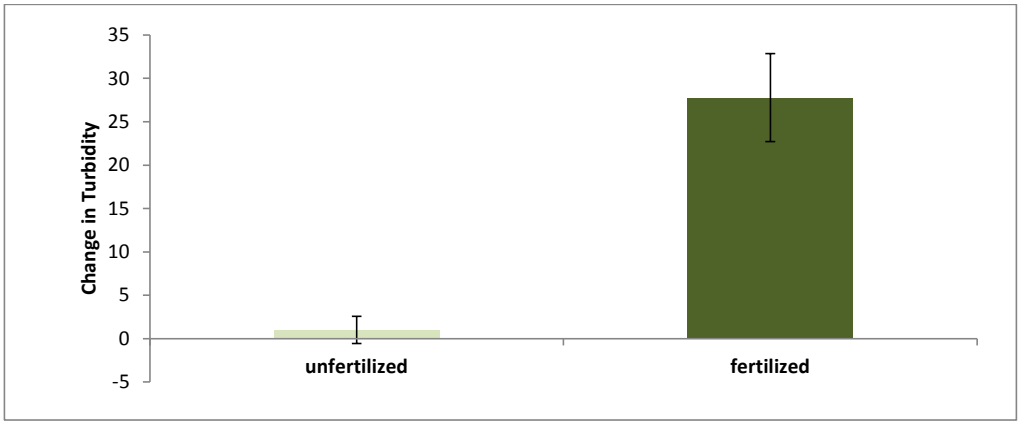


Figure 14: Change in turbidity from one month immediately before fertilization to one month after fertilization averaged by fertilization treatment. Error bars are standard deviation.

Structure and macrophyte primary productivity:

We found no difference in change in biomass from 2009 to 2010 between fertilized and unfertilized cells (n = 3,3; p = 0.19) (Figure 15). Similarly, we found no difference in emergent cover from 2009 to 2010 between fertilized and unfertilized cells (Figure 16).

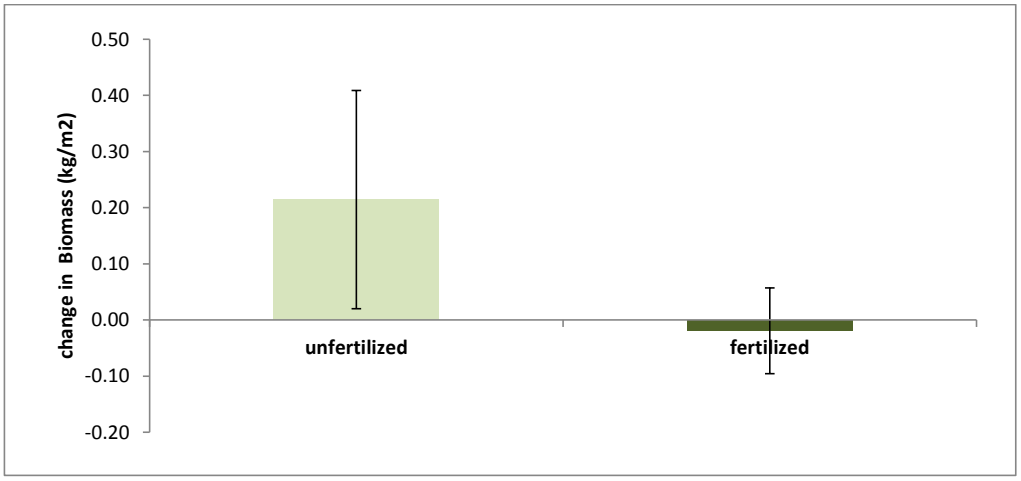


Figure 15: Change in biomass. End of season biomass in 2009 subtracted from end of season biomass in 2010 averaged by fertilized cells and unfertilized cells. Error bars are standard deviation.

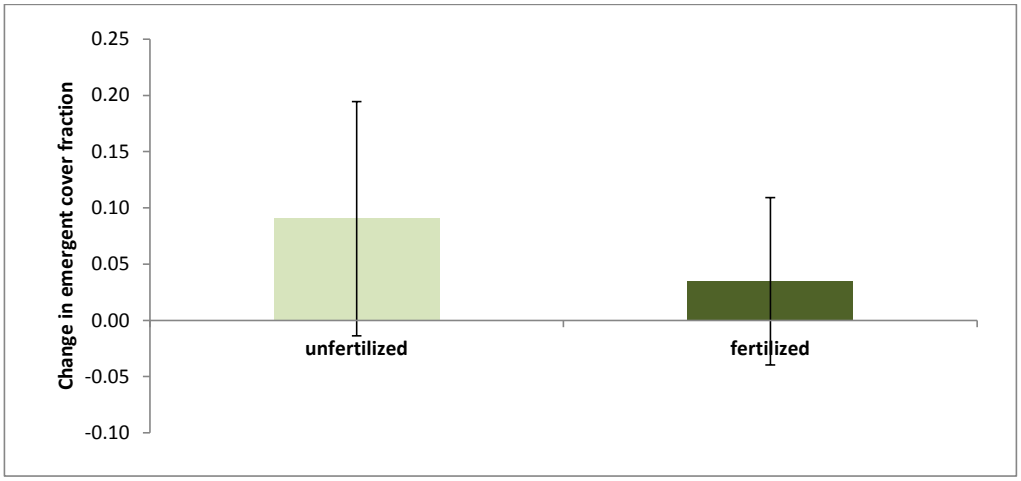


Figure 16: Change in emergent cover fraction calculated from emergent cover data in 2010 minus emergent cover data in 2009. Error bars represent standard deviation.

Nutrient uptake:

The absolute value of the decay constant for both N and P were not found to be correlated to SW diversity, change in biomass, change in aquatic GPP or change in percent emergent cover. Using the peak concentration and the bathymetry data, percent of load added to land and open water is reported in Table 8. Amount of water column N or P load were not causally related to change in aquatic GPP or change in macrophyte biomass.

Cell	Diversity	Water Volume (l)	Total Elemental N (kg)	Total Elemental P (kg)	Max [DIN] (mg/L)	Max [PO4] (mg/L)	Water Column N Load (kg)	Water Column P Load (kg)	Land N Load (kg)	Land P Load (kg)	% Total N received by water Column	% Total P received by water Column	Absolute value of N decay constant, r	Absolute value of P decay constant, r
2	High	660000	29.7	4.3	11.9	5.4	7.8	3.5	21.9	0.8	26%	82%	0.04	0.20
3	High	860000	29.7	4.3	4.0	9.1	3.4	7.8	26.3	-3.5	12%	181%	0.10	0.22
4	Low	350000	29.7	4.3	28.2	1.0	9.9	0.4	19.8	4.0	33%	8%	0.09	0.24

Comment [JP32]: I'm confused, how can we have 181% of P received by the water column? Something can't be correct here

Table 8: Summary of nutrient loading data.

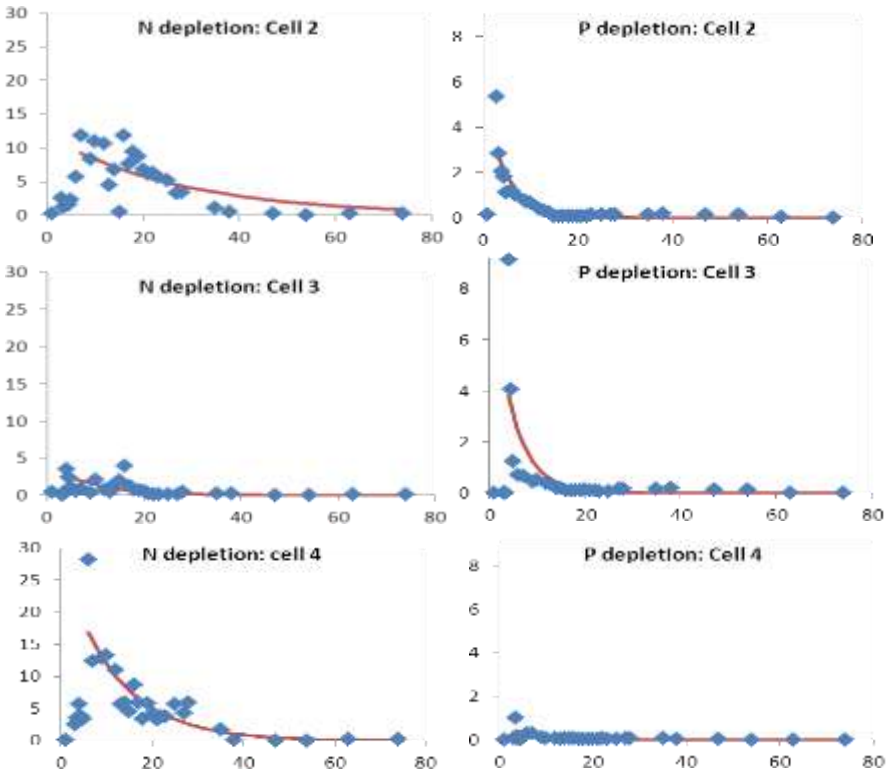


Figure 17: Nutrient depletion in all fertilized cells. Data points are shown in diamonds, fitted curves are shown with the line. All x axes are in days since fertilization. All Y axes are in mg/l.

Discussion:

The goal of this research was to simulate wetlands that receive nutrient loads from agricultural runoff and assess this response by understanding the relationship between restoration planting regime, plant biodiversity, measures of primary productivity, and measures of nutrient removal. Figure 1 within the introduction proposes two mechanisms by which biodiversity affects either above ground biomass

(mechanism A in figure 1) or aquatic primary productivity (mechanism B in figure 1). Results from the summer before fertilization, 2009, did not support the mechanism affecting the diversity of resource use (A) proposed in figure 1, but did support the mechanism affecting light availability (B). Results from the fertilization experiment, 2010, were inconclusive with respect to both mechanisms A and B. Figure 1 also proposes that the submerged aquatic community may compete with the macrophyte community for both light (mechanism B) and nutrients (mechanisms C and D). While the system was oligotrophic in, 2009, we could not expect to observe nutrient competition between macrophytes and aquatic primary producers (mechanisms C and D, figure 1). After fertilization, 2010, results were inconclusive with respect to the importance of aquatic versus emergent nutrient utilization.

Macrophyte biomass results from the summer before fertilization, 2009, were not consistent with the hypothesis that high diversity cells with a variety of life history strategies would be more effective at utilizing available resources for productivity (mechanism A, Figure 1). Above ground macrophyte biomass was not found to be different between high diversity and low diversity cells (Figure 9). An accepted explanation for this result is described by Huston (1994). Current dialogue in BEF theory is focused on two hypotheses: the complementary niche effect (Tilman and Downing 1994) and the sampling effect and (Huston 1994). The complementary niche effect ~~explains~~ assumes that a diversity of life history strategies will lead to an additive utilization of resources; this additive effect of several different niche types is equated to higher rates of primary productivity. The sampling affect hypothesis says that a more diverse ecosystem will, by probability, support more life history strategies that are individually more productive than other life history strategies. This hypothesis suggests that a highly diverse ecosystem can only be as productive as its most productive species. Therefore, Huston (1994) explains that the particular species composition alone may be a more important driver for measures of ecosystem primary productivity. In this case, there are several macrophyte species that are dominant in all ecosystems: *Juncus effuses, Ceratophylum, Elodea,Leerzia,*and *Phalaris arundinacea*. Assuming no complementary niche effect, the productivity and relative abundance of these dominant species may play a larger role on measures of macrophyte biomass than that of pure plant biodiversity in our experimental system.

Aquatic primary productivity results from the baseline data collected the summer before fertilization, 2009, were consistent with the hypothesis proposed in mechanism B (figure 1). High diversity cells had a higher abundance of emergent macrophytes which limited available light to aquatic primary producers; lower light levels meant lower rates of photosynthesis and respiration in the water column. Results from the aerial photograph analysis were consistent with the hypothesis that high diversity cells have a high density of emergent macrophytes (Figure 4); percent open water measured in 2008 is inversely correlated to diversity measured in 2008 (Figure 7). Aquatic GPP data from 2009 was consistent with the hypothesis that differences in aquatic metabolism is dominated by the availability of solar energy measured by percent open water; Figure 8 shows a strong positive correlation between aquatic GPP and total percent open water. These results suggest that there is a relationship between total plant biodiversity and aquatic GPP that is mediated by the amount of light that enters the water column.

The species composition found in high diversity cells ~~does~~ is, indeed, ~~provide distinct~~associated with distinct rates of aquatic productivity when compared against the low diversity cells. The term species composition is intentionally used because the amount of open water is the mechanism that we believe controls aquatic productivity not the diversity. Therefore, any combination of plants that create a low

Comment [JP33]: Reexplain mechanisms so that reader does not need to go back.

Comment [JP34]: See comment in introduction – good to introduce these distinctions there.

Comment [JP35]: Are you talking about niche or life history strategy, or are you using these terms interchangeably?

Comment [JP36]: But do we know that these are the most productive? There are also several dominant species only present in the high diversity cells – arrow head and water lily for instance. So I am not sure I follow your logic.

Comment [JP37]: But isn't this a function of biodiversity? If biodiversity results in a plant community that covers that water, then isn't this a function of biodiversity? Isn't that part of your model?

amount of open water could produce similar results. For example, a monoculture of *Nymphae* (emergent water lilies) may create very low rates of aquatic productivity, while a collection of *Ceratophylum, Elodea, and Naias* (submergent plants) would create high rates of aquatic productivity. In this case, high biodiversity simply provides the probability of including more emergent species. Indeed, it has been argued by Huston (1994) that high diversity systems simply provide higher probability of including individual species that fit the functional characteristic being measured.

Comment [JP38]: I'm not sure how deeply it makes sense to get into this debate. Some people argue that complementarity and sampling are both important components of species diversity. On one hand you have made the case that that species common to planted and unplanted cells may dominate and account for lack of differences in biodiversity, but then you are arguing that certain species in the biodiverse wetlands dominate and are a sampling effect., These arguments don't appear to be consistent to me.

While the system was oligotrophic, making distinctions between emergent community nutrient use and aquatic community resource use (mechanism C and D, figure 1) was not possible because nutrients were essentially never seen in the water column (Grossman 2008). Differences in competitive ability may still exist at an oligotrophic state, but we could not observe these nutrient flows without other techniques.

After fertilization, 2010, we found that the nutrient pulse affected rates of aquatic primary productivity; but the results were inconclusive with respect to which mechanisms (A,B,C,and D, figure 1) were most important. One of the problems we faced was a lack of recent measurements of ecosystem structure. Although biodiveristy and percent open water data for the years 2009 and 2010 had been collected, these data were not processed because of time constraints. Consequently, measures were taken to justify the use of proxies for these data in order to investigate the relationship between ecosystem structure and function after fertilization.

Grossman (2008) found that macrophyte biodiversity was stable from years 2005 to 2008. We used this data to make the claim that macrophyte biodiversity continued to be consistent thorugh 2011. We must be careful when claiming that biodiveristy remains stable after a large pulse of nutrients, but since changes in biodiversity are not expected to occur after one season (Benzing, personal communication) then this justification may be safe.

Percent open water measured by analyzing aerial photographs with GIS software provided the best estimation of the amount of aquatic community that is exposed to light. Percent emergent cover measured by observation during biodiversity surveys ~~seemed to provide an accurate proxy~~was strongly related to exposed water and therefore may serve as another good measure (Figure 11). Yet, we hestitate to consider percent emergent cover as a perfect inverse to amount of open water because how these two measurements were made. Emergent cover was done observationally in 9 of 18 total sample plots. Percent emergent cover was estimated by sight in each of the 9 sample plots and then averaged by cell. Because some of these plots are in seasonally dry areas of the wetlands, emergent cover may be over represented in the average by these upland sections. On the other, hand open water measurements from aerial photographs is an accurate measurement of where light can penetrate the water column from the vantage point of the sun. Future analysis should use up-to-date aerial photographs to get the best measure of open water.

With these caveats for measures of ecosystem structure in mind, we investigated the role of ecosystem structure on macrophyte aboveground biomass, aquatic primary productivity and rates of nutrient uptake after the fertilization event. Of the total fertilizer applied a median of 74% N and 12%P (Table 8) was calculated to be intercepted by macrophytes and/or immediately abosrbed by the system; yet, we did not observe that fertilized cells had differnces in change in plant biomass from 2009 to 2010 from unfertilized cells (Figure 15). This result is surprising as this system is highly oligotrophic; response to a nutrient load was expected. One potential methodological explanation for this data is that change in macrophyte

biomass was measured by subtracting biomass collected in August of 2010 from biomass collected in August of 2009. Macrophyte biomass may fluctuate from year to year due to other environment stressors besides nutrient regime. In order to account for this, we make two suggestions for future years of fertilization in these systems 1) nutrient pulse should be at least doubled what was added in 2010 in order to observe the affect of nutrient regime above year to year noise and 2) take biomass measurements at least twice a summer to calculate growth within a summer.

Comment [JP39]: I wonder how feasible this might be given how much work it was to do it once?

Ecological processes also provide potential explanation for this unexpected data. Primary producers may take up nutrients faster than they actually grow (Valiela 1995). This "luxury uptake" is thought to allow plants to store nutrients for growth for a later point in time when nutrients become scarce again. In our case, a potential nutrient sink could be in plant tissue, especially perrenial roots, that are storing nutrients for later years. We predict that future measures of biomass may reflect nutrients consumed during the first year of fertilization, 2010.

An alternative potential nutrient sink for the percent of total nutrients that were not observed in the water column is explained by Bridgham and Richardson (2003). Bridgham and Richardson (2003) measured rates of nutrient immobilzation via microbial decomposition of litter in peatlands; their results showed that litter with initially low endogenous nutrient concentrations had greater rates of N or P immobilaztion after fertilization. Bridgham and Richardson (2003) suggest that litter in an environment where decomposers are limited by nutrients can form a large potential nutrient sink upon fertilization. Furthermore, traditionally, the largest sink of short-term phosphorous storage exists in soils via adsortpion and exchange (Richardson and Vaithiyanathan 2009). Future studies may investigate techniques that assess the amount of nutrients adsorbed and immobilzed in soils.

Comment [JP40]: Seems like you could organize a single paragraph on possible fate of nutrients and invoke living biomass (above and belowground), organic matter and soil, denitrification – spell them all out as a group.

Within the macrophyte community, a lack of a clear response to the nutrient pulse leaves little to compare between high diversity and low diversity wetlands in the context of the divesity of resource use mechanism (A, in figure 1).

On the other hand, the affect of fertilization within the water column was observable in our other measure of primary productivty: aquatic photosynthesis and respiration. Of the total amount of nutrients applied, a medium of 26% N and 82% P were observed within the water column (Table 8). Within 32 and 5 days, N and P concentrations were at or below 10% of peak concentration respectively (Figure 17). Despite an apparently ~~c~~affective nutrient sink, aquatic primary productivity actually decreased in all fetilized cells (Figure12). This observation may be consistant with the hypothesis of emergent versus submergent nutrient competition and light availability (C in, Figure 1). We hypothesized that if the emergent community responded quickly to the nutrient pulse and grew proportionally, then the light may become limiting for aquatic primary producers. Yet we did not observe differences in emergent cover that support this hypothesis. Similar to biomass, emergent cover did not appear to respond to fertilization in a unified way (Figure 16). One obvious methodological explanation for these results is that the measure for sun exposed water was not the best measure available as explained earlier in this discussion. Although the best measure of sun exposed cover was not used, if the nutrient and light competition hypothesis was supported (mechanism C, Figure 1) we presume that this measure would at least show a consistant increase across all fertilized cells; this is not the case (see error bars in Figure 16). We suggest that an alternative mechanism is much more important for understanding the response to nutrients in the water column.

Comment [JP41]: Medium? Median? If median, median of what? Something is funky about eth 181% P

Sheffler et al (1993) provide an interesting example of a similar 'paradox of enrichment', where nutrient addition meant lowered aquatic primary productivity in shallow lake ecosystems. Sheffler et al (1993) propose two different equilibria for eutrophic systems. One of these equilibria, found primarily in highly eutrophied systems, has shown lower rates of aquatic primary productivity (Blindow et al. 2006). These systems have a dense layer of phytoplankton at the water surface. High rates of productivity become limited by nutrients because of rapid nutrient utilization and normal lake stratification that prevents nutrient mixing. The densely inhabited surface water creates low light attenuation to benthic environments where nutrients are replete. Consequently, nutrient limited surface producers and light limited benthic producers create an entire system that is less productive overall.

Consistent with the turbid water column hypothesis presented by Sheffler et al (1993), fertilized cells in our system were indeed more turbid than unfertilized cells (Figure 14). Yet Grossman (2008) found that our experimental system was both vertically and horizontally homogenous with respect to both nutrient concentrations and dissolved oxygen concentrations. Therefore, our system does not stratify in the same way as a lake, which makes this hypothesis invalid in our system. In fact, the assumption of homogeneity was made to make whole system measurements of primary productivity and nutrient concentration by taking samples from the same point in space. Nevertheless, we cannot dismiss the possibility that fertilization may have created a heterogeneous environment with respect to nutrients and dissolved oxygen in our system. Future studies should investigate the assumption of homogeneity after fertilization.

We hypothesized that rates of primary productivity measured in fertilized cells would be correlated to rates of nutrient uptake elucidating one of the potential nutrient sinks that characterize useful wetland ecosystem. In theory, because organisms often maintain relatively inflexible carbon to nitrogen to phosphorus ratios (Redfield ….), rates of water column nutrient uptake should be comparable to water column productivity. Yet in our case, primary productivity was lowered in fertilized cells when compared to unfertilized cells (Figure 12). Regardless to aquatic productivity results, rates of nutrient uptake were compared to several measures of ecosystem structure and function, but none proved to be related. Consequently, we cannot conclude anything about the importance of the proposed relationships between planting regime, ecosystem structure, ecosystem function and resulting ecosystem services. In fact, this may be the most important result. Regardless of restoration regime, wetlands are so capable that they can absorb nutrients in runoff simulated from agricultural water sheds 12.5 times larger than the wetland area in 32 days (N) and 5 days (P) without even seeing drastic differences in primary productivity.

Ecological explanation can be made for a loss of nutrients without an increase in primary productivity. Results from a similar restored wetland system provide a possible explanation for rapid nitrogen removal without substantial changes in primary productivity (Bachand and Horn 2000 a; Bachand and Horn 2000 b). In similar sized constructed surface wetlands, Bachand and Horn (2000 a) predicted that frequently elevated water temperatures, organically rich anoxic soils and high nitrate concentrations were ideal characteristics for bacterial denitrification. Indeed high rates of nitrate removal, 2800mg nitrate m^2 day^{-1}, were attributed primarily to denitrification, not vegetation (Bachand and Horn 2000 b). Using their rate of nitrate removal, and assuming all of the nitrogen load applied in our system ended up in anoxic soils –a conservatively false assumption– we calculated that it would take only six days to completely remove all of the nitrogen at that rate. This calculation is obviously very rough, but it does suggest that denitrification could play a significant role in the removal of nitrate from our system. Especially because

Comment [JP42]: ?I don't know what you mean by this word in this context.

Comment [JP43]: It is wonderful that you have thought has deeply as you have about all of the possibilities – this is an important part of the process. But the reality is that there is so much speculation on possible mechanisms in this discussion that I think the reader ends up confused. The explanation ends up being saturating. Better to just say where results do and do not support hypotheses.

Comment [JP44]: Redfield was focused on plankton. I don't think this applies to vascular plants. Again, I think this is great analysis to go through, but too much to present.

Comment [JP45]: Yes.

Comment [JP46]: Interesting idea!

summer water temperatures ranged between 59.6 and 90.0 Fahrenheit with an average of 72.3, and dissolved oxygen was below 2 mg/L more than 40% of the time during the summer in fertilized cells.

Ecological explanation may also be made for the observed decrease in aquatic primary productivity. Decrease in aquatic primary productivity must be due to a reduction of available resources. Based on several assumptions, I hypothesize that bacterial competition may provide an explanation for decreases in primary productivity. The first assumption is that bacteria in our system can out-~~-~~compete aquatic primary producers for available nutrients. This assumption has been observed in mesocosm studies (Caron et al 1988). The second assumption is that bacteria in our system have a biomass N:P ratio of 7:1 (Cleveland and Liptzin 2007). The third assumption is that nutrients entering the water column were at the same ratio as what was added to the entire system, 15.6N:1P. The last assumption is that although the system was initially oligotrophic, endogenous N and P cycling still occur and is continuously cycled through different organisms. With these assumptions, I suggest that bacteria consumed exogenous nitrogen. Because the exogenous N:P ratio (15.6:1) is higher than the bacterial N:P ratio(7:1) in order for bacteria to maintain their N:P biomass ratio consuming exogenous P is not enough. Therefore, there is a discrepancy of P to be consumed; presumably this P could come from initial endogenous P cycles that may have otherwise fueled primary productivity. Although a short search did not find any support for this hypothesis, I propose this mechanism as possible explanation to a rather perplexing set of results.

In conclusion, ~~while oligotrophic~~before fertilization when nutrient concentrations were low in all treatments, these systems showed ~~conclusive~~[?] results that suggest a negative relationship between plant biodiversity and aquatic productivity via competition for light. After ~~eutrophication~~nutrient addition, fertilized wetlands quickly removed nutrients and a decrease in aquatic primary productivity was observed with no apparent mechanism. For the ~~eutrophied~~ nutrient enriched systems, no conclusions could be made about why aquatic primary productivity decreased, how nutrients were removed without increases in productivity, or any of the relationships between ecosystem structure and function proposed in Figure 1. Yet, most importantly, this study reinforced other evidence that wetlands indeed are ~~great~~ effecetive at removing nutrients without damaging the ecosystem. In order to better understand the processes and relationships we expected to in this study, future studies should 1) apply at least twice as much nutrients as were applied in this study, 2) take biomass samples at least twice per summer, 3) separate emergent productivity from submergent productivity by separating submergent macrophytes from the measure of above ground biomass, 3) use soil organic matter, above ground biomass from emergent plants, and aquatic dissolved oxygen dynamics to estimate total ecosystem primary productivity and compare this to nutrient uptake, 4) investigate how to estimate changed in soil N and P with the methods available, and 5) use benthic dissolved oxygen chambers to estimate rates of aerobic respiration in the soil.

Comment [JP47]: This is a very interesting idea, but why would this REDUCE primary productivity?

Comment [JP48]: How

Comment [JP49]: ok

Comment [JP50]: Nice recommendations

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Appendix

Table 3: Wetland to Watershed Area Ratios

Wetland to Watershed Area ratio	Reference
.03	(Kovacic, Twait et al. 2006)
.12	(Lu, Wu et al. 2009)
.03	(Braskerud 2002)
.3	(Braskerud 2002)
.03	(McCartney 2010)
.04	(Kovacic, Twait et al. 2006)
.04**	(Kadlec and Knight 1996)
.04	Weighted Average*: The proposed value to use at the George Jones Wetlands

Table 3: Wetland to watershed area ratios are calculated by dividing the area of the wetlands by the area of the watershed that the wetlands are treating. **This value is a mean value from 85 treatment wetland studies compiled by the given reference. *The weighted average was used to account for the number of wetlands in the compiled reference.

Table 4: Phosphorus Loads in Agricultural Landscapes

Total Phosphorus	Total Phosphorus Loads in

Load Applied to Fields (kg/ha/yr)	Reference	Agricultural Runoff (kg/ha/yr)	Reference
16.18	(Domagalski, Ator et al. 2008)	.31	(Domagalski, Ator et al. 2008)
11.07	(Domagalski, Ator et al. 2008)	.17	(Domagalski, Ator et al. 2008)
		.08	(Alberts, Kitchen et al. 2006)
67.37	(D'Ambrosia, Ward et al. 2006)	1.3	(Alberts, Kitchen et al. 2006)
56.14	(D'Ambrosia, Ward et al. 2006)	.69	(Domagalski, Ator et al. 2008)
56.14	(D'Ambrosia, Ward et al. 2006)	7.8	(Domagalski, Ator et al. 2008)
84.0	(Quality 2008)	1.11	(Crumpton, Isenhart et al. 1993)
48.48	Average	4.0	(Kadlec and Knight 1996)
		1.93	Average: The loading value for the proposed research

Table 5: Nitrogen loads in Agricultural Landscapes

Comment [JP51]: No need to include so many redundant citations. Instead use footnotes within the table and then include these in a figure legend. So, for instance, mark data from (Domagalski, Ator et al. 2008) with a symbol in the column, then include the symbol next to the citation in the legend. Ask me if this is not making sense.

Total Nitrogen Load Applied to Fields (kg/ha/yr)	Reference	Nitrogen Form and Load in Agricultural Runoff (kg/ha/yr)	Reference
72.0	Contructed Wetlands for Water Quality inprovement	15.8 (NO_3-N and NO_2-N)	(Alberts, Kitchen et al. 2006)
150.0	Contructed Wetlands for Water Quality inprovement	15.5 (NO_3-N and NO_2-N)	(Domagalski, Ator et al. 2008)
84.0	(Borin and Tocchetto 2007)	4.9 (NO_3-N and NO_2-N)	(Domagalski, Ator et al. 2008)
32.0	(Borin and Tocchetto 2007)	12.9 (NO_3-N and NO_2-N)	(Fausey, Brown et al. 1995)
49.0	(Domagalski, Ator et al. 2008)	12.3 (NO_3-N and NO_2-N)	Average for NO_3 and NO_2
63.0	(Domagalski, Ator et al. 2008)	18.3 (Total N)	(Domagalski, Ator et al. 2008)
168.4	(D'Ambrosia, Ward et al. 2006)	9.6 (Total N)	(Domagalski, Ator et al. 2008)
16.8	(D'Ambrosia, Ward et al. 2006)	15.0 (Total N)	(Kadlec and Knight 1996)
84.2	(D'Ambrosia, Ward et al. 2006)	10.0 (Total N)	(Borin and Tocchetto 2007)
79.9	Average	13.2 (Total-N)	Average for Total N: Loading value for Proposed research.

Tables 4 and 5: Not all load values were measured in the same way, and therefore a large range of values were produced when they were converted into kg/ha/yr. The studies referenced above were selected for use in the final analysis because they are primarily studies from the mid-west. Studies that ~~obviously~~ produced outlier data in the compilation were not included.

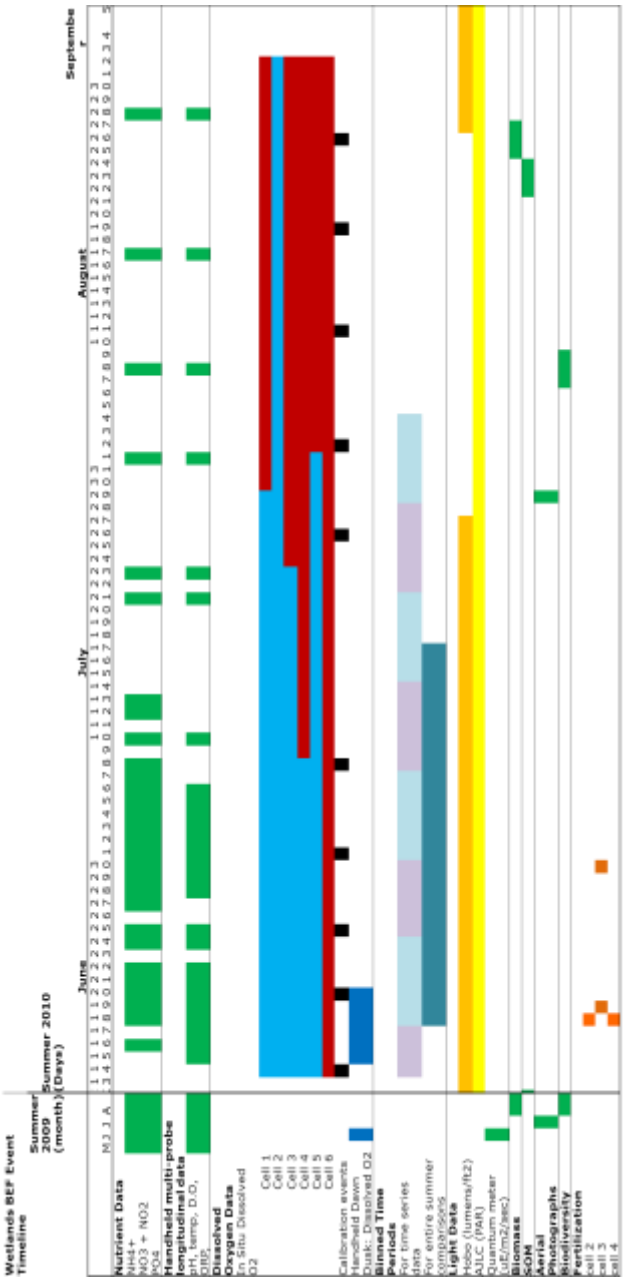

Figure 5: Data timeline of when data was collected . Data iollection is shown in solid colors. Dark red indicates data that was collected but was not good because of technical issues. Alternating blue and purple solid colors indicate binned time periods.