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**MARYLAND DEPARTMENT OF TRANSPORTATION
STATE HIGHWAY ADMINISTRATION**

RESEARCH REPORT

**THE EFFECTIVENESS OF AMENDMENTS IN PROMOTING
HYDRIC SOIL CONDITIONS IN MITIGATION WETLANDS**

**DR. ANDREW BALDWIN, PRINCIPAL INVESTIGATOR
DR. STEPHANIE YARWOOD, CO-PI
MR. BRIAN SCOTT, PHD CANDIDATE**

UNIVERSITY OF MARYLAND, COLLEGE PARK

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16. Abstract <p>Organic amendments did not promote hydric soil conditions in wetlands as originally hypothesized. Instead, soil saturation strongly determines if soils are hydric. Hydric soils can be tested using several different methods, and redox electrodes appeared to give more favorable test results than dipyrindyl strips in unsaturated conditions. IRIS can also be used to test hydric soils and gave favorable results, but those results cannot be compared directly to the redox electrodes and dipyrindyl. The research evaluated a broad range of wetland performance metrics, including soil bulk density, organic carbon storage, potential for methane generation, and plant growth and diversity. Overall, organic amendments often do very little. Sometimes there are benefits, like increased plant biomass, but the results can also be unfavorable, like loss of diversity and more invasive species. Often the perceived benefit (increased biomass) was of invasive species. Most organic matter amendments increase greenhouse gas (methane) production, but this can be mitigated by composting the material first and using a lower loading rate. Composted materials that have low pH value and are low in available nutrients (phosphorous and nitrogen) performed better than fresh, high nutrient options. Of the amendments used in the study, composted wood mulch and composted biosolids appeared the most favorable. The act of disturbing soil, in order to mix in amendments, also had negative side effects, so consideration should be given to placing amendments on the soil surface. The findings from the field and lab studies were generally consistent with a comprehensive literature review on the subject.</p>			
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Andrew Baldwin, Professor, University of Maryland. As Principal Investigator and a wetland scientist, Dr. Baldwin oversaw all aspects of the project, including development and implementation of research and managing project scope, schedule, and budget. In particular, he focused on the field-based components of the project. He also co-advised the graduate student, Brian Scott and co-authored journal manuscripts.

Stephanie Yarwood, Associate Professor, University of Maryland. As Co-PI and a microbial ecologist, Dr. Yarwood focused the laboratory studies and co-advised the graduate student. She also co-authored manuscripts for journal publication.

Brian Scott, Graduate Student, University of Maryland. As part of his Ph.D. research, Mr. Scott was responsible for developing research questions and methods with his advisors, and for the day-to-day coordinating and carrying out of field and laboratory studies, analyzing and interpreting data, and preparing and submitting manuscript drafts.

EXECUTIVE SUMMARY

This research evaluated the use of organic matter amendments (OM) for wetland restoration in Atlantic Coastal Plain soils. The research team was unable to document that OM was helpful in establishing hydric soil conditions, which was the primary purpose of the study. Instead, the research has shown that hydric soil conditions depended on hydrology. When soils were saturated, all hydric soil testing metrics passed, with or without amendments. As soils became unsaturated (oxic) the three hydric soils tests (IRIS film, redox, and α,α' -dipyridyl dye) were affected differently, but the effect did not depend on OM.

The research team also evaluated the broader question of whether or not OM amendments, sometimes required by the Maryland Department of Environment (MDE), are beneficial when constructing mitigation wetlands. For this question, the team evaluated a number of metrics, including root production, shallow (< 20cm) soil organic matter (SOM), deep (> 30 cm) OM, soil bulk density (Db), several metrics related to plant diversity, primary productivity (as aboveground plant biomass), and greenhouse gas production (methane (CH₄) and nitrous oxide (N₂O)). The amendments used were municipal waste biosolids (BLOOM[®]), composted wood mulch, hay, and cow manure. In most cases OM-amended plots were not distinguishable from unamended plots; however, there were some exceptions. High application rates of manure (3 – 6x the amount required by MDE) produced more plant biomass. However, the larger biomass was generally due to a significant increase in cattail growth, which reduced diversity. High application rates of manure also produced significant increases in greenhouse gases. Composted wood mulch increased the root to shoot ratio, retained the most OM, and the increase in methane gas production was moderate.

Site hydrology had a significant impact on the findings. Although the team intended to establish plots in 4 hydrologically equivalent Blocks, subsoil conditions varied, and Blocks were not hydraulically similar. Wetter Blocks always passed hydric soil tests, whereas the team saw several hydric soil test failures in the drier Blocks, and α,α' -dipyridyl was most sensitive (first to fail) in drying conditions. Wet conditions increased cattail growth and decreased plant diversity and richness and increased the release of greenhouse gases. Effects were evident when using high OM application rates.

In addition to the field study, reported here, the team prepared three manuscripts for publication. The first was a review paper that found topsoil (as OM) outperformed other OM amendments, and coarse grain, low SOM soils (common to the Atlantic Coastal Plain) do not benefit from OM compared to other soils. The second measured iron-oxide removal rates from IRIS tubes, which depended on hydrology (saturated soils reacted faster). The third summarized our 2018 – 2019 lab findings. The primary finding was that methane production significantly increases after prolonged inundation.

Overall, OM had few effects, good or bad, on wetland metrics. Negative side effects could be avoided by using moderate application rates and limiting the use of manure. While cattail growth was noted as a negative side effect, the initial growth of cattail appears to have been a consequence of soil disturbance when the team constructed plots, and high levels of nitrogen and phosphorus from manure accelerated cattail growth. Limiting disturbance during wetland

mitigation construction may help curb cattail prevalence. Given limited resources, focusing on site hydrology, rather than OM use, is more likely to improve wetland mitigation success.

Introduction

In 1978 the National Environmental Policy Act (NEPA) was established to identify and mitigate environmental impacts from projects such as highway construction (“National Environmental Policy Act” 1978). At the time, the ecosystem impacts of road construction were apparent but not well documented. Wetland ecosystems, in particular, received special attention because roads not only affect the area under construction but can influence entire watersheds by altering water flow (Adamus and Stockwell 1983; Shuldiner, Cope, and Newton 1979). Due to their unique interaction with the landscape, highways can either destroy, alter, or create wetland areas (Office of Technology Assessment 1984). In 2020, NEPA was updated in part because prior revisions failed to adequately address the exceedingly lengthy turn-around-time for environmental impact statements for highway projects (Council on Environmental Quality 2020).

In Maryland, NEPA is overseen by the MDE. To complement federal NEPA requirements Maryland enacted a wetlands protection act in 1996, the first of its kind in the US (Rubin 1997). Wetland mitigation in Maryland takes on extra significance due to Chesapeake Bay water quality protection requirements (Goldman and Needelman 2015). The Maryland Department of Transportation State Highway Administration (MDOT SHA) is the largest road builder in Maryland and therefore has the largest influence on NEPA implementation. The MDE and MDOT SHA often collaborate to improve NEPA compliance (Weber and Allen 2010). This study was made possible by an informal agreement where MDE modified their requirement for organic matter amendments (OM) for wetland mitigation (Walbeck, Clearwater, and Neff 2011).

This study was designed to provide data that could improve NEPA and MDE wetland mitigation compliance by addressing two ongoing issues raised by MDOT SHA: 1) the ability to meet hydric soil test metrics and 2) to identify the best performing OM amendment type and application rate. Wetland soils become hydric due to prolonged saturation and, as a result, begin reducing iron-oxides. Iron-oxide reduction can be measured using one of three tests: a chemical reaction with α, α' -dipyridyl dye, removal of iron-oxides from IRIS (Indicator of Reduction in Soils), and measuring the soil electrical (redox) potential. In lab and field studies the team examined what factors affect these three hydric soil tests. The team also examined the use of OM to determine their effect on a variety of wetland mitigation metrics. While the team considered which OM amendments would be the most beneficial toward developing hydric soils, many other important wetland evaluation metrics were also measured, including soil bulk density, plant growth, soil carbon accumulation, and production of greenhouse gases.

Methodology

Over the course of two years the team used lab and field studies to evaluate the use of OM on wetland mitigation. The MDOT SHA has existing specifications for Type A (manure) and Type C (compost) soil amendments (Maryland Department of Transportation State Highway Administration 2018). For Type A amendments the team used horse and cow manure in the lab study and cow manure (M) for our field study. For Type C amendments composted wood chips

(W) were used. Specifications for the cow manure and wood chips are shown in ([Appendix 1](#)). The team also used the leaf litter compost (LeafGro) as an additional Type C amendment. Two other amendments were evaluated: Biosolids (B) from DC Water's Blue Plains Advanced Wastewater Treatment Plant, the brand name is BLOOM®; and hay (e.g. Timothy grass). Hay is recommended by the Wetland Science Institute (Melvin 2003). The three treatment application rates evaluated were based on MDE recommendation of 60 cubic yards per acre (Walbeck, Clearwater, and Neff 2011) equivalent. In these studies, $1x = 60 \text{ yd}^3 \text{ acre}^{-1}$; $3x = 180 \text{ yd}^3 \text{ acre}^{-1}$, and $6x = 360 \text{ yd}^3 \text{ acre}^{-1}$. One exception was the use of hay in the field study, where $3x = 60 \text{ yd}^3 \text{ acre}^{-1}$. The research team chose lower application rates for hay based on earlier lab studies that showed it was more active than other amendments.

Lab study

Microcosm experiments were performed using 1000-mL straight-sided wide-mouth food canning glass jars. Each jar lid was precision drilled and fitted with an air-tight rubber septum. The microcosms were filled with water and homogenized soil from the Smith Farm site. If amendments were added, an equal quantity of soil was removed to make the soil volume the same. The team also conducted similar experiments using 40ml VOA vials ([Photo 1](#)).

Gas measurements were collected at varied intervals depending on gas production rate. A gas-tight syringe was used to measure the gas production volume and equilibrate the microcosm to atmospheric pressure. Then, a representative gas sample (0.01 - 1000 μL) was collected and analyzed for carbon dioxide, methane and nitrous oxide using a gas chromatograph (Varian Model 450-GC). Gas sampling continued for approximately 60 days.

Soil was sampled at the beginning and end of the incubation period. Soil extracts using KCL were analyzed for phosphorous using a Lachat Flow Injection Ion Chromatograph. Soil moisture content was determined by drying ~10 g of field-moist soil at 105°C for 24 h. Soil organic matter (SOM) was determined by loss-on-ignition (550°C for 2h).

Liquid supernatant was extracted at varied intervals and analyzed for pH, and ferrous and total iron. The pH was determined using an Orion 9142BN electrode. Ferrous iron was measured with a HACH DR4000 spectrophotometer. For total iron, thioglycolic acid (from CHEMetrics) was used to reduce soluble ferric to ferrous iron.

All data were analyzed using ANOVA contrasts (compared to the no-amendment control) to test for differences due to OM type, loading rate and soil type.

Field study

The team conducted the field study at the Smith Farm wetland mitigation site in Goldsboro, MD (Photo 2). A ditched and tile drained row crop farm, the site was converted to mitigation wetland in early 2017. Unusually heavy rains in 2018 delayed construction of the research plots until 2019. The total area of the site is 22.4 acres, but the research plots were limited to a contiguous 8.14-acre area that is regularly flooded. In lieu of a requirement for organic soil amendments, the MDE permitted the use of organic woody debris, allowing the research team to construct experimental plots using a total of 11 amendments (10 plus an unamended control). Each Plot

was replicated 4 times and grouped by Block (Photo 3). Additional photos of site construction are included as Photos 4 & 5. The OM type and loading rates are shown below.

Treatment	Loading Rate	# of replicates (1 per Block)
Control (C)	No amendments added	4
Organic matter		
Hay (H) (high C:N ~ 25)	9.6 Lb./plot or 0.2 bales/plot	4
	28.7 Lb./plot or 0.6 bales/plot (equivalent to the MDE recommended loading rate of 60 yd ³ acre ⁻¹)	4
	57.3 Lb./plot or 1.1 bales/plot	4
	Total: 382.3 Lb., 8 bales (50 Lb. ea.)	
Type A Composted Manure (M) (moderate C:N ~16)	4.8 ft ³ plot ⁻¹ (equivalent to the MDE recommended loading rate of 60 yd ³ acre ⁻¹) (36 gallons)	4
	14.4 ft ³ plot ⁻¹ (0.5 cy) + 6.7 gallons	4
	28.8 ft ³ plot ⁻¹ (1 cy) + 13.3 gallons	4
	Total: 232 ft ³ (includes 10% waste and 10% buffer)	
Type C Composted Wood (W) (high C:N ~ 25)	4.8 ft ³ ft ³ plot ⁻¹ (equivalent to the MDE recommended loading rate of 60 yd ³ acre ⁻¹)	4
	14.4 ft ³ plot ⁻¹	4
	28.8 ft ³ plot ⁻¹	4
	Total: 232 ft ³ (includes 10% waste and 10% buffer)	
BLOOM (B) (low C:N ~ 6) (2x6m plots)	4.8 ft ³ plot ⁻¹ (equivalent to the MDE recommended loading rate of 60 yd ³ acre ⁻¹) (36 gallons)	4
	Total: 19.2 ft ³ (includes 10% waste and 10% buffer)	
	Total	44

Microtopography has a significant influence on wetland development and adding OM changes the surface elevation. Some studies found that the apparent effects of OM were actually the result of changes in microtopography (Alsfeld, Bowman, and Deller-Jacobs 2009). In order to mitigate elevation effects, the team pre-excavated plots to a depth of approximately 15cm (Photo 5) and removed an equivalent amount of excavated soil prior to adding amendments (~ 7cm for the 6x application rate level). Each plot included a well to record hydrology and a metal base for gas measurements (Photo 6).

With the exception of the winter months (December – March), each plot was monitored at least monthly for all three hydric soil indicator methods: α,α' -dipyridyl, IRIS tubes, and redox potential (using platinum electrodes), in accordance with the MDE guidance (MDE 2016) and the Hydric Soil Technical Standard, Technical Note 11 (NRCS 2015). IRIS tubes were installed for 30-day periods during the growing season. Soil pH (necessary to evaluate redox potential) was also monitored.

Soil physical parameters were collected at multiple time points in each plot. The team measured bulk density at the soil surface by collecting a soil sample with a metal sleeve of known volume and measure the dry weight of the contents. Soil OM was measured by subjecting soil to combustion at 550°C for 2 hours. To determine root biomass, the team made root-ingrowth cores by filling 5 cm diameter mesh bags with peat. These cores were inserted prior to the growing season and removed near the end of the growing season, in August. Roots were separated from the peat, dried, and weighted. Aboveground biomass was also harvested in August from two randomly selected ½ m x ½ m squares in each plot. Biomass was then dried and weighed. The team estimated the percent cover for each plant species across the 2m x 5m plot (excluding the areas with the well and gas chamber base) using the ranges described in (Peet, Wentworth, and White 1998). Diversity indices were based on percent cover values. To measure greenhouse gases, the team placed a ½ m x ½ m x ½ m clear-sided chamber over the metal frame and collected the trapped gas in samples every 15 or 20 minutes for 1 hour.

Research Findings

Site Hydrology

The hydrology of the field site had a large impact on the findings. When the team originally set up test plots, the team located them within four Blocks (A – D), see Photo 3. In field studies such as these there are often slight, random variations between Blocks. The Plot elevations were similar so it was expected that the hydrology at each Block would be similar; however, this was not the case. The team noticed even during construction that Block D would dry out more quickly than other areas and Block C was continuously saturated. The team believed the differences in hydrology had to do with the sand content of the subsurface soils and have included additional information about hydrology in Appendix 2.

Because hydrology varied across Blocks, the team analyzed these data as multi-way ANOVA tests, where both, the Block and the treatment (amendment) as variables that may have caused changes in the results, were included. Even within Blocks the hydrology of individual Plots varied, so a “Water” ranking value for each site visit was assigned. If the surface of the soil in the Plot was dry it ranked “0”; wet soil ranked “1” and standing water (inundation) was ranked “2”. In some cases, the result of variations by OM (Plot) were corrected for “Water” rather than Block.

Hydric Soil Indicators

The research team did not find any field evidence that OM increased the potential to pass hydric soil indicator tests. Hydric soil tests include IRIS tubes, α,α' -dipyridyl strips, and redox potential. During the field test period, the site was often inundated, during which time all hydric soil tests were positive. There were several time windows when soils were not inundated, and the team redoubled our efforts to collect hydric soil testing data during those periods.

There is some evidence to indicate that α,α' -dipyridyl tests are more sensitive to soil drying than the redox potential. In mid-June, when water levels began to recede at the site (Appendix 2), both the IRIS and redox potential tests were initially positive. However, as the water receded the

team began to see negative α,α' -dipyridyl test results. Since test results for platinum redox electrodes continued to show hydric soil conditions, they may be a better method than α,α' -dipyridyl for hydric soils testing where water levels fluctuate. Appendix 3 summarizes the mid-June hydric soil test results.

The team found that, overall, hydrology was an important factor in hydric soils tests. Even though hydric soil test results were all positive (above or below the relevant threshold values) the team compared the numerical values for each test and determine if any OM amendment increased the redox, IRIS, or α,α' -dipyridyl test scores. Normally, dipyridyl testing criteria calls for 3 strips across the 30 cm depth, and at least two of the three must be positive to pass. So, the "score" is 0, 1, 2, or 3. To refine this scale for research, the team assigned "1" for a mild reaction and "2" for a positive reaction, so the score range would be 0 - 6. Hydric soil test values against hydraulic condition (Water) were also compared.

All hydric soil tests responded strongly to soil saturation (Water). P-values that are < 0.05 are usually accepted as "significant". On the other hand, hydric soil tests did not respond to OM. A p-value of 1.0 would mean there was no difference in the results. There was almost no difference (0.99) in IRIS tubes based on OM. In the 2020-Q1 report we reported that a high loading rate of (any) OM may improve the chances of passing the IRIS hydric soil test. However, with additional IRIS and hydrology data it appears that was a temporary condition that occurred only immediately after plot construction. There was a weak response (0.77) in redox potential. The α,α' -dipyridyl test is the most sensitive (0.69) to OM. In Appendix 3 it is noted that the α,α' -dipyridyl test is the most likely to result in a negative hydric soil test and redox may be preferred. However, this was with respect to changing the hydroperiod. It is unlikely the slightly different response to OM (0.77 vs 0.69) would be noticeable.

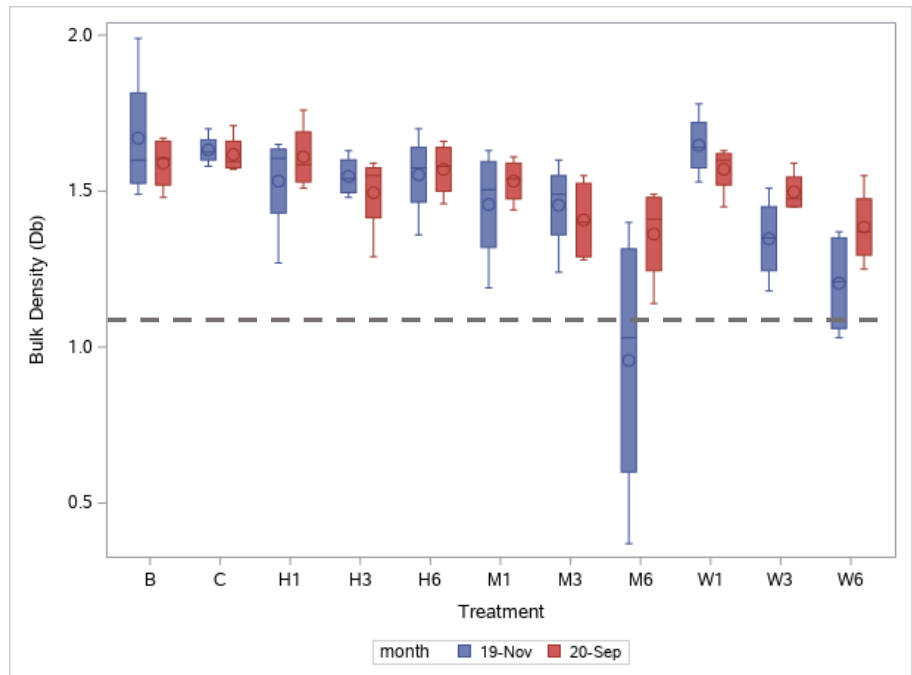
Test	Water		OM Amendment	
	P value	DF	P value	DF
IRIS	$< .0001$	5	0.99	10
Redox	$< .0001$	5	0.77	10
dipyridyl	$< .0001$	5	0.69	10

Successful hydric soil tests are less likely during periods when soils are in the process of drying, which occurred immediately after plots were constructed, and in mid-June. During these periods, the average α,α' -dipyridyl score in the unamended plots was $2.75 \pm 0.49SE$, not enough to have a passing score of 4.0. Adding OM did not always improve scores: the range of scores in the amended plots was 1.96 to 3.13. Without soil saturation, amending soils with OM did not result in any positive α,α' -dipyridyl test results.

Bulk Density

Bulk density was measured in November 2019 (after Plot construction), and September 2020. Bulk density values are shown on the adjacent figure.

There was an initial reduction in bulk density as a result of amending with OM, but only Plots M6 and W6 would be considered statistically significant. By September 2020, plots M6 and W6 remained statistically lower than the unamended (C) Plot.

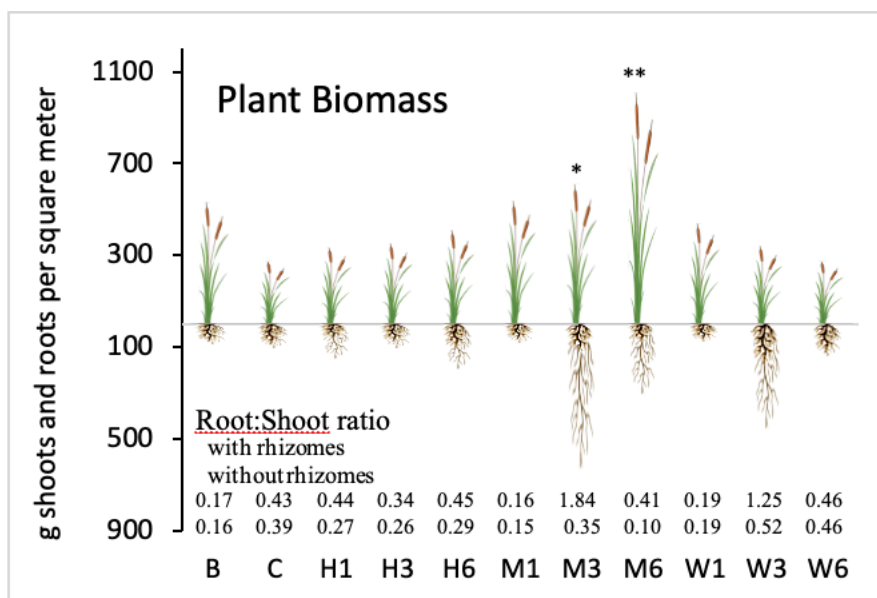


An important bulk density threshold is 1.3 g cm^{-3} , shown on the figure as a dashed line. Root growth is impeded above 1.3 g cm^{-3} , so the plots with high manure (M6) and high wood mulch (W6) may have benefitted root growth immediately after plot construction. However, after the first growing season the soil bulk densities in all plots were above the critical threshold, so the reduction in bulk density due to OM was transient.

Root and Shoot Biomass

The research team measured both above ground and below ground biomass. High application rates of manure (M3 and M6) significantly increased aboveground biomass, but OM did not have a significant change in belowground biomass. There were also no statistically significant differences in root to shoot ratios. As shown in the figure, the aboveground biomass varied between treatments, but only M3 and M6 were statistically significant (noted with *).

The team evaluated variation by Block, but differences were not significant. Although some above ground biomass values appear different, for example biosolids (B) had nearly double the average aboveground biomass as in the unamended control (C), the plot-to-plot variation was high, so we could not differentiate them statistically.



Manure consistently had higher biomass than other treatments and was also consistently higher in cattail coverage. Visually, cattail is taller than other vegetation and has broad, dark green leaves (Photo 7). So, it is tempting to conclude cattail produces more above ground biomass given the same aerial coverage. However, it was found that cattail had about the same mass per unit of area covered: at most 20% more.

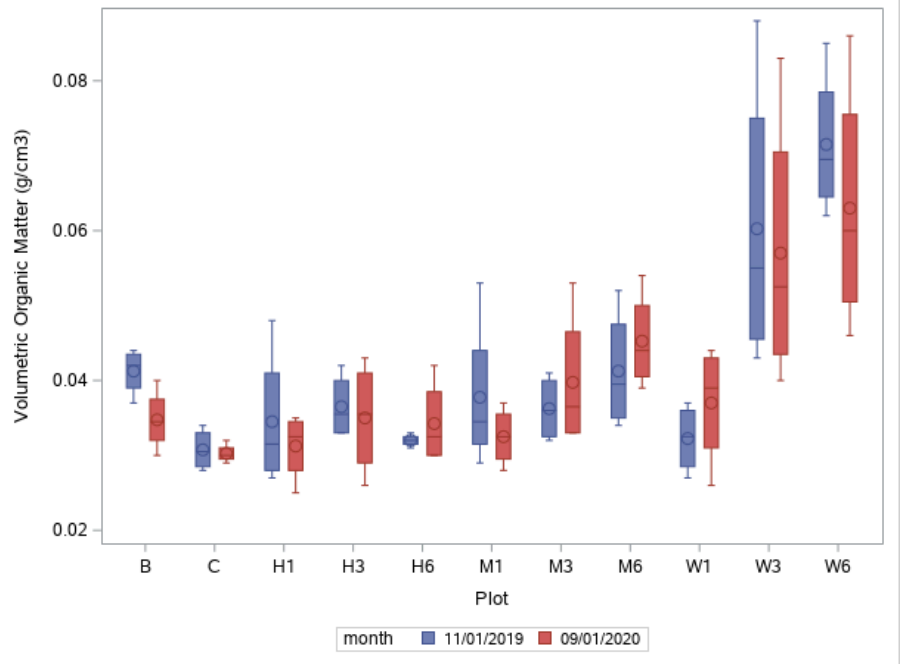
There were no statistical differences in root biomass. In some plots, cattails produce large rhizomes, which are much more massive than all the fine roots combined. Fine root biomass is shown in dark shading on the figure. In plots with large cattail stands (M3 and M6), rhizomes accounted for over 90% of the total root mass. However, as with aboveground biomass, variation was large so it cannot report a statistical difference between M3 and C total root mass, even though the M3 average is much greater.

The team also saw no statistical differences in root to shoot ratio compared to the unamended control (C). The figure above includes a table with root to shoot ratios with rhizomes, and without rhizomes (fine roots only). A higher root to shoot ratio, particularly fine roots, is an indication plants are investing more resources underground, which is considered good for soil health. Only two treatments, W3 and W6, had had a higher average root to shoot ratio than amended plots. A higher root:shoot ratio is a desired effect and may indicate nitrogen immobilization. The treatment with the lowest root to shoot ratios (M6) also showed high nutrient content, an expected outcome: M6 was high in both phosphorus (P) and ammonia (N).

Soil Organic matter (SOM)

The team measured SOM in November 2019 (after Plot construction) and September 2020. The SOM values, reported in g cm^{-3} to account for variations in bulk density, are shown on the adjacent figure. There is an obvious increase in SOM in November 2019 that reflects the addition of the amendments. However, by September of 2020, there was a decrease in SOM in most of the plots, including a small decrease in the unamended control plot (C).

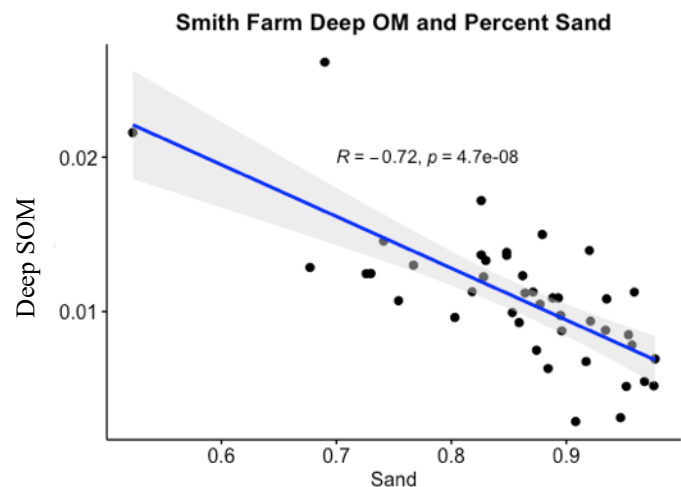
The most likely cause was disturbance (digging) when the research team constructed the plots, leading to increased microbial respiration and leaching losses. High application rates of wood mulch (W3, W6) had statistically higher levels of SOM compared to the unamended Plot and retained that SOM after one season of growth.



Smith Farm Deep SOM and Percent Sand

Deep Organic Matter

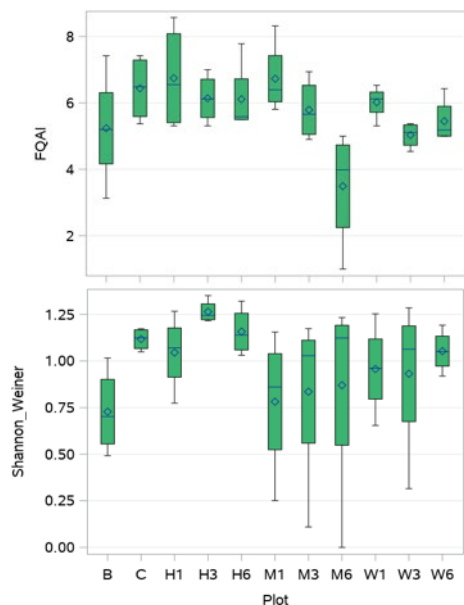
Some OM, whether from amendments, plant roots, or decaying plant matter, may have leached into the B horizon. Water at the site comes from surface sources, so there is persistent downward leaching of OM. The team measured SOM in deeper soils and found no statistically significant difference due to amendments or hydrology. This may have been in part due to the sandy texture of the B horizon soil. Sand is a poor medium for accumulating mineral organic carbon. There was a strong negative correlation between deep SOM and percent sand.



Measures of Plant Diversity

This research reports several metrics for plant diversity. The measures of diversity the team considered were the Simpson index, Shannon-Weiner Index, and Evenness. All these measures

were highly correlated ($r < 0.99$), so we are reporting only SWI. Similarly, the team measured species richness and also calculated the Floristic Quality Index (FQAI) which were also highly correlated ($r > 0.97$), so we are reporting only FQAI. The percent of facultative wetland plants, percent cover, and identification of dominant species, which are specified as mitigation evaluation criteria by the MDE are also presented.

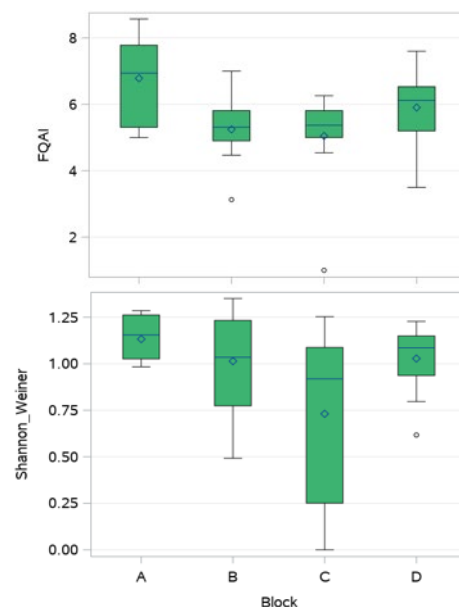


The Shannon-Weiner Index (SWI) values varied by Plot, but none of the differences were statistically different. The average SWI in the unamended Plots (1.12) was higher than most treated plots with the exception of high application rates of hay. A SWI of 1.12 is not atypical for wetlands and even healthy wetlands can be much lower. The average FQAI in the unamended Plots (6.4) is low for freshwater wetlands, indicating a disturbed condition. The high dose manure amended Plot was much lower (FQAI = 3.5).

Hydrology also affected FQAI, with the lowest FQAI in Block C, the plot with nearly continuous inundation. Both the SWI and FQAI values varied by

Block. Block C (wet) had lower SWI and FQAI, but the values were not statistically different. One cause of the reduced SWI and FQAI is the prevalence of cattail. Cattail was the dominant species and had the highest coverage in Block C.

Appendix 4 shows the dominant species by Block and Plot. All plant species we observed are listed in the table in Appendix 5. We also show a time sequences of plant growth at the site (Photo 9) and photos of plants in selected Plots in Block B along with the species distribution (Photo 10).

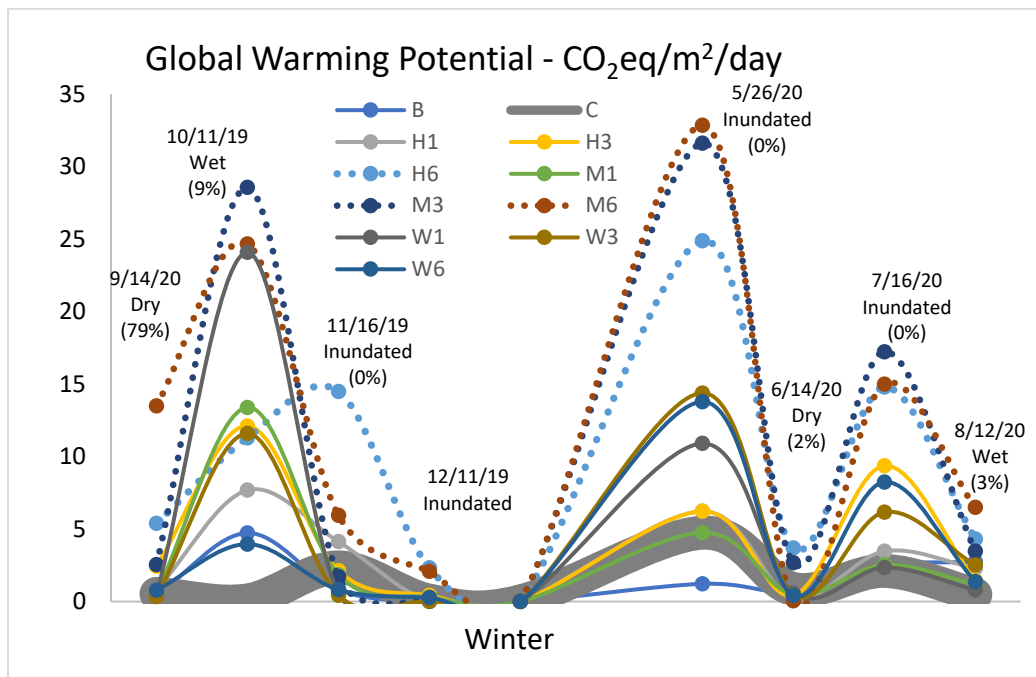


The MDE mitigation guidance requires thresholds be met for both the percent plant cover and facultative (FAC) species. Both metrics were well above the thresholds set by MDE for all plots, including the unamended plots. Still, the team evaluated whether or not there was any indication that OM increased total percent plant cover, or if there was an increase in obligate (OBL) and facultative (FACW) compared to FAC species. Neither were statistically different by Block or Plot. One notable difference was increased percent cover of cattail with high manure (M6). The team has specifically focused on cattail because it is considered invasive by the Interagency Review Team (IRT) performance standards, and MDE has expended resources to actively curb cattail growth.

Methane and Nitrous Oxide

The team measured both methane and nitrous oxide emissions starting in September 2019, several weeks after the plots were constructed, and continued monthly for one year, skipping winter months. Carbon dioxide, methane, and nitrous oxide are greenhouse gases, but differ in how much they increase warming, so the methane and nitrous oxide data was converted to carbon dioxide equivalents and combined. This value can also be referred to as the Global Warming Potential (GWP). The GWP data are shown on the figure (below). The unamended plot is shown with a thick dark grey line for reference. The three amendments with the highest greenhouse gas emission rates are shown with dotted lines. Numbers in parentheses represent the percent of the GWP that is from nitrous oxide.

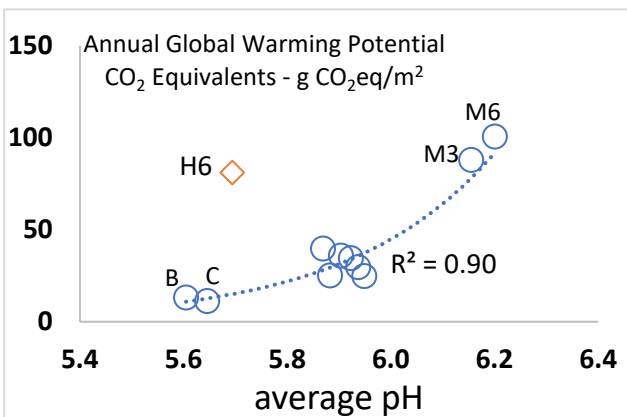
Methane generation occurred when soils were saturated and nitrous oxide when soils were unsaturated. Nitrous oxide is the stronger greenhouse gas than methane (263 CO₂eq vs 34 CO₂eq), but the amount emitted is usually much lower.



To get an overall picture of OM contribution to GWP, the annual emissions were estimated by assuming monthly GWP emission rates continued for 30 days. The estimated annual emissions for the unamended control (Plot C) was 11.2 g CO₂eq m⁻² yr⁻¹, which is low compared to similar wetlands (Nahlik and Mitsch 2010). High application rates of manure and hay had much higher greenhouse gas emissions whereas biosolids was similar to the unamended control.

Plot	GWP g CO ₂ eq m ⁻² yr ⁻¹
C	11.2
B	13.0
H1	24.8
M1	25.1
W6	29.6
H3	34.6
W3	35.9
W1	39.7
H6	81.2
M3	87.9
M6	100.6

The production of methane gas depends in part on the soil pH. Peak methane production is around pH 7 and decreases as pH decreases. This was also observed in our lab studies. Therefore, the annual GWP, most of which is methane gas, was compared against the average pH in each plot. Methane production matches the expected trend. The low methane production with biosolids (B) is likely because it maintained a low soil pH. Similarly, high pH is likely partly responsible for the high methane production from M3 and M6. The high application rate of hay (H6) did not fit the pH trend. The lab experiments showed that hay produced much more methane than other amendments, so high methane in H6 was consistent with the lab results.



Soil Disturbance: Soil disturbance is known to expose and release stored SOM. The team observed high losses in SOM, about 68%, as a result of the soil disturbance necessary to construct our experimental plots. The table below shows SOM results from the unamended control plot. SOM was measured in November 2019, shortly after plot construction, and then again in September 2020. The SOM values in November 2019 represent background SOM with few roots.

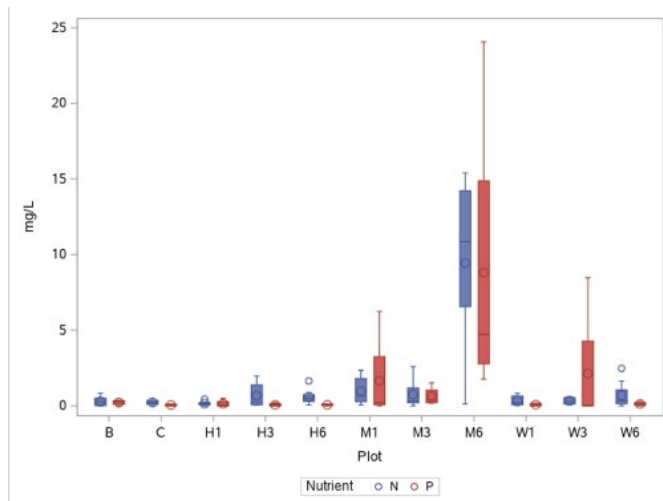
In September 2020, a large portion of the SOM was from plant roots. Assuming root ingrowth cores are representative of root density in the bulk soil, new roots made up on average ~21% of the SOM in the upper 10cm in September 2020. The remaining 23.9 (30.3 – 6.4 mg cm⁻³) represents how much of the original SOM remained.

Date	Total SOM (mg cm ⁻³)	OM from roots (mg cm ⁻³)	Net SOM (mg cm ⁻³)
November 2019	30.7	0*	30.7
September 2020	30.3	6.4	23.9
SOM lost	SOM lost (mg cm ⁻³)		6.8
	SOM lost %		21±18

* Value not measured. Area had been denuded. There was no visible evidence of live roots.

Soil disturbance appeared to be largely responsible for the re-growth of cattail following plot construction. Shortly before the construction activities, MDOT SHA had used herbicides to control cattail growth. Herbicide use had proven to be effective, as dead cattail stands are clearly visible in Photos 4 & 5. Despite removal of cattail rhizomes during construction, cattail regrew within the experimental Blocks. Soil disturbance extended beyond the fenced Block boundaries, and cattail re-growth was evident to the limit of disturbance (Photo 8). While high application rates of manure stimulated cattail, and high levels of hay led to cattail being the dominant species, these are secondary effects that followed cattail re-establishment where soil were disturbed.

Nutrients: Nutrient data was measured: nitrogen (N) as ammonia and nitrate, and phosphorous (P) as phosphate. This study reports P data from potassium sulfate soil extracts performed in the lab, and N data from wells installed in each plot. The data in this report represent a subset of all nutrient data collected to highlight the consistent pattern of elevated nutrient levels, particularly in the manure plots. Excess nutrients were likely responsible for the early season algal bloom (Photo 9).

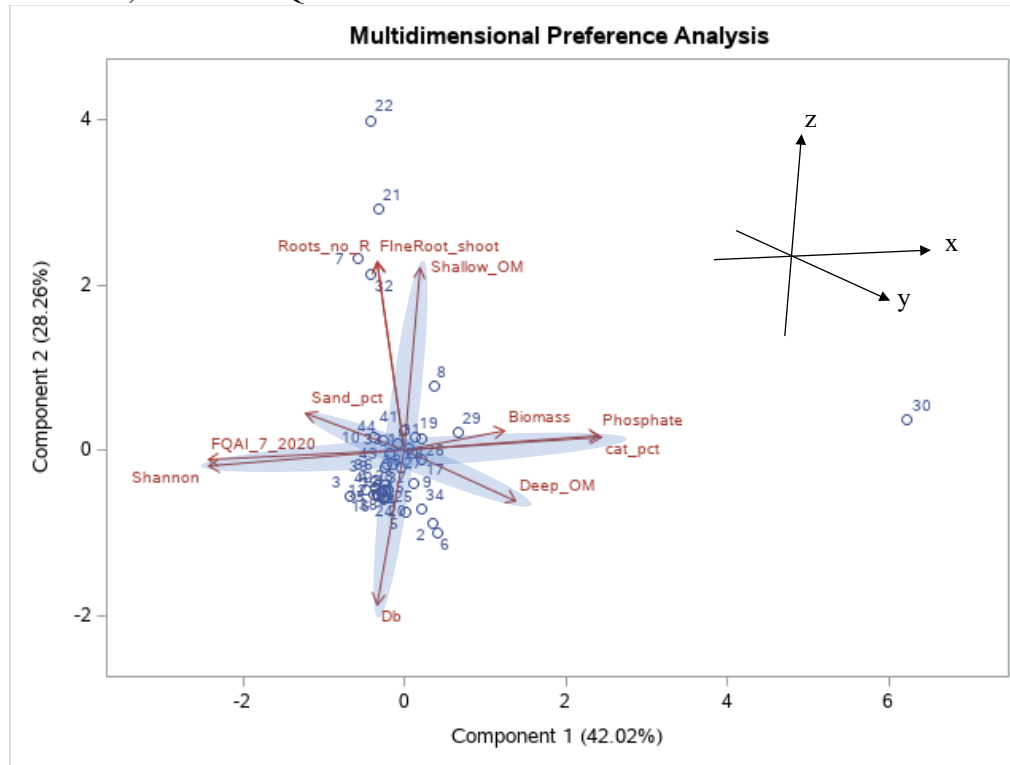


Nitrogen was not discharged from the site, but there was a small P discharge. In our experimental plots, nitrate values were low (< 0.1 mg/L), and amended plots were all much lower than the control plot. Organic amendments are known to promote denitrification; however, since nitrate was low in the control plot, nitrate is not an issue at the site. Nitrate in the adjacent drainage channel (marked with a red star in Photo 2), was 1 mg/L. Nitrate was below the detection limit in water discharging from the wetland. Like nitrate, ammonia was below the detection limit in water discharging from the wetland and would attenuate the low levels (0.04 mg/L) observed in the drain channel. The P concentration was 0.08 mg/L in the drain channel and was slightly higher (0.34 mg/L) in the site discharge. The background P levels from several MDOT SHA wells at the site was estimated to be ~ 0.2 mg/L. Well P levels, in the experimental plots, was below 0.2 mg/L in all cases except very high manure (M6). This suggests the site is reducing P, but P levels in M6 were too high to reduce below background levels.

Both N and P were elevated compared to the unamended control, particularly in the manure plots. Elevated nutrients are likely the cause of the cattail growth, which has been observed in other studies (Steinbachová-Vojtíšková et al. 2006). However, it is unable to determine from the data if this is from N or P or both. Note that both Type A and Type C composts, as described in the MDOT SHA Standard Specifications, are intended for use under a nutrient management plan, which may not be relevant in mitigation wetlands.

Multidimensional analysis: Multidimensional graphs are used to visualize the relationships between variables. In the figure below, there are three relationships that are immediately apparent. We have highlighted the relationships with shading for emphasis. The figure shows

many dimensions in two-dimensional space, but by visualizing the shaded areas as the three major axis (x, y, z) it shows which variables are related and which are not. The first relationship is sand percent and deep SOM. Sand is a poor medium to accumulate SOM. From the figure we can see that as sand percent increases, deep SOM decreases. The second group of relationships shows that the soil's bulk density (Db) is related to shallow SOM. As shallow SOM increases, the soil's bulk density decreases. The figure also suggests that root biomass is the primary reason for shallow SOM accumulation. The third relationship has to do with the nutrient phosphorus (P). High values of P are closely related to the increase in the percent cattail coverage and increases aboveground biomass. High P (and/or N) also corresponds to decreases in diversity measures, SWI and FQAI.



Related Findings and Publications

The research funding has supported two recent publications with several more pending (one or more reporting the results of this field study). The team prepared a review paper of OM use in wetland restorations, which was the first such publication. A second publication evaluated the use of IRIS tubes. A third paper summarized the results of the lab study on greenhouse gas emission potential. The team is also conducting a lab study using stable isotopes to evaluate the source of greenhouse gases which the team intends to publish. A brief summary of each manuscript is provided below.

The findings in the review paper (Scott et al. 2020) is a summary of prior research on the use of OM in wetland mitigation. There were three primary findings. First, topsoil, when used as an organic amendment, was clearly better than other OM amendments. Second, there are both benefits and drawbacks to using OM. Two of the most apparent drawbacks are loss of SOM and plant diversity. Third, using OM in soils low in SOM (< 2.5%) was no different than other soils.

This finding may have particular relevance to MDOT SHA mitigations since sandy, Atlantic Coastal Plain soils often have low SOM.

The paper about the use of IRIS tubes (Scott et al., SSSAJ, 2021) found that hydrology determines how fast the tubes react. In saturated soils, a 4% per day reaction rate was observed, and in soils that were only intermittently saturated, the rate was as low 1% per day, consistent with others findings (Castenson and Rabenhorst 2006). These two studies do not represent all potential conditions; however, it forms the basis of an expected reaction rate that can be verified with the collection of additional data. The minimum reaction of 30% is the threshold for a positive IRIS test, so this provides some indication of how long soils need to remain saturated to achieve a positive test: under completely saturated conditions one may expect a positive IRIS test within 10 days.

The third manuscript, which has been submitted for publication, was based on the initial lab studies. This work had three main findings. First, OM was not effective at promoting iron reduction, which is necessary to pass available hydric soils tests. An exception was using fresh hay, which did increase iron reduction. Second, OM can alter the soil pH. Lowering soil pH increases iron reduction and reduces methane production. Third, methane production began very soon, within a few days, after soils became inundated. After a period of about 40 days, the methane production rate increased by a factor 50, or more. The use of OM increased methane production and reduced the time it took to see the large increase in methane production rate. The use of OM in wetland restorations could significantly increase methane release.

The fourth manuscript will be a follow-up to the third manuscript. The team is using stable isotopes to investigate the microbial population dynamics that accompany the large shift in methane production. From this study the hope is to gain a greater understanding of the biogeochemical process that govern organic matter metabolism and methane production to inform better control of these processes.

Conclusions and Recommendations for Implementation

The conclusions and recommendations drawn regarding the use of OM in wetland mitigation from a compilation of the literature review, and field and lab studies.

One of the main findings from the literature review was that topsoil consistently performs better than other types of amendments.

- Recommendation: list topsoil as the preferred source of OM as specified in Section 701.03.02 of the Standard Specifications (State Highway Administration 2018).

Soils with low background SOM and high sand content, such as those on Maryland's Atlantic Coastal Plain (ACP), did not respond more favorably to OM amendments.

- Recommendation: Temper expectations for OM amendments in sandy soils.

Hydrology (hydroperiod and microtopography) impacted mitigation wetland development more consistently than OM amendments.

- Recommendation: Invest resources in establishing hydroperiods that mimic natural systems, with an adequate drainage outlet and periodic water drawdown to reduce methane production, control cattail growth, and increase diversity.

Hydric soil test procedures respond favorably to saturated conditions. In fluctuating water conditions, redox potential appeared to give more favorable results than α, α' -dipyridyl.

- Recommendation: Procure equipment for redox potential measurements in new mitigation wetlands. Platinum redox electrodes function best if they are left in place.

Soil disturbance can create negative mitigation outcomes, such as favoring undesired plant species and loss of background SOM.

- Recommendation: Use minimal disturbance practices to establish mitigation wetlands, such as ditch plugging.

Organic matter application rates of $60 \text{ yd}^3 \text{ acre}^{-1}$ appear to avoid some negative consequences that can be associated with OM use, although even this level may increase global warming potential of gases from mitigation wetlands.

- Recommendation: Consider $60 \text{ yd}^3 \text{ acre}^{-1}$ as an upper limit for OM application rates unless the amendment source is topsoil.

Of the OM amendments we evaluated, composted wood chips demonstrated the fewest negative consequences. Amendments with high nutrient content (N and P) favor undesired species growth and higher methane production.

- Recommendation: If OM amendments are used, screen them for free N and P content and favor materials that have been composted.

GLOSSARY

OM – Organic Matter Amendments. The use of the term “OM” in referring to soil amendments, such as manure or composted wood chips. Occasionally, the term “OM amendments” was used rather than just “OM”, if using “OM” was grammatically awkward.

SOM – Soil Organic Matter. The term “SOM” was used to refer specifically to the natural organic material that is part of the soil matrix, and is not OM that was added as an amendment. Once OM is added to soil, the assumption is that OM is eventually transformed into SOM. It is common in literature to refer to soil organic carbon, or SOC. The majority, approximately 60%, of SOM is SOC.

Hydric – The term hydric means soils that are wet (hydro = water). However, hydric has a specific definition related to wetlands that conveys the idea that wetland soils have unique characteristics.

IRIS – Indicator of Reduction in Soils. The acronym IRIS is specific to iron-oxide coated tubes or film used in hydric soils testing.

GWP – Global Warming Potential. Global warming is caused by certain “greenhouse” gases that are able to retain heat energy. Different gases have differing heat retention capacities and QWP is a mathematical process to normalize the heat retention capacities, so they are directly relatable. Carbon dioxide is a greenhouse gas, but it was not included in the analysis because photosynthesis converts carbon dioxide into plant material, which is then converted back to carbon dioxide by microbial respiration. Carbon dioxide, then, can be neutral with respect to GWP. The team considered only nitrous oxide and methane and GWP gases.

Water – The term “Water” (not water) was used as an experimental factor. Water is a categorical description of soils as being either Dry, Wet, or Inundated.

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PHOTOS

[Photo 1](#) – *Microcosm experimental setup*

1-liter jars and VOA vials showing gas sampling procedure. Bottom photo is of the stable isotope experiment setup.



Photo 2 – *Smith Farm wetland mitigation site*

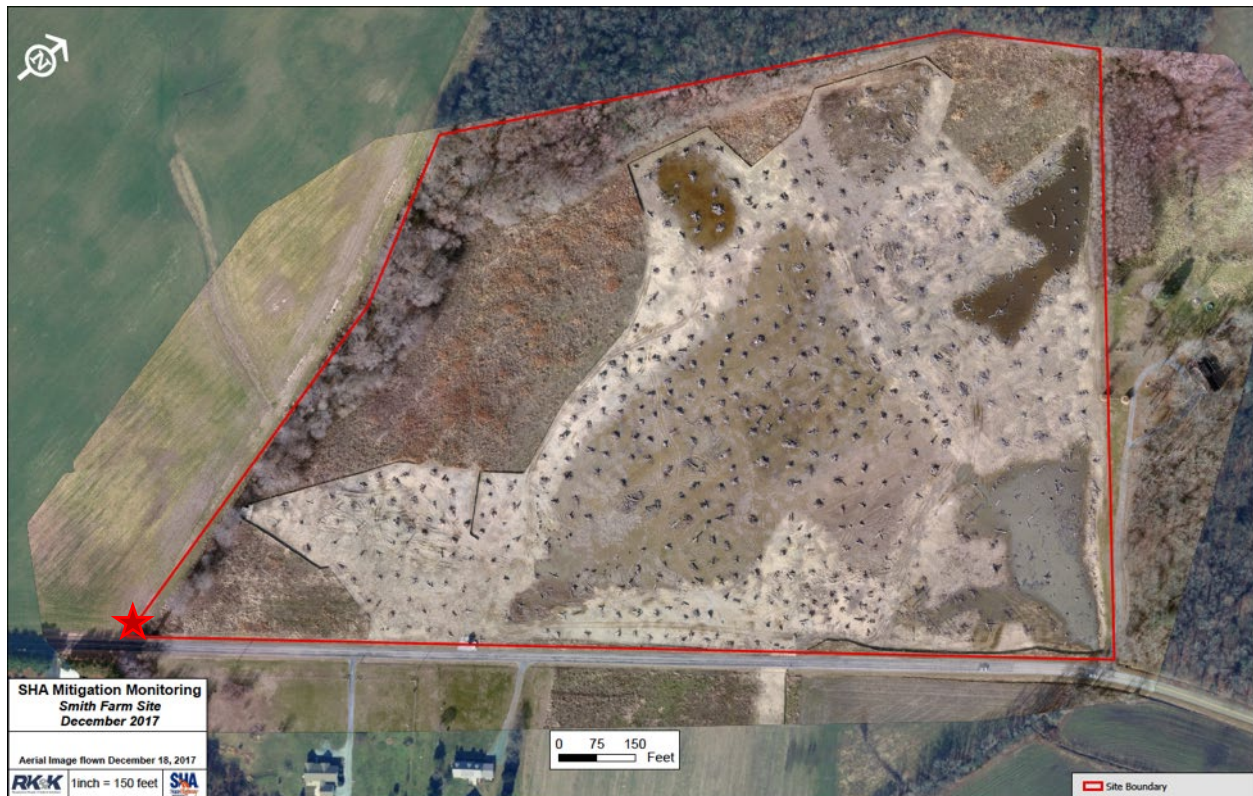


Photo 3 – Smith Farm site. April 17, 2020. Experimental Blocks are outlined in orange fencing.

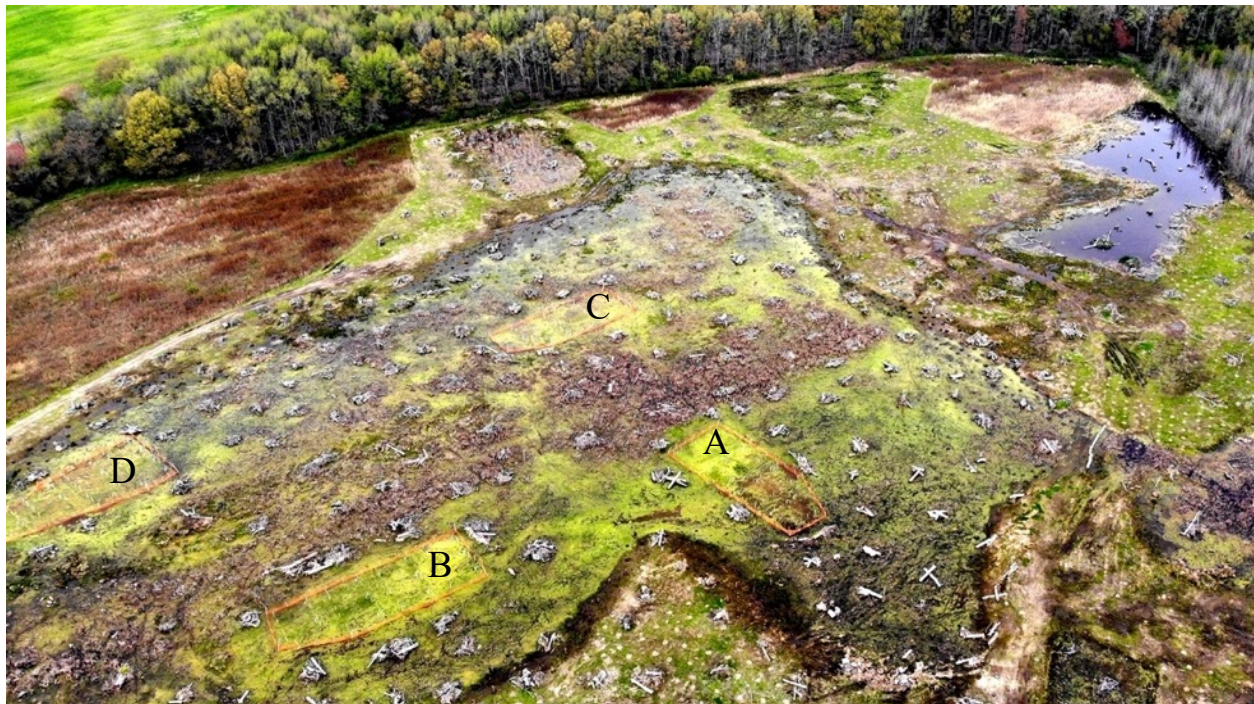
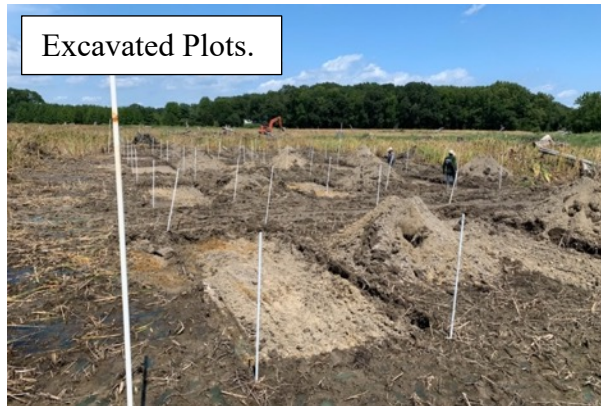


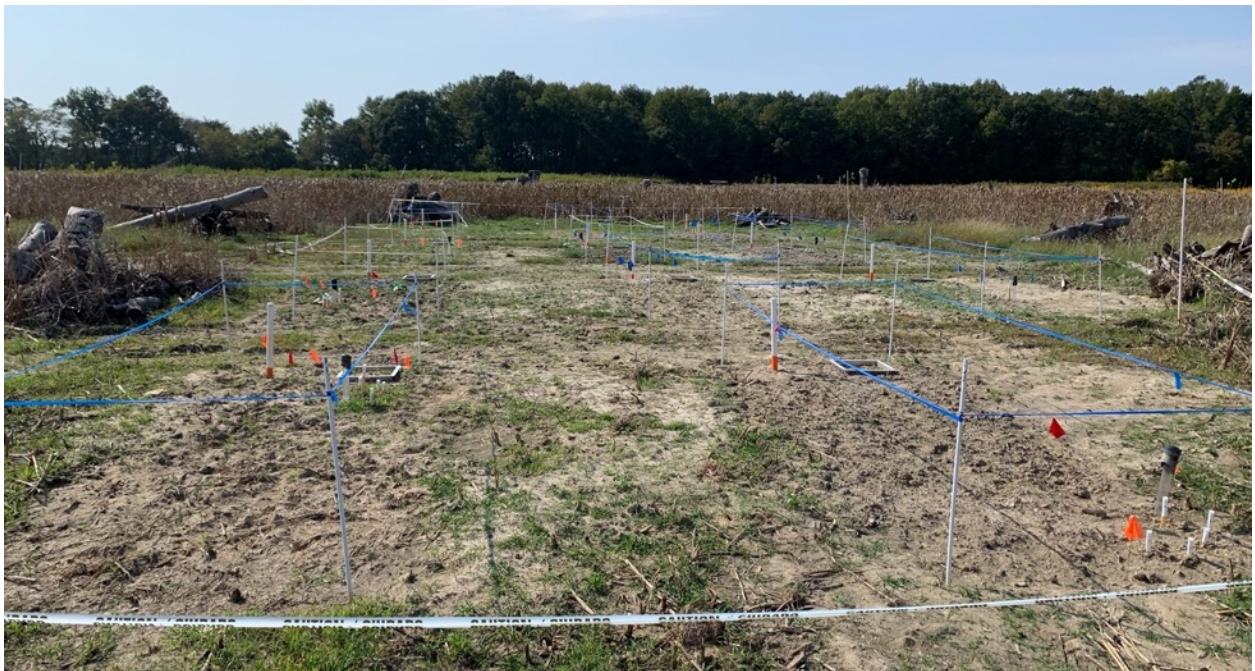
Photo 4 – Wetland drain structure.



Photo 5 – Plot Construction.



[Photo 6](#) – *Final Plot configuration*. Metal rectangular structure is a gas chamber base. The aboveground pipe is a monitoring well. Dimensions are 2 meters x 6 meters.

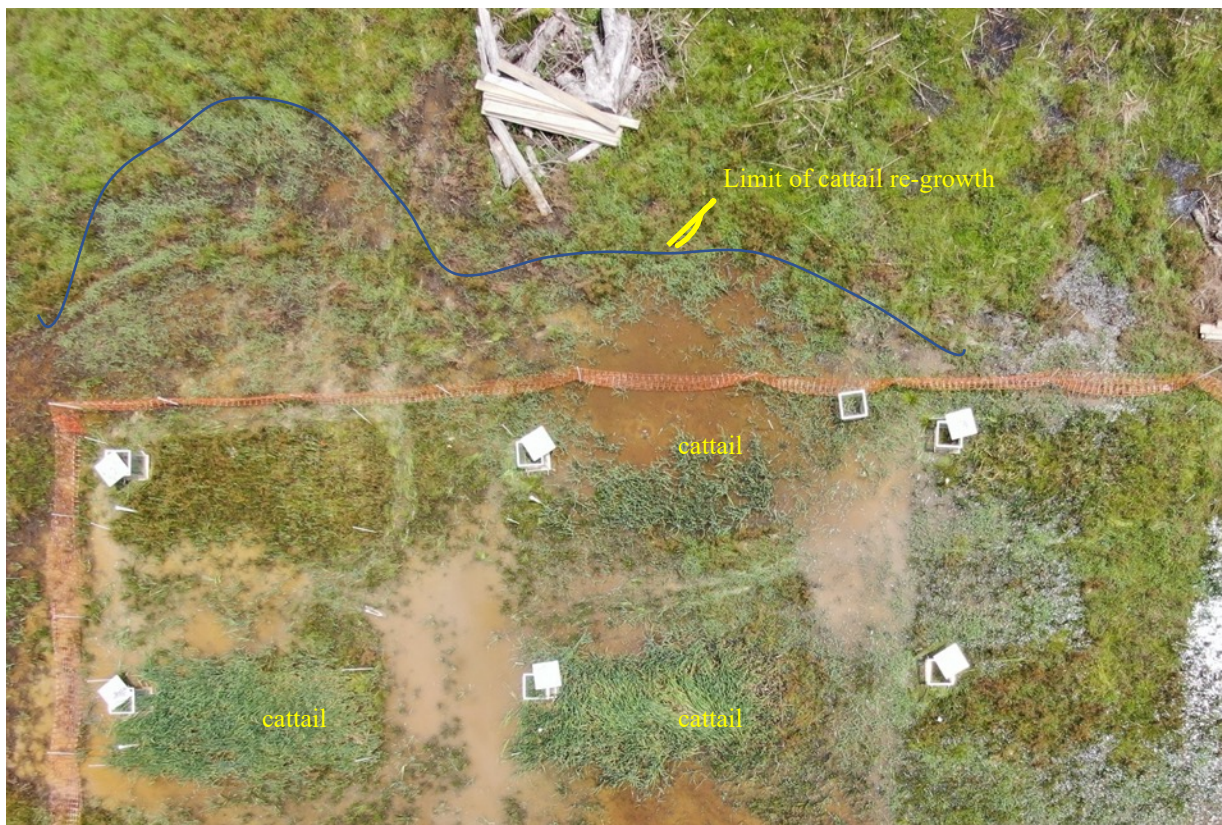


[click here to return](#)

Photo 7 – Block C on 7-28-2020. Large cattail stands are apparent in the Manure Plots (M1, M3, M6).

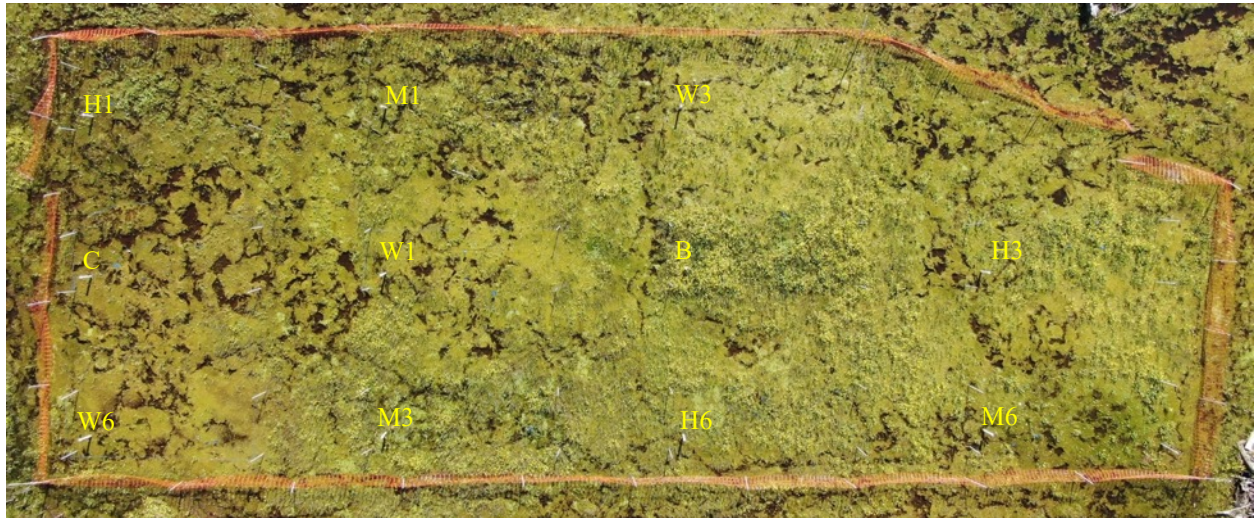


Photo 8 – Block C on 7-17-2020. Cattail re-growth in disturbed areas.

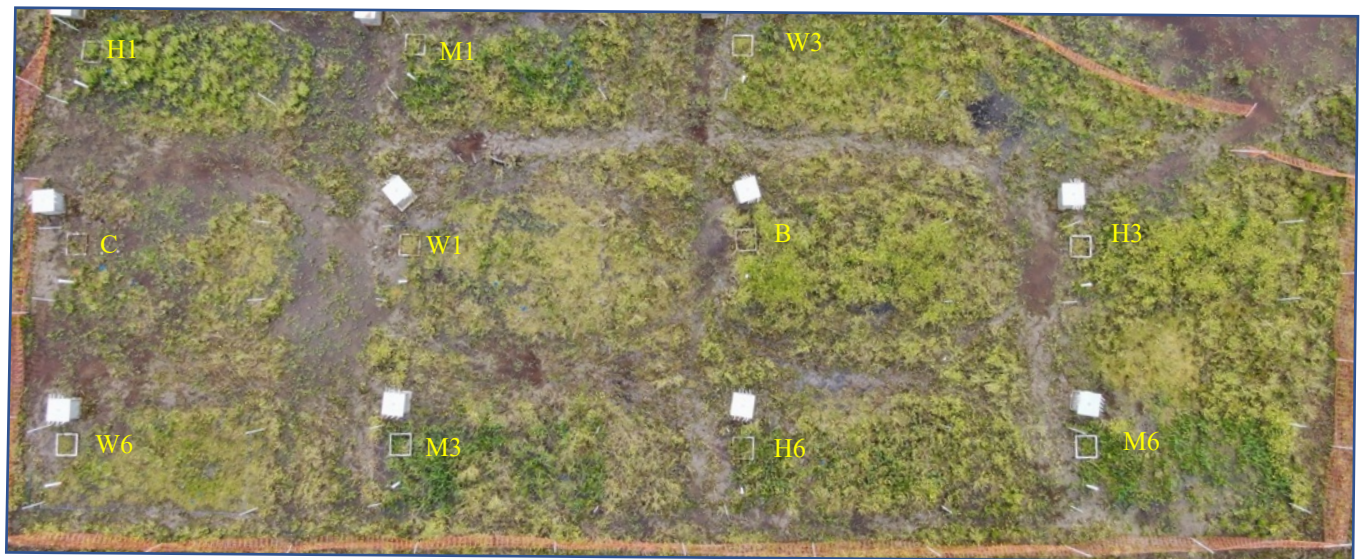


[Photo 9](#) – *Time sequence* for selected days – Block B. Images are high resolution: details can be observed at high zoom.

May 10, 2020 – Green areas are mostly [algal bloom](#). Yellow flowers, particularly evident in Plot B, are buttercup.



June 4, 2020 – Block B. Algal bloom dissipating. Cattail growth more evident in manure Plots.



June 26, 2020 – Block B. Significant cattail growth evident in manure Plots. Some early season plants (e.g. Buttercup) senescing under drying conditions.

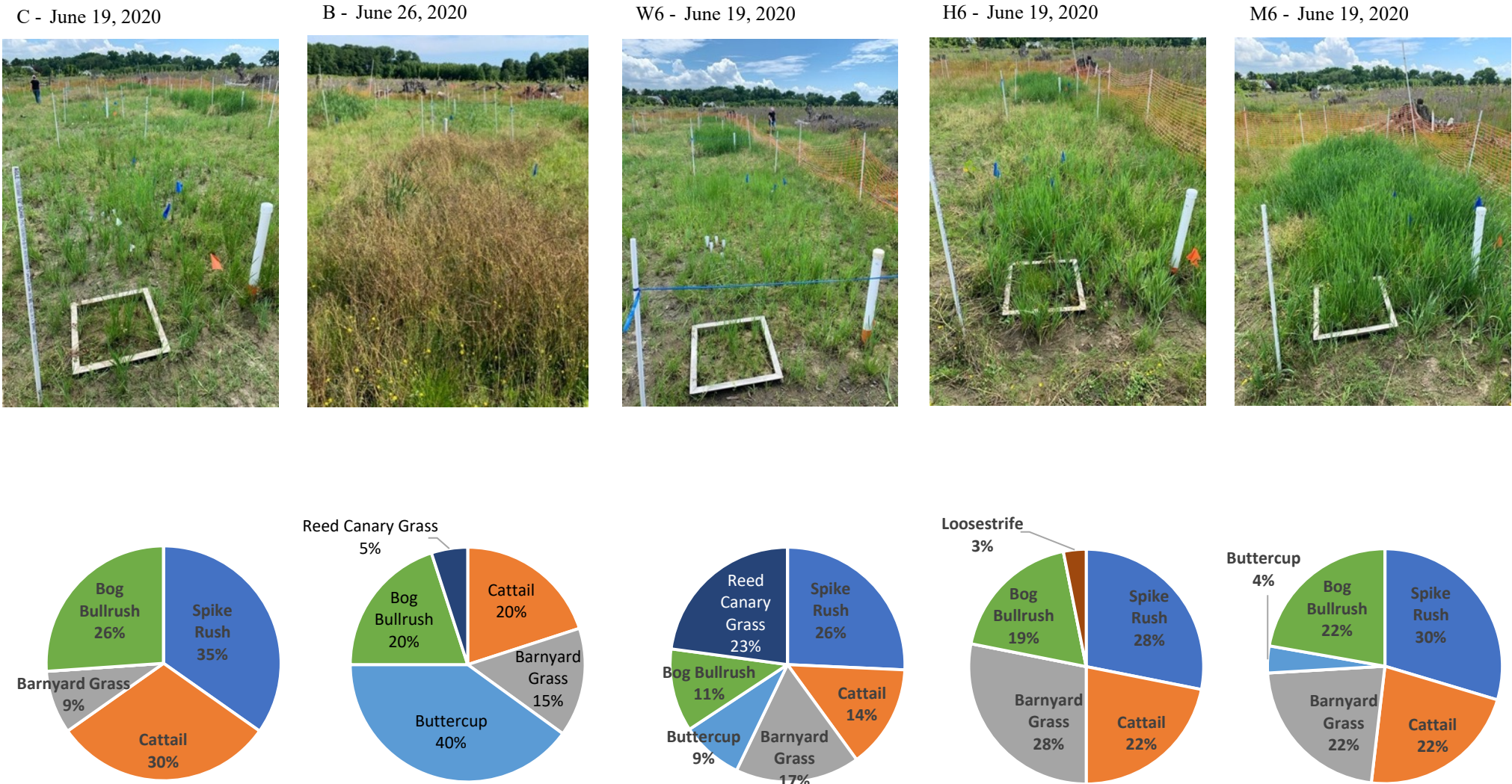


July 28, 2020 – Block B. All Plots, including unamended Control, showing robust growth. Darker green areas in manure Plots are cattail.



Photo 10 –*Selected Plots in Block B*

Plant species distribution from July 28, 2020. The pie charts are included as a visual aid and represent the species present in each photo in Block B in July. A more representative overall distribution of plants by amendment is shown in [Appendix 4](#).



APPENDIX 1 - SHA Compost Classifications

Type A Compost for Smith Farm (Manure)

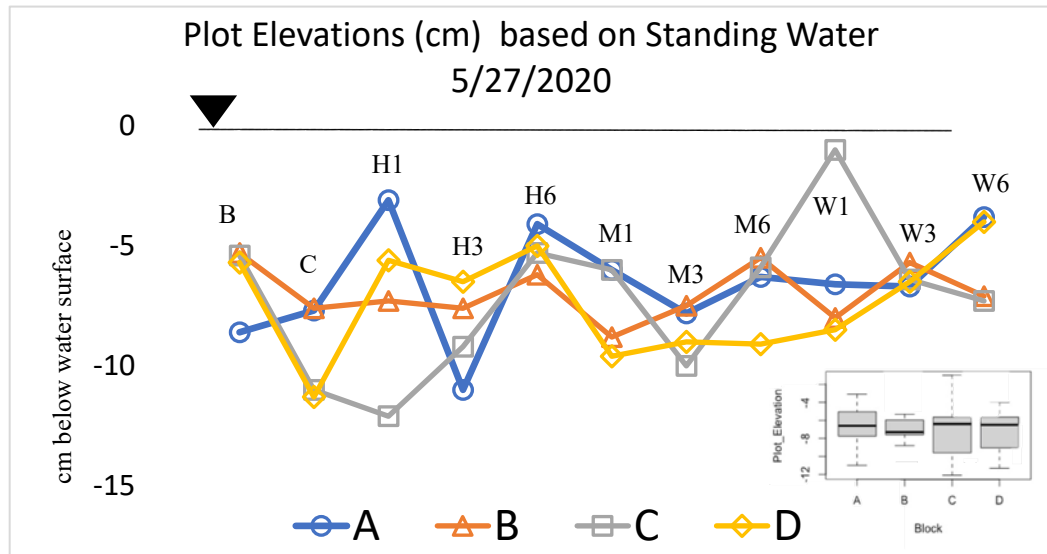
Parameter	Requirement	Proposed Compost
pH	6 – 7.5	7.6
Salt content	< 4 mmhos/cm	0.35
Moisture	30 – 55%	40.5
Passes 1" sieve	--	100%
Passes ½" sieve	100% min	--
Passes 3/8" sieve	--	80%
Passes #4 sieve	90% max	43%
Passes #40 sieve	25% max	14%
Passes #200 sieve	2.2% max	0.5%

Type C Compost for Smith Farm (Mulch)

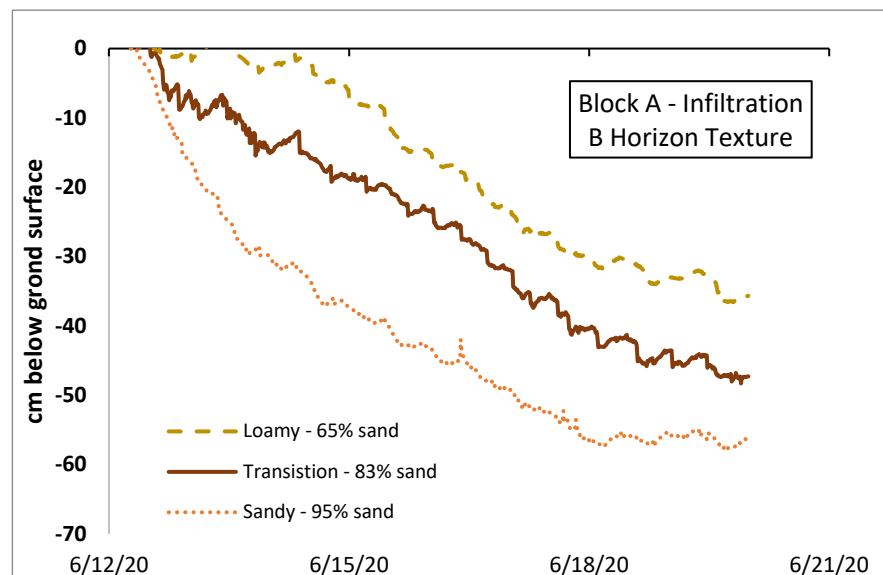
Parameter	Requirement	Proposed Compost
pH	5 – 8	6.9
Salt content	< 10 mmhos/cm	4.3
Moisture	30-55%	39.5%
Passes 6" sieve	100%	100%
Passes ¾" sieve	Minimum 75%	88%

APPENDIX 2 – Site Hydrology

Conditions at this field site led to differences in the hydrology of the test Plots. The site was graded to create a “bowl” in the center, where all the Plots were located. The team selected Plot locations with similar elevation. The figures below show the similarities in Plot elevations in the four Blocks. Based on elevation, all Blocks should have similar hydrology.

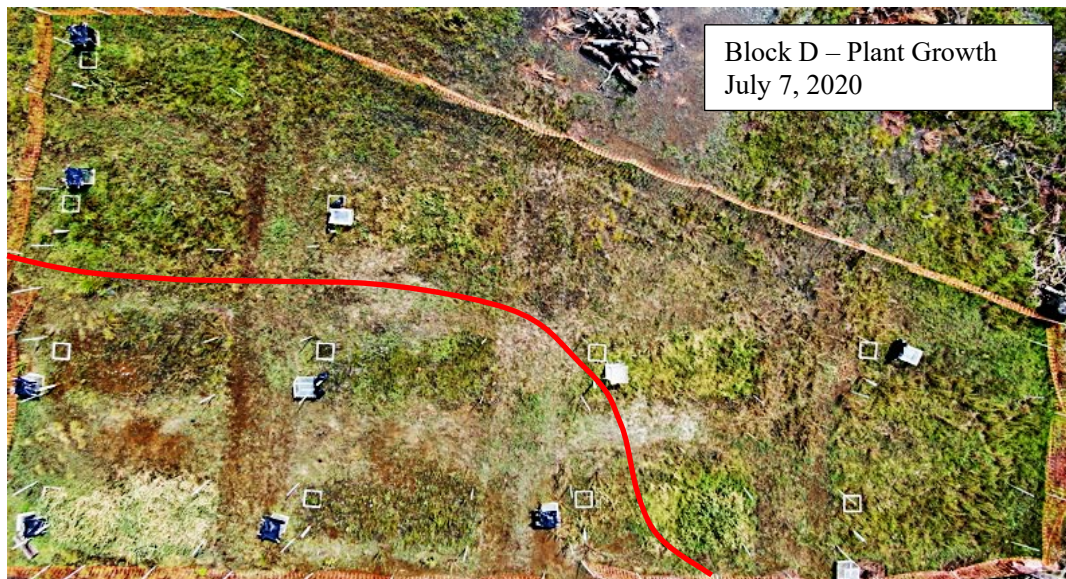


Variations in the subsurface soil texture is the most likely cause of hydrologic variations between Blocks. Saturated conditions at this site depend on surface water (as opposed to rising and falling groundwater levels). How long an area stays saturated depends in part on the surface water percolation rate. For most of the study there was standing water at the site; however, in mid-June rainfall levels were low enough that we were able to record falling water levels. During that time, we had installed water level pressure sensors in all the Plots in Block A. Water levels fell more rapidly in Plots that had sandy B horizon soils.

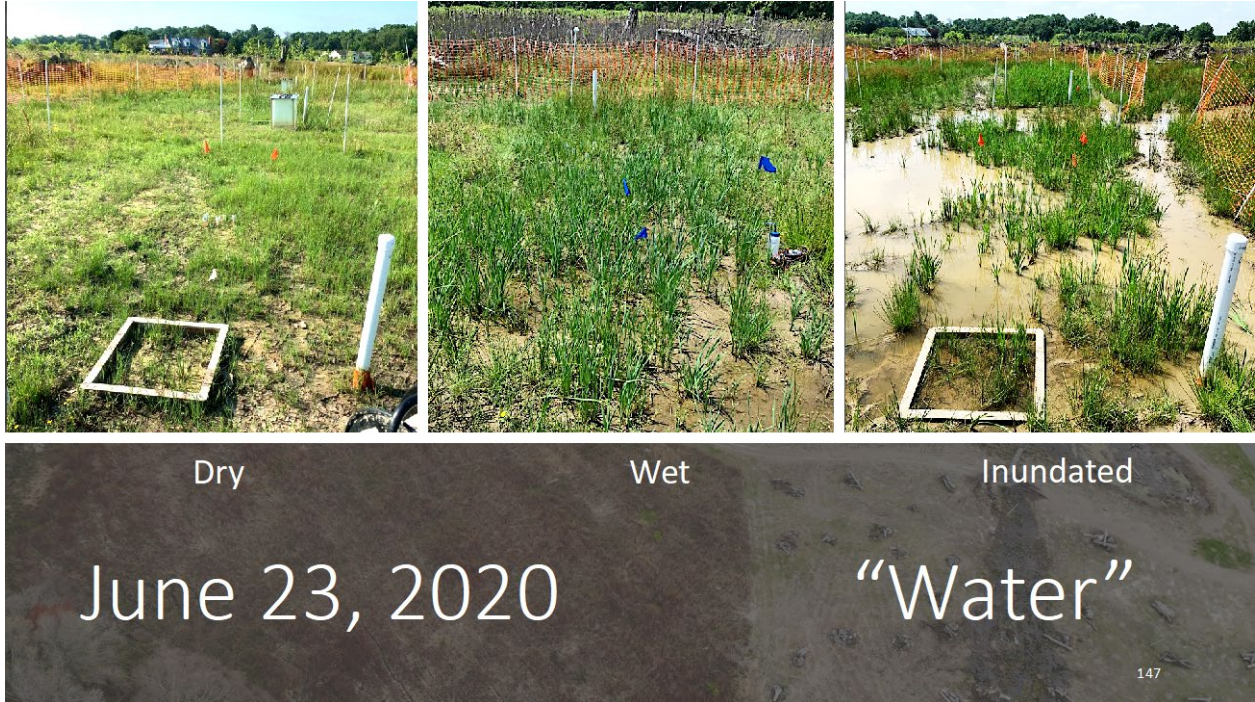


The soil texture transition in Block A corresponds to USDA Soil Conservation Service soil series designations. The July 2016 Phase II Wetland Mitigation Report by Johnson, Mirmiran & Thompson also identified the soil series transitions.

There is a similar soil series transition that straddles Block D. The team was unable to record water levels in this Block during a period of infiltration; however, plant growth showed a clear indication of the soil series dividing line. In June 26, 2020, cattail was still green in the M6 plot (lower left corner) but by July 7 it had deteriorated with age.



The team grouped the results by Block. However, the hydraulic condition of each Block was different. Even within the same Block the hydraulic condition could vary. In order to account for varied hydraulic conditions, the team created a three-category variable named “Water”. Dry = 0 (surface cracking and topsoil visibly not wet); Wet = 1 (soil was wet); Inundated = 2 (at least 1/2 of the plot was inundated).



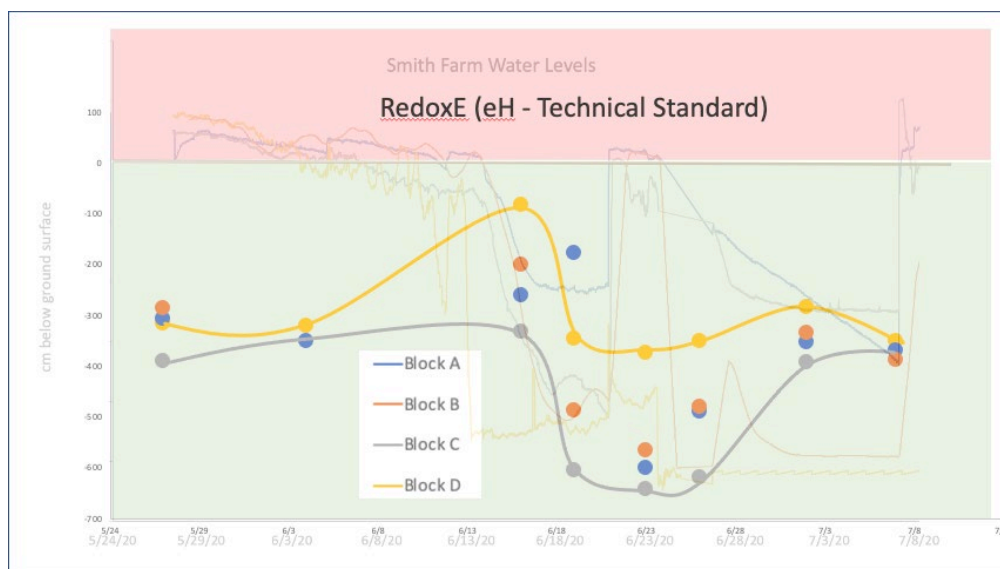
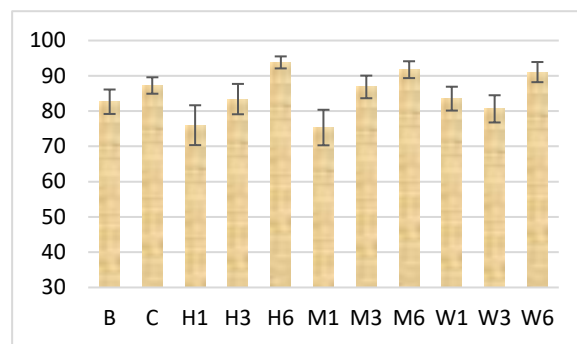
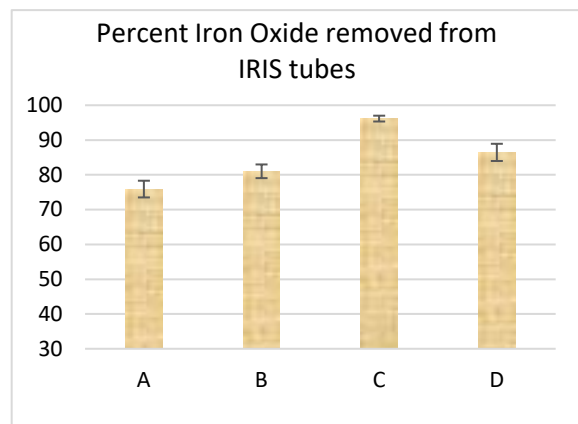
APPENDIX 3 – Hydric soils testing

IRIS film. In mid-June, water levels at the site began receding and soils started to become oxic, which affects the hydric soil indicator tests. IRIS tubes were installed on June 16, after water levels had fallen, to capture this change. However, by the time the team retrieved the IRIS tubes in early July there had been a rainfall event at the site and all the IRIS tubes soils at the site tested positive (> 30% iron removal). Block C remained inundated the entire period, but other Blocks experienced dry soil conditions. The difference between Block C and the other Blocks is small, but statistically significant.

There were also apparent differences based on the OM. Hay removed the most iron oxide, consistent with our lab results, but none of the treatments were significantly different from the plot with no amendments (Plot C).

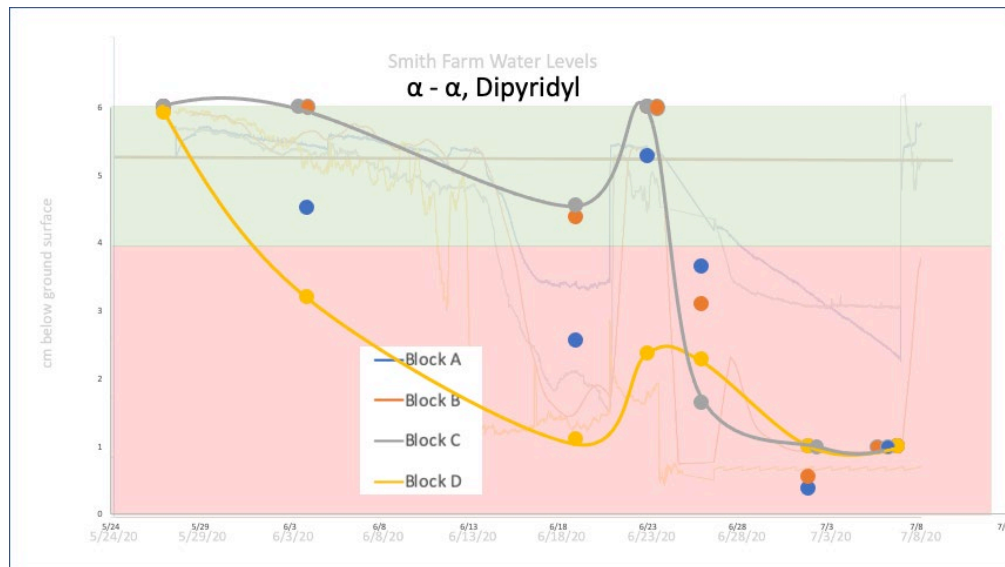
Redox and α, α' -dipyridyl dye

The team measured redox potential in mid-June when water at the site receded. The redox potential responded to changes in water levels, but throughout the period all test results were positive. A positive test is an Eh value lower than the Technical Standard, shown on the figure below. Red shading is a negative test result, green shading is a positive result.



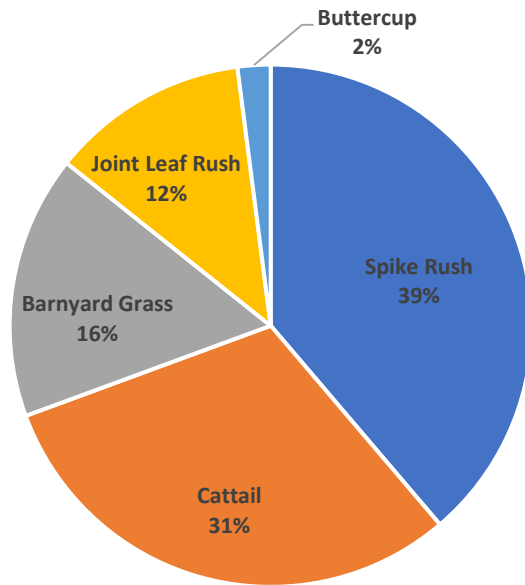
The reaction to α, α' -dipyridyl dye during the same period was also measured. The α, α' -dipyridyl test responded to changes in water levels. Under saturated conditions test results were positive. As water levels fell, negative test results were observed.

The α, α' -dipyridyl test is popular because it is inexpensive, easy to use, and provides immediate results. However, in the field study, this test was the most likely to yield a negative result.

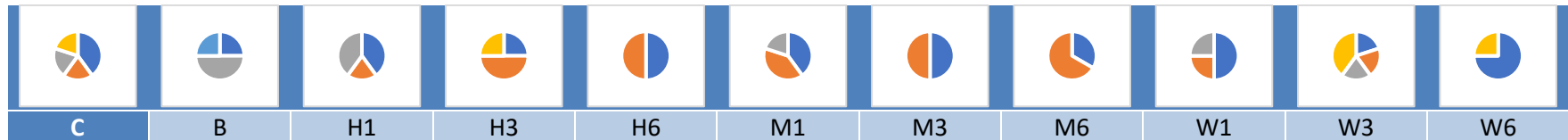


APPENDIX 4 – Dominant Plant Species

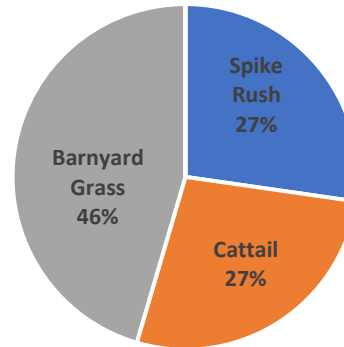
Dominant Species
Overall



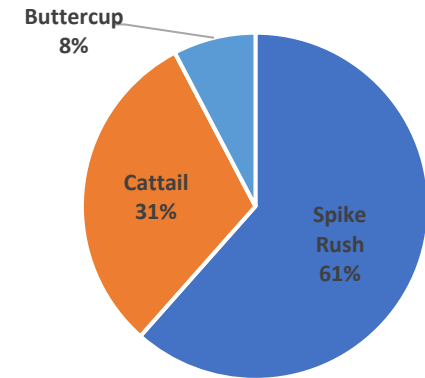
Dominant Species by Plot



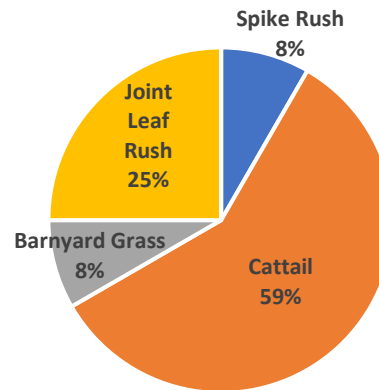
Block A



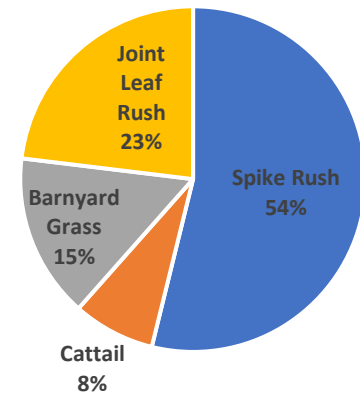
Block B



Block C



Block D



APPENDIX 5 – Plant Species Identified Within Test Plots

Common Name	Scientific Name	Authority	CoC¹	Native/ Introduced	Wetland Indicator status
Broadleaf Cattail	<i>Typha latifolia</i>	L.	1	Native	OBL
Spike Rush	<i>Eleocharis palustris</i>	Britton	5	Native	OBL
Jointleaf Rush	<i>Juncus articulatus</i>	L.	3	Native	OBL
Buttercup	<i>Ranunculus repens</i>	L.	2	Introduced	FAC
Bog Bullrush	<i>Schoenoplectus mucronata</i>	J. Jung & H.K. Choi	2	Introduced	OBL
Pannicgrass	<i>Panicum dichotomiflorum</i>	Michx.	0	Native	FACW
Duckweed	<i>Lemna minor</i>	L.	3	Native	OBL
Nodding Beggarticks	<i>Bidens cernua</i>	L.	3	Native	OBL
Water plantain	<i>Alisma subcordatum</i>	Raf.	2	Native	OBL
Barnyard grass	<i>Echinochloa crus-galli</i>	P. Beauv.	2	Introduced	FACW
Reed Canary Grass	<i>Phalaris arundinacea</i>	L.	0	Native	OBL
Common Rush	<i>Juncus effusus</i>	L.	1	Native	OBL
Switchgrass	<i>Panicum virgatum</i>	L.	4	Native	FAC
Seedbox	<i>Ludwigia alternifolia</i>	L.	3	Native	OBL
False pimpernel	<i>Lindernia dubia</i>	Pernell	2	Native	OBL
Curly Dock	<i>Rumex crispus</i>	L.	2	Introduced	FAC
Valley Redstem	<i>Ammannia coccinea</i>	Rpttb.	7	Native	OBL
Loosestrife	<i>Lythrum salicaria</i>	L.	0	Introduced	OBL

¹ Coefficient of Conservatism (Andreas, Mack, and McCormac 2004). Some plants not listed were assigned based on professional judgment.

APPENDIX 6 – *Map showing Atlantic Coastal Plain*
Findings from our studies would be most applicable in this region.

