ABSTRACT

Title of Dissertation:	ORGANIC MATTER SOIL AMENDMENTS, ANOXIC SOIL BIOGEOCHEMISTRY AND WETLAND RESTORATION
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Organic Matter (OM) amendments are often used in wetland restoration – a practice required in Maryland and other states. This work summarizes a literature review and lab and field experiments to evaluate the consequences of OM amendment use. The literature review showed that although OM use is widely accepted, the evidence that they are effective is weak, and there can be negative effects. Transplanted topsoil is much more effective than allochthonous OM (e.g., manure). OM amendments were largely ineffective in a field study conducted on a mitigation wetland in Caroline County, MD, and negative consequences were possible, although composting the OM relieved negative effects. One example of ineffectiveness: OM is not needed to develop anaerobic conditions in saturated soil. While in some cases OM seems to be a benefit, as in aboveground biomass production, this is usually accompanied by a loss of diversity and it selects for undesired and invasive species. One of the negative

consequences OM is the increased production of methane, a greenhouse gas, which became the focus of this work. Two lab microcosm studies and a field study revealed that rewetting dried soils (as in after mitigation wetland construction) immediately releases small amounts of methane, and methane sharply increases after about 7 weeks. Using OM affects methane production in two ways. First, overall methane production usually increases. Second, the time frame before there is a sharp increase in methane production is shorter, from ~7 weeks to as little as 1 or 2 weeks. These effects are somewhat reduced with composted OM. Using a Stable Isotope Probing microcosm study, the work also helped to identify the archaeal and bacterial taxa that are responsible for the sudden increase in methane. Methanosarcina is likely the primary taxa responsible for methane generation. Understanding the conditions that result in methane emanating from wetlands could lead to practices that reduce its release into the atmosphere, where it contributes to global warming. Methane is a more potent greenhouse gas than carbon dioxide, but is short lived, so controlling methane emissions can have a more immediate effect on climate change.

ORGANIC MATTER SOIL AMENDMENTS, ANOXIC SOIL BIOGEOCHEMISTRY AND WETLAND RESTORATION

by

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Dedication

With sincere gratitude to my co-advisors, Drs. Stephanie A. Yarwood and Andrew H. Baldwin, and my committee members Drs. Martin Rabenhorst, Margaret Palmer and Patrick Megonigal.

There are so many people who inspired or helped me on my PhD journey. Too many to mention. So, instead, I offer this one story.

In June of 1982 I received a surprising letter. I immediately ran to Mr. John Friend's house. He was my high school chemistry teacher. He'd want to know. As he thumbed through the letter his eyes got bigger and bigger. I had aced the Advanced Placement Chemistry test. "So what now?", I asked. "Now," he said, "now you can do anything you want". The trouble was, I was 18 years old and I didn't know what I wanted. My parents had left me to care for my grandmother and I felt stuck. College didn't seem like an option. Classes would be starting in just a few weeks and I hadn't applied anywhere or even taken the SAT. Three days later I received another surprising letter. Northern Arizona University (NAU) had offered me a full scholarship with credit for freshman chemistry. Mr. Friend had walked my test results over to the chemistry department.

Now jump forward to June 2015 (32 years later). I was back in my hometown, Flagstaff, this time to attended John Friend's memorial. I never got to thank him,

properly, for what he had done for me. I thought I would feel sad, but I didn't. Instead, I felt aroused, determined to make him proud and fulfill the potential he had seen in me. I decided right then to do what he, and many other mentors, had encouraged: pursue a PhD.

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List of Abbreviations

Anaerobic methane oxidation (amo). The term amo here includes anaerobic oxidation of the methyl group in acetate.

Analysis of Variances (ANOVA). A statistical method used to combine similar data by pooling the variances in measurements and then comparing averages.

Buk Density (D_b).

Carbon Dioxide (CO_2).

Carbon Use Efficiency (cue). The amount of metabolized carbon incorporated as cell mass (as opposed to respired).

Ferrous Iron (Fe^{2+}). A soluble form of iron that is usually only present in soils as a result of microbially mediated reduction of iron-oxides.

Floristic Quality Index (FQAI). A diversity measurement that takes into account the ecological value of each species. Invasive (undesired) species have low value and native and/or rare species have high value.

Global Warming Potential (GWP). A normalized measure of heat absorbing capacity of carbon dioxide, methane, nitrous oxide, and other greenhouse gases.

Humin. Soil hydrocarbons that include aromatic and carbohydrate carbon with humic and fulvic acids.

Hydric soil. A soil that formed under conditions of saturation, flooding or ponding long enough during the growing season to develop anaerobic conditions in the upper part (official USDA/NRCS definition).

Indicator of Reduction in Soils (IRIS). PVC tube or film painted with iron or manganese oxide. Under anaerobic conditions, biogeochemical reactions remove the paint.

Maryland State Highway Administration (SHA).

Methane (CH_4).

Nitrous Oxide (N_2O).

Nitrogen (N).

Organic Matter (OM). Organic carbon originally derived from plants, including manure. Unlike SOC, OM is distinct and physically separate from bulk soil.

Organic Matter Amendments. The following amendments were used in the experiments: C – Control (no amendments); Biosolids from the Blue Plains wastewater treatment facility, brand name Bloom (B); Composted manure (M); Hay (H); LeafGro, a commercial compost product (L); wood chips, usually used as a mulch (W).

Phosphate (P).

RedoxE. A numerical value representing the difference between the standard reference line value and the measured redox voltage at the field pH.

Shannon-Weiner Index (SWI). Measure of plant diversity using the formula:

Soil Organic Carbon (SOC). Carbonaceous compounds, from organic sources, that are integrated into a soil matrix.

Soil Organic Matter (SOM). A measured value, through loss on ignition by heating soil to 550^{o}C. SOM includes roots and measures non-carbon oxidizable elements.

Chapter 1: Introduction

The other evening, over dinner, I was discussing Jared Diamond's "Guns, Germs, and Steel" with a friend. She claimed the book's central theme was the ongoing struggle for imperialistic power, a thought which never occurred to me. I see the book as a history of human development and a window into land-use changes as we have mined carbon and nutrients from the world's soils, as described by Sanderman, Hengl, and Fiske (2017). Yes, we did read the same book. My incentive to begin a PhD program was a desire to rejuvenate depleted soils. Much more attention is directed toward improvements in foliage (Matzek, Warren, and Fisher 2016), but soils are the foundation (Richardson et al. 2016).

I grew up at the base of Mount Elden in Flagstaff, Arizona. Although not as imposing as the 12,633-foot Humphrey's peak, the mountain still rises an imposing 2,000 feet above its 7,000-foot John Galbraith t base. Despite the large elevation change it is within the vegetation zone for ponderosa pine trees. In 1977 Mount Elden burned (Photo 1.1). Within two days all the trees were gone. Ponerosa pines seeds need fire to germinate, but nothing re-grew. There was a monumental effort to replant trees, which failed. A study showed the fire was so hot it had sterilized the soil, and heavy rains a few days after washed away what little topsoil remained (Falk, Watts, and Thode 2019). Forty years later (Photo 1.2) few trees had regrown: sterilized soil can take decades to centuries to recover (Falk, Watts, and Thode 2019).

The disciplines required to understand soil revitalization are multifarious and can be perceived, like Diamond's book, from various perspectives: pedology, microbiology, (wetland) ecology, biogeochemistry, and even a bit of environmental history. Not being able to strictly identify with any of these disciplines I describe myself as an edaphologist, interested in the effect of soil on living systems.

One of the most commonly accepted measures of soil health is soil organic carbon (SOC) (Stewart et al. 2018). The term SOC, as it is used there, means organic carbon that is integrated into the soil matrix through biogeochemical processes. Very different from, say, burying tree trunks (Zeng 2008). Even roots are not SOC (by the definition used here) because they are distinct organic entities found within soil. The very nature of organic carbon has been, until recently, described in overly simplistic, reductionist terms such as humin (Rice 2001). This research seeks to evaluate the use of organic carbon amendments to enhance soil restoration and reveal at least a small measure of the complexity of organic carbon and how it interacts with minerals and microbes. Even organic carbon seems an inappropriate term, so the term organic matter (OM) is used, in part to differentiate this material from SOC. The composition of OM and biogeochemistry of SOC represents a research *science* topic. However, an equally important applied aspect is ecosystem (soil) restoration. Fortunately, the research and applied science interests are a good match.

Growing up in Arizona I did not expect to be in Maryland studying the biogeochemistry of wetlands. On the surface Arizona appears to be mostly desert, yet it

has a vast network of wetlands, mostly in small streams or are an artifact of human activities. Maryland has an entirely different environment where wetlands are integrated into the landscape and are still widespread even though the majority have been drained for agriculture. In both places wetlands are vital natural resources that clean and store fresh water, provide wildlife habitat, and are diverse recreation areas. Wetlands are so vital that the United States adopted a national "no-net-loss" policy to ensure that for every wetland removed for development, a new one is built to take its place (Executive order 11990). The no-net-loss policy has a large impact on the Maryland State Highway Administration (SHA) who is responsible for constructing mitigation wetlands. SHA provided much of the funding for this work. One goal was to discover more effective construction methods. Another, to determine whether or not using OM amendments, like manure or wood mulch, were effective toward developing the necessary wetland soil chemistry (given the term "hydric") and if amendments were generally beneficial. To address these issues, we performed a comprehensive literature review of previous research, and lab and field studies, designed to help guide the SHA toward selecting the most effective amendment. What we found instead was that for the most part OM amendments have little to no effect, and when there is an effect, it can be often as not negative. For example, OM amendments can release excess methane, a greenhouse gas.

The United Nations has declared 2021 – 2030 the decade of restoration, and the Biden administration has also made ecosystem restoration a priority. Through Executive Order 14008, President Biden calls for restoration of 30% of our land and waters by

2030. It is a sound sentiment, but may be a monumentally bad idea. Without sound principles guiding restoration outcomes the results may be ineffective or even deleterious (K. Suding et al. 2015). Maintaining wetlands often results in an enormous financial burden to control invasive species (Pimentel, Zuniga, and Morrison 2005) and States are expected to bear most of the costs (Hiatt et al. 2019). This is why some States (poorer, more rural) often appear opposed to nature conservation when in fact they are simply financial pragmatists who would otherwise enjoy widespread conservation measures. And where exactly will the land come from? Historically land was taken at the expense, often of their lives, of the people living there (Jacoby 2014). In order for wetlands to be effective they need to be in appropriate locations (Wintle et al. 2019), but the most beneficial and least expensive land resources rarely overlap (International Institute for Sustainability, Figure 1.1). Given these constraints, it is vital we use effective methods of wetland restoration. However, numerous studies suggest we aren't doing a great job (J. Brown and Norris 2018; Moreno-Mateos et al. 2012; Hoeltje and Cole 2007; Burgin 2009; Xu et al. 2019). Embracing a 30% by 2030 policy could push us to do shoddy work in non-ideal locations that bear few ecosystem services. In order to limit restoration failures (Mitsch and Wilson 1996), we need to better understand how wetlands work, what restoration methods will be the most cost effective, and avoid negative outcomes, even in the face of critics (Carpenter 1996, Cabin 2007). The objective of this thesis has been to evaluate if amending soils with OM is a strategy we should continue.

The thesis consists of six chapters, including this introduction. Chapter 2 is a literature review, published in Restoration Ecology. Chapter 3, published in SSSA Journal, is a field study using iron-oxide coatings to test for hydric soils. Chapter 4, findings from a lab study regarding methane emission potential from saturated soils, has been submitted to Biogeochemistry and is under review. Chapter 5 is a summary of field study results. A report summarizing these findings has been submitted to the SHA, which is available online1. Chapter 5 is a substantial re-write of the SHA report, more suited to a scientific audience and one step closer to publishing these findings. Appendix 2 includes information that was not known or available at the time of these earlier publications and provides more context for the publications and how they fit within the overarching thesis theme, something which continuously evolved. Chapter 6 is the results from a stable isotope probing study not yet submitted for publication. Included, also, is a brief writeup on wetland history (Appendix 1). Appendix 3 describes possible future research topics.

1 https://www.roads.maryland.gov/OPR_Research/MD-21-SHAUM5-11_Hydric-Soil-Conditions-Phase-II_Report.pdf

Figures

Figure 1.1 – Areas of the eastern United States where ecosystem restoration would maximize carbon sequestration, increase biodiversity, or be inexpensive to implement. Note there is little overlap.

Source: International Institute for Sustainability

High carbon sequestration potential

High potential to increase biodiversity

Areas where conservation and restoration would be relatively inexpensive

Photos

Photo 1.1 – Radio Fire on Mount Elden, 1977.

Photo 1.2 – Mount Elden 40 years after the fire. Few trees have regrown.

Chapter 2: The role of organic amendments in wetland restorations

** Previously Publish in Restoration Ecology **

Abstract

At the present rate of loss (since 1990), half of the remaining wetlands worldwide will be developed within ~ 140 years, underscoring the importance of improving the creation and restoration of wetlands. Organic amendments are sometimes used during wetland creation. To evaluate the effectiveness of adding organic amendments we used a combined numerical method to assign "scores" on five categories of evaluation metrics: plant growth, soil properties, carbon accrual, denitrification, and anaerobic processes (e.g., redox potential). We found that amendments identified as "topsoil" scored measurably higher and had consistently more positive values with fewer negative results compared to amendments identified as "allochthonous organic matter". Organic amendments had about the same effect on soils with low soil organic carbon (<2.5%) compared to soils richer in organic carbon. Organic amendments are not uniformly effective, and in some cases may have negative side effects. For example, allochthonous organic matter often resulted in a loss of plant diversity. These outcomes along with site conditions should be evaluated before using organic amendments.

Introduction

Wetlands provide beneficial ecosystem services such as water purification, groundwater recharge, streamflow maintenance, wildlife habitat, and carbon (C) storage

(Clarkson, Ausseil, and Gerbeaux 2013; Saha 2016). Worldwide, as much as 87% of natural wetlands have been lost. The rate of loss since 1990 has been 0.57% year^{-1}, somewhat lower than the post-World War II peak of 1.34 % per year (Davidson 2014). The continued loss is alarming, considering that there are fewer wetlands to lose and we now understand their importance to society as demonstrated by the 1975 Ramsar Convention, an international treaty promoting wetland conservation. Replacing them has proved challenging. Restored wetlands may be lower in plant abundance and species richness (Moreno-Mateos et al. 2012) and lack the β-diversity of natural sites (Price, Spyreas, and Matthews 2019). It may take decades to develop soil properties, such as bulk density (Db), comparable to natural sites (K. Ballantine and Schneider 2009). High organic carbon content is ubiquitous in natural wetlands (L. Yu et al. 2017) and wetlands are thought to be a potential long-term carbon sink for excess atmospheric carbon dioxide (Mitsch et al. 2013). However, a review by Trettin and Jurgensen (2002) did not find any long-term studies confirming an increase in soil C after forested wetland restoration. A more recent meta-analysis reported that soil carbon recovery in restored wetlands, in general, is inefficient (Xu et al. 2019). One long term study estimated marsh soils would require 124 and 54 years to develop carbon (C) and nitrogen (N) reservoirs comparable to levels in natural sites (Noll, Mobilian, and Craft 2019). Restored wetlands can have impaired C, N and phosphorus (P) cycling (Hossler et al. 2011) and lower denitrification rates (Bruland, Richardson, and Whalen 2006). Similar shortcomings have also been reported by federal and state agencies (Fennessy et al. 2004; Stelk et al. 2017). In general,

we are falling short of replacing wetland acreage and function (Campbell, Cole, and Brooks 2002; Burgin 2009; Hossler and Bouchard 2010; Jones et al. 2018).

A critical question in restoration science is whether or not a given approach, such as the use of organic amendments, accelerates recovery of functions that are comparable to natural systems. Due to the long recovery period for wetlands it has been challenging to demonstrate restoration goals are being met, leading some to suggest a guiding image rather than a predetermined fixed endpoint (M.A. Palmer et al. 2005; Wohl, Lane, and Wilcox 2015). Unfortunately, studies that evaluate the use of organic amendments in wetland restorations do not address these larger restoration questions and instead focus on intermediate evaluation metrics, which may support long term restoration goals.

A recent review of wetland restoration practices (Richardson et al. 2016) identified hydrology and microtopography (heterogeneity) as important factors for restoration success. The study also identified organic amendments as an additional factor and invited a more in-depth review of this topic. Organic amendments accelerate the reclamation of non-wetlands for agricultural purposes and can lead to improved primary productivity (Larney and Angers 2012), so they may improve wetland establishment and function. Kentula (1997) suggested using organic amendments in wetland restorations to provide opportunities for study and we believe there are now a sufficient number of studies available to synthesize and evaluate their usefulness.

Amendments can come from a variety of sources, including composted plant materials, sewage sludge, or salvaged topsoil. We distinguish between two amendment

types: topsoil (TS), which may include salvaged marsh soil or upland topsoil, and allochthonous organic matter (alOM). There was an insufficient number of studies to compare alOM types (e.g. compost and manure). Many early marsh restoration studies reported favorable results using TS as an organic amendment (Clewell 1981; Erwin et al. 1984; S. C. Brown and Bedford 1997). Erwin and Ronnie Best (1985) reported that TS favored "desired" plant species. In their two-year study, both species richness and percent cover (of desired species such as Pontederia cordata) improved and were able to out-compete undesirable Typha latifolia (cattail). The use of TS can also improve seedling emergence (Burke 1997). In contrast, adding N and P in the form of alOM can create a priming effect that releases bound nutrients, particularly P, from over-fertilized agricultural soils (Ohno and Crannell 1996; Ann, Reddy, and Delfino 1999; Doherty et al. 2014) which favors non-native species (Venterink et al. 2003; K. N. Suding et al. 2005) and reduces diversity (Russell and Beauchamp 2017). Based on these studies we hypothesized that TS would be more favorable than alOM as an organic amendment in wetland restorations. Since all organic amendments provide degraded soils with carbon and nutrients, a common indicator of soil quality (Bünemann et al. 2018), we also hypothesized that amendments would have a greater impact on soils low in organic carbon (Richardson et al. 2016; Stelk et al. 2017).

Methods

We conducted a quantitative review of the literature using methods similar to Pickering and Byrne (2014). Google Scholar was used for our initial search, which

included the terms: “wetland”, “restoration”, and “organic amendments”. We then considered literature that was cited in and cited by articles in this subset. This generated an extensive list from which we selected peer-reviewed scientific studies in twenty-three published manuscripts from 1977 through May 2019 (Table A5.2.1). We included three dissertations and one Master’s thesis, which have not been published in the peer-reviewed literature, because they contained key findings or present additional data for sites from published studies. Studies included experiments that were manipulated at a small scale (e.g., 1 m^2) or entire sites where the author compared their findings either to unmanipulated sites constructed around the same time or to nearby reference sites. Most sites were freshwater palustrine wetlands, but there were a variety of hydrogeographic settings (Table A5.2.1).

We adopted a scoring approach similar to Margaret A. Palmer, Hondula, and Koch (2014) using the evaluation metrics provided in each of the publications reviewed. All evaluation metrics were given equal weight. We sorted metrics into five general categories: a) plant responses, b) soil physical properties, c) increase in total C, d) denitrification, and e) anaerobic processes, which fall within the key ecosystem attributes defined by the Society for Ecological Restoration (McDonald et al. 2016). When an amendment showed a positive, statistically significant change in some metric compared to an unamended control, we assigned a point value of +1 (see Table A5.2.2). We assigned a neutral value (0) when there was no difference between the amended sample and the control. A negative value (-1) meant the result was opposite of that defined as a

positive value. Many studies made repeated measures and we used the largest time increment to assign values. A score represents an average of values by category: the sum of all values in each category divided by the total number of measurements in that category. The net score is the average of all values from all five categories for a given group (all studies, TS, alOM, < 2.5% SOM). A score of +1 means all the individual values in a given category was +1. Each value for a given study was made up of metrics that were assigned +1, 0 or -1, but the values could be fractional, for example if the metric root growth was +1 and the metric shoot growth was 0, then the plant response value would be +0.5). Scores did not meet the requirements for an ANOVA analysis, so we used Wilcoxon rank sum tests for statistical comparisons.

Evaluating scores alone can be ambiguous. For example, a score of +0.25 could represent the average of four values: In the second case there are negative effects, and if those effects were a key metric, like biodiversity, it may be prudent to avoid using amendments. Since scores do not reflect negative effects, we present a qualitative evaluation of the data based on an enumeration of positive, neutral and negative values. In the example above, the first set of values would have a negative value frequency of 0 /4 = 0%, whereas the second set of values would be 1 / 4 = 25%. So, the second set of values may be interpreted as less favorable even though the score is the same. Table A5.2.3 shows all scores and value enumerations.

Results

We present results that measure the effect of organic amendments on each of the five wetland restoration evaluation categories: plant responses, soil physical properties, increase in total C, denitrification, and anaerobic processes, and also the use of TS versus alOM and soils with low SOC. The term SOC is used here as the authors' measure of the C content in the upper layer of the soil where organic amendments were added (see Table A5.2.1). Soils are considered low in SOC if they are less than 2.5%.

Use of Topsoil

Topsoil scored significantly higher than alOM (W = 1449, p = 0.007), supporting our first hypothesis. The net TS score for all categories (+0.53) was significantly higher than the average (+0.30; W = 1141.5, p = 0.03). The net alOM score (+0.22) was below average (), but the difference was not statistically significant (W = 4276.5, p = 0.31). The use of TS had a low incidence of unfavorable outcomes. Out of 29 studies using TS, only one had a negative value ($\frac{-1}{29} = -3\%$; Figure 2.2A). In contrast, alOM had a much higher negative value frequency, $\frac{-14}{75} = -19\%$, and less than half of the values were positive. Topsoil scored higher than alOM in each of the five categories: plant responses (+0.26 versus +0.14), soil properties (+0.47 vs. +0.30), carbon accumulation (+0.75 vs. -0.06), denitrification (+1.0 vs. +0.46) and anaerobic parameters (+0.50 vs. +0.42; Figure 2.1). The score for carbon accumulation using TS was significantly higher than alOM (W = 465, p = 0.01).

Soils with less than 2.5% SOC

We hypothesized that scores would be higher in soils that initially had low (< 2.5%) SOC; however, this was not always the case. The net score for low SOC soils (+0.22) was not statistically different (W = 3000, p = 0.98) than the net score for all studies (+0.30; Figure 2.1). We also expected the frequency of positive values would be greater than average, but soils with low SOC had almost the same positive value frequency of $\frac{32}{57} = +56\%$ compared to the average for all studies ($\frac{57}{103}$) = +55%; Figure 2.2A). The scores for low SOC soil was almost the same for the categories of plants (+0.15 vs. +0.14) and soil properties (+0.39 vs. +0.35; Figure 2.1). Adding amendments to low SOC soil did improve the scores (although not statistically significant) for SOC accumulation (+0.30 vs. +0.18) and increased denitrification (+0.81 vs. +0.53). The anaerobic parameters category scored particularly low (+0.16) versus the score for all studies (+0.44), primarily due to elevated methane production. We considered the possibility that low SOC soil could require a higher amendment dose, therefore, we evaluated effectiveness by amount added using our scoring system. We did not observe a consistent correlation with dose for any of the categories (Figure A5.2.1).

Plant Responses

To evaluate plant responses, investigators used metrics including measures of survival, diversity, and biomass. Overall, the response of plants to amendments was minimal with a score of +0.14 (Figure 2.1). Plants had a frequency of positive values of

$\frac{8}{19} = 42\%$; (Figure 2.2B). We considered the plant subcategories of survival, diversity, and biomass separately. Subcategory metrics are shown in Table A5.2.2 by varied shading. The use of organic amendments improved plant survival (+0.39; Table A5.2.4). However, diversity had a negative score (-0.10) and a high frequency of negative values $\frac{-4}{8} = -50\%$. Plant biomass was largely unaffected by the use of organic amendments (+0.07).

Soil Physical Properties

Soil physical properties include bulk density (Db), soil moisture, and macro and micro-nutrients. Note that we included all measures of N as a macro-nutrient except nitrate, which we discuss separately under denitrification. Organic amendments improved soil properties and had a score of +0.35 (Figure 2.1). The frequency of organic amendments improving soil properties was $\frac{7}{29} = +24\%$, and had a frequency of negative values of ($\frac{-2}{29} = -7\%$; Figure 2.2B).

Carbon

Soil organic carbon was the single most commonly reported value and was included in 26 studies. The addition of an organic amendment to a mineral soil results in an immediate increase in SOC, so we assigned a positive value only when the increase was sustained. Including data from a Master's thesis (Bergschneider 2005) and a graduate dissertation (E. Ott 2018) was particularly helpful in evaluating SOC because it allowed us to construct a chronosequence using data from multiple studies. In this case there was

an initial increase in SOC with the addition of organic amendments, but SOC consistently declined over time (Figure 2.3B). When averaged across all studies, the score for carbon accumulation was low (+0.18; Figure 2.1) and had the highest frequency of negative values $\frac{-7}{27} = -26\%$; Figure 2.2B).

Amendola et al. (2018) reported that in general SOC increases with increasing clay content (as well as being strongly influenced by pH, hydro morphology and aluminum and iron content). We mined data from Amendola's source materials, which showed coarse-grained, or sandy, soils correlated better with low SOC (Figure A5.2.2). Unfortunately, there was an insufficient number of studies to apply a scoring analysis separately to restorations using organic amendments in sandy soils.

Denitrification

Denitrification is cited most often as a treatment goal for constructed wetlands, which came into use in the early 1960s and research on the subject has increased exponentially since the early 1990s (Zhi and Ji 2012). Wetlands not specifically designed for denitrification often receive elevated nitrate inputs due to widespread non-point sources (Cherry et al. 2008; Kaushal and Belt 2012), thus denitrification is important in these systems as well. Although denitrification is widely studied, we found only six restoration studies that evaluated the effect of organic amendments on denitrification. In two of the studies (Bruland, Richardson, and Daniels 2009; Morrissey and Franklin 2015), the investigators had control plots, so we were able to use a simple comparison of statistical significance to assign values. Sutton-Grier, Ho, and Richardson (2009) varied

amounts of amendments and used a regression analysis of denitrification versus SOC, which showed an increasing trend with dose ($r^2 = 0.37$). However, this was not helpful for our scoring method, so we assigned values separately each of the 19 measurements, four of which had denitrification rates that were lower than the sample with the lowest total C, which we identified as the control (Table A5.2.3). Due to the ways denitrification can be measured and the wide variation in denitrification rates in natural systems, evaluating denitrification rates is nuanced. Details on denitrification rate comparisons are provided in Table A5.2.5. The denitrification score for all studies was +0.53 (Figure 2.1) and there were no reported negative side effects of adding organic amendments (Figure 2.2B).

Anaerobic Processes

For our final category, anaerobic processes, we included oxidation-reduction potential (E_h), redoximorphic features, methanogenesis, and microbial biomass. We have included microbial biomass in this category and make the argument that anaerobic indicators are microbially driven. However, microbial biomass does not necessarily represent anaerobic organisms, so this category could be considered "other". The overall score for anaerobic soil processes was +0.44 (Figure 2.1) and had a low incidence rate of negative values, $\frac{-2}{20} = -10\%$ (Figure 2.2B). Topsoil (+0.50) and alOM (+0.42) were similar to the overall score (+0.53). Surprisingly, organic amendments were ineffective at improving anaerobic processes in low SOC soils having a low score of (+0.16).

One of the requirements for the formation of anaerobic redox conditions in soils is the presence of an oxidizable carbon source (L. M. Vasilas and Vasilas 2011). Therefore, we expected organic amendments to reduce the soil $E_{H,}$ but this was not the case. Gray (2010) evaluated E_H by subjecting organic matter amended soil columns (several sources of hay and wood chips) to periodic saturation. Initially, amended columns had a lower E_H compared to the unamended control. However, after several cycles, the unamended control had lower E_H (Figure A5.2.3) so we assigned a negative value for this study. Ott (2018)[57] evaluated E_H in amended soils at a restored field site. Low levels (up to 112 Mg/ha) of alOM (wood and yard waste) decreased the soil E_H resulting in positive values; but higher loading rates increased soil elevation, which allowed the soils to drain and become aerated, increasing E_H so here we assigned negative values. Similarly, we expected organic amendments to stimulate redoximorphic feature development. Gray (2010) observed no difference in redoximorphic features as a result of amendments after one year. E. Ott (2018) saw redoximorphic features in both amended and unamended soils developed after 15 years. Amended soils were dark in color, a positive hysteresis effect of added alOM but unamended soils were gleyed from the loss of iron oxides under comparably low SOC. Both are (equal) hydric soil indicators (Wakeley, Livchar, and Noble 2010) so these were assigned neutral values. Both Gray and Ott used low SOC soil. These two studies suggest that, overall, organic amendments are not needed to lower E_h or develop redoximorphic features.

Methanogenesis was considered in four of the studies from two publications (Winton and Richardson 2015; K. A. Ballantine et al. 2015). The score for methanogenesis alone was -0.75, a potential negative side effect of adding organic amendments.

Microbial biomass was a commonly reported metric and had a score of +0.18 for all studies. Although recent work has verified the efficacy of the standard microbial biomass assessment, chloroform fumigation, in saturated soils (Oren et al. 2018), it is nonetheless a dubious metric in anoxic soils because it can't differentiate between denitrifiers, iron-reducers, methanogens, or even aerobic organisms from unsaturated areas of the soil or rhizospheres.

Discussion

We have employed a scoring method to assess the overall effectiveness of organic matter soil amendments in wetland restorations. This approach enabled us to combine dissimilar metrics and weigh positive and negative outcomes. Organic amendments may provide some modest improvements in common short-term evaluation metrics like denitrification, but there are also potential negative effects, most notably loss of biodiversity. Such trade-offs are common in ecosystem restoration.

Topsoil reduced the potential for negative effects and had consistently higher scores in all the categories we evaluated, which included plant growth, soil physical properties, carbon accumulation, denitrification and, anaerobic properties. We have shown that TS organic amendments scored significantly higher ($W = 1449$, $p = 0.007$)

than alOM. We expected organic amendments to have a larger positive effect on soils with low initial SOC, but this was not always the case. We have shown that using amendments in soils with low SOC is statistically no different ($W = 3000$, $p = 0.98$) than other, higher SOC soils. There are key limitations to our approach. For example, Bruland, Richardson, and Daniels (2009) showed that amendments may reduce p-sorption, but this only occurred at high doses, a detail obscured in aggregate scores. Numerical scoring fails to capture details that could explain a given value. For example, in counting positive and negative outcomes for TS use, we observed only one negative value, so we reported $\frac{-1}{27} = -4\%$. However, the negative value could have been due to factors other than TS, such as the ages of the sites. Another limitation is that our findings may overstate organic amendment benefits since they are based on scientific publications, which tend to be biased toward positive results (Fanelli 2012).

Organic amendments resulted in a positive overall score for plants (+0.14), primarily due to increased plant survival. Plant survival can be a major obstacle to restoration success (O'Brien and Zedler 2006) and can have an indirect effect on the development of biomass and diversity. We saw improved plant survival (+0.39) due to studies using TS, which accounted for 3 of the 4 positive values. The fourth, Stauffer and Brooks (1997), saw an increase in survivability with an alOM amendment (leaf litter compost) and the authors attributed this to improved water holding capacity. While the water holding capacity of mineral soils can be increased by adding alOM as a source of SOC, a recent meta-analysis found that SOC improved water holding capacity at only a

small percentage of sites and those increases were insignificant (Minasny and McBratney 2018).

Organic amendments resulted in a score of +0.35 for soil properties, in large part due to reductions in soil bulk density (Db). High Db can limit root growth (E. C. Wolf, Rejmánková, and Cooper 2019). The root penetration limit for *unsaturated* soils is approximately 1.3 Mg/m^3 (Dexter 2004). Data for saturated soils is limited. E. C. Wolf, Rejmánková, and Cooper (2019) performed a study by physically compacting saturated soils (no amendments) and observed a reduction in the growth of *Scirpus microcarpus* above 1.3 Mg/m^3, which at least does not contradict the threshold from unsaturated soils studies. The results in Wolf et al. (2019)[74] were reported in MPa, which we converted to Mg/m^3 using the relationship derived in Mirreh and Ketcheson (1972). Restored wetlands consistently have higher Db than their natural counterparts, 1.2 vs. 0.6 g/cm^3 (Campbell, Cole, and Brooks 2002). While many of the studies we reviewed reported reductions in Db, very few were below 1.2 g/cm^3, and none came close to 0.6 g/cm^3. Therefore, the values for Db may have artificially inflated the score for soil properties. Bulk densities approaching those of natural wetlands may require decades to develop (K. Ballantine and Schneider 2009) and there is no evidence to date that amendments support long-term Db reductions. Organic amendments may not be necessary to reduce bulk density since all soils in the studies we reviewed were below critical rooting thresholds without amendments.

Soil aggregate formation is a primary contributor to decreasing Db and helps stimulate SOC accumulation (Larney and Angers 2012; Obalum et al. 2017) but we know of no studies linking organic amendments and aggregate formation in restored wetlands. One possible study would be to evaluate different organic amendment types. In unsaturated soils, easily decomposable material had a strong, short-term effect on aggregate formation whereas more composted material has a reduced but more persistent effect (Haynes and Naidu 1998; Abiven, Menasseri, and Chenu 2009). Additional research into soil aggregation could increase understanding of the long-term processes of SOC accumulation and reduction in Db. Micro-aggregate formation has recently been tied to wet-dry cycles (Krause et al. 2019); therefore, research into aggregate formation in saturated soils may help both wetland and non-wetland contexts.

There was a large, statistically significant ($W = 465$, $p = 0.01$) effect on long-term C storage for TS (+0.75). However, initial increases from the use of alOM were lost over time resulting in a score of -0.06. Therefore, we considered what factors could account for these results. Aggregation has been suggested as a primary factor in long term C storage in tidal freshwater wetlands (Maietta et al. 2019). Soil texture may affect SOC storage since the sand fraction of soil does not contribute to aggregation (Elliott et al. 1991). Amendola et al. (2018) reported that SOC increases with increasing clay content (lower sand content), as well as being strongly influenced by pH, hydro morphology and aluminum and iron content. The use of amendments, particularly clay-rich TS at sites with sandy soil, may be highly effective and could be an important area for future study.

Our results show that organic amendments improve denitrification (+0.35), particularly in low SOC soils (+0.81). However, our scoring does not address the underlying mechanism. Wetlands are effective at removing nitrate from water sources and a labile C source such as methanol can drive denitrification (Gersberg, Elkins, and Goldman 1983). Organic amendments may also increase denitrification (Ingersoll and Baker 1998; Burchell et al. 2007). When considered as a source of labile C, the C:N ratio of the organic amendment can be low and still be effective: C:N ratios above five maximize denitrification rates (Ingersoll and Baker 1998) . The C:N ratio in all the studies we reviewed were greater than nine. Even organic matter sources such as chicken manure (Tiquia and Tam 2000) and biosolids (Alam, Fakhru'l-Razi, and Molla 2003) that are known to have elevated N contents typically have C:N ratios greater than five. Thus, virtually any organic matter source would have maximum denitrification rates when acting as a C source. However, the increased denitrification from labile C does not last (Gabor et al. 1994; Pal et al. 2010) and continued labile C inputs from macrophytic vegetation is more sustained and efficient(Lin et al. 2002).

Beyond being a labile C source, organic amendments may increase denitrification rates by being an ongoing source of elevated nitrate. Higher N loading generally results in higher denitrification rates in a broad range of ecosystems (Seitzinger et al. 2006). In tidal freshwater wetlands, denitrifier populations increased with higher nitrate loading, but not with organic amendments (Morrissey and Franklin 2015). Organic amendments may elevate nitrate indirectly. Luo et al. (2018) saw improved denitrification in a staged

oxic-anoxic system with added rice husk. In the oxic stage, the rice husk increased the population of nitrifying bacteria, which elevated nitrate levels and stimulated denitrification in the subsequent anoxic stage. If organic amendments are increasing denitrification by supplying N, this mechanism would rely on an oxic-anoxic interface, which in a restored wetland would occur within root zones (Seitzinger et al. 2006) or as a result of hydrologic cycling (Wu et al. 2014). Microtopography increases the oxic/anoxic interface area caused by hydraulic cycling.

Amendments are often not necessary to stimulate anaerobic conditions such as lowering redox potential and the development of redoximorphic features and may cause excess methane generation. Wetlands can be a significant source of methane and may make up to 30% of global methane emissions (Bridgham et al. 2013). The presence of SOC may lead to methane production if soils remain inundated (Martins et al. 2017; McNicol et al. 2017; Mander et al. 2018). Methane emissions could be controlled by reducing the amendment application rate (Lou et al. 2007; Winton and Richardson 2015). Another alternative, not yet investigated, would be using an amendment with reduced pH. Low pH has been shown to suppress methanogenesis (Z. P. Wang et al. 1993; Ye et al. 2012).

While our review is focused on organic amendments, we found that they can create microtopography which acts as a confounding factor. For example, Bailey, Perry, and Daniels (2007) showed increased tree size with amendment dose, but the authors determined the observed effect was due to a corresponding increase in soil elevation.

Dickinson (2007) compared the effect of microtopography and alOM (yard waste compost) on root growth. Root dynamics in the early part of the growing season responded to microtopography with mounds improving root length and count. However, later in the season elevation became insignificant and the alOM amended plots improved root metrics. Roots, most importantly fine roots, play an important role in belowground SOC formation and nutrient accumulation (J Patrick Megonigal and Day 1988). Several studies compared organic amendments and microtopography (Pietrzykowski, Daniels, and Koropchak 2015; Alsfeld, Bowman, and Deller-Jacobs 2009; Doherty and Zedler 2015). Therefore, we performed a separate scoring analysis on this subset that included microtopography (not shown) and microtopography consistently scored higher than amendments. Other studies have shown that enhancing microtopography can result in greater species richness, percent cover, prevalence of hydrophytic vegetation and increased soil moisture (K. F. Moser, Ahn, and Noe 2009), higher plant diversity(K. Moser, Ahn, and Noe 2007; Sleeper and Ficklin 2016), higher seedling growth and plant survivability (Titus 1990), and enhance nitrogen cycling (K. L. Wolf, Ahn, and Noe 2011). Microtopography generates a range of moisture and physical habitat regimes in close proximity. This provides varied conditions for plant establishment and growth leading to greater productivity and diversity. Intermixed aerobic-anaerobic conditions also support different microbial communities and functions. Seed viability, an important factor in plant survivability, depends on moisture content (Titus 1990; Budelsky and Galatowitsch 1999), water depth, and saturation duration (Seabloom, van der Valk, and

Moloney 1998; Baldwin, Egnotovich, and Clarke 2001). A targeted study using organic amendments across a microtopographic gradient may help clarify the relative contributions of these two factors.

Figures

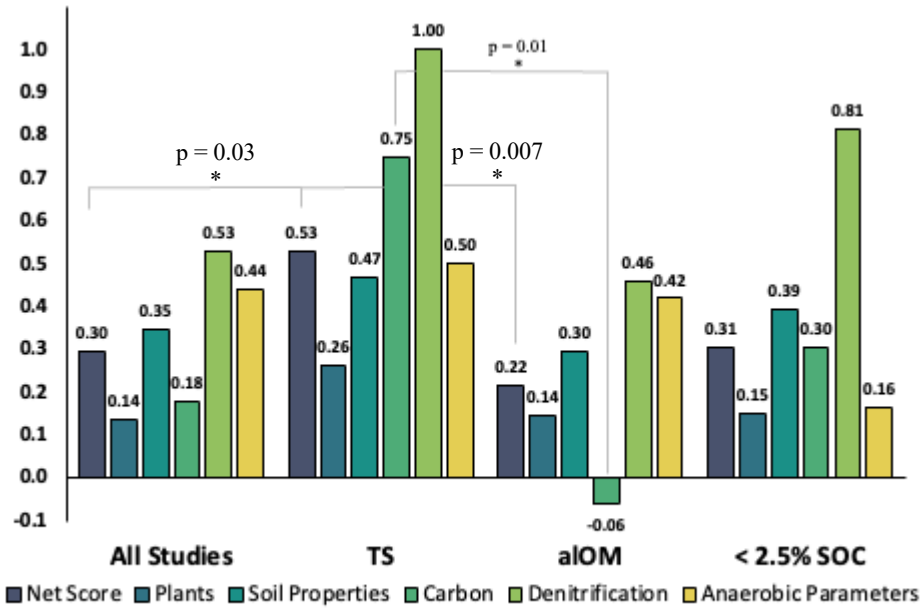


Figure 2.1 – Summary of scores for organic amendment use in wetland restorations. Scores are the average of individual values for reported metrics (e.g. Shannon diversity index). A list of metrics, by category, used to generate values is shown in Table A5.2.2. Negative values were possible if the unamended control was better (e.g. higher diversity) than the amended plot, so the range of possible scores is -1 to +1. The net score is the average of values from all 5 categories (Plants, Soil Properties, Carbon (accrual), Denitrification and Anaerobic Parameters).

TS = Topsoil; alOM = allochthonous Organic Matter; soils with < 2.5% Soil Organic Carbon (SOC) prior to soil amendments. p values from Wilcoxon rank sum test.

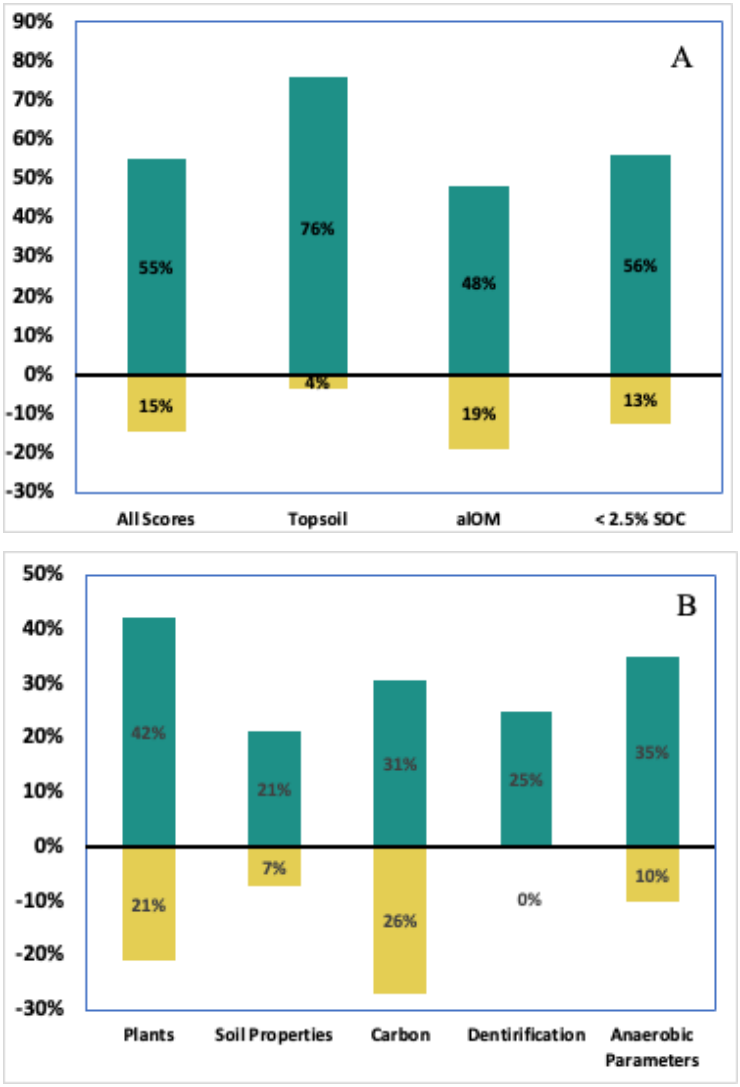


Figure 2.2 – Percentage of values that were positive (above the axis) or negative values (below the axis) after the addition of soil amendments during wetland restoration. The percent of neutral values (not shown) bring the total to 100%. A positive value means the organic amendment produced a positive result compared to a control (as defined on Table A5.2.2). A neutral result meant there was no statistically difference. A negative value means the control produced a positive result compared to the organic amendment.

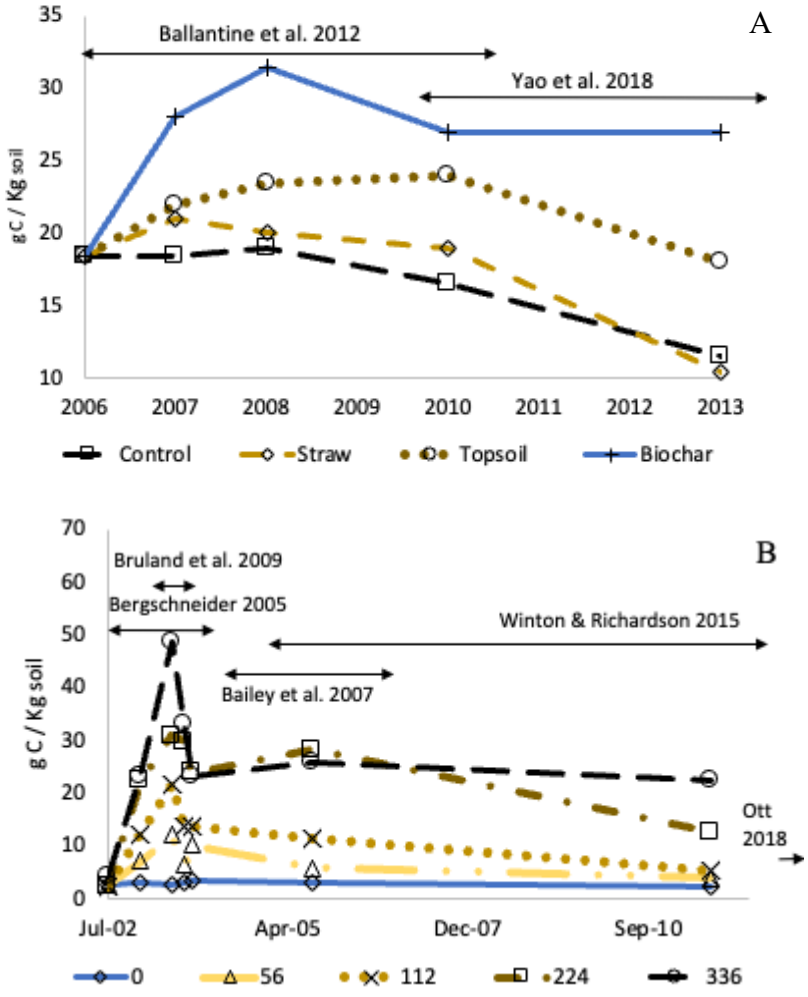


Figure 2.3 – Percentage Chronosequences of SOC content following organic amendments at the same study site.

Plots for 2.3B were originally set up by Bergschneider (2005) and later sampled by others (Bruland, Richardson, and Daniels 2009; Bailey, Perry, and Daniels 2007; Winton and Richardson 2015). Units for SOC are in g C / Kg soil. E. Ott (2018) also sampled these plots but used different units (total biomass, not just SOC), so we did not include the values on the figure. However, based on the ratio of SOC in the amended plots versus the control the data suggests a continued declining trend.

Chapter 3: Macro and Microscopic Visual Imaging Tools to Investigate Metal Reducing Bacteria in Soils

** Previously Publish in SSSAJ **

Abstract

Indicator of Reduction In Soils (IRIS) technology is an important tool for identifying hydric soils, but it does not allow the user to monitor in real time. IRIS uses metal-oxide coatings on a poly vinyl chloride surface that, under anaerobic conditions, are removed to varying degrees over a 30-day incubation period, during which time the user is not cognizant of the outcome. We document the viability of an alternative IRIS approach using clear-IRIS tubes, made from cellulose acetate butyrate, that can be continuously monitored in-situ with a Wi-Fi-enabled video camera. This work shows that IRIS and clear-IRIS tubes are statistically equivalent. Manganese-oxide coated clear-IRIS tubes correlated well with IRIS tubes ($r = 0.79$) and ferrous-oxide had a high correlation ($r = 0.97$). A time-series analysis showed that rain-driven soil saturation induced IRIS metal-oxide reduction and controlled the rate. Clear-IRIS tubes enable remote sensing of metal-oxide removal over time.

Introduction

The Indicator of Reduction in Soils (IRIS) is one of three U.S.-approved techniques (National Technical Committee for Hydric Soils (NTCHS). 2015; NRCS 2010) used to demonstrate soils are reducing (Castañeda, Luna, and Rabenhorst 2017; Berkowitz and Sallee 2011; Hodges et al. 2018). Iron-oxide IRIS rely on iron reduction (B. J. Jenkinson

and Franzmeier 2006; Castenson and Rabenhorst 2006). Iron-oxide IRIS tubes are prepared by applying an ~ 40:60 iron-oxide suspension of ferrihydrite (F) and goethite (G) to the surface of a poly vinyl chloride (PVC) pipe. The tubes are then inserted into the soil, and, if soils are reducing, the iron-oxide coating is converted to soluble ferrous iron (Fe^{2+}), leaving the tube white. Under the NTCHS procedure, IRIS remain in the ground for a period of 30 days before removal and inspection. Manganese-oxide IRIS function similarly but allow detection of more mildly reducing conditions (Dorau, Eickmeier, and Mansfeldt 2016; M. C. Rabenhorst and Persing 2017).

We present an extension to IRIS that uses an in-situ camera to monitor metal-oxide reduction with clear-IRIS tubes. The main advantage of clear-IRIS is the ability to observe iron reduction over time. We painted minirhizotron tubes, which are used to non-destructively monitor roots in situ in upland (Hendrick and Pregitzer 1996) and wetland(Iversen et al. 2012) environments. We hypothesized that the clear-IRIS would be statistically equivalent to IRIS, which we tested by installing both types of tubes at a field site and monitoring the IRIS paint removal over time.

The removal of iron-oxides from the surface of IRIS is assumed to be a microbially mediated process (B. J. Jenkinson and Franzmeier 2006). Microbes access iron-oxide in a variety of ways, including the use of extracellular iron-chelating chemicals (siderophores), electron shuttling via reducible organic molecules, nanowires, and direct contact with the iron-oxide surface (Melton et al. 2014; Sivan, Shusta, and Valentine 2016; Dorau, Eickmeier, and Mansfeldt 2016) proposed two mechanisms for

the removal of manganese-oxides from IRIS: abiotic exchange of Fe^{2+} ions with manganese or phytosiderophores from plant roots. Manganese-respiring microbes can also remove manganese-oxides (Myers and Nealson 1988). We attempted to identify the microorganisms responsible for the removal of metal-oxide from the IRIS. We hypothesized that bacteria would form a biofilm on the metal-oxide rich surface, similar to biofilms observed on the surface of metal pipes (Usher et al. 2014), which we tested by incubating IRIS painted glass microscope slides under saturated conditions in the lab.

Methods

Approach

The primary purpose of this work was to determine if clear-IRIS tubes could be used in place of opaque PVC IRIS tubes. The first step was to verify that IRIS paint would adhere to the tube surface. While we found the IRIS paint adhered to cellulose acetate butyrate, other materials would need to be independently tested. We compared metal-oxide coated IRIS tubes to clear-IRIS tubes in a field experiment at a recently constructed mitigation wetland. After installing all the IRIS tubes, we pulled 5 replicate IRIS tubes at various time intervals and compared the average metal-oxide removal percentage with that of the clear-IRIS tubes, which were video-logged at the same time. The time interval was chosen so all IRIS tubes were pulled prior to 100% metal-oxide removal, and the maximum time interval in our experiment was 28-days. We conducted pilot tests using a minirhizotron camera, but this was unsuitable for IRIS analyses due to the extreme image processing time (Vincent et al. 2017), so we constructed an alternative

camera. The purpose of the field study then focused more on whether the new camera design, which had lower image resolution, was still suitable to analyze the IRIS tubes.

This study also included an evaluation of metal-oxide paint removal rates. By pulling IRIS tubes at various time intervals, we were able to observe whether other monitored parameters, soil temperature and water level, were affecting removal rates. We also evaluated one possible mechanism of metal-oxide removal - the establishment of microbial colonies on the paint surface. Using a clear media for metal-oxide paints (glass microscope slides) it is possible to observe paint removal as it is occurring, so we set up glass jar mesocosms in the lab and incubated saturated soil. Once iron-oxide removal was evident (Figure A5.3.1) we removed the slides and looked for evidence of microbial surface colonization.

Study Area

The field study was conducted at a 13-acre wetland mitigation site located at the Beltsville, Maryland, USDA Agricultural Research Center (Figure A5.3.2a). The site was a forested wetland until the 1950's, when it was drained for farmland conversion. In the early 1980's farming ceased and the site was used as a wastewater discharge area, and then later abandoned in the late 1990's. Construction of the mitigation wetland began in 2016 using salvaged wetland soil from off-site and composted wood chips for topsoil, leading to a relatively homogeneous A horizon (~15 cm) acting as a mulch over the original site soils. The modified soil horizon details are provided in Table A5.3.1. Four locations were randomly selected based on a 6m x 6m grid of the site. Each IRIS

deployment covered an area about 2m x 2m within the selected grid location. Because of significant vertical relief, the site has a series of terraced ponds with interconnected drainage channels. Two plots were located adjected to ponded areas (Pond-A and Pond-B), one along an intermittent drainage channel (Intermittent) and one in an upland area (Upland). We also carried out a pilot test one year earlier. Locations are shown on Figure A5.3.2b**.**

Preparation of Iron and Manganese-oxide Paints and IRIS tubes

We prepared iron-oxide paint as described in Martin C. Rabenhorst and Burch (2006). Ferric chloride was titrated with KOH to pH 12 to form ferrihydrite (Fe_2O_3). The ferrihydrite ages at this pH and converts to goethite (α-FeO(OH)) over time. Ideal IRIS paint is a 60:40 mixture of ferrihydrite:goethite (F:G). Manganese-oxide (birnessite: $(Mn^{4+}, Mn^{3+})_2O_4 \cdot 1.5H_2O$) paint was made as described in Rabenhorst and Persing (2017). Potassium permanganate is reduced in the presence of sodium lactate (6:1 molar ratio). The lactate catalyzes the production of a crystalline form of birnessite that adheres to IRIS surfaces. The birnessite crystals do not contain organic carbon (lactate), which may act as a microbial substrate (verified with a LECO thermal combustion analyzer, data not shown). IRIS tubes were ½" schedule 40 PVC pipe, and clear-IRIS tubes (5.08 cm diameter) were cellulose acetate butyrate. Iron-oxide paint adhesion onto the clear-IRIS tubes was comparable to PVC, whereas manganese-oxide paint required mild sanding with 400 grit sandpaper. Clear-IRIS tubes were given longitudinal and

circumferential registration marks (every 2 cm) with a permanent marker to identify the location during imaging**.**

Soil Incubations, Slide Preparation and Imaging

We performed laboratory mesocosm incubations to test for microbial colonization of metal-oxide surfaces using three different soil types. The first soil was a sandy clay loam surface horizon from the USDA site, where the field study was performed. The second soil was a sandy clay loam from the Salineta saline wetland in northern Spain (Castañeda, Herrero, and Conesa 2013). The third soil, a loamy sand, was from the surface horizon of a recently constructed mitigation wetland in eastern Maryland (39.031227°, -75.794501°). Soils were sieved to 2 - 5 mm, as necessary, to remove large particles. Glass slides were prepared by applying several coats of one of five metal-oxide paints. Manganese-oxide and iron-oxide preparation methods are described above. Iron-oxide pH and curing times were modified to produce three different mineral ratios: > 95% ferrihydrite; > 95% goethite and the standard IRIS F:G ratio. We also prepared a series of slides with lepidocrocite, prepared as described in Schwertmann and Fechter (1994). We verified mineral formulations with X-Ray Diffraction (Figure A5.3.3). We placed glass slides on the wall of the glass jar with the iron-oxide surface pointing in, then filled the jars with soil until the slides were covered, holding them in place. We could observe the metal-oxide removal through the glass jar wall. We incubated jars in the dark at 20^{o}C. To increase the reaction rate, we added hay leachate as a soluble, labile

carbon substrate. We used the leaching ratio from McMahon et al. (2005), 18 g in 3000 mL deionized water shaken at 5ºC for 24 h and diluted this leachate 10:1 with tap water.

The removal of metal-oxides from the slide surfaces became visually apparent after 3-6 days. After trying many dyes, we preferred methylene blue for microscopic analysis. Common dyes, such as crystal violet and malachite green, reacted with the iron-oxides, sometimes creating crystal structures that could be misidentified as filamentous bacteria. Pink dyes such as safranin did not provide sufficient contrast against the yellow/orange metal-oxide background. We attempted fluorescent in-situ hybridization (FISH) using Pacific Blue dye with procedures adapted from Eickhorst and Tippkötter (2008). We also harvested DNA from slides as described in Rahlff et al. (2017) using a stainless-steel razor as a squeegee.

Field Procedures

We installed IRIS tubes by making a 1.9 cm diameter hole with a soil probe before inserting. The installation depth was 50 cm. For the clear-IRIS tubes, we made a 4.85 cm pilot hole, slightly smaller than the clear-IRIS tube diameter, with an auger to a depth of 40 cm, then smoothed the pilot hole with a beveled-end pilot tube. Leaving the pilot tube in overnight eased subsequent clear-IRIS insertion and minimized paint abrasion. Foam insulated 1-quart buckets with brick weights held down the clear-IRIS tubes, avoiding buoyant lifting and reducing solar heat gain. Each plot contained two iron-oxide clear-IRIS tubes, two manganese-oxide clear-IRIS tubes, 25 iron-oxide IRIS and 25 manganese-oxide IRIS tubes (5 per time point). We monitored tubes at various

times over a 28-day period. We removed IRIS tubes in replicates of five (unless otherwise noted) after reviewing clear-IRIS tube images. We calculated the average percent paint removed from the five replicate tubes from the 15cm zone within the upper 30cm with the most paint removed, as stipulated in the NTCHS Technical Standard (National Technical Committee for Hydric Soils (NTCHS). 2015).

We monitored each plot for groundwater elevation and temperature using HOBO ONSET UL20 data loggers for the duration of the experiment at 15-minute intervals. We recorded redox (E_H/pH) at three time points per plot at two depth intervals: 5 - 10 cm and 20 - 25 cm. Redox potential was measured as described in Rabenhorst et al. (2009). In order to provide signal stability, 5 platinum electrodes were left in the soil for a minimum of 4 hours, usually overnight. We measured the soil reactivity to α, α' dipyridyl paper (Macherey-Nagel) at the beginning, and end of the monitoring period. Soil for the dye tests and morphologic descriptions came from a soil biscuit at least 30 cm deep**.**

Camera and Image Processing

Prior to the main experiment, we performed a pilot test using clear-IRIS tubes, but image processing times were excessive. We reduced image processing time by switching to a borescope camera (DEPSTECH) with a 198^o wide-angle lens (Vorida). The borescope camera creates a video image of the entire tube circumference. Four mini-LED flashlights (SanSiDo Bullet) provided illumination (Figure 3.1). The borescope camera has on-board lights, but they create glare when using a wide-angle lens. We have since created a custom 3-D printable housing and replaced the lights with miniature LEDs.

Details for camera construction provided in Figure A5.3.4. The borescope camera transfers images via WiFi to a smartphone. We obtained a video image of the clear-IRIS tube by advancing the camera at a constant rate of 0.9 cm sec^{-1}. Since a smartphone viewscreen is rectangular, coverage can be improved by rotating the camera by 90-degrees and collecting a second video. Videos were converted to a single image using an interactive web tool developed at the Spanish National Research Council – Experimental Station of Aula Dei. Within the program, a user identifies sequential circumferential rings, and the tool stacks and flattens the images to create a single view of the entire tube (Figure A5.3.5). We obtained IRIS tube images using a custom-modified flatbed scanner (Dr. Martin Rabenhorst). The scanner rolls the tube in front of the imaging camera until a full 360-degree image is collected. On the final day of the experiment, the clear-IRIS tubes were removed and scanned.

We quantified paint removal from clear-IRIS and IRIS tubes by pasting images in a Microsoft Excel spreadsheet using an 11w X 24h cell grid. Although one may use an arbitrary cell grid dimension, our cell grid layout corresponds to 17 mm x 12 mm, which is the same size as a minirhizotron view window and matches the parameters in our pilot test. Upon visual inspection, we assigned a value of "1" if the paint was removed and "0" if the paint was still present (Figure 3.2). Manganese-oxide tubes may experience abiotic iron substitution, which is easily visible as a color change: this was also counted as paint removed. We modified the image settings to improve contrast (Table A5.3.2); however, optimal settings may vary depending on the user. We formatted the spreadsheet to

automatically identify the 15 cm region with the highest percentage of paint removed(National Technical Committee for Hydric Soils (NTCHS). 2015).

To compare the clear-IRIS to the IRIS tubes, we used a Pearson correlation, including data from the pilot test (iron-oxide IRIS n = 25; manganese-oxide IRIS n = 27). We also tested to see if paint removal could be represented as a linear function with time, as suggested by M. C. Rabenhorst and Persing (2017) using simple linear regression of paint removal versus time separately for iron and manganese tubes at each plot location. We compared temperature data using time series clustering as described in Montero and Vilar (2014). All statistical calculations were performed in R (R Core Team, 2013).

Results and Discussion

The primary purpose of our study was to compare clear-IRIS tubes to standard IRIS tubes. The amount of paint removal was statistically equivalent for clear-IRIS and IRIS tubes (Figures 3.3a & b). A Pearson correlation between the iron-oxide IRIS tubes was 0.96 (p = 2E-15, Figure 3.3a). The Pearson correlation between manganese-oxide IRIS tubes was 0.75 (p = 4E-6, Figure 3.3b). This shows the reaction rates were similar and our camera afforded images of sufficient clarity to identify areas where paint had been removed (Figures 3.2a &b). Iron-oxide coated tubes are easier to interpret than manganese-oxide tubes. It is difficult to distinguish manganese-oxide from the soil background, and abiotic replacement with iron-oxide results in a mosaic of colors (Figure 3.2b).

The investment of labor and capital associated with the two methods is similar. Initially, we successfully performed a pilot test using a minirhizotron camera; however, the image processing time was so high (> 12 hours per data point) that we would have abandoned the work had we not developed an alternative camera and image processing system (~ 30 minutes per data point). We built the camera using commonly available components for under $100. The camera specifications are shown on Figure A5.3.4. Although image resolution is significantly lower, it was sufficient for our purpose.

Clear-IRIS is a step toward a remote sensing, change detection approach to monitoring the soil redox condition (Hussain et al. 2013). Remote sensing, which includes object-based images, has seen an increase in popularity and utility, including wetland studies (Dronova 2015). Scientists often use a destructive sampling approach to evaluate a time series change detection. Destructive sampling is appropriate in a laboratory because steps are taken to make experimental units homogeneous. However, in a field setting this would not be a true change detection because it cannot account for spatial variability. Spatial variability can give rise to impossible scenarios, such as an apparent reduction in the percent paint removal with time. Our results show that local heterogeneity did not compromise overall results, reinforcing the findings by M. C. Rabenhorst and Persing (2017), who compared the change in iron-oxide to manganese-oxide IRIS tubes over time.

During our experiment, both temperature and soil saturation varied. The metal-oxide removal rate for both iron-oxide IRIS and manganese-oxide IRIS tubes has been shown

to vary with temperature (Dorau, Papenfuß, and Mansfeldt 2018). We logged the temperature in each of the plots at 15-minute intervals (Figure A5.3.6). The soil temperature in our plots varied from ~ 9 - 13^{o}C (Figure A5.3.6), sufficiently above biological zero to promote microbially mediated iron-reduction (M. C. Rabenhorst 2005). Time series clustering showed there was no significant difference in temperature between plots ($p > 0.991$), so it is unlikely temperature was a factor in the different paint removal rates between plots. Rainfall changed both the soil temperature (Figure A5.3.6) and the degree of soil water saturation in the target zone (0 – 30 cm bgs; Figure 3.4). Metal-oxide paint removal rates fit the expected pattern based on soil saturation (Hodges et al. 2018): higher saturation resulted in higher removal rates (Table 3.1). Iron-oxide tubes (r^2 range: 0.71 and 0.94) and manganese-oxide tubes (r^2 range: 0.48 and 0.84) fit a linear model (Table 3.1). Our observed metal-oxide removal rates compared favorably with Rabenhorst and Persing (2017). Iron-oxide removal rates were 1.1 – 4.3 % day^{-1} compared to 2.0 % day^{-1} in Rabenhorst and Persing (2017). Manganese-oxide removal rates were 3.0 – 8.4 % day^{-1} compared to 2.75 % day^{-1}.

Metal-oxide removal rates, however, were not strictly linear but dynamic following soil-saturation (Figure 3.4), which varied between plots. The deep zones in plots adjacent to standing water (Pond-A and Pond-B) were saturated throughout their 21-day insertion period and iron-oxide removal were highest (84 % and 75 %, respectively). The Pond-A shallow zone, which was adjacent to a spring fed pool, was saturated the first 10 days, when most of the paint removal occurred (23 %): final removal was 27 %. The shallow

zones of the other three locations experienced fluctuating water levels, and as a result paint removal was low (0.9 – 16 %). The Intermittent deep zone was saturated the first 9 days when most of the paint removal occurred (43 %): final removal, after 22 days, was 56 %. The Upland deep zone did not see significant paint removal the first was 22 days (6 %), but following a rain event, which saturated the deep zone, paint removal increased to 27 % in the subsequent 6 days. In all cases, paint removal rates were higher during periods of saturation, consistent with other IRIS studies that show rainfall events can induce iron reduction (Hodges et al. 2018) and soil saturation is the main factor affecting ion-oxide paint removal (Bryant 2010). One other factor, necessary for microbial activity responsible for paint removal, is a labile carbon source. We assumed carbon was not a factor due to the recent construction which resulted in a homogenous, carbon rich surface horizon.

We also measured the soil's redox condition with α, α' dipyridyl dye and E_h electrodes. For dipyridyl, we applied the NTCHS guidance requiring a 66% response (2 of 3 test strips) for a positive reaction. For E_h measurements, we compared the E_h and pH values to the NTHS technical standard line. Values above the line were considered not reducing and values below reducing. Values more than 100 mV below the technical standard line were labelled strongly reducing. In general, both tests were consistent with our IRIS results.

We attempted to visually verify and identify the bacteria responsible for the removal of metal-oxides on IRIS surfaces but were unsuccessful. Using methylene blue, we

observed the presence of stainable organic material on the metal-oxide surfaces but found no visual evidence of microbial cells (Figure A5.3.1). Similarly, we did not observe surface-bound organisms using FISH. Downie et al. (2018) had previously used fluorescent spectroscopy to observe bacteria grown on similarly prepared metal-oxide coated slides; however, their study did not use in-situ soil conditions but instead used cultured *Geobacter sulfurreducens*. Material removed from our slide surfaces contained trace amounts of DNA, but it was present in insufficient quantities to amplify and sequence. With no evidence of surface-bound bacteria, our results suggest an indirect electron transfer model, such as cytochrome or flavin embedded extracellular material or electron shuttling (Markelova et al. 2017; M.E. Hernandez and Newman 2001; Uchimiya and Stone 2009; Strycharz-Glaven et al. 2011).

We have shown that clear-IRIS tubes perform similarly to standard IRIS tubes and may be a useful complement to existing IRIS technologies, particularly in its ability to provide temporal redox information, a limitation identified by Dorau, Papenfuß, and Mansfeldt (2018). Clear-IRIS tubes can remain in place, enabling the user to take repeated images over time. There are several advantages of this approach: 1) the clear-IRIS tube could be used as a sentry to alert the user that the IRIS tubes are ready for retrieval, which could be particularly useful if reducing conditions do not develop within 30 days of the original installation date, or if the paint is removed from the tube prior to the 30-day waiting period. 2) Clear-IRIS tubes present the opportunity to monitor metal-oxide removal rates and correlate removal to triggering events, like temperature, rainfall,

or changes soil saturation due to fluctuating groundwater levels. 3) the user may elect to leave the tubes in place and use them to monitor root growth or other soil properties after the metal-oxide paint has been removed. An important limitation of this technology is the oxygen permeability of cellulose acetate butyrate (Quintero et al. 2014), which in some cases resulted in re-precipitation of iron-oxides on the clear-IRIS tubes surface. Another limitation is precipitation of opaque iron-sulfides where sulfur is present (Vaughan et al. 2016). Using an approach such as ours, clear-IRIS tubes may provide an opportunity for remote sensing and time-series redox data collection.

Figures

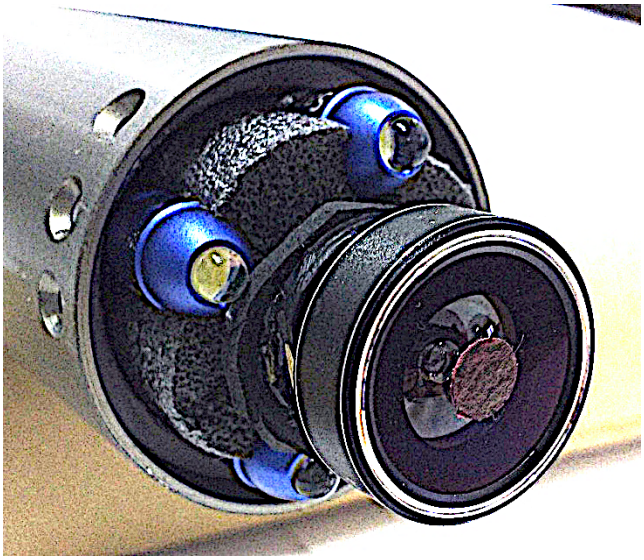


Figure 3.1 – Camera constructed for clear-IRIS tube images.

A wide-angle lens was attached to a borehole camera. Illumination was provided by mini-flashlights. The center of the lens, not used for image processing, was blotted out to reduce glare.

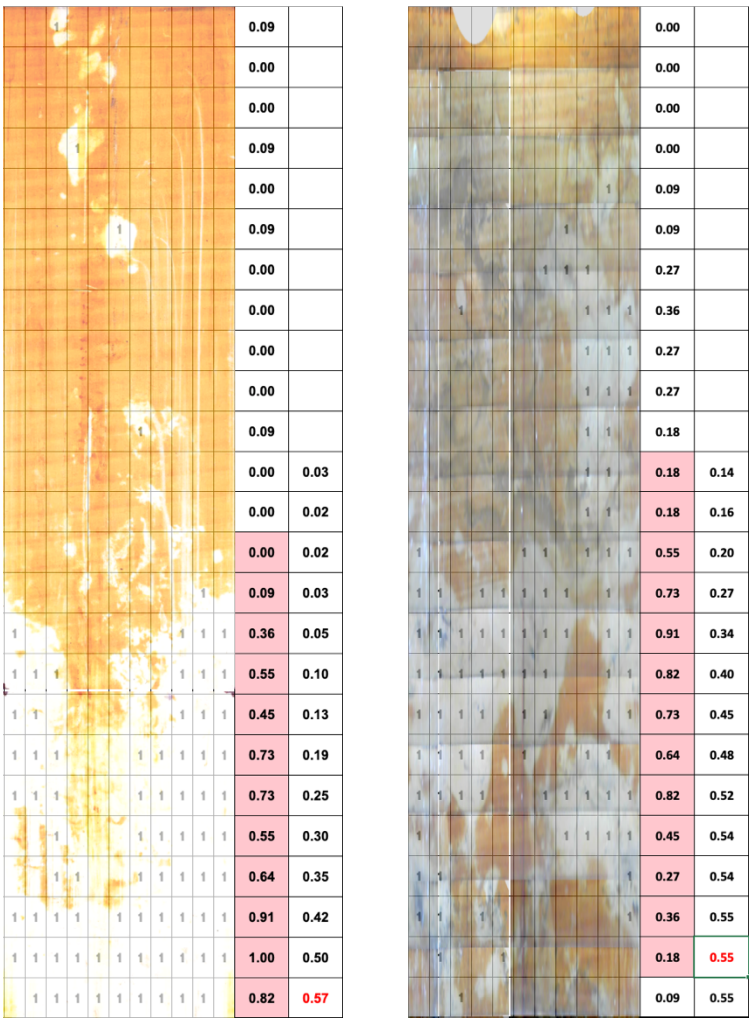


Figure 3.2 – Sample images of a scanned metal-oxide IRIS tubes (left) and a clear-IRIS tube (right) taken from the same plot at the same time.

a – Iron oxide

A value of "1" was assigned if iron-oxide was removed. Values to the right of the image is the percent removal from that interval. The shaded area is the 15 cm interval with the highest average paint removal (highlighted in red in the column to the far right). Intermittent plot after 17 days.

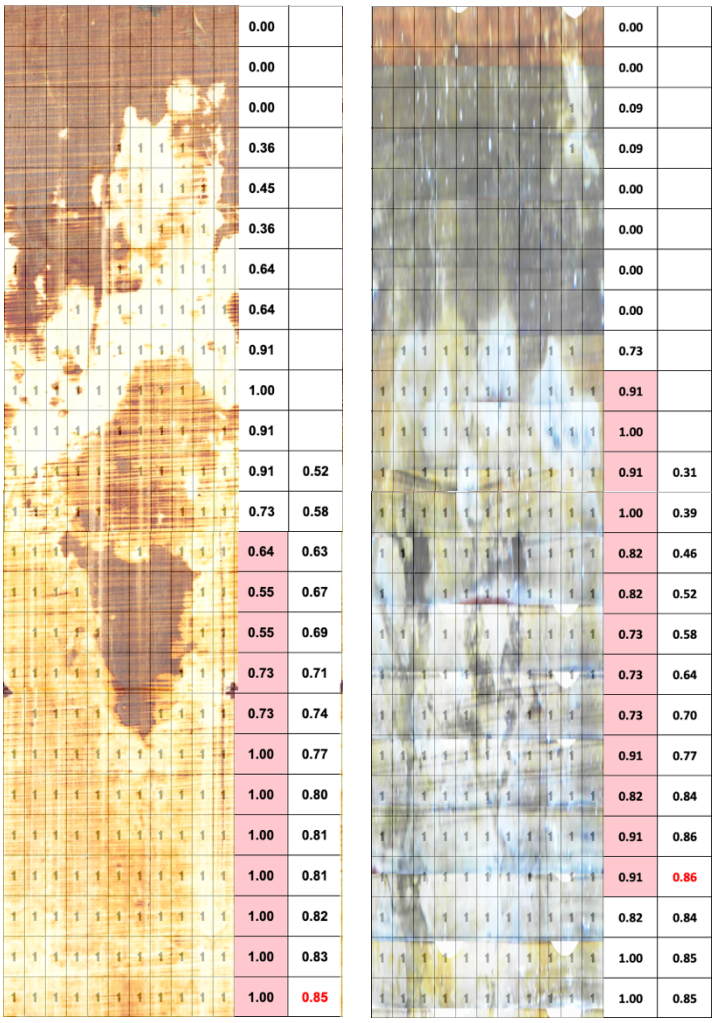


Figure 3.2 – Sample images of a scanned metal-oxide IRIS tubes (left) and a clear-IRIS tube (right) taken from the same plot at the same time.

b – Manganese oxide

A value of "1" was assigned if manganese-oxide was removed or replaced with iron. The yellow color is from abiotic iron-oxide replacement of the manganese-oxide. Values to the right of the image is the percent removal from that interval. The shaded area is the 15 cm interval with the highest average paint removal (highlighted in red in the column to the far right). Pond-B (t = 7 days).

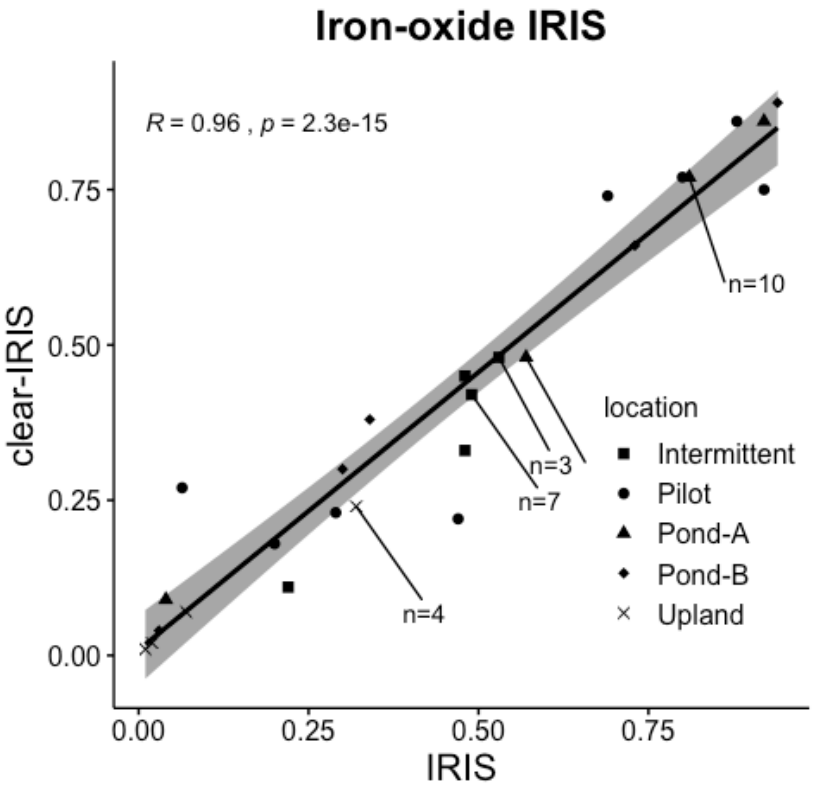


Figure 3.3 – Comparison of metal-oxide coating removal from IRIS and clear-IRIS tubes. a = Iron oxide

Each data point is taken from the same location at the same time and represents the average of two clear-IRIS tubes and several IRIS tubes. n = 5 for IRIS tubes, except where noted.

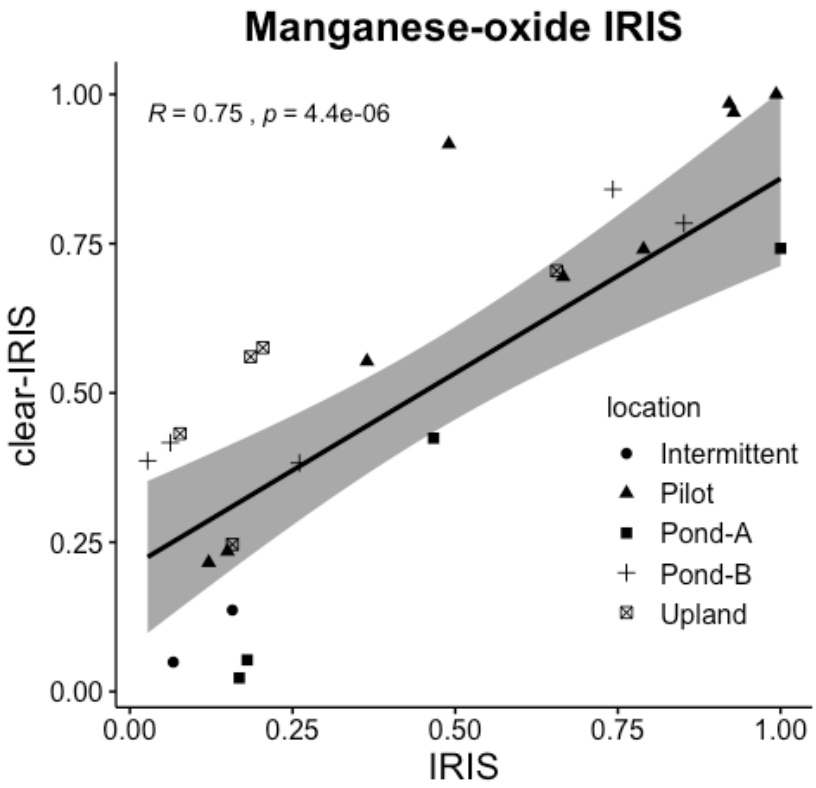


Figure 3.3 – Comparison of metal-oxide coating removal from IRIS and clear-IRIS tubes.

b = Manganese oxide

Each data point is taken from the same location at the same time and represents the average of two clear-IRIS tubes and five IRIS tubes.

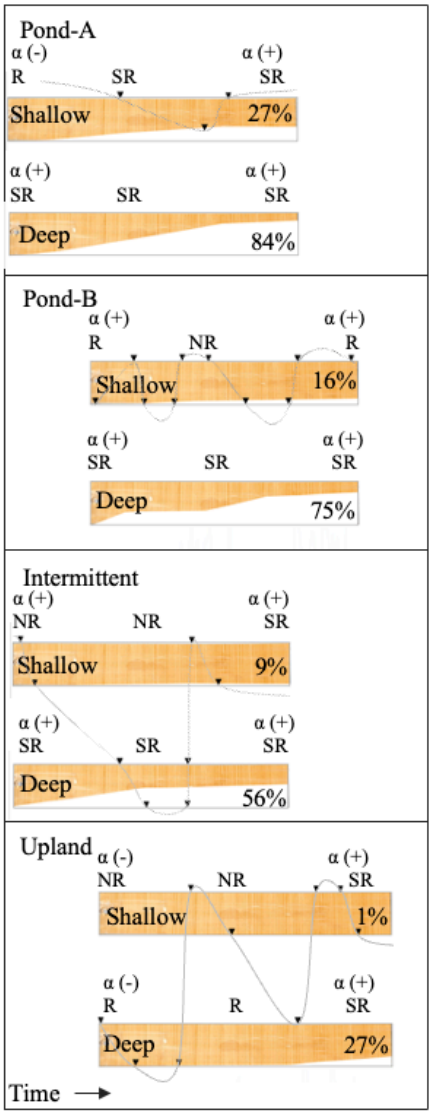


Figure 3.4 – Iron-oxide removal from IRIS tubes (orange band) between April 2 to May 3.

Final percent removal shown on the right. Shallow interval is 5 – 10 cm below ground surface (bgs) and Deep interval is 20 – 30 cm bgs. Water levels (---) are shown where they cross interval boundaries. α (-) = negative reaction to dipyridyl, R = Reducing (E_h below technical standard), NR = not reducing, SR = Strongly reducing, > 100mV below technical standard.

Tables

Table 3.1 – Metal-oxide paint removal rates by plot.

Removal rates were the slope coefficient from a simple linear regression of percent removal vs. time. Hydraulic condition was inferred from water level curves (Figure 3.4).

Plot	Hydraulic Condition	Fe Paint Removal rate (percent day^{-1})	Mn Paint Removal rate (percent day^{-1})
Pond-A	Saturated	4.3 $r^2 = .94$, $p < .001$	8.4 $r^2 = .82$, $p < .001$
Pond-B	Saturated	4.0 $r^2 = .93$, $p < .001$	8.1 $r^2 = .84$, $p < .001$
Intermittent	Intermittent saturation	1.7 $r^2 = .71$, $p = .01$	6.1 $r^2 = .59$, $p = .015$
Upland	Limited saturation	1.1 $r^2 = .88$, $p < .001$	3.0 $r^2 = .48$, $p = .015$

Chapter 4: Quantification of potential methane emissions following oxic soil inundation with organic matter amendments

** Submitted to Biogeochemistry – currently under review **

Abstract

Methane (CH_4) emissions are a potent contributor to global warming and wetlands can be a significant CH_4 source. In a microcosm study we evaluated how the practice of amending soils with organic matter as part of wetland restoration projects may affect CH_4 production potential. Organic amendments including hay, manure, biosolids and wood mulch were evaluated at three different levels. Using 1-liter glass microcosms, we measured the production of biogenic gases over 60 days in two soils, a sandy loam (SL) and a sandy clay loam (SCL). Fresh organic amendments increased CH_4 production, leading to potentially higher global warming potential and wetland C loss, and was more pronounced in the SL. We observed biogenic gas production in two sequential steady state phases: Phase 1 produced some CH_4 but was mostly carbon dioxide (CO_2) followed by Phase 2, two to six weeks later, with much higher total gas and nearly equal amounts of CH_4 and CO_2. The CH_4 from the SCL soil ranged from 0.003 – 0.8 cm^3/Kg/day in Phase 1 to 0.75 – 28 in Phase 2 and the SL range from 0.03 – 16 cm^3/Kg/day in Phase 1 to 1.8 – 64 in Phase 2. Adding fresh organic matter (e.g. hay) resulted increased whereas in some cases composted organic matter decreased Fe^{2+} and CH_4. Methanogenesis normally increases following the utilization of reducible iron; however, we observed

instances where this was not the case, suggesting other biogeochemical mechanisms must be contributing to the shift in gas production.

Introduction

The ecological benefits of wetlands are well documented, including their role as carbon sinks to stabilize global climate (Mitsch, Bernal, and Hernandez 2015). Driven in part by this ecological contribution, from 1970 to 2015 new (human-made) wetlands have increased 233% (Darrah et al. 2019). Between 2004 and 2009 the United States saw a net gain of 16,670 hectares of freshwater wetlands: 360,820 hectares of new wetlands to offset 344,140 hectares of existing (carbon-sink) wetlands that were destroyed (Dahl 2011). Although human-made wetlands may effectively sequester carbon (C), it may take hundreds of years to offset their radiative forcing due to methane (CH_4) emissions (Neubauer 2014). With such a large number of new human-made wetlands, and their potential to increase global warming, it is vital to consider factors that contribute to CH_4 emissions.

Organic amendments such as straw, wood mulch, manure, and biosolids, mixed into the soil, are thought to accelerate C storage by enhancing the conversion of plant-derived compounds to microbial residues (Richardson et al. 2016). In the United States organic amendments are often required in mitigation wetlands; however, there has not been a systematic evaluation of the biogeochemical consequences of amendments like CH_4 emissions. Microbial residues, largely aliphatic-C from cell membrane lipids, can accumulate under anoxic conditions and are not directly accessible by methanogens

(Chen et al. 2018). Belowground plant materials are preferentially converted to soil organic carbon (SOC) (Mazzilli et al. 2015). Before contributing to SOC, standing litter in natural wetlands is partially decomposed by fungi (Kuehn et al. 2011), and further decomposed by aerobic bacteria (Yarwood 2018). In saturated soils root residues of wetland plants contain suberin and cutin (Watanabe et al. 2013), which persist, reducing biogenic gas production (Mikutta et al. 2006). Allochthonous organic amendments are derived from above-ground material, but they have been subjected to wetland biogeochemical processes. Studies suggest these materials are less amenable to soil C stabilization compared to natural plant inputs and may increase CH_4 production (B. Scott et al. 2020). In addition to increasing CH_4 production directly, organic amendments may cause SOC priming that produces additional CH_4 (Nottingham et al. 2009), and can lead to an increase in iron reduction and iron toxicity (Saaltink et al. 2017).

Iron (Fe) oxides play multiple roles in anoxic soils, being both an electron acceptor for organic C metabolism (Straub, Benz, and Schink 2001), and a stabilizing agent for SOC on mineral surfaces (Lehmann and Kleber 2015). As a metabolite, Fe reduction competes with CH_4 production (Huang, Yu, and Gambrell 2009) and can facilitate sulfur recycling (which also competes with CH_4 production) in freshwater sediments (Hansel et al. 2015). However, some recent literature suggests the relationship of iron reduction and methanogenesis is more complex. Some methanogens appear capable of switching between methanogenesis and iron reduction (Sivan, Shusta, and Valentine 2016). In cultures with *Methanosarcina acetivorans*, adding iron oxides

increased methane production[167], presumably by the utilization of a metabolic pathway where electron flow is bifurcated with some electrons going toward iron reduction to increase energy yield (Zhuang et al. 2015; Prakash, Chauhan, and Ferry 2019). In systems that are near pH neutral, Fe reduction does not necessarily have an energetic competitive advantage over CH_4 production (Bethke et al. 2011). In addition to influencing metabolic pathways, metal-oxide surfaces can stabilize organic matter, making it less bioavailable, which can affect both Fe reduction (Poggenburg et al. 2018) and C mineralization (Amendola et al. 2018; Lalonde et al. 2012). Due to the influential role of iron reduction on biogeochemistry, it is one of the primary methods for determining if soils are hydric National Technical Committee for Hydric Soils (NTCHS 2015), a key indicator of wetland success under mitigation guidelines.

We carried out a lab experiment using organic amendments commonly used in wetland restoration (biosolids (Bloom®) - B, manure - M, composted yard waste (LeafGro®) - L, wood chips - W, and hay - H) and measured how they affected CH_4 production and Fe-reduction. A series of 1-liter glass-jar microcosms were incubated with two different soils from recently created freshwater wetlands. The microcosms were kept under anaerobic conditions to compare the ability of these substrates to support anaerobic metabolism. We hypothesized that organic amendments would stimulate dissimilatory Fe-reduction in soils (measured as soluble ferrous iron, Fe^{2+}). Further, we hypothesized that amendments promoting Fe reduction would limit methanogenesis. We also tested differences between cured (i.e., aged/composted) and uncured (fresh) organic

amendments and hypothesized that uncured amendments would increase Fe reduction due to the presence of more labile, soluble, compounds. In the United States organic amendments are often required in mitigation wetlands. However, there has not been a systematic evaluation of whether or not amendments promote hydric soil conditions (Fe reduction), or may lead to Fe toxicity (from Fe reduction), or may increase CH_4 production.

Materials and Methods

Microcosm Setup

Saturated incubations were established using soil from two recent mitigation wetlands located in Maryland, USA. The first site (76°50'40.35"W, 38°47'5.41"N) was most recently a horse pasture and will be referred to as SCL denoting the texture (sandy clay loam). The second site (75°47'40.20"W, 39°1'52.42"N) was most recently a corn/soy farm with tile drains and was likely a wetland prior to conversion to farmland. The second site will be referred to as SL (sandy loam). Both sites had been recently graded to establish wetland topography, so the upper portion of the soils, where soil samples were collected, were mixed endo- and umbr-aquic horizons but with no ped structure. Soil was collected from these surface horizons to a depth of 15 cm, a typical depth for mixing-in organic amendments, sieved (2mm) and homogenized prior to use. Additional soil information is shown on Table A.5.4.1.

Microcosm experiments were conducted in 1000-mL glass straight-sided wide-mouth food canning jars. Each microcosm had a total of 600cc of solid material and was

filled with water for a total volume of 660cc. The volumes needed to be precise in order to facilitate headspace and liquid sampling and allow space for soil expansion. When amendments were added, an equal volume of soil needed to be removed so the total volume of solid material was a constant 600cc. At the start of the experiment, the headspace was purged with nitrogen gas. The incubation temperature was 20^{o}C. Jar lids had precision drilled holes fitted with grey butyl rubber stoppers, making it possible to non-destructively remove the overlying liquid (for Fe and pH analyses) using a 7.5 cm needle. Since the head-space pressure increased due to biogenic gas production, atmospheric pressure was re-established during gas sampling events by piercing the septa with a 24-gauge needle connected to a 50mL gas-tight syringe. This procedure allowed us to record the total volume of gas produced and collect gas samples (0.01 - 1000 μL) under atmospheric pressure (Figure A5.4.1). A small coating of silicone applied to stoppers after piercing prevented leaks. All microcosm trials were run with three replicates except where noted.

Microcosm Experiment 1

We measured CH_4 and Fe^{2+} production with various organic amendments, including composted yard waste (L), composted wood chips (W), class 1 biosolids - (B), manure (M), and hay (H) at three treatment levels: 8.8% (v/v), 26%, and 53%, in two soils, a SL and a SCL. We used horse M for the SCL incubations and cow M for the SL incubations. This matched the wetland mitigation conditions at each field location. The treatment levels reflect the Maryland Department of Environment (MDE)

recommendation for wetland restoration (60 cubic yards per acre assuming a 6” mixing depth) = 1x, 3x, and 6x the MDE recommended level. All amendments were sieved to 5mm. Hay was chopped with a Wiley mill, blended, or cut with scissors until it could easily pass a 5mm sieve.

Microcosm Experiment 2

We measured CH_4 and Fe^{2+} production using cured (aged) and uncured (fresh) organic materials. We used two amendments, B and M. The two cured materials were from the same two sources as the fresh material but had been cured for a minimum of 3 months. We added the same amount of amendment to each microcosm based on organic matter (OM) content. Each amendment was evaluated for OM by loss-on-ignition (LOI) (550°C for 2h). Based on the percent OM we adjusted the amount of amendment so the final dose was 20g OM/ 600 cm^3 soil. The microcosm setup was the same as Experiment 1 except we used the same volume of soil (600 cm^3) in all microcosms. These microcosms were incubated for 13 days and sampled periodically for Fe^{2+} and biogenic gases.

Microcosm Experiment 3

We measured a) CH_4 and b) Fe^{2+} production as a function of pH. We used H leachate as a substrate (McMahon et al. 2005). We leached 5.63 g H with 125 cm^3 cold de-ionized water, shaking horizontally at 5^{o}C for 24 hours. The leachate was filtered to 20 μm and immediately placed into jars with 600 cm^3 SL soil and incubated for 22 days.

The pH was adjusted to target levels of 5.6, 6.1, and 6.6 using a non-substrate buffer: 2-(N-morpholino) ethanesulfonic acid (MES). To determine the necessary concentration of MES, we titrated SL (pH 5.8) to our maximum desired pH (6.6). We determined that the buffering capacity of the soils corresponded to ~ 2 mN in the 125 cm^3 of liquid (leachate volume), so we prepared microcosms using 125 cm^3 of 20 mN MES buffer.

Microcosm Experiment 4

We measured Fe^{2+} production using leached H as a substrate (as in Experiment 3) but compared these finding to those with unleached H, and the H residuals.

Soil, Liquid, and Gas Analyses

Prior to the start of the experiments, we analyzed the SL and SCL for soil texture, percent soil C, and extractable iron. Soil texture was determined by adding 50 g soil to a 1000 ml cylinder with 0.5% hexametaphosphate. Sand settled after 1 minute and silt after 24 hours. Soil moisture content was determined as weight loss of approximately 5 g of soil dried at 105ºC for 48 hours. We determined percent soil C using thermal combustion analysis at 950°C on a LECO CHN-2000 analyzer (LECO Corp., St. Joseph, MI). Iron extractions were performed sequentially with 1 M hydroxylamine hydrochloride (HHCL) in 25% v/v acetic acid; 50 g / 1 sodium dithionite in solution 0.35 M ace-tic acid / 0.2 M sodium citrate buffered to pH 4.8; 0.2 M ammonium oxalate / 0.17 M oxalic acid (pH 3.2) (Poulton and Canfield 2005). The HHCL extraction targets bioavailable iron, primarily ferrihydrite and lepidocrocite. Dithionite also includes more crystalline iron

oxide forms, hematite and goethite. Oxalate includes the bioavailable iron oxides and magnetite.

Throughout the experiments we measured Fe^{2+}, pH, and biogenic gases in the headspace. In some cases, Fe^{2+} and pH were measured only at the end of the incubation. Using a 3" needle, we extracted 0.3 - 1 cm^3 (for Fe^{2+}) and 1 cm^3 (for pH) of the supernatant liquid to avoid disturbing soil in the jars. Samples of liquid supernatant were removed during gas sampling, when atmospheric pressure was maintained, to avoid loss of biogenic gases and atmospheric contamination. For the final sample point the jar contents were thoroughly mixed prior to sampling. Ferrous iron in supernatant liquid was measured with a HACH DR4000 spectrophotometer. The spectrophotometer was also used to measure Fe in the Fe-oxide extractions. Prior to analysis, extracted Fe-oxides were reduced by adding thioglycolic acid. To confirm the spectrophotometer accuracy, a subset of samples was also analyzed on a PerkinElmer PinAAcle 900T atomic absorption spectrometer. An Orion 9142BN electrode was used to determine pH.

Gas samples were collected in 12 cm^3 N-purged exetainer vials and analyzed by injecting 5 cm^3 into a Varian Model 450-GC gas chromatograph. Since sample volume was typically 1 cm^3 or less, 5 cm^3 nitrogen gas was added to the vials immediately prior to analysis for CO_2 and CH_4, and measured concentrations were corrected for dilution and prior headspace gas concentrations. For fluorescent spectral scans dissolved organic matter was extracted from organic materials with 1:10 solid (weight) / deionized water (volume) for 24 hours and filtered to 0.45 μm (Fischer et al. 2020). After diluting

samples, emission spectra were recorded using an Aqualog fluorometer (Horiba Scientific; Edison, NJ).

Data Analyses

Unless otherwise noted, statistical determinations were done using ANOVA in R or SAS. The Fe^{2+} concentrations were evaluated using contrasts for each of the amendments compared to the control using the multcomp package. The gas curves were modelled as piecewise, bimodal linear functions using the R "Segmented" package (Muggeo 2008). Breakpoints were determined using the total gas curves but, in some cases, Segmented could not identify a breakpoint in the total gas curve, so CH_4 curves were used as noted in Figures A5.4.2 & 3. Gas curves from H amendments did not fit a piecewise model and were modelled as sigmoidal functions using the SSgompertz function in R. However, Ssgompertz is sensitive to data scatter, particularly at the beginning and end of the curve, so in two cases, the total gas and CO_2 curves for H6x in the SL, we fit the data with a power function in Excel.

Results

Experiment 1a: Effect of organic amendments and soil type on CH_4 gas production

The addition of organic amendments increased CH_4 production (Table 4.1). The amount of the increase depended on the soil texture, the incubation time point when CH_4 samples were collected, amendment type, and dose. Methane gas production occurred in

two distinct steady-state gas production periods, which we identified as Phase 1 and Phase 2 (Figure 4.1). Therefore, we reported Phase 1 & 2 total gas production rates, as well as CH_4, CO_2, and the breakpoint (Table 4.1). Individual gas curves are shown in Figures A5.4.2 (SCL) and 3 (SL). Some CH_4 was produced almost immediately upon inundation (Phase 1), but after the breakpoint (40 days in both the SL and SCL soils), there is a large increase in CH_4 as well as an average 4.7x ± 1.9 increase in total gas production (Table 4.1). In general, the SL soil produced 2.4 times as much CH_4 as the SCL (Figure 4.2). In the SCL soil, CH_4 production in Phase 1 was 0.003 cm^3/Kg/day and with amendments increased to as much as 0.8 cm^3/Kg/day (Table 4.1a). In Phase 2 CH_4 was 1.9 cm^3/Kg/day and with amendments increased to as much as 28 cm^3/Kg/day (Table 4.1b). In the SL soil, amendments increased CH_4 from 0.04 to 16 cm^3/Kg/day in Phase 1 and from 1.8 to 64 in Phase 2.

Gas production rates increased with amendment dose. With the exception of L in the SL, all amendments reduced the time required to transition from Phase 1 to Phase 2 (i.e. the breakpoint). Biosolids caused the largest shift, decreasing the breakpoint to as little as 5 days. While amendments generally increased CH_4 production there were exceptions. Low doses of cured amendments (L and W) had lower CH_4 production rates than unamended soil: L1 in Phase 1 in both soils; L3 in the SL; L3 in the SCL (Phase 2 only); W1 in the SCL (Phase 2). Biosolids (B1) also lowered CH_4 production rates in both soils (Phase 1) (Table 4.1a).

Using fresh H, biogenic gas production followed a sinusoidal pattern and we reported maximum CH_4 production rate at the inflection point (Table 4.1c). Hay was prone to floating and at higher doses and was present in the water column above the surface (not in contact with soil). In the instances where this occurred (H3 and H6 in the SCL), there was a decrease in overall gas production rate and very low CH_4 – much lower than unamended soils (Table 4.1c and Figures A5.4.2z & 3z).

Experiment 1b: Effect of organic amendments and soil type on Fe^{2+}

The type and dose of organic amendments affected total soluble Fe^{2+} production, compared to the unamended control, in a limited number of cases (Figure 4.3, Table A5.4.3). In the SL soil, L caused a decrease ($p < 0.05$) in supernatant Fe^{2+} concentrations whereas H increased supernatant Fe^{2+} in both soils ($p < 0.05$). In a separate set of experiments, we documented the relationship between supernatant Fe and pore water Fe (Figure A5.4.11). Soil type affected the amount of soluble Fe^{2+} produced ($p < 0.05$). We did not see a difference in Fe^{2+} in the unamended microcosms even though the SCL had 2.2x the amount of hydrochloramine hydrochloride extractable Fe (FeHHCl) compared to the SL and had 7.6x more dithionite extractable Fe (Table A5.4.1). Of the FeHHCl in soil, 19% or less in the SCL and 61% or less in the SL was reduced to Fe^{2+}. Hay was an exception, where up to 155 % of the FeHHCl in the SCL and 236 % in the SL was reduced to Fe^{2+} (Table A5.4.3). During the SL soil incubations, aqueous Fe^{2+} was measured simultaneous to CH_4 production. In the H and M treatments, there was a marked increase in CH_4 production when Fe^{2+} became asymptotic. However, with the

other amendments, Fe^{2+} production continued or even increased during periods of high CH_4 production. Figure 4.4, which shows two examples that highlight this pattern, is a subset of the complete set of curves in Figure A5.4.6.

Experiment 2a: Effect of cured versus fresh organic amendments on CH_4 gas production

In Experiment 1a, it appeared that curing may have had an effect on CH_4 production. Fresh H produced the most CH_4. The H1 trials had maximum CH_4 production rates of 18.2 and 27.8 cm^3/Kg/day in the SCL and SL soils, respectively (Table 4.1c). The H3 and H6 doses would likely have been higher had some portion of the H not floated. The M6 trials produced the most CH_4 at 27.7 and 64.0 cm^3/Kg/day in the SCL and SL soils, respectively. Of the amendments used, M was cured the least (after fresh H, which was uncured). LeafGro, a commercial composted yard waste, was cured the most and produced very little CH_4, in some cases less than the controls. Since we could not specify precisely how long the organic material had been cured, we conducted a separate experiment with organic materials of known curing periods (at least 90 days), using B and M. Rather than use the same volumetric quantities, we used the same dose based on OM content. The results confirmed that curing has a strong influence on CH_4 production. Methane production was much higher using fresh material in both cases and cured material sometimes decreased CH_4 production (Table 4.2).

Experiment 2b: Effect of cured versus fresh organic amendments on Fe^{2+} production

In Experiment 1b, we observed that curing also had an effect on the amount of Fe^{2+} produced. Hay was the only amendment that produced significantly more Fe^{2+} and L produced a significant reduction in Fe^{2+} (Figure 4.3). In Experiment 2 we used biosolids (B) and manure (M) that had been cured at least 3 months. Whether the material had been cured had a strong influence on Fe^{2+} production and Fe^{2+} was higher using fresh material in both cases (Figure 4.5).

Spectral Analysis: Effect of organic amendments and soil type on CH_4 gas production

We observed differences in CH_4 and Fe reduction rates when using organic material that had been cured versus uncured. The fluorescent spectral signatures of the cured materials (B and M) were similar as were the signatures of fresh material (Figure A5.4.7). The fluorescent signatures varied due to curing, but not due to the source material. The difference in signatures was indicative of higher concentrations of organic (humic) acids and lower nominal oxidation state in the cured materials. We considered other organic matter characterization methods such as the material's carbon to nitrogen ratio, but we did not find another reliable predictor of CH_4 and Fe^{2+} production other than curing.

Experiment 3: Effect of pH on a) CH_4 and b) Fe^{2+} production

The soil pH affected both CH_4 and Fe^{2+} production. In Experiment 1, we observed that on Fe^{2+} varied with pH in the SL soil ($p<0.001$; Figure A5.4.8a), but there was little

variation in the SCL ($p=0.45$; Figure A5.4.8b). In order to isolate the effect of pH, we performed experiment 3 using a single substrate (H leachate) in the SL soil. Higher pH increased the CH_4 production rate in both Phase 1 and 2 (Table 4.3) and reduced the production of Fe^{2+} (Figure 4.6).

Experiment 4: Effect of H fractions and pH on a) CH_4 and b) Fe^{2+} production

In Experiment 4 we measured Fe^{2+} produced from H, H leachate, and the H residuals (Figure 4.7). The H residuals appeared to produce more Fe^{2+} than the leachate. However, as noted on the figure, separate leached fractions changed the system pH. Using the results from Experiment 2, we predict that at comparable pH there would have been no difference in Fe^{2+} production between H, H residuals, and leachate (Figure A5.4.9). Therefore, we re-evaluated the results from Experiment 2b, correcting for pH and confirmed that the organic material age accounts for differences in Fe^{2+} production (Figure A5.4.10). Similarly, we considered whether pH affected the out-come of Experiment 1 results. However, a MANOVA analysis of the Experiment 1 data (Table A5.4.4) indicated that pH had a small effect ($p=0.30$) compared to organic matter type and dose ($p<0.0001$).

Discussion

Net CH_4 emissions are a primary factor that determines whether a wetland is a C sink or contributes to long term global warming(Neubauer and Verhoeven 2019). Soil management practices, such as wetland restoration methods, can have a large impact on

CH_4 production and total greenhouse gas emissions (Paustian et al. 2016). Our data indicate that organic amendments used in mitigation wetlands can have a large influence on CH_4 production. Organic amendments that had been cured (L and W) only slightly increased CH_4 emissions, whereas fresh material (M and H) resulted in large increases (Table 4.1). This is consistent with field studies where comparable cured amendments (composted wood and yard waste), did not result in increased CH_4 emissions (Winton and Richardson 2015), but straw (K. A. Ballantine et al. 2015) and peat bales (H. E. Green 2014) increased CH_4 emissions. Organic material is commonly cured, or composted, to remove plant pathogens (Noble and Roberts 2004) and to reduce the amount of cellulosic material (Hubbe, Nazhad, and Sánchez 2010), which competes for oxygen, contributing to phytotoxicity (Saidpullicino, Erriquens, and Gigliotti 2007; Hu et al. 2011). Curing produces humic acids and increases the nominal oxidation state (NOSC) of C (Guo, Liu, and Wu 2019). When cured material is then subjected to anaerobic conditions, less CH_4 is produced (Yao and Conrad 1999).

Following soil inundation, we observed two distinct gas production phases (Phase 1 and 2). This pattern is difficult to distinguish in unamended soils but has been reported previously (Yao and Conrad 1999). The breakpoint was similar to other studies: from 5 – 36 days in a study by Yao and Conrad (1999) and 5 – 45 days in our study (Table 4.1). The Phase 2 CH_4 production rates in unamended soils were 0.96 – 3.98 cm^3/Kg/day in Yao and Conrad (1999) and 1.82 – 1.94 in our study (Table 4.1). There are several known causes of this gas production pattern. One is the lag period required to re-establish

populations of methanogenic bacteria, which become dormant under oxic conditions and doubling times for regrowth can be on the order of days (Jabłoński, Rodowicz, and Łukaszewicz 2015). In our study, B had the earliest onset of Phase 2 CH_4 production (Table 4.1b), possibly due to elevated levels of dormant methanogens. Another cause for the two-phase gas production is the depletion of bioavailable iron-oxides, which are suppress methanogens (J. P. Megonigal, Hines, and Visscher 2004). However, some of our data seemed to contradict this model. Figure 4.4a shows that the trial with the amendment M1 (for example) fit the expected pattern – ferrous iron in the supernatant plateaued at about the same time as the breakpoint, after which methane increased. Figure 4.4b shows that with W3 soluble iron continued to be produced well after the breakpoint, and the amount of bioavailable iron used during the course of the incubation was less than 28 ± 4% (Figure 4.4, Table A.5.4.3). We also looked at CO_2:CH_4 ratios. As with iron-oxide utilization, we would expect the CO_2:CH_4 ratio to be near 1:1 after the methane breakpoint Bridgham et al. (2013). However, we observed notable exceptions (also discussed in Bridgham et al. 2013). The SCL L1 trial had a ratio of 73:1 after the breakpoint (Table 4.1b), yet still had the characteristic shift to higher overall gas production (4.67x). Other trials (L3, L6, W1, B1, C, and W1-3 in the SL soil) showed similar unexpected behaviours, but to a lesser degree. Therefore, there are likely underlying mechanisms that contributes to the breakpoint other than depletion of iron-oxides and a shift to methanogenesis. One possible explanation put forth in other work is that redox dynamics can be controlled by the presence of microsites (Yang et al. 2017).

In our experiments, the anomalous trials listed here produced low levels of biogenic gases, which decreased the incidence of bioturbation through the release of subsurface gas bubbles. In other trials, when the headspace pressure was relieved in order to collect samples, gas ebullition occurred, to the point of effervescence with H, which may have disrupted microsites.

The increased gas production from organic amendments was more pronounced in SL compared to SCL, where there was 2.4x higher CH_4 and 2.6x higher gas production (Figure 4.1a & b). We observed a more pronounced effect than a recent rice field study where there was more methane from SL soils versus SCL; although in that study results were not statistically significant (Kim et al. 2018). Yagi and Minami (1990) observed that compost (approximate dose the same as our 1x treatment) increased respiration rates by 1.8x in a SCL versus a loam soil. Maietta, Hondula, et al. (2020) observed that respiration rates were higher in a sandy loam soil compared to a silty clay, with and without 3.3% & 23% wetland hay. Thus, we might conclude coarse grained (sandy) soil textures emit more methane; however, there are a number of investigations where this was not the case (Yagi and Minami 1990; Glissmann and Conrad 2002) and additional studies would be needed to isolate this variable.

We considered the gas production from H microcosms separately because they followed a different pattern than the other amendments (Table 4.1), but the pattern was similar to other studies using hay (Glissmann and Conrad 2002) and wetland hay (Maietta, Monsaint-Queeney, et al. 2020). Our study adds to these findings by observing

that H produced very low CH_4 in the water column (after floating) compared to being mixed with soil (Table 4.1c). This may merit further study because if this is generally true, applying fresh organic matter as a mulch, rather than mixed into the soil, could greatly reduce the adverse consequence of increased CH_4 emissions.

Reduction of Fe-oxides occurs in saturated soils in the presence of an organic substrate and is a key biogeochemical process in wetland soils. With sufficient time, hydric soils may develop redoximorphic features from Fe reduction; however, studies have not shown lasting redoximorphic development due to organic amendments(Gray 2010; E. T. Ott et al. 2020). Organizations responsible for constructing mitigation wetlands have an interest in documenting Fe reduction prior to redoximorphic feature development as evidence soils that are hydric. Some mitigation wetland practitioners experience challenges meeting hydric soil testing standards. Although reports in the scientific literature are rare, there are examples of sites meeting vegetation and hydrology wetland indicators, but not hydric soils (Berkowitz, Page, and Noble 2014). Both the soils we tested produced Fe^{2+} and would have passed hydric soils tests without the aid of an amendment.

We observed that fresh organic matter resulted in increased Fe^{2+} compared to cured organic matter (Figure 4.3), likely due to the presence of labile carbon, allowing access to more crystalline Fe-oxides (Lentini, Wankel, and Hansel 2012). Fresh material such as hay has been promoted as a soil amendment in wetland construction (Melvin 2003). In some soils Fe-reducing bacteria using fresh organic matter amendments could

access crystalline Fe making it more bioavailable. However, without an anoxic/oxic cycle, increased Fe^{2+} production could lead to Fe^{2+} toxicity and ferrolysis (Kirk 2004), similar to the way fresh organic matter leads to SOC priming (Blagodatsky et al. 2010). Ferrolysis occurs when bioavailable Fe-oxides are reduced to Fe^{2+} and are subject to hydraulic transport. We observed that cured amendments, like L, lowered Fe^{2+} concentrations (Figure 4.1), possibly due to the presence of humic acids that are generated during curing (Guo, Liu, and Wu 2019). Humic acids often contain insufficient biogeochemical energy to drive dissimilatory Fe reduction (Keiluweit et al. 2017), chelate Fe^{2+}, removing it from the liquid phase (Catrouillet et al. 2014), and create insoluble precipitates(Shimizu et al. 2013).

Regulating Fe^{2+} production, through the selection of the appropriate OM amendment, could influence the growth of wetland plants. For example, rice growth may be stimulated under low Fe^{2+} doses of 1 mg/L (Müller et al. 2015), but higher doses can produce detrimental Fe plaque (Pereira et al. 2014). Some native wetland species are adapted to high Fe^{2+} concentrations. *Juncus effusus* growth is stimulated at 25 mg/L Fe^{2+} (Deng, Ye, and Wong 2009). North American native reed *Phragmites australis* ssp. *americanus* was stimulated at 11 mg/L Fe^{2+} from ferrous sulfate (Willson et al. 2017), but the invasive Eurasian lineage of *Phragmites australis* seedling growth was inhibited by Fe^{2+} as low as 1 mg/L (Batty 2003). Soils high in free Fe^{2+} adversely affected *P. australis* growth by creating an Fe-oxide plaque on roots (Saaltink et al. 2017).

Our results show that pH has a significant effect on both the production of Fe^{2+} (Figure 4.3) and CH_4 (Table 4.3). Between pH 5.6 and 6.6, the lower pH produced more Fe^{2+} and less CH_4, consistent with thermodynamic predictions(Ye et al. 2012). Hydrogenotrophic methanogens can maximize CH_4 production at pH 5 (Bräuer, Yavitt, and Zinder 2004). In rice paddy soils, CH_4 emissions had a clear peak at pH 7, but almost none below pH 5.5 (Z. P. Wang et al. 1993). The strong effect of pH underscores the need to take this parameter into account when interpreting data from experiments evaluating Fe-reduction and methanogenesis. Attempting to control the pH of soils could potentially introduce confounding effects. We used an MES buffer with 10x the quantity we estimated from a soil titration and still saw shifts in the pH after incubation. With a high residual soil acidity, the amount of buffer needed to control soil pH may increase the ionic strength to a level that could influence cellular sorption to mineral and Fe-oxide surfaces(Mills et al. 1994) as well as enzyme activity (Leprince and Quiquampoix 1996).

Implications

In our experiment, we saw that organic amendments can increase CH_4 production, particularly after extended anaerobic periods. We quantified methane production potential from several organic amendments, and in Chapter 5 show that these results are useful in predicting field methane production, even though in the field values are much lower, likely due to methanotrophic activity. There is mounting concern that CH_4 from mitigation and created wetlands may result in net global warming for decades to centuries (Neubauer 2014). Our results suggest that not only do organic amendments increase CH_4

gas production overall, but uncured amendments can also decrease the time it takes before there is a large increase in both total gas production and CH_4. Methane production is not constant and dramatically increases after several weeks. Therefore, it may be possible to limit CH_4 by designing systems with shorter flooding or saturation periods, alternating with drier conditions, a strategy that has been proposed for rice paddy fields (Souza 2021). Our lab study demonstrates the potential for significant CH_4 emissions, but in a real system, methanotrophic activity could attenuate CH_4 some of the emissions (Chowdhury and Dick 2013); however, this would not decrease the overall C loss from soils, it only changes the pathway. If organic amendments are to be used, cured amendments may be preferrable because they are not as prone to high CH_4 generation and may attenuate Fe^{2+} toxicity. Amendments that lower the soil pH increases Fe reduction and limits methanogenesis (Marquart et al. 2019). When deciding whether or not the use of organic amendments for wetland mitigation is beneficial, or necessary, consideration should be given to whether or not the material has been cured, the material pH, the soil texture, and expected hydroperiod.

Figures

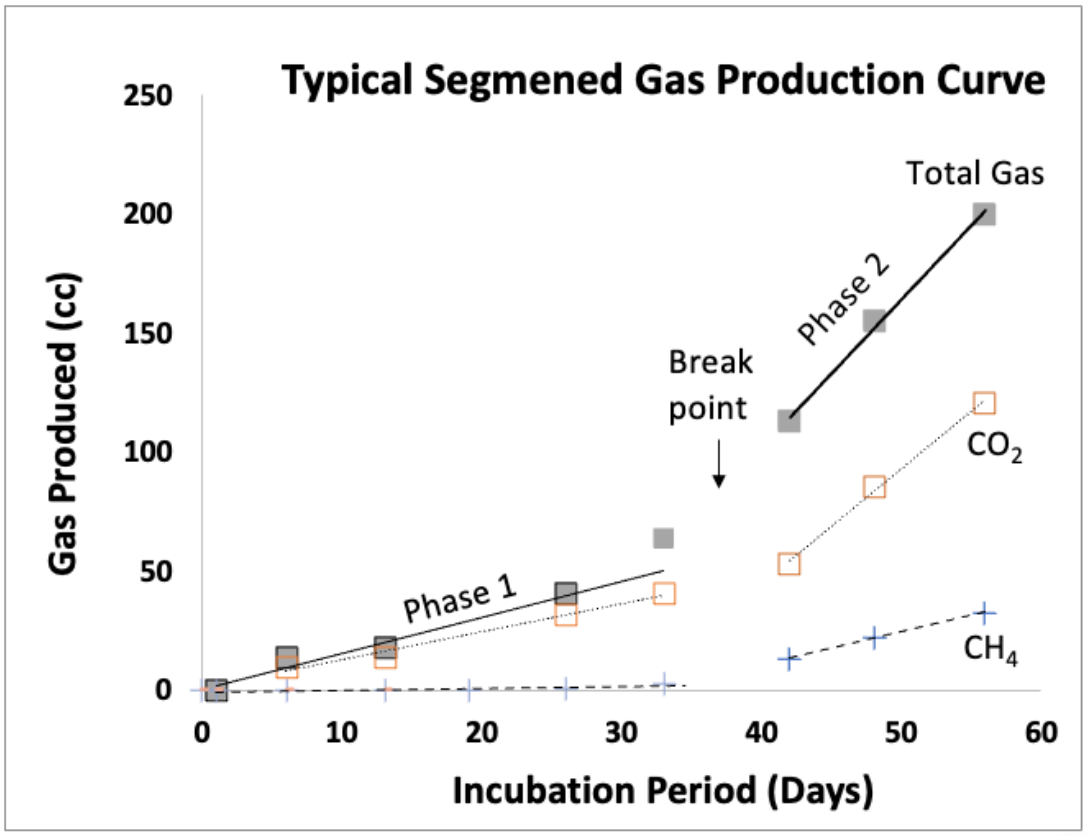


Figure 4.1 – Typical gas saturated soils amended with organic matter (All Experiments). Gases were best modeled using a segmented linear function. After a breakpoint the average total gas production increases by a factor of 5 whereas there is a sharp increase in methane production. Note that hay amended trials exhibited a typical sinusoidal pattern.

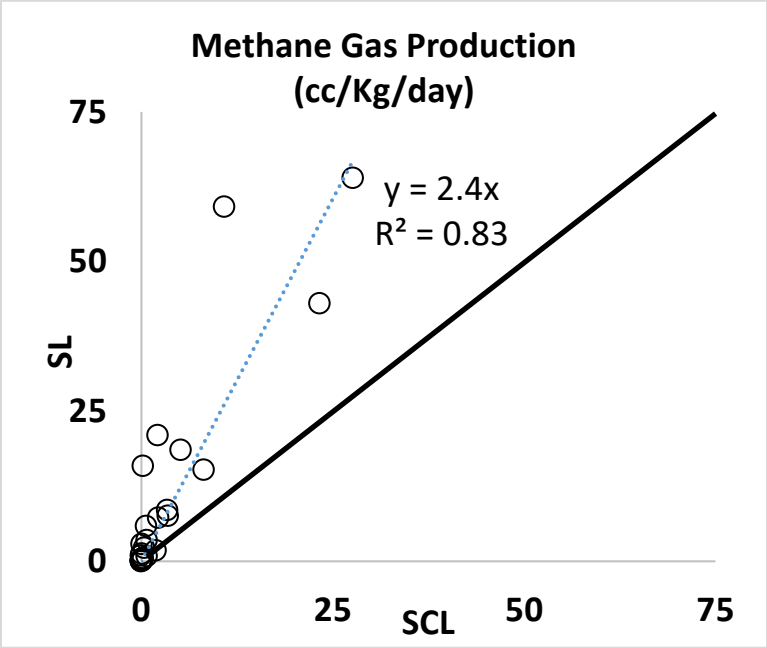


Figure 4. 2 – Experiment 1. Biogenic methane gas production rate in the SL soil versus the SCL mesocosms.

The SL mesocosms had, on average, 2.4 times higher gas production than the SCL.

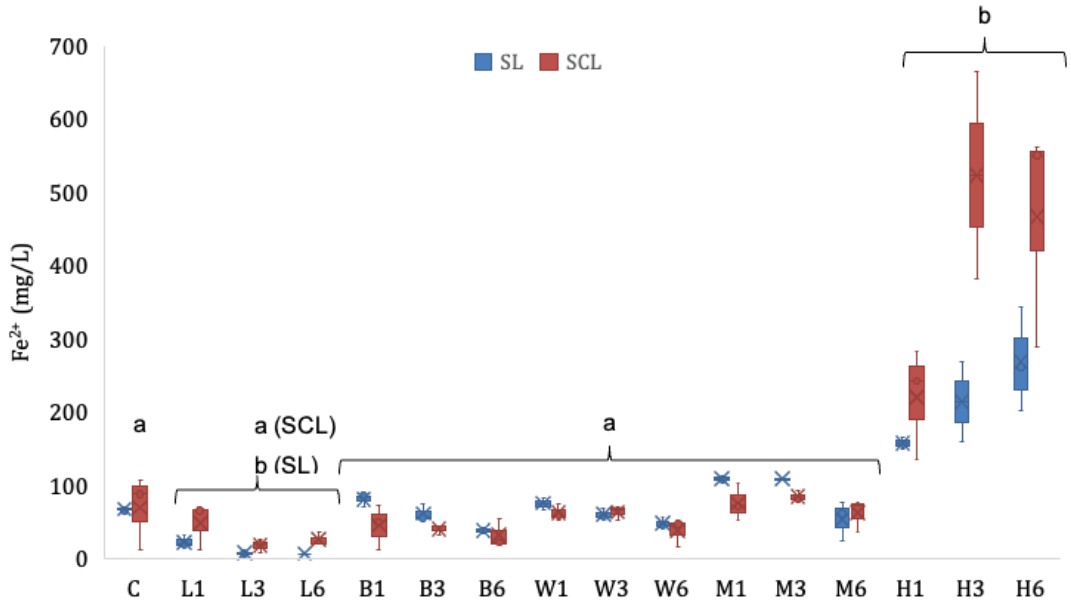


Figure 4. 3 – (Experiment 1b) Ferrous iron (Fe^{2+}) concentration in the liquid phase at the end of the incubation period.

Microcosms receiving different organic amendment types and levels in Sandy Clay Loam (SCL) and Sandy Loam (SL) soils. C = no amendment control, L = LeafGro (yard waste), B = biosolids, W = wood chips, M = manure, H = hay. Numbers signify treatment level (1, 3, or 6 times amount of organic matter equivalent to 60 yd^3 / acre to a depth of 6 inches). Different lower-case letters signify differences ($p < 0.05$) based on contrasts compared to C and brackets signify all results in the bracketed group were not statistically different. Hay increased total Fe^{2+} production compared to the C in both soils, and L decreased total Fe^{2+} production compared to C (SL only).

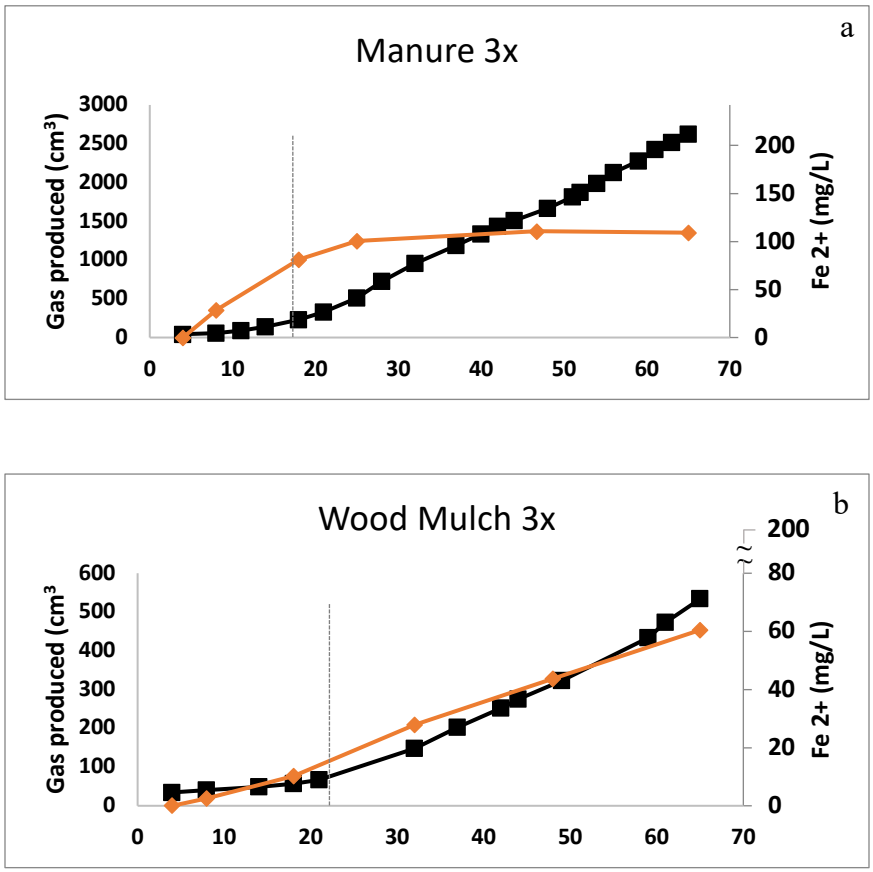


Figure 4. 4 – (Experiment 1b) Ferrous iron (Fe^{2+}) and CH_4 in selected microcosms. Depletion of Fe coincided with the breakpoint with M3, but not with W3. Other examples of this pattern are shown in Figure A5.4.6. The maximum value on the secondary x-axis is the maximum expected Fe^{2+} concentration based on the HHCL extraction.

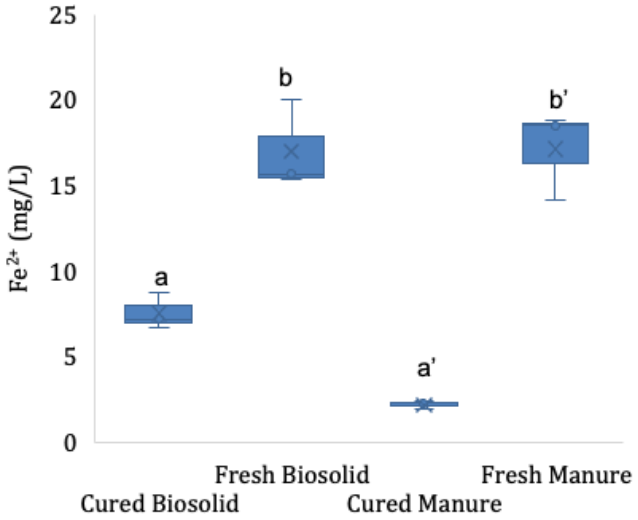


Figure 4. 5 – (Experiment 2b) Ferrous iron (Fe^{2+}) concentration in the liquid phase at the end of the incubation period (13 days).

Incubation was carried out with cured and uncured biosolids (B) and manure (M) in SL soil. Letters indicate a difference at $p<0.001$.

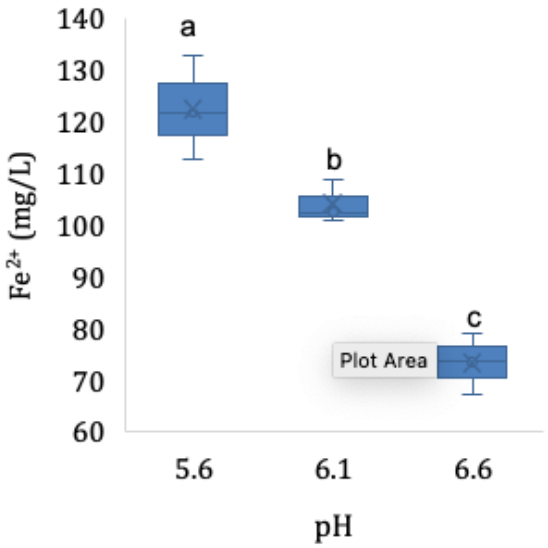


Figure 4. 6 – (Experiment 3) Ferrous iron (Fe^{2+}) concentration in the liquid phase with varied in of microcosms receiving H in Sandy Loam soils.

Letters indicate a difference at $p<0.05$.

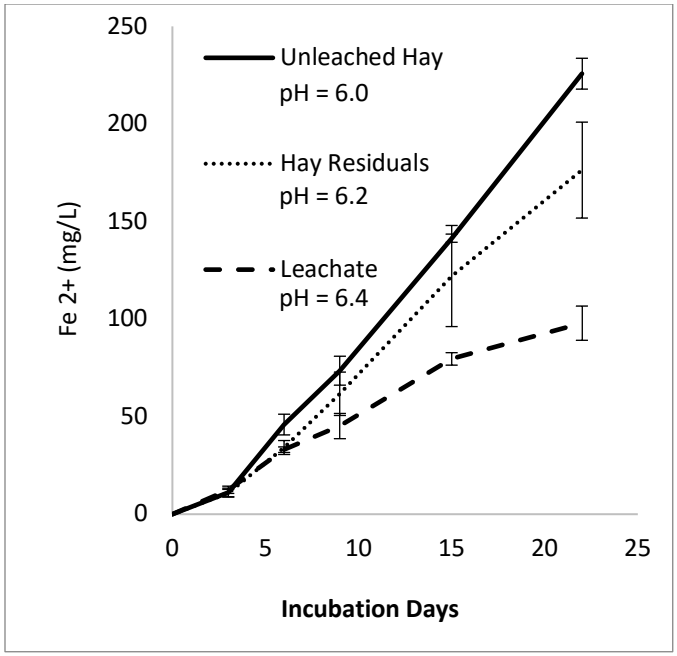


Figure 4. 7 – (Experiment 4) Ferrous iron (Fe^{2+}) concentration in the liquid phase with H as substrate.

Tables

Table 4.1a – (Experiment 1a). Carbon dioxide (CO_2), methane (CH_4) and total gas production pre and post breakpoint. Phase 1.

Incubations of different organic amendment types and levels in silty clay loam (SCL) and sandy loam (SL) soils. Instances where organic amendments did not increase CH_4 production are bolded. Note: CO_2 : CH_4 ratios are based on calculated gas production rates, not total gas produced.

			CO_2		CH_4		Total Gas		
Soil	Treatment	Soil (g)	cm^3/day	cm^3/Kg/day	cm^3/day	cm^3/Kg/day	cm^3/day	cm^3/Kg/day	CO_2:CH_4
SCL	Control	621.63	0.97	1.56	0.002	0.003	0.99	1.59	520.0
SCL	B1	425.24	1.53	3.61	0.08	0.18	4.13	9.70	20.1
SCL	B3	544.53	1.50	2.76	0.44	0.80	3.85	7.06	3.5
SCL	B6	468.02	2.09	4.46	0.06	0.13	3.53	7.55	34.3
SCL	M1	583.40	0.74	1.27	0.02	0.04	1.33	2.27	31.8
SCL	M3	495.56	1.79	3.61	0.32	0.64	2.05	4.13	5.6
SCL	M6	394.39	1.49	3.77	0.12	0.30	4.35	11.03	12.6
SCL	L1	586.46	0.83	1.42	0.001	**0.001**	0.85	1.45	1420.0
SCL	L3	516.34	0.89	1.72	0.01	0.01	0.91	1.77	172.0
SCL	L6	410.17	0.67	1.63	0.04	0.09	0.80	1.95	18.1
SCL	W1	593.36	1.00	1.68	0.01	0.01	0.92	1.56	168.0
SCL	W3	539.61	0.98	1.81	0.10	0.19	1.39	2.58	9.5
SCL	W6	457.42	1.03	2.25	0.11	0.24	1.29	2.81	9.4
SL	Control	634.60	0.50	0.79	0.03	0.04	0.56	0.88	19.8
SL	B1	606.80	1.25	2.06	0.02	0.04	4.13	6.80	51.5
SL	B3	551.50	1.57	2.84	0.44	0.79	2.92	5.29	3.6
SL	B6	467.87	2.08	4.44	0.59	1.27	3.81	8.15	3.5
SL	M1	619.92	2.62	4.22	0.58	0.93	3.49	5.63	4.5
SL	M3	588.37	4.48	7.61	3.44	5.85	9.42	16.02	1.3
SL	M6	540.93	8.63	15.95	8.59	15.87	17.92	33.13	1.0
SL	L1	600.10	0.35	0.58	0.02	0.03	0.73	1.22	19.3
SL	L3	530.30	0.61	1.15	0.02	0.03	0.78	1.46	38.3
SL	L6	425.87	0.62	1.47	0.11	0.26	1.66	3.89	5.7
SL	W1	603.27	0.98	1.62	0.06	0.10	1.55	2.56	16.2
SL	W3	538.77	1.42	2.64	0.20	0.36	2.14	3.98	7.3
SL	W6	442.57	3.05	6.88	0.24	0.54	3.23	7.31	12.7

Table 4.1b – Carbon dioxide (CO_2), methane (CH_4) and total gas production pre and post breakpoint. Phase 2.

		CO_2		CH_4		Total Gas					
Soil	Treatment	cm^3/day	cm^3/Kg/day	cm^3/day	cm^3/Kg/day	cm^3/day	cm^3/Kg/day	CO_2:CH_4	Break Point	r^2	Ph 2: Ph1
SCL	Control	2.06	3.31	1.20	1.94	2.54	4.09	1.7	40.0 ± 4.5	0.959	2.57
SCL	B1	5.58	13.13	1.47	3.45	5.49	12.91	3.8	29.3 ± 1.9	0.987	1.33
SCL	B3	3.74	6.86	4.45	8.17	9.48	17.40	0.8	20.1 ± 3.4	0.974	2.46
SCL	B6	7.42	15.85	10.90	23.29	18.20	38.89	0.7	10.3 ± 2.4	0.994	5.15
SCL	M1	2.26	3.88	1.29	2.22	5.82	9.97	1.7	40.2 ± 2.1	0.997	4.39
SCL	M3	4.64	9.37	5.39	10.89	10.69	21.58	0.9	20.8 ± 0.8	0.997	5.23
SCL	M6	5.85	14.83	10.91	27.67	19.69	49.93	0.5	22.1 ± 3.2	0.956	4.53
SCL	L1	3.85	6.57	0.05	**0.090**	3.96	6.76	73.0	32.2 ± 1.6	0.966	4.67
SCL	L3	4.21	8.16	0.39	**0.75**	4.54	8.79	10.9	32.0 ± 2.2	0.983	4.97
SCL	L6	5.90	14.39	0.92	2.24	6.95	16.95	6.4	32.0 ± 3.7	0.923	8.68
SCL	W1	1.56	2.63	0.27	**0.460**	3.22	5.42	5.7	34.0 ± 3.7	0.986	3.48
SCL	W3	1.93	3.58	1.90	3.52	4.51	8.35	1.0	24.2 ± 3.1	0.989	3.23
SCL	W6	2.19	4.79	2.36	5.15	6.22	13.60	0.9	13.0 ± 2.4	0.981	4.84
SL	Control	1.00	1.58	1.16	1.82	3.11	4.91	0.9	40.0 ± 3.2	0.957	5.55
SL	B1	4.44	7.31	5.16	8.50	10.19	16.79	0.9	8.6 ± 3.0	0.880	2.47
SL	B3	8.76	15.89	8.42	15.28	16.12	29.23	1.0	4.7 ± 1.8	0.989	5.53
SL	B6	12.61	26.96	20.15	43.07	40.39	86.33	0.6	9.1 ± 1.2	0.992	10.59
SL	M1	8.64	13.93	13.03	21.02	19.41	31.30	0.7	16.7 ± 0.7	0.998	5.56
SL	M3	15.23	25.88	34.77	59.10	50.79	86.33	0.4	17.2 ± 1.5	0.992	5.39
SL	M6	29.50	54.53	34.62	64.00	84.92	156.98	0.9	29.4 ± 1.4	0.974	4.74
SL	L1	1.35	2.24	1.71	2.85	3.76	6.26	0.8	38.3 ± 1.2	0.992	5.12
SL	L3	2.27	4.27	1.86	3.50	4.82	9.09	1.2	40.5 ± 2.0	0.977	6.22
SL	L6	4.25	9.99	3.07	7.21	7.15	16.78	1.4	44.8 ± 1.3	0.988	4.31
SL	W1	2.10	3.48	1.32	2.19	3.47	5.76	1.6	25.6 ± 7.6	0.762	2.25
SL	W3	6.58	12.22	4.05	7.51	9.46	17.56	1.6	23.2 ± 2.3	0.974	4.41
SL	W6	10.10	22.83	8.23	18.60	16.22	36.65	1.2	23.2 ± 1.1	0.991	5.02
									AVERAGE		4.7
									STDEV		1.9

Table 4.1c – Carbon dioxide (CO_2), methane (CH_4) and total gas production with hay amendment. The amendment hay (H) floated to the surface in the H3 and H6 trials in SCL.

Sigmoidal curve values			CO_2		CH_4		Total Gas		
Soil	Treatment	Soil (g)	cm^3/day		cm^3/day	cm^3/Kg/day	cm^3/day	cm^3/Kg/day	CO_2:CH_4
SCL	H1	573.03	9.70	16.93	10.40	18.15	18.40	32.11	0.93
SCL	H3	477.85	7.50	15.70	0.02	0.04	9.90	20.72	0.002
SCL	H6	334.20	6.60	19.75	0.09	0.27	6.70	20.05	0.01
SL	H1	582.57	8.90	15.28	16.20	27.81	18.40	31.58	0.88
SL	H3	478.00	20.80	43.51	12.20	25.52	36.80	76.99	0.33
SL	H6	321.13	14.70	45.78	13.20	41.10	35.60	110.86	0.37

Table 4.2 – (Experiment 2a). Methane gas data for incubations with fresh and cured organic matter in SL (Experiment 1).

Control data (*) from Experiment 1a (Table 4.1) included for reference. Letters indicate a difference at $p<0.001$.

	Phase 1	Phase 2
Treatment	Methane (cm^3/Kg/day)	Methane (cm^3/Kg/day)
Control*[a]	0.04	1.8
Cured Biosolids[a]	0.003	0.37
Fresh Biosolids[b]	3.29	17.48
Cured Manure[a']	0.22	5.4
Fresh Manure[b']	3.85	42.36

Table 4.3 – (Experiment 3). Methane gas data versus pH.

Microcosms receiving H in Sandy Loam soils (Experiment 3). Letters indicate a difference at $p<0.001$.

pH	Phase 1 CH_4 (cm^3/Kg/day)	Phase 2 CH_4 (cm^3/Kg/day)
5.6[a]	0.44	10.6
6.1[b]	1.0	13.0
6.6[c]	1.8	13.8

Chapter 5: Organic Matter Amendments in Mitigation Wetlands – a Field Study

Abstract

We conducted a field study to evaluate the use of organic matter (OM) amendments in establishing mitigation wetlands. Overall, OM had little to no effect on wetland performance metrics. In the cases where OM showed an improvement in some metric, e.g., plant growth, this was often accompanied by a negative side effect, in this case loss of diversity and promotion of undesired species. In some cases, there were clearly negative side effects, for example, increased methane generation. Negative side effects could be avoided by using moderate application rates and composting the OM. We were unable to document that OM was helpful in establishing hydric soil conditions, which was the primary question addressed in the study. Instead, hydric soil conditions depended on hydrology. The OM amendments included municipal waste biosolids (B), composted wood mulch (W), hay (H), and cow manure (M). Application rates were 60, 180 and 360 yd^3 / acre. Cattail was a nuisance species at this site, promoted by soil disturbance (when adding OM amendments) and high levels of nitrogen (N) from manure. Given limited resources, focusing on site hydrology, rather than OM use, may be more likely to improve wetland mitigation success.

Introduction

It is common practice in the United States, and required in Maryland (Walbeck, Clearwater, and Neff 2011), to add OM amendments to soil when constructing mitigation wetlands. The Maryland State Highway administration (SHA) constructs over half of the mitigation in the state and tasked us with conducting field and lab studies to help them select the most effective amendment. We conducted a field study to evaluate OM amendments for the SHA and prepared a report of our findings. The findings have been redrafted into a format more suited for the science community.

Numerous studies of OM use have been conducted with mixed results (B. Scott et al. 2020). In this study we added to previous work by conducting a large-scale field experiment with a comprehensive list of common metrics in a single study. The total area of the study was ~ 0.14 acres spread out over an 8.1-acre site. We attempted to include all the field parameters that have been reported previously (B. Scott et al. 2020), or at least as many as were practical. We also targeted, in the field study, the effect of OM amendments on developing hydric soil conditions, which had not been studied previously. Wetland soils become hydric when they are under anaerobic conditions for extended periods. Anaerobic soils reduce iron-oxide, ubiquitous in soil, which can be measured using one of three tests: a chemical reaction with α,α'-dipyridyl dye strips (dipyridyl), removal of iron-oxides from Indicator of Reduction in Soils (IRIS), and measuring the soil electrical (redox) potential, or E_H (National Technical Committee for Hydric Soils (NTCHS 2015).

We evaluated four different OM types: (manure (M), wood mulch (W), hay (H), and biosolids (B)) at three different application rates, similar to the OM used in Chapter 4. We hypothesized amendments would increase plant growth and percent coverage. We also hypothesized that amendments (at least one amendment) would increase iron reduction at the site, and would positively affect all three methods of identifying hydric soil conditions: lower E_H, increase ferrous iron (Fe^{2+}) production (dipyridyl) and increase iron removal from IRIS. We hypothesized the soil bulk density (Db) would decrease, and SOM would increase. We also monitored free phosphorous (P) and N (as nitrate and ammonia) that were present in some amendments to determine what affect they had on plant growth. We also considered soil moisture (the effect of OM on soil water retention) as an additional parameter, but excluded it because the site was often inundated and we acceded to reduced sampling opportunities due to the Covid-19 outbreak. Based on the results from the lab study (Chapter 4), we hypothesized that the field methane production would be similar to the lab results, with some amendments (H, M, B) increasing methane and some (W, at low application rates) would reduce methane (CH_4).

Methods

Study Location

The field study was performed on a recently constructed mitigation wetland located in Goldsboro, MD (39° 1'52.04"N, 75°47'39.43"W, Photo 5.1). The site was formerly a ditched and tile drained row crop farm that was converted to a mitigation wetland in early 2017 and research plots were constructed in September 2019. The total

area of the site is 22.4 acres, but research plots were limited to a contiguous 8.14-acre area that is regularly flooded.

The OM amendments used in the study include materials for which the SHA has existing specifications: Type A (manure) and Type C (compost) (State Highway Administration 2018). To represent these OM types we used local cow manure (M) and composted wood chips (W), both provided by the contractor who assisted with plot construction. Specifications for the cow manure and wood chips are included in the SHA report. We also used biodsolids (B) from DC Water's Blue Plains Advanced Wastewater Treatment Plant (brand name Bloom®) and hay (H - e.g. Timothy grass). Amending soils with (wetland) H is recommended by the Wetland Science Institute (Melvin 2003). The three treatment application rates we evaluated were based on the Maryland Department of Environment (MDE) recommendation of 60 cubic yards per acre (Walbeck, Clearwater, and Neff 2011), so the dose rates were: 1x = 60 yd^3 $acre^{-1}$; 3x = 180 yd^3 $acre^{-1}$, and 6x = 360 yd^3 $acre^{-1}$. One exception was H, where 3x = 60 yd^3 $acre^{-1}$. Lower H application rates were used in the field study because the earlier lab study (Chapter 4) showed it produced much more CH_4 than other amendments and high application rates tended to float out of the soil when inundated.

Each amendment was applied to 2m x 6m plots (n=4). Plots were grouped, based on geography, by Blocks (Photo 5.1). Plots were we pre-excavated to a depth of approximately 15cm. Before amendments were added, an equivalent amount of excavated soil was removed to limit mounding. Each plot was divided into 12 @ 1m x

1m subplots. One subplot was dedicated for a well to record water levels and a second sub-plot had a ½m x ½m metal base (Kestha 2019) for gas measurements (Photo 5.2).

We monitored the five categories of field parameters described in Chapter 2: plants, soil physical properties, organic carbon, denitrification, and anaerobic processes. The category anaerobic processes are further defined here by the subcategories redox indicators and greenhouse gases (nitrous oxide (N_2O) and CH_4).

Plants

We monitored 3 plant parameters: aboveground biomass, below-ground biomass (roots), and cover percent for each species present. Samples for above-ground biomass were harvested once in August 2020 from two non-adjacent 0.5 m x 0.5 m subplots and samples were dried until the weight was stable. Belowground biomass was estimated using 2 peat filled 5 cm mesh bags that were harvested in September 2020. Roots were separated into three size categories: > 2 mm (rhizomes), 1 - 2mm (coarse) and < 1 mm (fine) and recorded in three 10-cm increments (0-10 cm bgs, 10 - 20 cm, and > 20cm. We estimated the percent cover for each plant species across a 2m x 5m area for each plot, which excluded the subplots with the well and gas chamber base. Percent cover was quantified using the standard cover class ranges(Peet, Wentworth, and White 1998) and range midpoint values were used for downstream calculations (diversity and Floristic Quality Index - FQAI). We calculated Shannon Diversity using the formula:

$$-1 * sum\left(\left(\frac{percent\ cover(i)}{total\ percent\ cover}\right) * \left(Ln\left(\frac{percent\ cover(i)}{total\ percent\ cover}\right)\right)\right)$$

The FQAI (Andreas, Mack, and McCormac 2004) was calculated as:

$$sum((coefficient\ of\ conservation(i))/sqrt(richness))$$

Soil physical properties

Bulk density (Db) at the soil surface was calculated by collecting a soil sample with a metal sleeve of known volume and measuring the dry weight of the contents. Soils were dried at 105ºC for at least 48 hours, or until there was no further weight loss. We also measured the surface soil strength (penetration resistance) using a pocket penetrometer. We measured percent sand by wet-sieving to > 0.125 mm to remove silt and clay.

Organic Carbon

The gravimetric SOM was measured by heating a known mass of soil at 550ºC for 2 hours and measuring the mass loss. The soils had been pre-dried by heating to 105ºC. The precent OM was then converted to volumetric OM content by multiplying by Db (Howard et al. 2014). We measured shallow and deep OM separately. Shallow OM was in the upper 15 cm of the soil (the amended zone) and deep OM was > 30cm.

Denitrification (and Phosphorus Retention)

Nitrate, ammonia, and phosphate samples were collected from the wells in each plot and placed into a pre-acidified (hydrochloric acid) container to pH < 4 and then frozen prior to analysis. Samples were run on a Lachat ion analyzer.

Anaerobic Properties – Greenhouse Gases

To measure greenhouse gases, We placed a ½ m x ½ m x ½ m clear-sided chamber (Kestha 2019) over a metal frame and collected the trapped gas every 15 or 20 minutes for 1 hour. Gas samples were collected in 10 cm^3 Exetainer vials and analyzed

on a Varian Model 450-GC. Gas production rates were estimated from linear regression curves. The gas production rates (nitrous oxide (N_2O) and methane (CH_4)) were considered to be de-minimus if the slope was negative or the r-squared value was less than 0.1. The CO_2 slope was usually negative due to photosynthesis, so this was not reported. For reporting purposes here both N_2O and CH_4 were converted to CO_2 equivalents (Rodhe 1990).

Anaerobic Properties - Redox Indicators

Redox indicators included those that are specified as hydric soil technical standards (NTCHS 2015). Redox indicators include: dipyridyl, IRIS tubes or film, and E_H. We had planned to monitor clear IRIS tubes as a follow-up on the study described in Chapter 3, but this work was curtailed due to Covid-19 restrictions. The standard dipyridyl procedure calls for test strip scoring system: +1 is positive and +0 is negative. Two out of three positive results are a passing score (soils are anaerobic). We expanded the scoring to give higher resolution for study purposes: +0 (negative), +1(slight reaction), +2 (strong reaction) so the scale was 0 - 6. We used both IRIS tubes (Castenson and Rabenhorst 2006) and film (Martin C. Rabenhorst 2018). Normally, films are inserted to 50 cm and the top 30 cm used for reporting. At this site soil may become cemented and sometimes it was not possible to penetrate soils to 50 cm even with a 110V power drill. However, in all cases, the insertion depth was at least 15 cm - the same as the amended depth. We recorded the E_H using a platinum tipped electrode and standard reference electrode (M. C. Rabenhorst 2009). The E_H values need to be interpreted in the context of the soil pH and compared to a standard reference. To obtain numbers that

could be compared statistically, E_H values were converted to "RedoxE". The RedoxE is the measured E_H minus the Technical Standard value at the field pH. Using this system positive values are more oxidizing (failed hydric soil test) and negative values are more reducing.

The three redox tests are similar in that they reflect soil potentials indicative of active iron reduction; however, they differ temporally and are strongly influenced by hydrologic condition. Dipyridyl and E_H measure the soil condition at a specific point in time and IRIS is an average over a 30-day period. For most of the study, soils were inundated, by as much as 13cm of water. For a brief period during the study soils became dry and oxic, during which time the sampling frequency was increased from monthly to weekly. To reflect the temporal soil hydrologic condition, we recorded (in addition to groundwater levels) a numeric (categorical) value (Water) based on whether any given plot was inundated (+2), saturated (wet)(+1), or dry (oxic)(+0) (Photo 5.3). Partial increments were possible: for example, if half of the plot was saturated it would have a Water value of 1.5.

Results and Discussion

Plants - Root and Shoot Biomass

Above-ground biomass increased with the addition of M and B, but not H or W (Figure 5.1). The likely reason for the differences was increase in available nutrients from the OM. Manure had elevated N and biosolids elevated P. The M amended plots had elevated N and statistically higher biomass. The M plots were also dominated by cattail. These findings are consistent with previous work. Nutrient enrichment results in higher

biomass (S. C. Brown and Bedford 1997; Steinbachová-Vojtíšková et al. 2006) and dominance of cattail (S. C. Brown and Bedford 1997). Cattail is taller and darker than other vegetation, so it is critical not to rely on visual observations. Due to its size, cattail appears to produce more biomass given the same coverage; however, in the study cattail had no more than 20% more mass, which is within the range of variation of cover classes (Peet, Wentworth, and White 1998). Nutrient enrichment is not the only factor that may favor cattail. Inundation and high litter accumulation may also be causes (Vaccaro, Bedford, and Johnston 2009). Prior to setup of the field plots, cattail had become dominant at the site, and although it was being actively controlled through the use of herbicides there had been enough growth prior seasons to build up a layer of mulch. Cattail was also clearly influenced by disturbance. Due to herbicide use, cattail was not regrowing at the site when plots were constructed, but began sprouting again in all areas that had been cleared for the study. Disturbance is often cited as favorable to invasive species (Johnson 2016; Cordell et al. 2016; Lang et al. 2015). Biosolids also increased above-ground biomass, but in this case the excess nutrient was P (not N) and cattail did not dominate.

Below-ground biomass (roots) did not vary with amendments (Figure 5.2). Although the average biomass for W3 and W6 amended plots (numbers signify dose) were higher, due to high variability neither were significant at $p < 0.05$. We calculated root biomass values excluding rhizomes. In plots with cattail growth (M3 and M6), rhizomes accounted for over 90% of the total root mass and were several orders of magnitude greater in mass than medium and fine roots. We considered root biomass at

three depths; 0 – 10 cm; 10 – 20 cm; > 20 cm, and in three different root size categories, large (rhizomes), medium (> 1mm) and fine (< 1mm) but did not observe any differences due to OM amendments. Few studies consider root growth in wetlands with OM amendments, but those that do report root growth reduced (Dickinson 2007).

The root:shoot ratio in some plots was different than the unamended control (C) although none were statistically different at $p < 0.05$ due to high variability. Wood mulch (W3 (0.52) and W6(0.46)) had a higher average root:shoot ratio than unamended plots (0.39). A higher root:shoot ratio is a desired effect as a buffer against nutrient stress (Bornette and Puijalon 2011). Treatments with the lowest root:shoot ratios (B(0.16); M1(0.15); M6(0.1)) were those that had elevated nutrients.

Plants - Diversity

We calculated several metrics for plant diversity: Simpson index, Shannon-Weiner Index (SWI), and Evenness. All these measures were highly correlated ($r > 0.99$), only SWI is reported here. Similarly, species richness and Floristic Quality Index (FQAI) (Andreas, Mack, and McCormac 2004) were also highly correlated ($r > 0.97$), so only FQAI is discussed. Other metrics, percent of facultative wetland plants, percent cover, and identification of dominant species, which are specified as mitigation evaluation criteria by the MDE, are included in the SHA report.

SWI variation by plot was minimal. The average SWI in the unamended Plots (1.12) is somewhat low for wetlands (Bailey, Perry, and Daniels 2007; Anderson and Cowell 2004; L. Green and Duguid 2020; Havens, Varnell, and Bradshaw 1995) but even healthy wetlands can be lower (Stauffer and Brooks 1997). Based on several

reviews(Bedford, Walbridge, and Aldous' 1999; B. Scott et al. 2020) We expected the elevated nutrient plots (B and M) to have lower average SWI. This was the case but none of the differences had $p < 0.05$: B (0.73, $p = 0.06$); M1 (0.78, $p = 0.10$), M3 (0.84, $p = 0.16$), M6 (0.87, $p = 0.22$). There were difference in SWI by Block. Block C had lower SWI ($p < 0.05$) (Figure 5.3). Block C, the wettest Block and nearly always inundated, was dominated by cattail.

Manure decreased the FQAI. The M6 plots had a lower FQAI (4.28) compared to the overall average (6.92: $p < 0.05$). This is consistent other findings, where species richness decreased with elevated N (from manure) (Bedford, Walbridge, and Aldous' 1999). Species richness has also been reported to vary with net productivity, where there was a drop-off in richness as net productivity increased above 400 g m-2 (Moore and Keddy 1988). This value was consistent with the findings where only M6 plots had average productivity > 400 g m-2 and also had significantly lower diversity. Adding nutrients, especially N, is beneficial in a monocultural agricultural setting, but the benefit of increased productivity in a wetland is a trade-off with decreased diversity. Plant diversity improves the ability of wetlands to predictably purify water (Cardinale 2011; McGrady-Steed, Harris, and Morin 1997). Hydrology also affected FQAI, with the lowest FQAI in Block C (Figure 5.4). Both the SWI and FQAI values varied by Block. Block C (wet) had lower SWI and FQAI, but the values were not statistically different. Sites with varying hydroperiod can increase diversity (Russell and Beauchamp 2017). 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. The removal of invasive cattail can increase diversity and richness (Lishawa et al. 2019).

Soil physical properties - Bulk Density

One of the characteristics of mature wetland soils is low (< 0.5 g cm^3) bulk density (Db) soils (Bantilan-Smith et al. 2009; Fenstermacher et al. 2016; Noll, Mobilian, and Craft 2019). Low soil Db allows easier root penetration and is associated with the accumulation of SOC (Chaudhari et al. 2013). OM amendments are known to decrease Db in upland settings (Rivenshield and Bassuk 2007); however, in wetlands the benefits are less clear. OM amendments offer a short-term reduction in Db by displacing minerals with less dense OM. Under natural conditions, Db reductions in hydric soils evolve through a very different process than simple mineral soil displacement - a process that requires decades (J. Brown and Norris 2018). Nevertheless, the bulk density was measured in November 2019 (immediately after Plot construction), and September 2020 (Figure 5.5). 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 1.65 g cm^{32}, shown as a dashed line on Figure 5.5, where there is no impediment to root growth. All samples were below this

[2] At the clay content at my field site (15%, Appendix 3), root growth is impeded above 1.65 g cm^3 (Dexter 2004).

line, even prior to OM addition, so in this sandy loam soil there was no meaningful benefit of Db reduction.

Soil physical properties - Soil Strength

The soil strength is another parameter (besides Db) that can be used to estimate resistance to root growth. There is a strong correlation between root elongation rates and soil strength (Day, Seiler, and Persaud 2000; E. C. Wolf, Rejmánková, and Cooper 2019). At the study site, soils became cemented (Cheng, Cord-Ruwisch, and Shahin 2013) as they dried and the penetration resistance, measured with a pocket penetrometer, was > 0.43 MPa. However, once the soil became inundated it became fluidized, reducing the penetration resistance effectively to zero. All OM amendments reduced cementation (penetration resistance) of dried soils. However, if reducing the penetration resistance of the soils during dry periods had a meaningful effect on plant and root growth and soil development, it was not evident.

Organic Carbon - Soil Organic matter (SOM)

Adding OM amendments to soils increases the SOM by definition. It is the most commonly reported value in OM amendment studies (B. Scott et al. 2020), but is not useful in monitoring soil development or health over the short term. Therefore, an alternative approach to calculated the OM amendment contribution to SOC is presented. Some SOC was lost due to soil disturbance. Soil disturbance, necessary to incorporate OM amendments, can expose and release SOC (Fenstermacher et al. 2016). In November 2019, shortly after the plots were constructed, the SOC in the unamended plots was 30.7 ± 2.7 mg cm^3. In November 2020, the SOM was 29.1 ± 1.4. Although there appeared to

be a slight loss of SOM, it was within experimental error. However, new root growth accounted for 6.4 ± 3.3 mg/cm^3 of the SOC in November 2020. Therefore, the SOC (not including roots) was 22.7 ± 4.7 mg/cm^3, or 8.0 ± 7.4 mg/cm^3 of the original SOC was lost. This means for an OM amendment to have a net positive impact on SOC content, the soil would need to contain at least 38.7 (30.7 ± 8.0) mg/cm^3 of SOC. The high doses of wood much (W3 and W6) had 44.5 ± 25.8 and 51.2 ± 11.2 mg/cm^3 non-root SOC; therefore, only high doses of wood mulch had a lasting increase in SOC. However, this finding needs to be qualified. The particle size of wood chips in the mulch is large. It appears, in soil OM measurements, as SOC, but it is not incorporated into the soil structure, per the definition used here.

Organic Carbon - Deep Organic Matter

We hypothesized that 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. We 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 (Amendola et al. 2018). There was a strong negative correlation between deep SOM and percent sand (Figure 5.6).

Anaerobic Properties - Methane and Nitrous Oxide

Greenhouse gas production varies greatly based on the OM amendment. We calculated the Global Warming Potential (GWP) in CO_2eq / m^2 / yr based on a sum of the

monthly methane and nitrous oxide emissions. Over 95% of the GWP was from methane, and nitrous oxide was only present immediately after plot construction when the site was transitioning from dry to saturated soils. Nitrous oxide emissions are highest when soil saturation is in flux (Maria E. Hernandez and Mitsch 2006; Mander et al. 2011). A chart showing the monthly greenhouse gas emissions is included in the SHA report. The estimated annual emissions for the unamended control (Plot C) was 4.1 Kg CO_2eq / m^2 / yr, which is low compared to similar wetlands (Nahlik and Mitsch 2010). Biosolids had a comparable rate of 4.8 Kg CO_2eq / m^2 / yr. Manure had the highest greenhouse gas emissions (M3 = 32.1 and M6 = 36.7 Kg CO_2eq / m^2 / yr). We previously reported the effect of OM amendments on methane (Chapter 4) and since then other reports (Rubin, Anderson, and Ballantine 2020) have shown increased methane emissions with composted manure and their reported value of 17.1 Kg CO2 eq / m^2 / yr was similar to the field results (Table 5.1). We observed high methane emissions with manure in the lab study (Chapter 4), but in the field manure may not be the only cause since high doses of manure also cause aggressive growth of cattail, and cattail is known to increases methane emissions (Lawrence et al. 2017). In the lab study H produced the most methane and the field results are consistent when taking into account that the field dose was reduced: H6 (field) = H3 (lab).

The production of methane gas depends in part on the soil pH (Z. P. Wang et al. 1993). Peak methane production is around pH 7 and decreases as pH decreases, as observed in the lab studies (Chapter 4). Similarly, there was a strong correlation between the annual GWP and pH (Figure 5.7), which we also observed in Chapter 4, where

methane production was affected by pH. 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. Hay, which produced the most methane in the lab study, produced high levels of methane in the field even at comparatively low pH.

We used the same amendments in the field study and lab study (Chapter 4), and are able to compare emissions to evaluate if the field study results are representative of field conditions. Field and lab methane emissions were converted to cc/Kg/day and are plotted on Figure 5.13. There is a strong correlation (0.9) between field and lab results. Methane emissions from the lab microcosms were about 7.5 times higher than in the field, likely due to the activity of methanotrophic bacteria.

We have documented methane emissions that resulting from adding OM in a wetland restoration but we recognize the effect may be temporary. In one study, OM did not increase CH_4 after wetlands had been restored 10 years (Winton and Richardson 2015).

Anaerobic Properties - Hydric Soil Indicators

We did not find any field evidence that OM increased the potential to pass hydric soil indicator tests. Hydric soil testing was the primary reason SHA funded the work and further discussion is included in the SHA report. However, one observation merits discussion and is included under the hydrological considerations section.

Hydrologic Considerations

Hydrologic setting

Conditions at the field site led to differences in the hydrology of our test Plots. The site was graded to create a "bowl" in the center, where all our Plots were located. We selected locations within the bowl that had similar elevation (Figure 5.8) and so expected similar hydrology. For most of the study period the site was inundated. However, several times the water level receded and this occurred at different rates such that Block D would become dry but Block C was always saturated. Different water infiltration rates could be observed withing Blocks. 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 where water levels fell more rapidly in Plots that had sandy B horizon soils (Figure 5.9). 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. Resulting differences in vegetation are evident across the soil series dividing line (Photo 5.4). We saw similar differences in vegetation in Block D (not shown).

Hydrology and hydric soil test response

We measured the E_H and dipyridyl in mid-June, when water at the site receded. The E_H responded to changes in water levels, but throughout the period all test results were positive (Figure 5.10a). We also measured the reaction to dipyridyl during the same period (Figure 5.10b). The dipyridyl test responded to changes in water levels. Under

saturated conditions test results were positive. As water levels fell, we began to observe negative test results. The α,α'-dipyridyl test is popular because it is inexpensive, easy to use, and provides immediate results. However, in our field study, this test was the most likely to yield a negative result.

The dipyridyl test is an abiotic chemical oxidation reaction of Fe^{2+}, a by-product of iron reduction. The oxidation rate of ferrous iron is pH dependent, and at neutral pHs (and higher) the reaction is nearly instantaneous (Stumm and Sulzberger 1992). At acidic pHs the abiotic oxidation rate progressively decreases, about 100x per pH unit. However, around pH 5 and below microbial oxidation of Fe^{2+} becomes favorable (Meruane and Vargas 2003) . The pH range at the site during the sampling period when soils were unsaturated was 5.0 – 6.7, and Fe^{2+} would theoretically be stable. Still, Fe^{2+} was rapidly oxidized at the site and there was no correlation between pH and dipyridyl test results. The E_H measurements responded slower when soils became unsaturated, and indicated anaerobic conditions the throughout the study period, even when dipyridyl tests indicated the soils were aerobic. It is these relative rates of reaction, particularly the slow response of E_H, that may merit further study as discussed in Appendix 3.

Hydrology and methane release

We 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 values were converted to carbon dioxide equivalents and combined using the conversions: nitrous oxide = 263 CO_2eq,

methane = 34 CO_2eq. The carbon dioxide equivalent values are shown on Figure 5.11. The values on Table 5.1 were calculated from values in Figure 5.11 assuming constant emission levels over the a 30-day period. To evaluate the effect of hydrology on methane emissions, we focused on the period from 5/26/2020 to 8/12/2020 (Figure 5.12), the only period when the site was not continuously inundated. Maximum greenhouse gas (methane) emissions occurred on 5/26/2020. Methane production depends in part on soil temperature. The soil temperature increased in subsequent sampling events (Figure 5.12), so, based on temperature alone, methane emissions should have increased (Yvon-Durocher et al. 2014). Methane emissions on 6/14/2020 were very low because water levels had receded below the surface, allowing soil to begin to become oxic. On 7/16/2020 and 8/12/2020 soils were inundated and temperatures were the highest; however, methane emissions were still low. This is likely because antecedent water levels were low. The soil had recently been oxic, and full methane generating capacity had not recovered. This is consistent with our general finding in Chapter 4, that the duration of inundation may have a large effect on methane production.

Conclusions

According to the MDE wetland mitigation guidance, OM amendments are "needed to meet hydric soil characteristics and maintain the desired plant species". In this field study, neither of these outcomes were observed, except in some cases with composted OM. The study was conducted during a higher-than-normal rainfall period (Appendix 2) and Plots were normally inundated. During a brief dry period, approximately two months, water levels receded and we were able to compare the results

of the three hydric soil testing methods (even though all tests would have been considered "positive"). Amendments had no effect on the hydric soil test results; however, the hydrology, which varied by Block, did affect results. Amendments affected plant growth, but did not maintain desired species. At this site cattail (*typha glauca*) was an undesired species and was being actively controlled through the use of herbicides. Our Plot construction resulted in cattail re-growth and manure amendments, due to elevated levels of nitrogen, increased cattail growth. Most amendments, biosolids, manure, and hay, increased the percentage of invasive species: none had a statistically significant effect on floristic quality, although averages were slightly lower with OM. Composted OM (wood chips) improved root growth at the W3 treatment level and slightly reduced the average percentage of invasive species, although not by a statistically significant amount. High doses (W3, W6) of composted wood chips were also able to offset SOC losses due to disturbance-induced priming. OM amendments also increased methane release. Overall, composted material appears more suited as a wetland OM amendment, but avoiding soil disturbance to add OM may be better.

Figures

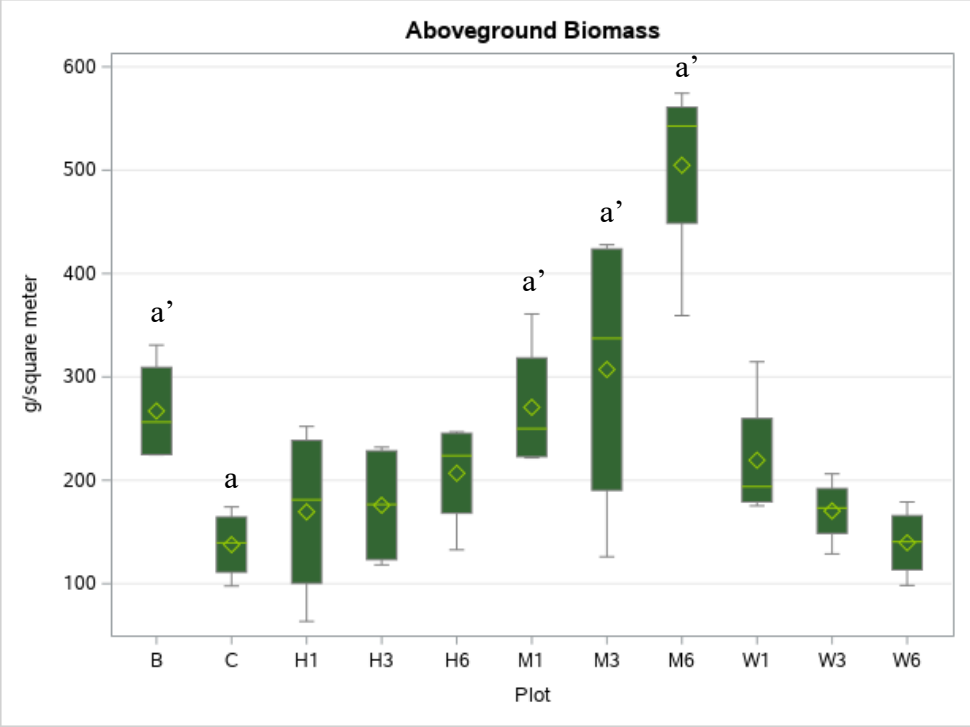


Figure 5.1 – Aboveground Biomass.
a is the control plot (no amendments). a' signifies values where $p < 0.05$.

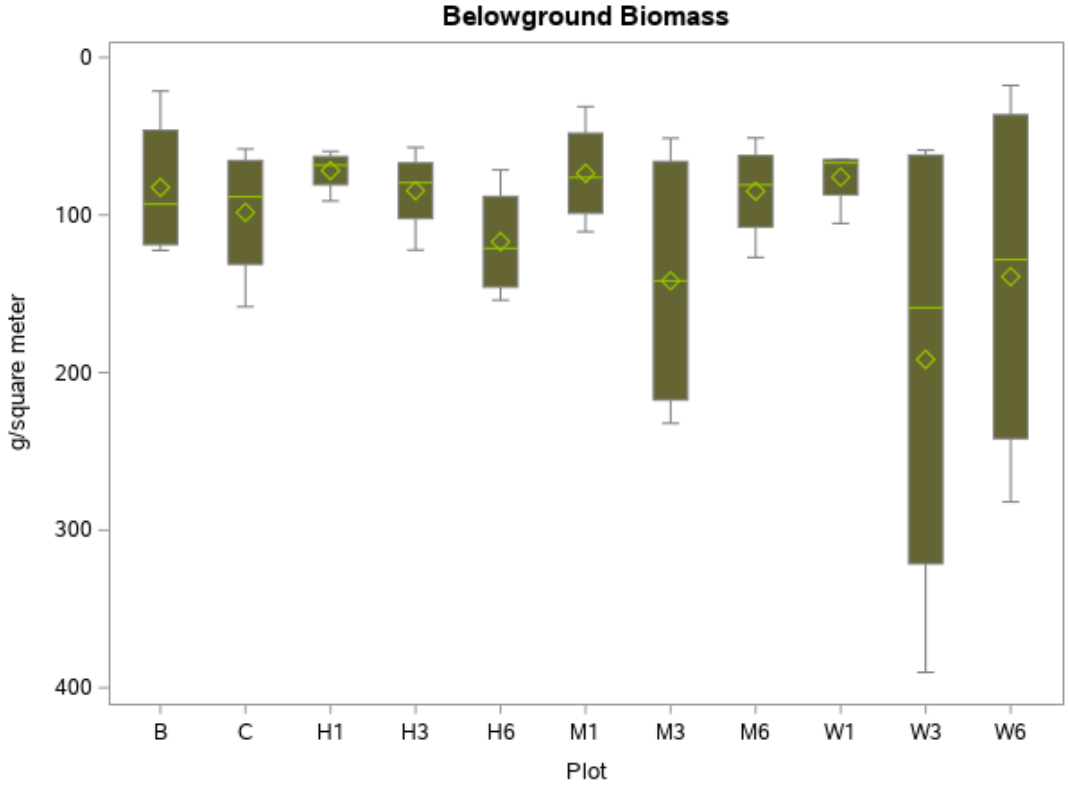


Figure 5.2 – Below-ground (root) biomass.
There were no differences in values at $p < 0.05$.

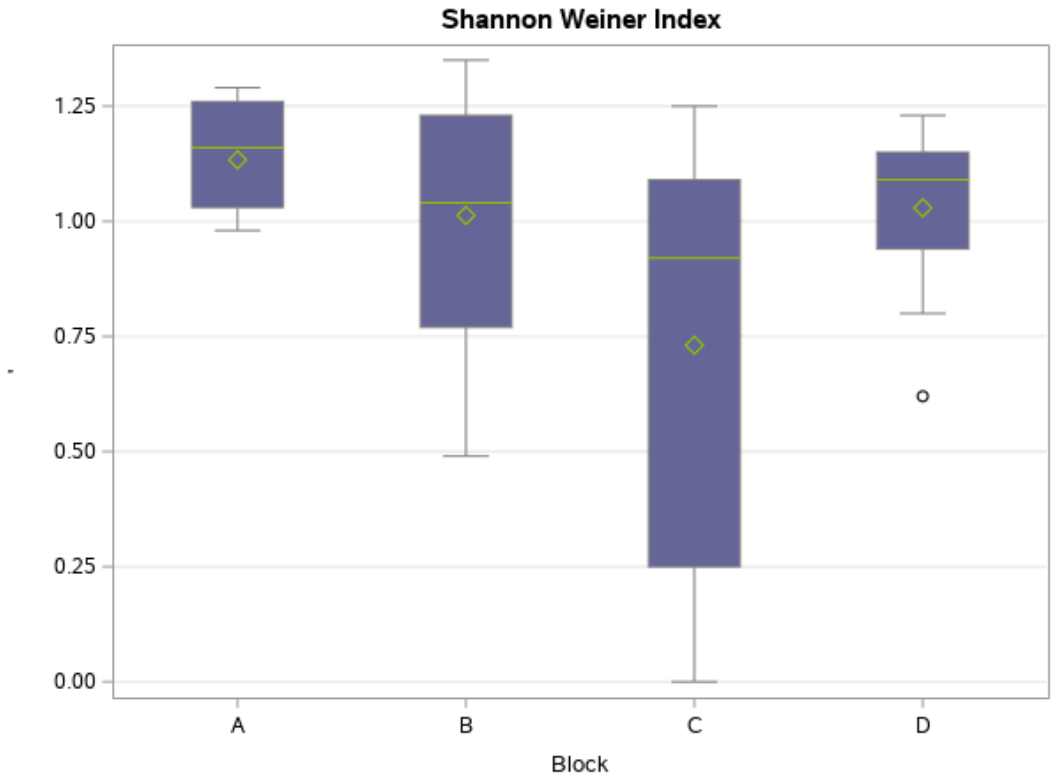


Figure 5.3 – SWI by Block.

Despite the wide variation, Block C, which was constantly inundated, has statistically lower ($p = 0.01$) SWI than Block A, which experienced more wet/dry cycles.

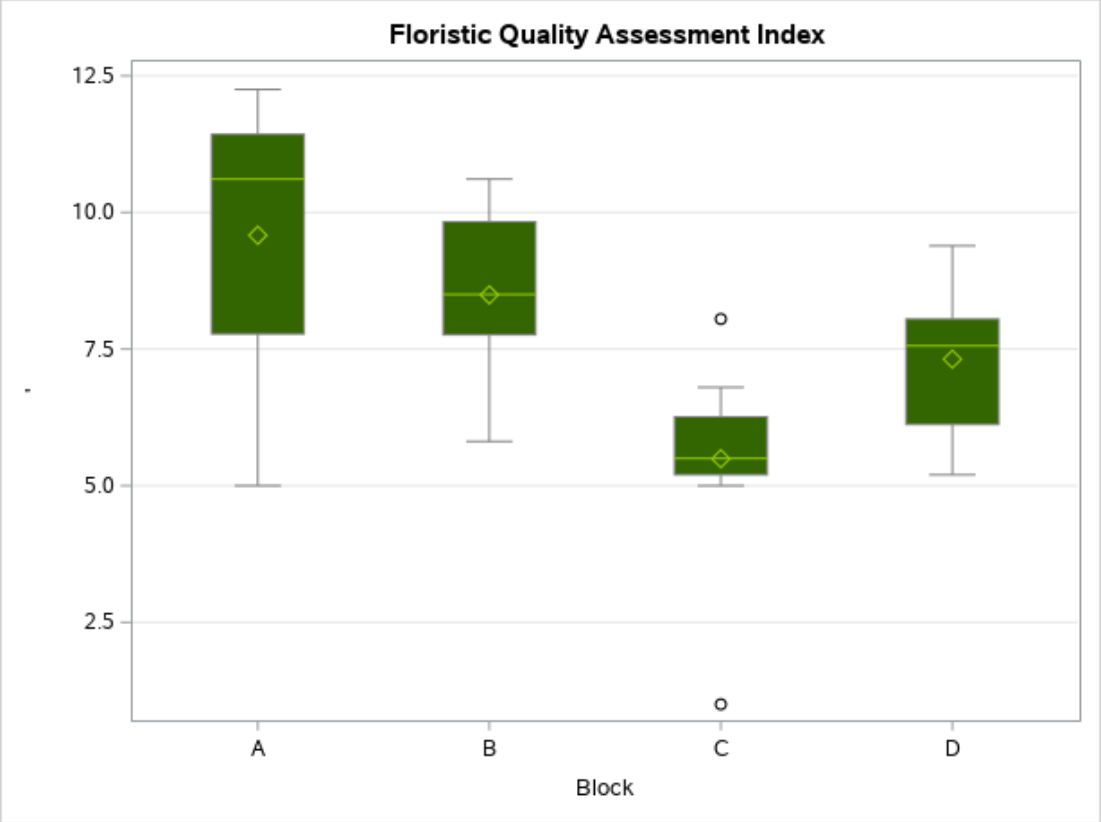


Figure 5.4 – FQAI by Block

Block C, which was constantly inundated, had lower FQAI, primarily because of the dominance of cattail (T*ypha glauca*).

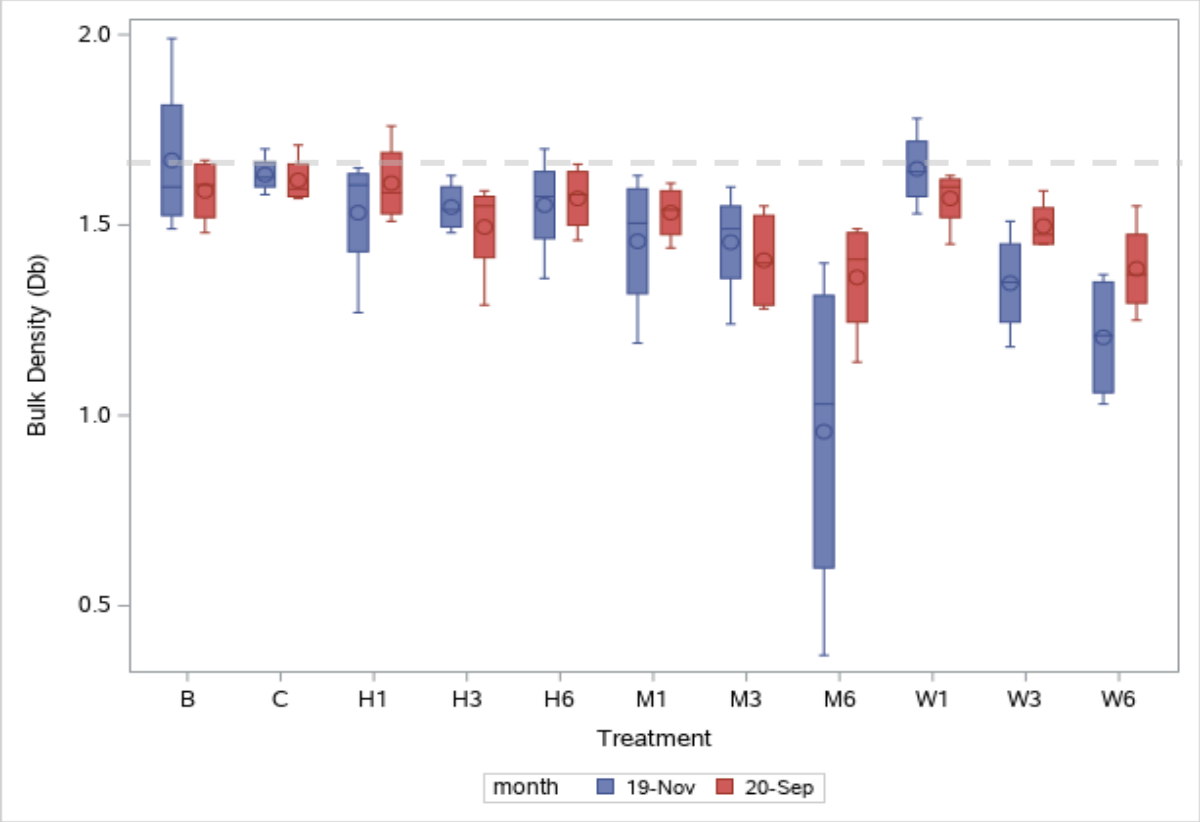


Figure 5.5 – Bulk Density by Plot.
Dashed line is 1.65 g cm^3 below which root penetration is unrestricted (Dexter 2004).

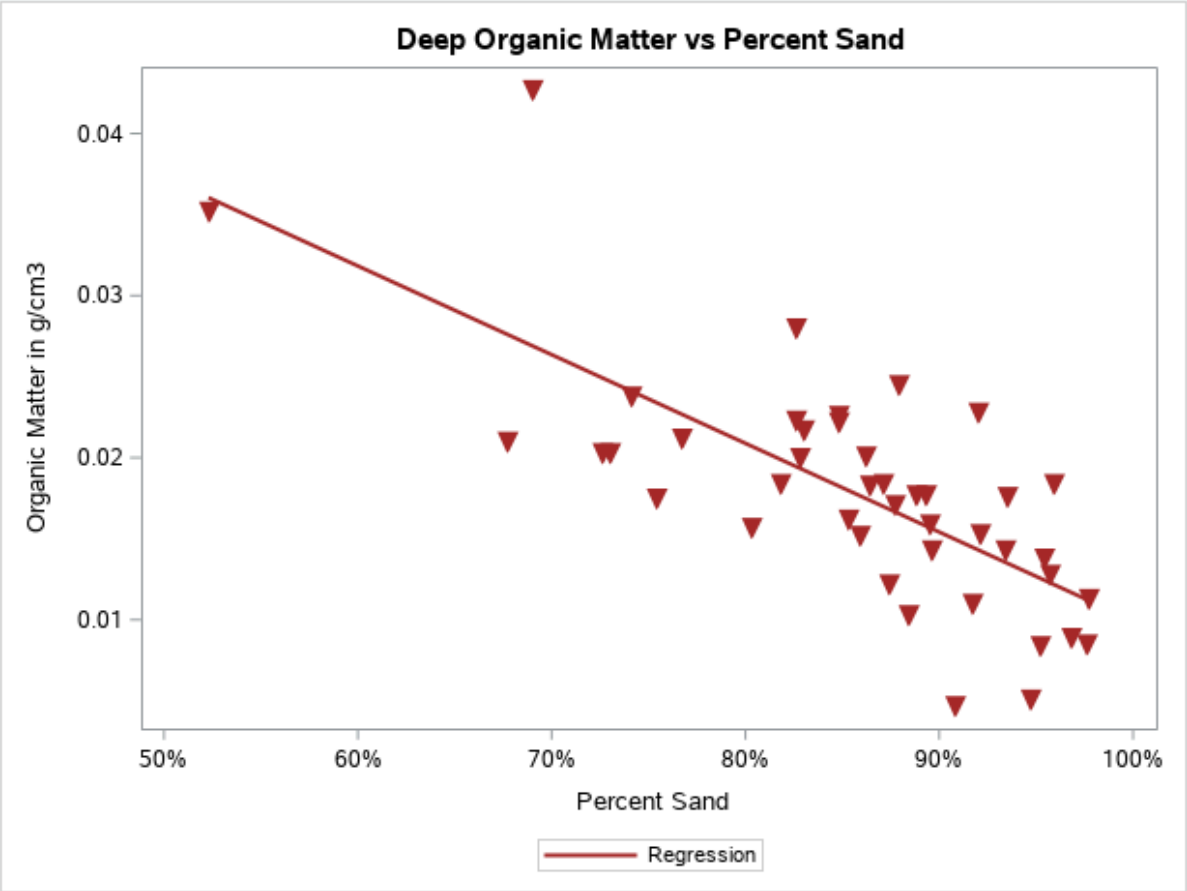


Figure 5.6 – Correlation between OM and percent sand below 15cm (OM amendment depth).

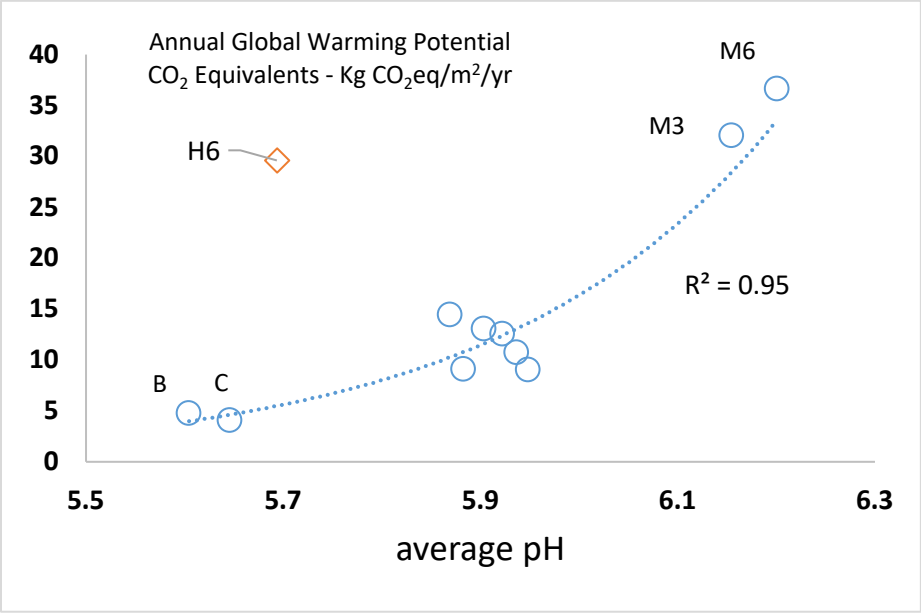


Figure 5.7 – Annual global warming CO_2 equivalents versus pH.

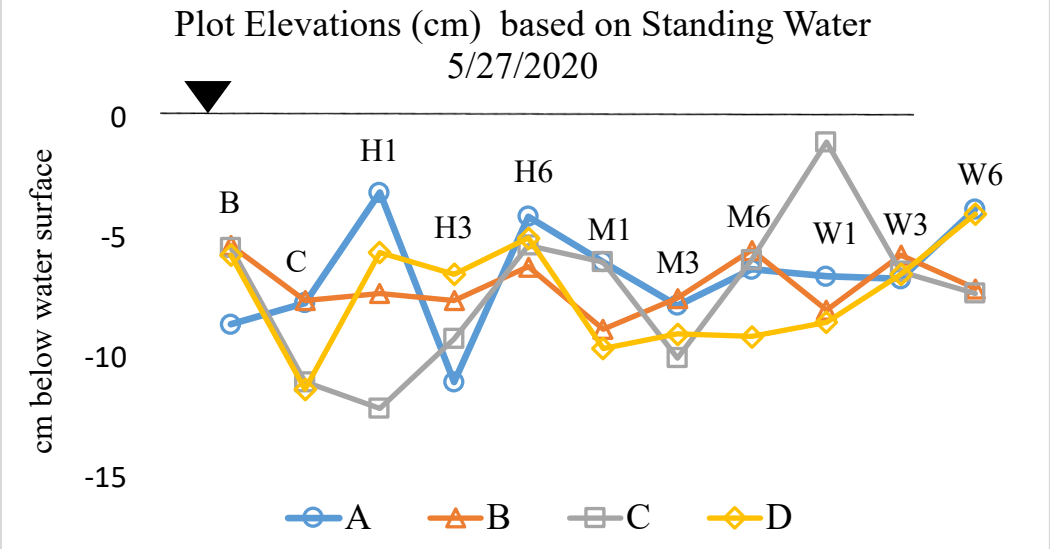


Figure 5. 8 – Plot elevations.

There was no difference in the average elevation by Block (A,B,C,D).

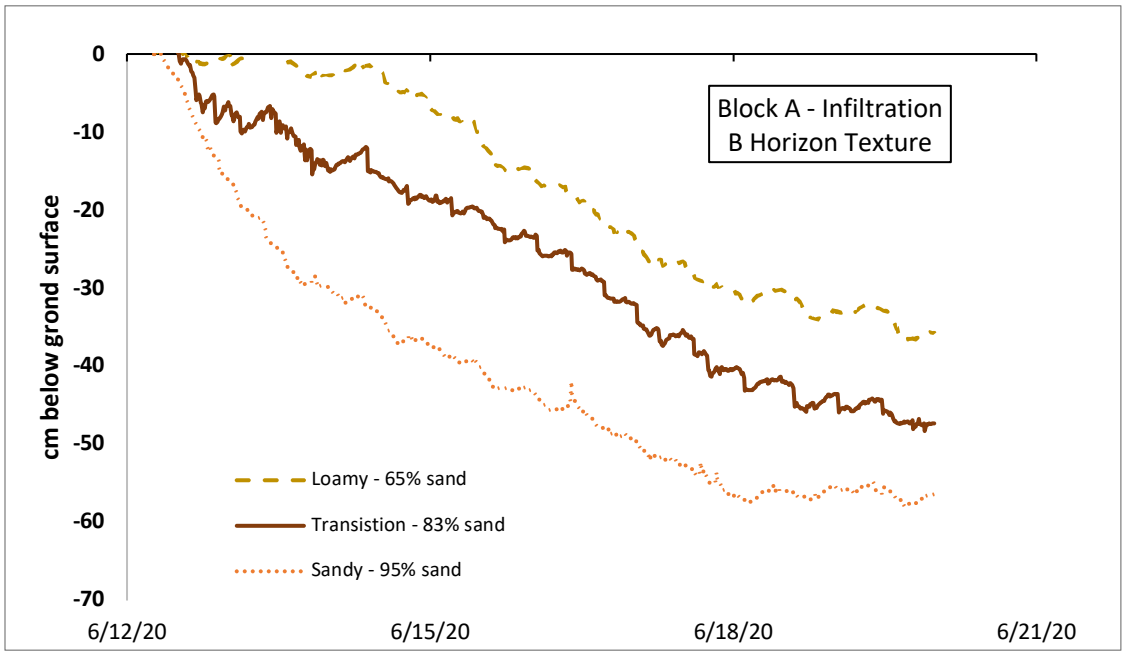


Figure 5. 9 – Varied water infiltration rates in Block A.
At this location, water infiltration rates corresponded to the sand percent in the B horizon.

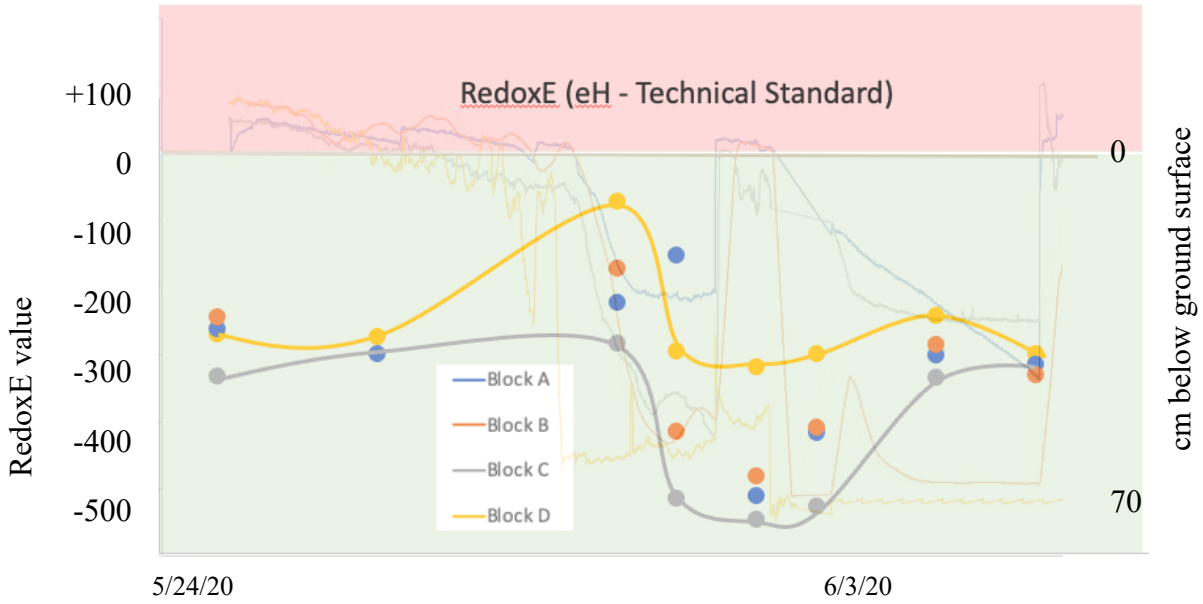


Figure 5. 10a – Response of E_H to changing water levels.

Water levels appear (shaded) in the background. Negative test results (E_H value higher than Technical Standard) is shaded in red, green shading is a positive test result.

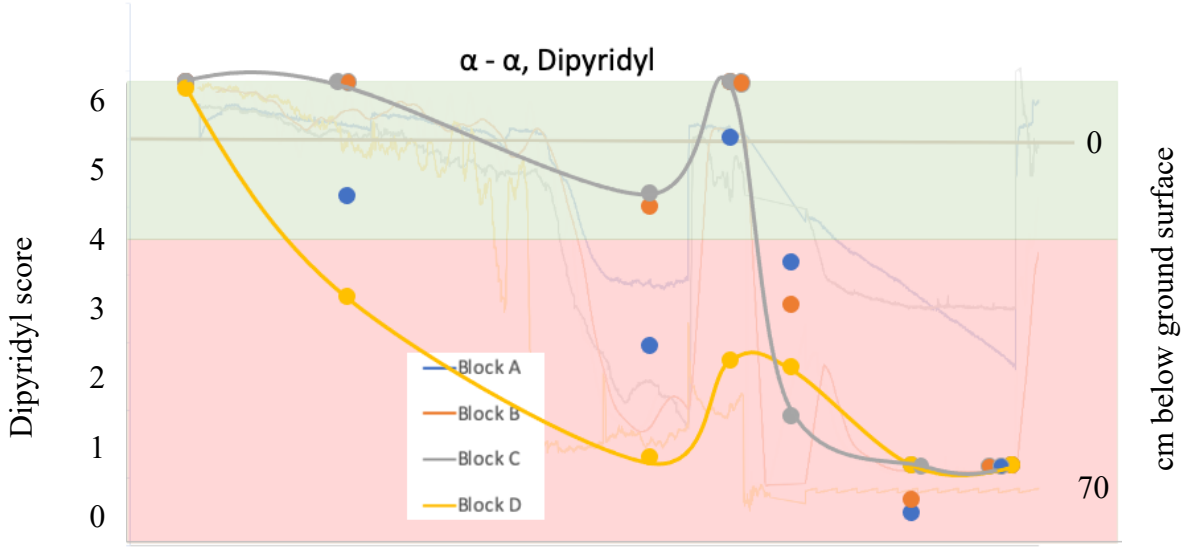


Figure 5.10b – Response of dipyridyl to changing water levels.

Red shading (score < 4) is the equivalent of a negative test result, green shading is a positive test result.

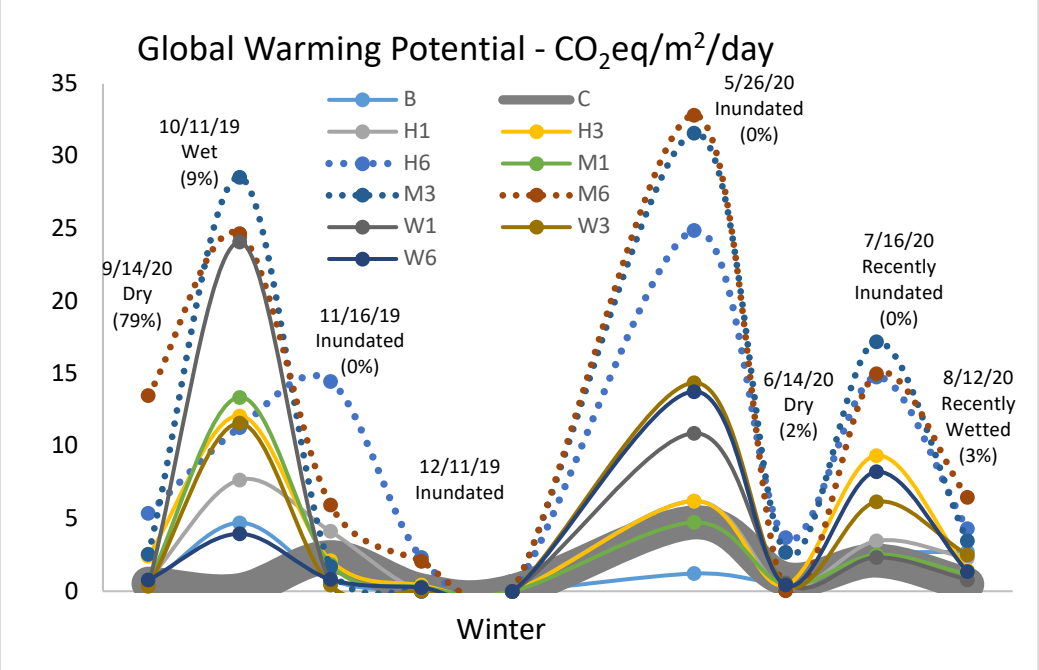


Figure 5. 11 – Global warming potential from nitrous oxide and methane in carbon dioxide equivalents.

Value are averages (across the 4 Blocks). Numbers in parenthesis represents the relative percent of nitrous oxide, the balance is methane.

Dry, Wet, and Inundated are the hydraulic conditions at the time of gas sampling.

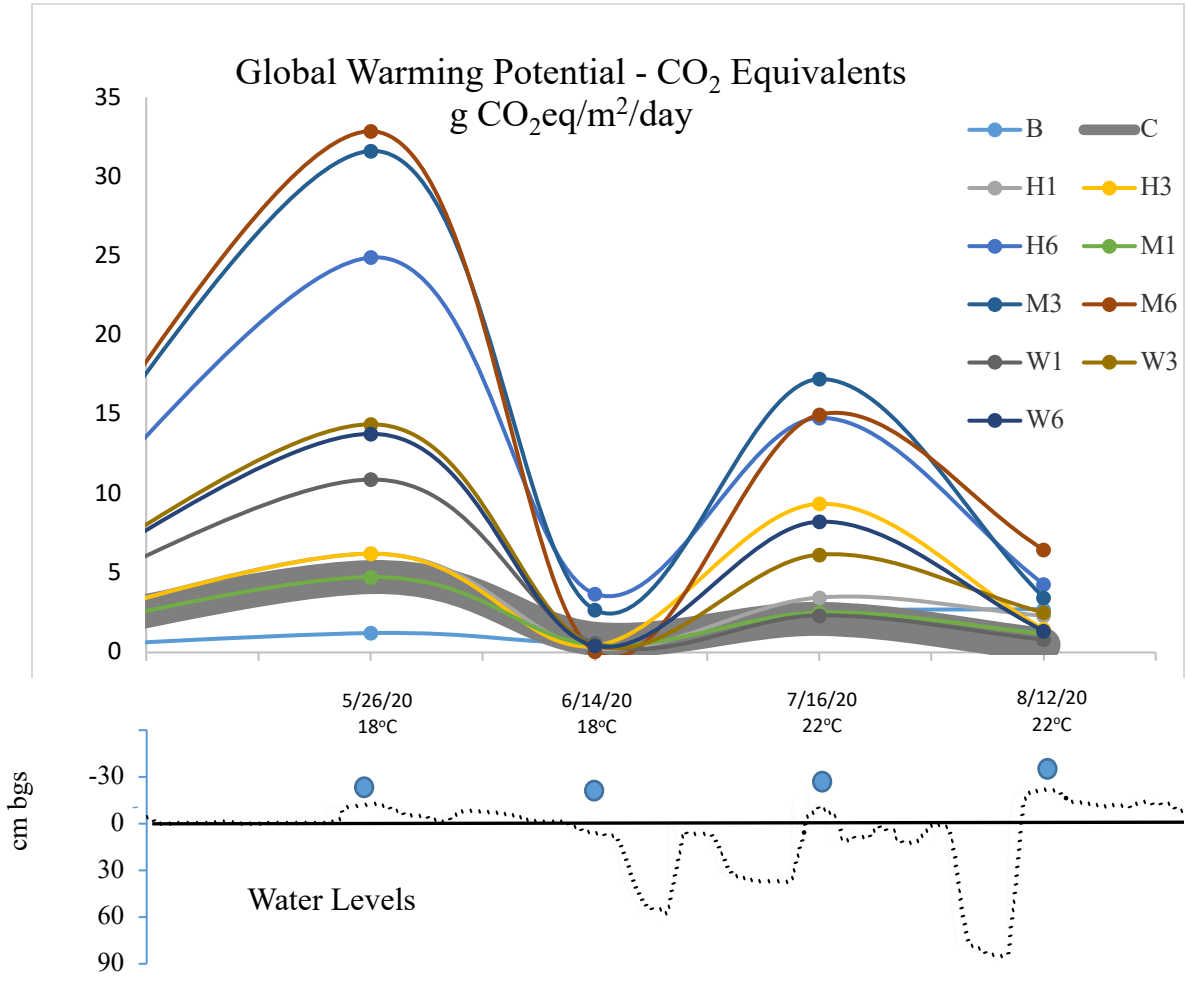


Figure 5. 12 – Carbon dioxide equivalents plotted with water levels and temperatures.

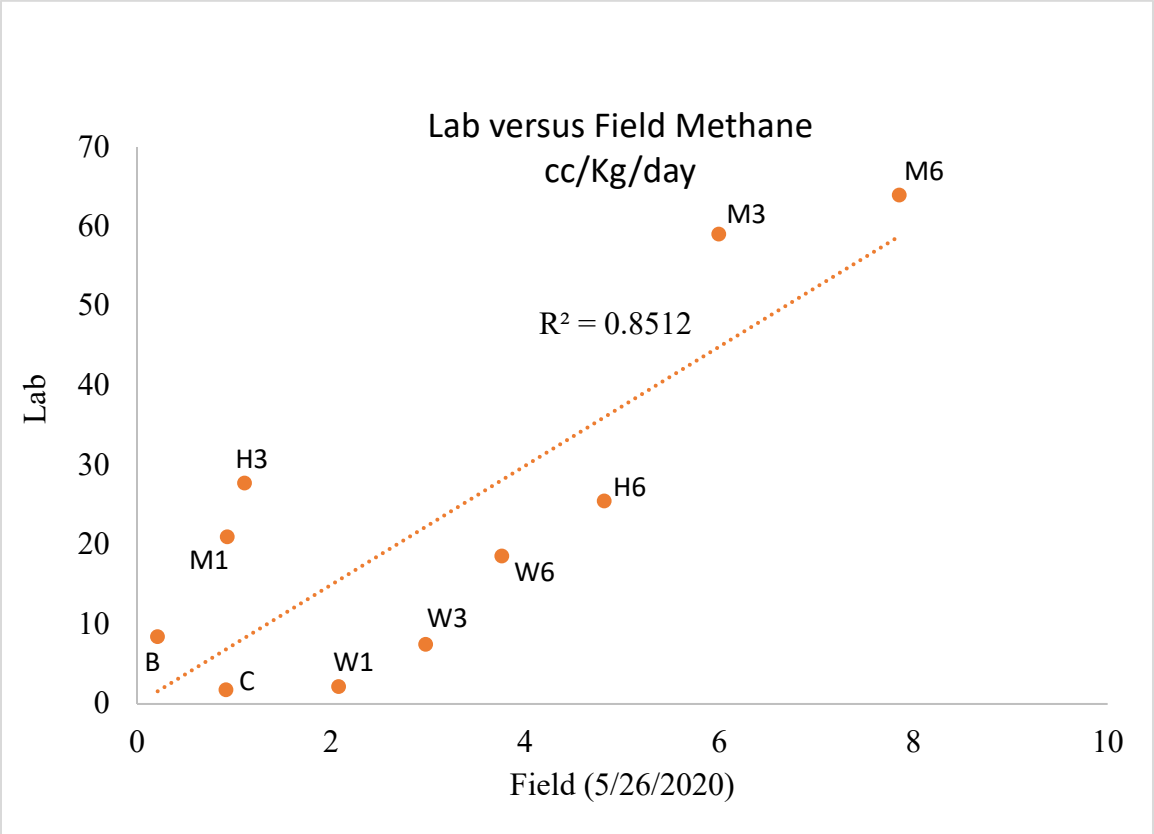


Figure 5. 13 – Comparison of field and lab methane emissions.

Tables

Table 5.1 – Estimated annual greenhouse gas emissions based on monthly sampling. Plots included OM amendments: C – Control (no amendment); B – Biosolids; M – composted manure; H – Hay; W – Composted wood chips. Number signifies loading rate.

Plot	Kg CO2eq /m^2 /yr
C	4.1
B1	4.8
H1	9.06
M1	9.15
W6	10.8
H3	12.6
W3	13.1
W1	14.5
H6	29.6
M3	32.1
M6	36.7

Photos

Photo 5.1 – Site for field study.

The four blocks are outlined in orange. Each Block contains 11 treatment plots, randomly distributed.

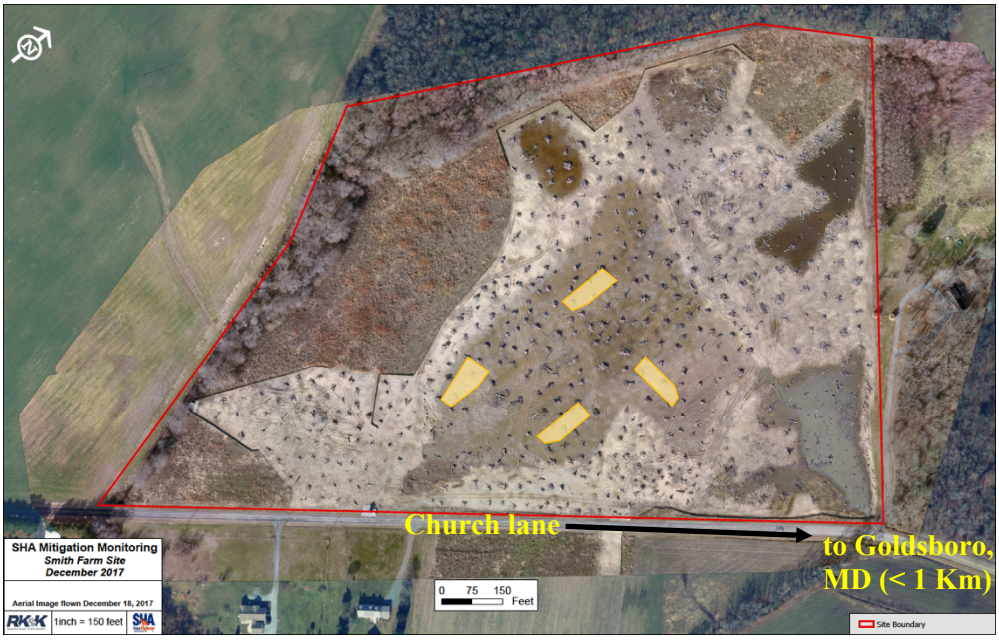


Photo 5.2 – Final Plot configuration.

Metal rectangular structure is a gas chamber base. The aboveground pipe is a monitoring well. Outside dimensions are 2 meters x 6 meters.

Photo 5.3 – Varied hydrology conditions at the Smith Farm site.

I recorded the categorical blocking variable “Water” to reflect this temporal condition.

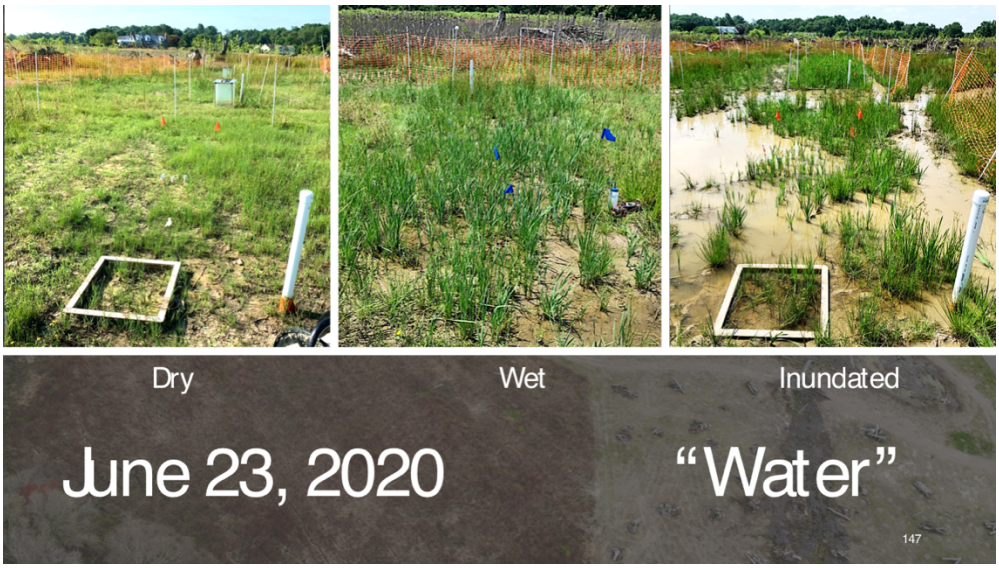


Photo 5. 4 – Changes in vegetation across soil series withing Block A.

In June 26, 2020, cattail was still green in the M6 plot (lower left corner) but by July 7 it had senesced.

Chapter 6: Methanosarcina dominate soils and increase methane production weeks after rewetting

Abstract

After rewetting dried soils, methane production begins almost immediately, then increases sharply after 3 – 7 weeks, a point in time identified as the methane breakpoint. A shorter time interval usually corresponds to adding a labile substrate. Using Stable Isotope Probing (SIP) we measured changes in the microbiome before and after the breakpoint. The archaea *Methanosarcina* appeared to be responsible for the majority of the methane production as abundance was strongly correlated (r = 0.999) with methane production rates. Labeled acetate ($^{13}CH_3{}^{12}COOH$: Ac)-fed incubations revealed evidence of methyl oxidation (likely by *Methanosarcina*) and soil organic carbon (SOC) priming, which was released primarily as methane (not carbon dioxide). Glucose (Glu)-fed incubations were similar to acetate fed incubations, except there was a delay while Glu was being fermented to Ac. We could not identify a clear pattern in the bacterial microbiomes that would account for the methane breakpoint. A better understanding of the dynamics of methane generation in saturated soils may help lead to practices that limit the unintended release of this greenhouse gas.

Introduction

Methane from wetlands makes up approximately 10% of total anthropogenic emissions[131]. Methane is a greenhouse gas that is 28 times more heat absorbing than carbon dioxide. There has been increasing acceptance that global warming, due to carbon dioxide and methane emissions, is causing harm and needs to be addressed (IPCC 2021).

As a greenhouse gas carbon dioxide receives more attention because the atmospheric concentration is 200x higher than methane; however, methane emissions have been increasing faster (Rigby et al. 2008; Ruddiman et al. 2008) and increased anthropogenic methane emissions results in more stress on global systems that attenuate methane. Atmospheric carbon dioxide can persist for hundreds or thousands of years, but methane persists for about 9 years (Prather, Holmes, and Hsu 2012). Therefore, controlling methane would have a much more immediate effect on the climate (Abernethy et al. 2021), (Montzka, Dlugokencky, and Butler 2011). In order to limit methane from wetlands we need a greater understanding of the factors that affect methane production.

Methane is produced under anaerobic conditions by a complex consortium of microbial activity (McInerney, Sieber, and Gunsalus 2009). Fermentative bacteria break down complex organic molecules like cellulose, a polymer of glucose, into simpler molecules: e.g. formate, acetate, CO_2 and H_2 (Kotsyurbenko et al. 2019). Methane producing organisms, usually archaea, then use these simple organic molecules as substrates. There are two main methanogenic pathways: hydrogenotrophic and acetoclastic. Hydrogenotrophic organisms use hydrogen gas to reduce carbon dioxide $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ and acetoclastic organisms ferment acetate CH_3COOH -> CO_2 and CH_4. The two are affected differently by environmental factors such as temperature and pH (Kotsyurbenko 2005). Many of the chemical reactions used by methanogens are energetically unfavorable and organisms must rely on specific environmental conditions (Bethke et al. 2011) and/or syntrophic relationships (Kotsyurbenko et al. 2019) to realize a net energy gain. Methanogens are strict anaerobes

and inactive in aerobic soils, which includes drained wetland soils. However, after soils become saturated and oxygen is purged from the system, methane production begins almost immediately. Immediate methane production has been observed in bulk aerobic soils that experience heavy rain events (Teh, Silver, and Conrad 2005), but the level of methane production is generally low. Methane then increases sharply after extended inundation (Chapter 4).

There are many factors that influence the amount of methane released from wetlands, including plants, attenuation by methanotrophic bacteria, pH, and temperature (Bubier and Moore 1994). Hydrologic factors also affect methane: water level (Limmer, Evans, and Seyfferth 2021; Calabrese et al. 2021), water level fluctuations (Y. Zhang et al. 2020), inundation frequency (Altor and Mitsch 2006) and inundation duration (Yuan et al. 2020). Methane is produced during rice cultivation where organic soil amendments are sometimes used to increase rice yields; however, increased methane has been observed with manure (van der Gon and Neue 1995) and rice straw (Yuan et al. 2020; Souza 2021). A recent field study demonstrates the methane breakpoint, and how manipulating inundation duration can be used to control methane. Researchers found that inundation of no more than 35 days, followed by drainage, reduced methane emissions. In fields that included rice straw amendment, the inundation period before methane increased was about 20 days (Souza 2021).

In a previous laboratory study (Chapter 4) we observed a consistent pattern in methane production rates following dried soil inundation. After about 40 days there was a breakpoint and methane suddenly increased. After the breakpoint, total gas production

increases by a factor of 2 – 5x and methane production rates increased by several orders of magnitude. The time to reach the methane breakpoint decreases with the addition of an OM amendment, but once the breakpoint is established it is consistent and predictable. Here, we examine the microbial populations before and after the breakpoint. We hypothesized that the microbial community would be characteristically different (rather than an increase in microbial populations). To test this, we devised a ^{13}C Stable Isotope Probing (SIP) experiment, modeled after the study in Chapter 4, to investigate the microbial communities that control methane generation before and after the breakpoint. We used two ^{13}C labeled chemical substrates: acetate (Ac) and glucose (Glu). The Ac trial represents the condition where this substrate, used by acetoclastic methanogens, has built up in the system and the Glu trial represents the conditions where substrates must first be fermented. We considered whether the use of specific substrates, Ac and Glu, may artificially alter the microbial community behavior or composition. Artificially elevated substrate concentrations, such as Ac, can affect enzyme activity (German, Chacon, and Allison 2011) and the use of specific substrates at elevated concentrations may alter the rhizosphere microbiome, a condition plants produce, in the form of exudates (Zhalnina et al. 2018). To determine if Ac and Glu altered the microbiome we ran a set of ^{12}C hay amended trials for comparison. The use of ^{13}C Ac and Glu not only allowed us to identify active microorganisms, but also track the production of biogenic gases.

Methods

The methods used in this study were designed to be comparable to the Chapter 4 lab study. The initial experiments were done in 1L glass jars with a nitrogen purged headspace. Here, we used a stable isotope probing (SIP) technique with two ^{13}C labeled substrates: Glu ($^{13}C_6H_6O_2$) and Ac ($^{13}CH_3{}^{12}COOH$), and (^{12}C) hay. Instead of 1 liter glass jars, incubations were carried out in 40ml VOA vials with Teflon septa (Figure A5.4.1). The smaller size was to accommodate the use of costly ^{13}C substrates. Using ^{13}C substrates also allowed us to qualitatively track substrate respiration by examining $^{13}CO_2$ and $^{13}CH_4$. Each microcosm had 15 g soil, 15 cm^3 water and 0.06 g substrate (Ac, Glu, or hay), which resulted in 20.9 cm^3 of headspace in each vial.

Headspace pressure increases due to biogenic gas production so atmospheric pressure was re-established periodically by piercing the septa with a 23-gage needle. A small coating of silicone applied to the septa after each piercing prevented leaks. Prior to the gas sample events the VOAs were shaken to release gas trapped in the soil matrix, which in some trials made up as much as 90% of the gas produced. When gas samples were collected the septa were pierced with a 24-gauge needle connected to a 20 cm^3 gas-tight syringe (Figure A5.4.1). This procedure allowed us to estimate the total volume of gas produced and equalize with atmospheric pressure. With the gas-tight syringe remaining in place, 5 – 20 cm^3 of nitrogen gas was added to the two connected chambers, mixed, and a 5 cm^3 sample was collected for analysis. For the ^{13}C gas analysis, a separate 5 cm^3 sample was collected. Regular gas analysis was performed on a Varian Model 450-GC gas chromatograph and the ^{13}C samples were sent to the UC Davis stable isotope lab.

Following gas sampling, DNA was extracted from soils using a Dneasy PowerMax soil kit. The manufactures procedures were followed, except samples were incubated for 20 minutes at 38^{o}C after surfactant addition to increase DNA recovery, which was very low in the sandy soils used in the study. We also increased the amount of soil used for DNA extractions from the recommended 10g to 20g. In order to have sufficient soil two vials were needed for soil extraction, so with 4 VOAs we had only duplicate DNA samples, but 4 replicates for gas sampling. After extraction, the DNA was purified and concentrated using a Zymo genomic DNA cleaner and concentrator kit. Approximately 1 μg of DNA was recovered and loaded into tubes with a 1.725 g/ml cesium chloride solution for separation and spun at 164,000 g for 56 hours (Neufeld et al. 2007). Heavy DNA was separated, pooled, and precipitated with 30% polyethylene glycol (6000) + glycogen solution, washed with 70% ethanol, and re-suspended with 10 mM Tris, 1 mM EDTA buffer. Samples from the H amended trials were handled the same way except the light DNA fraction was used. The precipitated DNA was cleaned and concentrated with a Zymo DNA concentrator kit. The DNA was prepared for 16s rRNA sequencing using the Illumina platform with adapters targeting the 515/805 base pair region. PCR amplified DNA was cleaned using AMPure XP beads and indexed with a Nextera XY 96 kit. Samples were submitted to the University of Maryland School of Medicine Institute for Genomic Sciences for sequencing. Downstream processing was carried out using the DADA2 pipeline (Callahan et al. 2016), which identifies abundance and taxa at the genus level.

Samples (DNA and headspace gas) were collected at pre-determined times. A preliminary trial run showed the same bi-modal gas production pattern We observed in Chapter 4: two quasi-linear gas production phases (Figure A5.4.5). The breakpoint between phases was approximately 20 days for both Ac and Glu (H did not exhibit a discreet breakpoint). Samples were collected on days 14 and 43 (Ac), 7 and 23 (Glu), and 10 and 15 (H). Immediately after gas sampling, the 4 replicate VOA vials were sacrificed for DNA extraction.

Results and Discussion

This experiment was modeled after a prior experiment (Chapter 4) that was conducted in 1 liter glass jars. In order to accommodate small substrate quantities, we first verified that results were similar in 40ml VOA vials. The key characteristic trait in the original experiment was a breakpoint in total and methane gas production rates after about 40 days (Figure A5.4.5). Adding an organic amendment reduced the breakpoint and increased gas production. The preliminary trials with ^{12}C Ac, Glu and H in 40-ml VOAs and the breakpoints were consistent: ~ 40 days (unamended) and ~20 days with Ac and Glu. Another key trait was an increase, after the breakpoint, in total gas production of 2 - 5 times, with a much larger increase in methane. The 40 ml VOA preliminary trials matched that pattern with one difference: the Ac amended trials had relatively high methane production prior to the breakpoint (Table 6.1). Since acetate is a substrate for acetoclastic methanogens, and Ac would not normally be available in the system for some time after inundation, this result was not surprising. However, there was

still a breakpoint: a 5x increase in total gas production, so the accumulation of Ac (after the breakpoint) cannot be the (only) trigger that causes this shift.

Methanosarcina were the dominant taxa in the Ac trials and became dominant in the Glu trials after the breakpoint (Figure 6.1). This may be due in part to having started with dried soils. Due to the high sand content of the soils used in this study, the field - moisture content was 2.7% by weight. *Methanosarcina* have a competitive advantage in this context because they have a superior ability to survive desiccation (Fetzer, Bak, and Conrad 1993; Kendrick and Kral 2006; Yao and Conrad 1999). Using Ac enriched for *Methanosarcina* compared to Glu (Figure 6.1). *Methanosarcina* (and *methanosaeta*) are able to utilize acetate as a substrate (James G. Ferry 2020) but *Methanosarcina* is more competitive when acetate concentration is high (J G Ferry 1992, Qiu et al. 2019). With Glu *Methanosarcina* did not become dominant until after the breakpoint (Figure 6.1), likely due to fermentation of Glu to Ac, and *methanosaeta* were absent with Glu (Table 6.2). The relative abundance of *Methanosarcina* and *methanosaeita* is typical: they are often found together (Drake, Horn, and Wüst 2009; Narrowe et al. 2017; Ralf Conrad 2020) but *methanosaeta* is less abundant.

Methanosarcina, the most abundant archaea by at least an order of magnitude, were likely responsible for the majority of the observed methane production. The abundance of this taxa had a strong correlation ($r = 0.99$) with methane production rates (data not shown). *Methanosarcina* has a higher Ac utilization rate (μmax) than many other methanogens (Jetten, Stams, and Zehnder 1990). *Methanosarcina* abundance that

coincides with high methane generation rates has been observed by others (Qiu et al. 2019).

In this study, *Methanosarcina* abundance and methane production rates increased as saturation duration increased, e.g., after the breakpoint. In field studies, *Methanosarcina* are dominant in wetland zones that are continuously saturated (Maietta, Hondula, et al. 2020). Similarly, field studies document the increase in *Methanosarcina* after a breakpoint. In one rice paddy soil study, the relative abundance was 6%–13% during the first 30 days, then there was a sharp increase to 46% (Qiu et al. 2019). Another study showed the shift where *Methanosarcina* were dominant after ~20 days with rice straw amended soil (R Conrad and Klose 2006). A number of studies have observed an increase in methane after a breakpoint of ~ 20 - 40 days (Maietta, Hondula, et al. 2020; R Conrad 1999; Zhao et al. 2020; T. Zhang, Liu, and An 2020; RoyChowdhury et al. 2018; Weimer and Zeikus 1978; Ramakrishnan et al. 2001).

There was a distinct shift in the bacteria community structure before and after the breakpoint (Figure 6.2). Using Ac, pre-breakpoint bacteria were dominated by four genera: *Clostridium sensu-stricto (CSS)-13*, *WCHB1-32*, *Chungangia*, and *Fonticella*, which made up 61% of the bacteria abundance. These same four genera became the most abundant taxa (61%) with Glu post-breakpoint, likely because the Glu was being fermented to Ac (Table 6.3). The dominant Glu pre-breakpoint taxa, *Sphingomona*, *CSS-10, CSS-1 and CSS-10*, all Glu fermenting bacteria, were still present post-breakpoint as the next most abundant taxa (Figure 6.2). With Ac the bacterial community structure was largely unchanged. The bacterial and archaeal microbiomes were similar to those

observed in rice paddy research (Lu et al. 2020), suggesting it is a good analog for more general wetland contexts.

In attempting to characterize the archaeal and bacterial communities before and after the methane breakpoint there are a number of inherent limitations. Unidentifiable taxa (genus level) (Fierer 2017) represented a large percentage: 41% of archaea and 34% of bacteria. Even with genus identified, there are still issues with gene copy number bias (Louca, Doebeli, and Parfrey 2018) and the inability to definitively identify function. In the post-breakpoint Ac trials, the bacteria with the highest abundances were *pseudomonas* and *pseudarthrobacter*, thought to be strict aerobes, or factitive aerobes able to utilize nitrate. However, we were not able to completely eliminate the possibility of sample contamination. *Pseudomonas* may have been present in the heavy DNA fraction due to its GC content (Hungate et al. 2015); however, this cannot explain why abundance increased with time with Ac (Table 6.3). *Pseudomonas* are able to grow on acetate at a microbial fuel cell anode without a traditional terminal electron acceptor (Mutyala et al. 2021). *Pseudarthrobacter* have been observed as the dominant taxa in coal where there was there was active coal to methane conversion (B. Wang et al. 2019). This was a comparative analysis, so identifying specific taxa and their function was not as crucial; however, it is important to evaluate if the substrates Ac and Glu altered the microbial community compared to a natural substrate such as H. The H amendment did not appear to alter the archaeal community: the taxa present and clone abundance were similar (Table 6.2). Bacterial microbiomes were different. With H there was an absence of *Clostridium sensu-stricto-13*, which was the dominant taxon with both Ac and Glu (Table

6.3). A non-dimensional plot of bacterial abundance shows the distinct differences between Ac, Glu and H (Figure 6.3). This response to H amendments, little change in archaea and change in (rare) bacterial taxa, has been observed in straw fed anaerobic digesters (Sun et al. 2015).

We were able to follow CO_2 and CH_4 production through the use of the ^{13}C labeled substrates Glu and Ac and (Table 6.1). The use of Glu ($^{13}C_6$) as a substrate resulted in SOC priming. Prior to the breakpoint 56.6% of the methane produced was not labeled. Glu priming did not produce $^{12}CO_2$ - only 5.6% and 7.2% before and after the breakpoint. Cellulose (polymerized glucose) causes priming and loss of SOC through microbial respiration (Blagodatsky et al. 2010). Rice straw can stimulate SOC priming and result in release of CH_4(Ye and Horwath 2017). Thus, in anaerobic systems, the addition of a soil amendment may preferentially convert SOC to CH_4. Studies of SOC priming assume loss of SOC as CO_2 (Bernal, Megonigal, and Mozdzer 2017); however, if a significant fraction is being lost as methane the negative impact on the soil carbon budget and global warming potential is worse.

In the Ac amended trials only the methyl group was labeled ($^{13}CH_3{}^{12}COOH$), so production of $^{13}CO_2$ is an indication of anaerobic methyl oxidation (amo). AMO represented ~20% of the total CH_4 produced before and after the breakpoint (Table 6.1), which is further evidence that *Methanosarcina* were the main producers of CH_4. *Methanosarcina* are mixotrophs and conserve energy by acetate fermentation, methanol reduction, and hydrogenotrophic CO_2 conversion (Weimer and Zeikus 1978). A study by Weimer and Zeicus (1978) saw the same level methyl oxidation (18 – 37%), but only

when grown under a nitrogen atmosphere, as was the case in the lab experiments (Weimer and Zeikus 1978).

Conclusions

The results from this experiment are preliminary, and have not undergone formal review and development. Nevertheless, we can make some general conclusions. In this follow-up experiment to Chapter 5, where we saw a distinct shift in methane and total gas expression after a breakpoint, *methanosarcina* appear to be largely responsible for the majority of methane gas production. *Methanosarcina* have a very high relative abundance in all samples and their absolute abundance numbers strongly correlate with methane generation rates. The anaerobic oxidation rate of methyl carbon also matches other researcher's observations for *methanosarcina* metabolic pathways. Therefore, a shift in *methanosarcina* populations likely contributes to the breakpoint. Characterization of microbial genus was less revealing. Mostly dominated by fermentative bacteria, this group responded to the substrate available (hay, acetate or glucose), and there did not appear to be a pattern that would account for the sharp breakpoint.

Figures

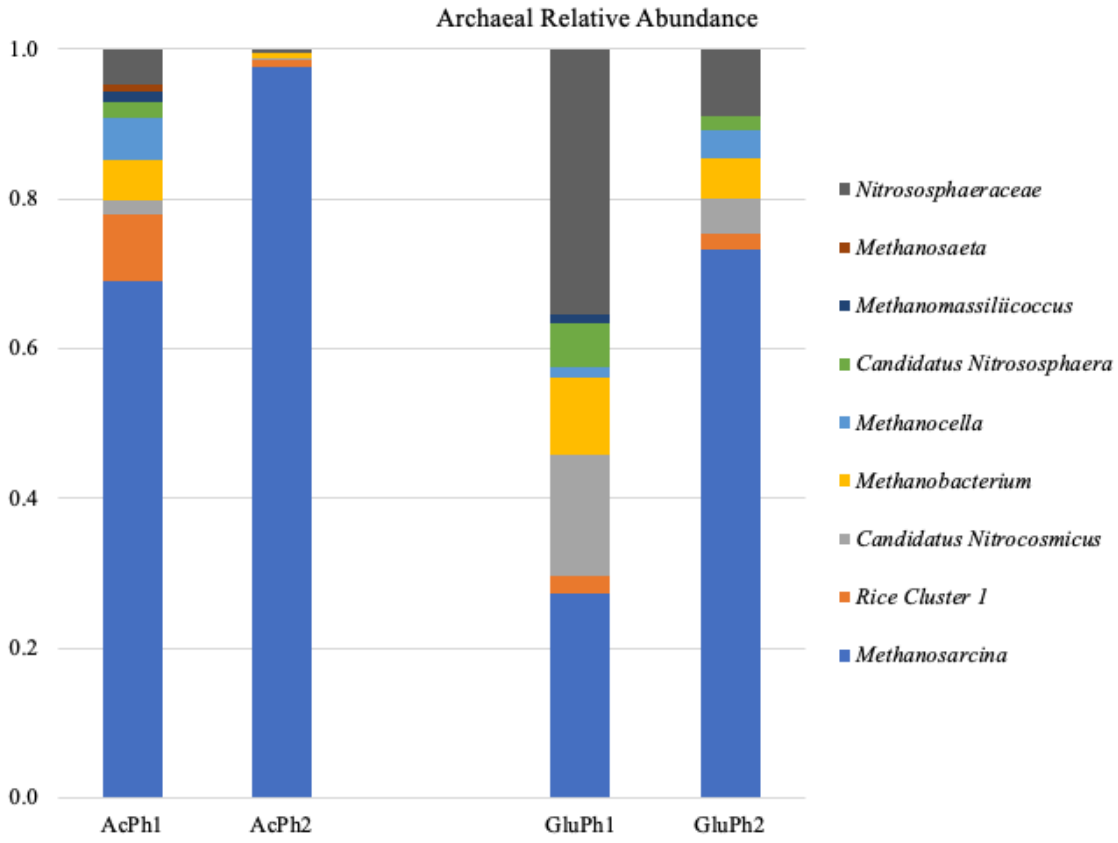


Figure 6.1 – Archaeal abundance before (Ph1) and after (Ph2) the breakpoint. Ac = Acetate; Glu = Glucose. *Nitrosphaeraceae* (family) shown where genus was not identified. Archaea not identified at the family level have been omitted.

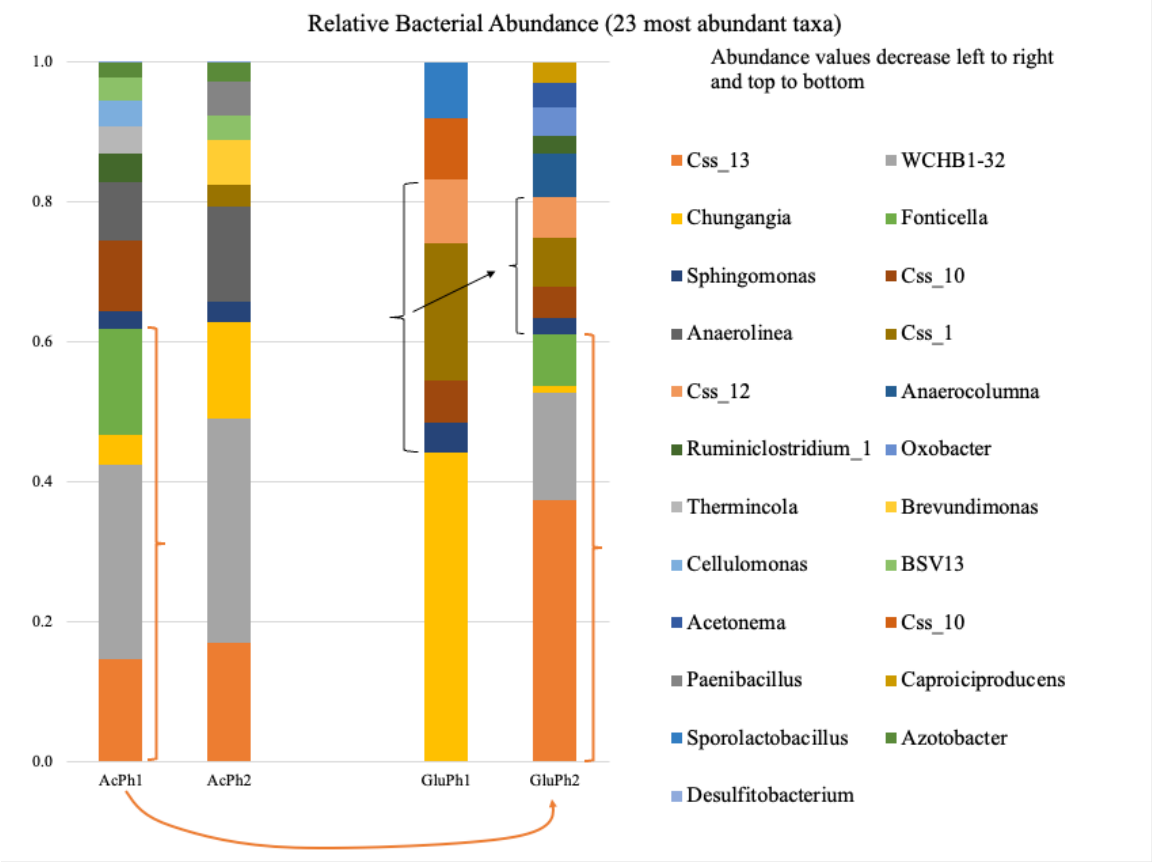


Figure 6.2 – Identification of top 23 bacterial taxa before and after the breakpoint. Ac = acetate fed and Glu = glucose fed. Ph1 = prior to methane breakpoint and Ph2 = after. The Ac fed trials were very similar, with a decrease in clone abundance. In the Glu fed trials the pre-breakpoint dominant taxa were maintained, but displaced by more dominant taxa after the breakpoint: *CSS-13 (clostridium), WCHB1-32, Chungangia and Fonticella*. As Glu was fermented to Ac, there is a clear shift to the dominant acetate utilizing taxa.

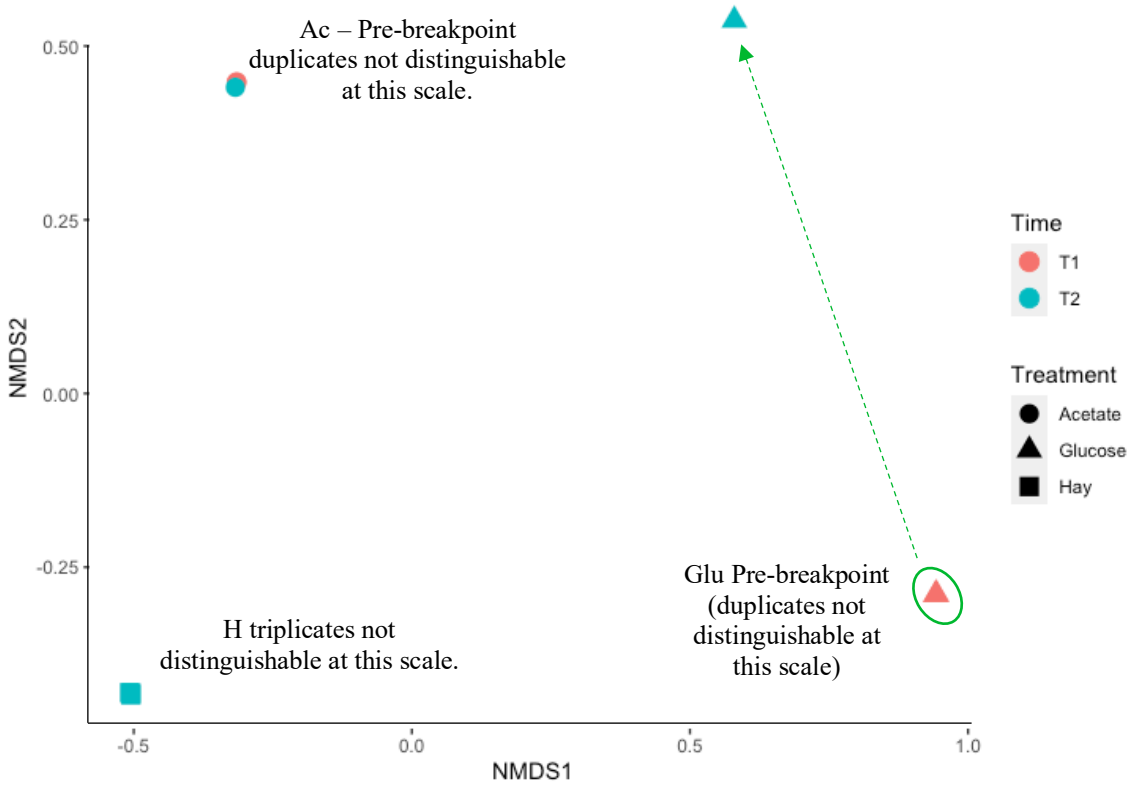


Figure 6.3 – Non-dimensional scale plot of bacteria.

T1 = Pre-breakpoint; T2 – post-breakpoint

Tables

Table 6.1 – Gas production with ^{13}C labeled substrates, ^{13}C Acetate and Glucose.

		before methane breakpoint		after methane breakpoint	
Treatment	**Methane Breakpoint (days)**	**carbon dioxide (cm^3/Kg/day)**	CH_4 **(cm^3/Kg/day) or % enrichment**	**carbon dioxide (cm^3/Kg/day)**	CH_4 **(cm^3/Kg/day)**
Control	42	4.39 ± 0.22	0.0065 ± 0.0013	22.6 ± 12.0	2.3 ± 0.2
^{13}C Acetate*	~20	23.1 ± 1.9	11.0 ± 1.4	96 .2 ± 13.8	100.1 ± 26.6
^{13}C enrichment (%) or $^{13}CO_2$	--	23 ± 1 %	89%	18 ± 1 %	77 ± 2 %
^{13}C Glucose	~20	28.6 ±	0.023 ± 0.006	38.8 ± 0.01	14.6 ± 1.4
^{13}C enrichment (%) or $^{13}CH_4$	--	94.8 ± 0.3 %	43.4 ± 0.1	92.4 ± 0.3 %	89.0 ± 0.2

Table 6.2 – Archaeal abundance.

Archaea	Abundance		
Genus	**Hay**	**Ac**	**Glu**
Methanosarcina	7,151	10,804	2,256
Candidatus Nitrocosmicus	342	77	101
Rice Cluster 1	196	290	83
Methanocella	192	131	120
Methanobacterium	142	173	239
Methanomas siliicoccus	136	34	10
Methanosaeta	108	35	0
Candidatus Nitrososphaera	79	77	101
Methanosphaerula	18	N/A	N/A
Unknown	492	154	560

Table 6.3 – All bacteria taxa by substrate and time.

T1 is pre-breakpoint and T2 is post-breakpoint.

Taxa	Acetate (Ac)			Glucose (Glu)			Hay (H)		
	T1	T2	Total	T1	T2	Total	Total	Grand Total	
NA	42559	20146	62705	12249	25827	38076	40057	140838	34%
WCHB1-32	8556	5814	14370	96	4693	4789	19547	38706	9.3%
Clostridium sensu-stricto-13	4463	3052	7515	653	11347	12000	0	19515	4.7%
Pseudarthrobacter	529	16590	17119	104	60	164	372	17655	4.2%
Chungangia	1347	2497	3844	4789	301	5090	8676	17610	4.2%
Clostridium sensu-stricto-10	3077	191	3268	942	1327	2269	5152	10689	2.6%
Pseudomonas	235	8895	9130	262	94	356	433	9919	2.4%
Fonticella	4627	310	4937	0	2219	2219	2611	9767	2.3%
Ruminiclostridium 1	1255	233	1488	0	772	772	4769	7029	1.7%
Anaerolinea	2604	2449	5053	202	429	631	1019	6703	1.6%
Clostridium sensu-stricto-1	563	578	1141	2118	2141	4259	474	5874	1.4%
BSV13	1042	618	1660	0	409	409	1502	3571	0.85%
Sporomusa	39	0	39	65	164	229	3277	3545	0.85%
Flavisolibacter	1257	178	1435	176	149	325	1672	3432	0.82%
Clostridium sensu-stricto-12	138	53	191	997	1780	2777	436	3404	0.81%
RB41	1086	158	1244	659	722	1381	623	3248	0.78%
Sphingomonas	775	537	1312	483	756	1239	469	3020	0.72%
Aquitalea	0	0	0	0	0	0	2691	2691	0.64%
Anaerocolumna	66	0	66	500	1902	2402	174	2642	0.63%
Leptolinea	1198	453	1651	81	127	208	705	2564	0.61%
Candidatus Udaeobacter	435	188	623	592	656	1248	506	2377	0.57%
Oxobacter	432	354	786	0	1201	1201	311	2298	0.55%
Azospirillum	103	769	872	0	0	0	1318	2190	0.52%
Mobilitalea	303	0	303	0	111	111	1662	2076	0.50%
Clostridium sensu-stricto-8	434	0	434	220	222	442	1024	1900	0.45%
Christensenellaceae R-7 group	340	499	839	0	191	191	816	1846	0.44%
Thermincola	1193	98	1291	0	166	166	280	1737	0.42%
Anaerovorax	115	101	216	0	304	304	1138	1658	0.40%
Paenibacillus	208	911	1119	168	114	282	170	1571	0.38%
Brevundimonas	310	1148	1458	38	55	93	0	1551	0.37%
Ruminococcaceae UCG-014	216	0	216	529	159	688	617	1521	0.36%
Cellulomonas	1111	118	1229	0	61	61	225	1515	0.36%

Taxa	Acetate (Ac)			Glucose (Glu)			Hay (H)		
	T1	T2	Total	T1	T2	Total	Total	Grand Total	
Magnetospirillum	0	15	15	0	0	0	1449	1464	0.35%
Geobacter	78	45	123	99	77	176	1129	1428	0.34%
Methylocystis	323	95	418	157	300	457	519	1394	0.33%
Tellurimicrobium	671	170	841	90	169	259	272	1372	0.33%
Anaeromyxobacter	585	196	781	0	134	134	418	1333	0.32%
Bacillus	291	43	334	221	326	547	447	1328	0.32%
Caproiciproducens	90	10	100	103	907	1010	180	1290	0.31%
Clostridium sensu-stricto-9	289	186	475	0	698	698	107	1280	0.31%
Candidatus Solibacter	654	38	692	96	122	218	340	1250	0.30%
Candidatus Koribacter	468	141	609	144	147	291	333	1233	0.29%
Desulfitobacterium	637	139	776	0	161	161	273	1210	0.29%
JSC-12	582	401	983	0	0	0	197	1180	0.28%
Herbinix	177	0	177	0	78	78	907	1162	0.28%
Acetonema	0	0	0	41	1079	1120	0	1120	0.27%
Phycicoccus	408	104	512	147	280	427	156	1095	0.26%
ADurb.Bin063-1	207	199	406	151	87	238	438	1082	0.26%
Bryobacter	463	59	522	132	196	328	209	1059	0.25%
LD29	0	159	159	0	503	503	373	1035	0.25%
Sporolactobacillus	0	0	0	880	115	995	0	995	0.24%
Gemmatimonas	426	65	491	191	159	350	132	973	0.23%
Terrabacter	273	86	359	135	165	300	306	965	0.23%
Methylobacter	88	327	415	112	130	242	299	956	0.23%
Curvibacter	0	0	0	0	0	0	950	950	0.23%
Xylophilus	439	94	533	0	77	77	333	943	0.23%
Clostridium sensu-stricto-5	33	12	45	407	218	625	271	941	0.23%
Anaerosporomusa	25	36	61	0	70	70	796	927	0.22%
Lentimicrobium	65	486	551	0	30	30	331	912	0.22%
Nubsella	142	78	220	356	189	545	134	899	0.21%
Chryseobacterium	312	367	679	13	11	24	158	861	0.21%
Azotobacter	657	202	859	0	0	0	0	859	0.21%
Aquisphaera	117	29	146	180	373	553	128	827	0.20%
Gaiella	95	46	141	222	265	487	195	823	0.20%
Hydrogenispora	279	71	350	57	148	205	217	772	0.18%
Hyphomicrobium	127	140	267	71	242	313	184	764	0.18%
Bradyrhizobium	107	115	222	148	142	290	251	763	0.18%
Sedimentibacter	158	123	281	0	0	0	460	741	0.18%
Mycobacterium	147	94	241	193	251	444	31	716	0.17%
mle1-7	205	50	255	78	168	246	200	701	0.17%

Taxa	Acetate (Ac)			Glucose (Glu)			Hay (H)		
	T1	T2	Total	T1	T2	Total	Total	Grand Total	
Ruminococcaceae UCG-010	322	41	363	0	184	184	153	700	0.17%
Ellin6067	174	96	270	0	189	189	236	695	0.17%
Microvirga	161	14	175	137	75	212	288	675	0.16%
JGI_0001001-H03	307	0	307	124	193	317	49	673	0.16%
Herbaspirillum	26	0	26	0	0	0	622	648	0.15%
Gracilibacter	111	155	266	0	110	110	256	632	0.15%
Sporobacter	32	0	32	0	33	33	539	604	0.14%
Phenylobacterium	209	191	400	45	70	115	73	588	0.14%
Pleomorphomonas	168	380	548	0	0	0	40	588	0.14%
Pseudacidovorax	0	0	0	0	0	0	586	586	0.14%
Singulisphaera	140	78	218	136	185	321	25	564	0.13%
Streptomyces	127	50	177	96	113	209	176	562	0.13%
Pseudolabrys	98	118	216	78	168	246	81	543	0.13%
Methylomicrobium	78	45	123	113	101	214	198	535	0.13%
Tumebacillus	145	31	176	0	281	281	73	530	0.13%
Clostridium sensu-stricto-3	89	0	89	168	116	284	123	496	0.12%
Anaerobacterium	264	102	366	0	61	61	67	494	0.12%
Caldicoprobacter	225	151	376	0	89	89	0	465	0.11%
Clostridium sensu-stricto-6	0	0	0	186	260	446	10	456	0.11%
Methyloversatilis	0	0	0	0	0	0	453	453	0.11%
Chthoniobacter	111	25	136	67	91	158	144	438	0.10%
Acidothermus	52	0	52	153	199	352	20	424	0.10%
Pir4_lineage	61	0	61	118	177	295	63	419	0.10%
Galbitalea	151	132	283	83	30	113	0	396	0.09%
Rhodomicrobium	130	67	197	45	107	152	43	392	0.09%
Alsobacter	245	117	362	0	0	0	28	390	0.09%
Desulfosporosinus	107	0	107	0	0	0	282	389	0.09%
Massilia	115	74	189	0	0	0	200	389	0.09%
Leptolyngbya ANT.L52.2	184	139	323	0	15	15	39	377	0.09%
Psychrobacillus	0	365	365	0	0	0	0	365	0.09%
Ornatilinea	183	143	326	0	15	15	23	364	0.09%
Ruminococcaceae UCG-012	63	0	63	128	148	276	24	363	0.09%
Rhodoplanes	85	0	85	47	97	144	129	358	0.09%
Cylindrospermum PCC-7417	115	71	186	67	103	170	0	356	0.09%
Conexibacter	39	13	52	97	176	273	28	353	0.08%
Nostoc PCC-7524	162	22	184	63	103	166	0	350	0.08%
Haliangium	222	0	222	51	48	99	21	342	0.08%

Taxa	Acetate (Ac)			Glucose (Glu)			Hay (H)		
	T1	T2	Total	T1	T2	Total	Total	Grand Total	
Aquabacterium	0	0	0	0	0	0	338	338	0.08%
Methylophilus	255	61	316	20	0	20	0	336	0.08%
Arcticibacter	34	299	333	0	0	0	0	333	0.08%
Methylorosula	80	48	128	45	54	99	105	332	0.08%
Pseudonocardia	66	0	66	116	138	254	12	332	0.08%
Ruminococcus 1	0	0	0	0	0	0	320	320	0.08%
Nocardia	159	28	187	35	66	101	30	318	0.08%
Aneurinibacillus	71	116	187	39	33	72	51	310	0.07%
Ruminococcaceae NK4A214 group	0	0	0	0	130	130	169	299	0.07%
Undibacterium	0	0	0	0	0	0	296	296	0.07%
Oryzihumus	112	28	140	51	50	101	50	291	0.07%
Opitutus	74	0	74	20	0	20	192	286	0.07%
Novosphingobium	40	0	40	0	0	0	241	281	0.07%
Nitrosospira	69	27	96	64	118	182	0	278	0.07%
Acidovorax	0	0	0	0	0	0	271	271	0.06%
Mangrovibacter	59	19	78	0	0	0	182	260	0.06%
Nitrospira	99	16	115	0	83	83	62	260	0.06%
Telmatospirillum	0	0	0	0	0	0	254	254	0.06%
Anaerosinus	0	0	0	106	69	175	77	252	0.06%
Acidibacter	86	43	129	24	80	104	17	250	0.06%
Skermanella	47	0	47	72	45	117	79	243	0.06%
Defluviicoccus	58	28	86	49	105	154	0	240	0.06%
Aphanizomenon NIES81	157	28	185	43	0	43	11	239	0.06%
Pelosinus	28	32	60	20	29	49	130	239	0.06%
Gemmata	94	20	114	0	13	13	110	237	0.06%
Acetivibrio	0	0	0	0	30	30	206	236	0.06%
Clostridium sensu-stricto-7	210	0	210	0	26	26	0	236	0.06%
Archangium	75	27	102	47	83	130	0	232	0.06%
Ruminococcaceae UCG-013	0	0	0	0	143	143	89	232	0.06%
Rhodopseudomonas	0	231	231	0	0	0	0	231	0.06%
Porphyrobacter	99	0	99	43	86	129	0	228	0.05%
Methylobacterium	74	24	98	0	0	0	129	227	0.05%
Planococcus	139	0	139	29	56	85	0	224	0.05%
Ramlibacter	154	0	154	0	0	0	67	221	0.05%
Vulgatibacter	65	0	65	0	0	0	156	221	0.05%
Rhodococcus	0	173	173	21	26	47	0	220	0.05%
Acinetobacter	0	34	34	0	0	0	179	213	0.05%
Aestuariimicrobium	198	0	198	0	0	0	14	212	0.05%

Taxa	Acetate (Ac)			Glucose (Glu)			Hay (H)		
	T1	T2	Total	T1	T2	Total	Total	Grand Total	
Anaerosporobacter	0	0	0	0	0	0	212	212	0.05%
Syntrophomonas	0	63	63	0	29	29	120	212	0.05%
Cylindrospermum NQAIF308	105	0	105	49	48	97	6	208	0.05%
Intrasporangium	111	44	155	20	0	20	25	200	0.05%
Paucimonas	49	87	136	0	38	38	25	199	0.05%
Anabaena PCC-7122	66	28	94	20	44	64	39	197	0.05%
1959-1	57	0	57	41	83	124	15	196	0.05%
Herminiimonas	56	79	135	0	59	59	0	194	0.05%
Quadrisphaera	0	0	0	72	120	192	0	192	0.05%
Aetherobacter	0	0	0	71	118	189	0	189	0.05%
Pir2_lineage	38	0	38	22	61	83	68	189	0.05%
Noviherbaspirillum	0	0	0	0	0	0	185	185	0.04%
Sporacetigenium	0	0	0	0	10	10	175	185	0.04%
Lysobacter	94	21	115	41	28	69	0	184	0.04%
Nonomuraea	33	0	33	37	65	102	49	184	0.04%
Nodularia PCC-9350	95	32	127	24	32	56	0	183	0.04%
Brevibacillus	0	0	0	0	0	0	176	176	0.04%
Rhodoblastus	62	51	113	0	16	16	45	174	0.04%
HAVOmat113	76	70	146	0	0	0	26	172	0.04%
Pseudobacteroides	0	0	0	0	0	0	172	172	0.04%
Sphingobacterium	103	0	103	0	0	0	66	169	0.04%
Marmoricola	0	0	0	40	60	100	66	166	0.04%
Streptosporangium	24	0	24	25	63	88	50	162	0.04%
Ercella	51	25	76	3	0	3	80	159	0.04%
Pedobacter	32	104	136	23	0	23	0	159	0.04%
Thermomonas	56	18	74	30	39	69	14	157	0.04%
Caulobacter	0	58	58	0	0	0	98	156	0.04%
Ideonella	62	16	78	0	0	0	75	153	0.04%
Raineyella	58	0	58	0	31	31	61	150	0.04%
Gemmatirosa	57	0	57	0	92	92	0	149	0.04%
Denitratisoma	80	11	91	0	25	25	30	146	0.03%
Intestinimonas	42	20	62	0	0	0	84	146	0.03%
Microbispora	52	0	52	0	35	35	55	142	0.03%
HSB OF53-F07	27	0	27	42	46	88	26	141	0.03%
Aminobacter	30	107	137	0	0	0	0	137	0.03%
Piscinibacter	0	0	0	0	131	131	0	131	0.03%
Trichormus HINDAK 2001-4	75	24	99	0	32	32	0	131	0.03%
Anaerospora	0	0	0	0	16	16	114	130	0.03%

Taxa	Acetate (Ac)			Glucose (Glu)			Hay (H)		
	T1	T2	Total	T1	T2	Total	Total	Grand Total	
Duganella	0	0	0	0	0	0	130	130	0.03%
Renibacterium	0	128	128	0	0	0	0	128	0.03%
Paenisporosarcina	0	68	68	54	0	54	0	122	0.03%
Variovorax	33	88	121	0	0	0	0	121	0.03%
MTP1	51	33	84	0	12	12	22	118	0.03%
Roseomonas	53	47	100	18	0	18	0	118	0.03%
Phyllobacterium	46	71	117	0	0	0	0	117	0.03%
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	57	40	97	19	0	19	0	116	0.03%
Syntrophorhabdus	56	0	56	0	11	11	48	115	0.03%
Arthronema	64	47	111	0	0	0	0	111	0.03%
SAG_12.89									
Neorhizobium	0	0	0	0	0	0	111	111	0.03%
Haloplasma	60	48	108	0	0	0	0	108	0.03%
Ammoniphilus	41	0	41	21	45	66	0	107	0.03%
Bosea	0	89	89	0	0	0	18	107	0.03%
Stenotrophobacter	0	0	0	64	0	64	43	107	0.03%
Fodinicola	72	34	106	0	0	0	0	106	0.03%
Verrucosispora	67	0	67	0	38	38	0	105	0.03%
Dyadobacter	0	85	85	0	0	0	19	104	0.02%
Oxalophagus	0	0	0	0	0	0	104	104	0.02%
Lutispora	0	0	0	0	102	102	0	102	0.02%
Nakamurella	66	31	97	0	0	0	0	97	0.02%
Blastococcus	23	9	32	35	27	62	0	94	0.02%
Solirubrobacter	0	0	0	29	65	94	0	94	0.02%
Paramesorhizobium	0	0	0	0	0	0	92	92	0.02%
Dendrosporobacter	0	0	0	0	90	90	0	90	0.02%
Pirellula	34	9	43	24	22	46	0	89	0.02%
Cohnella	37	29	66	0	0	0	21	87	0.02%
Mesorhizobium	0	87	87	0	0	0	0	87	0.02%
Propionicicella	0	55	55	0	32	32	0	87	0.02%
Roseiarcus	55	30	85	0	0	0	0	85	0.02%
Vicinamibacter	85	0	85	0	0	0	0	85	0.02%
Actinotalea	43	0	43	0	41	41	0	84	0.02%
Pelotomaculum	0	11	11	0	17	17	56	84	0.02%
Rugosimonospora	0	0	0	0	29	29	55	84	0.02%
GWD2-49-16	37	15	52	0	0	0	31	83	0.02%
Candidatus Latescibacter	0	12	12	10	19	29	40	81	0.02%
TG-45	43	38	81	0	0	0	0	81	0.02%

Taxa	Acetate (Ac)			Glucose (Glu)			Hay (H)		
	T1	T2	Total	T1	T2	Total	Total	Grand Total	
Fimbriiglobus	60	14	74	5	0	5	0	79	0.02%
Candidatus Soleaferrea	0	0	0	0	0	0	78	78	0.02%
Cuspidothrix	56	22	78	0	0	0	0	78	0.02%
LMECYA 163									
Greenland-10	54	24	78	0	0	0	0	78	0.02%
Milano-WF1B-03	0	0	0	0	0	0	78	78	0.02%
Ralstonia	0	0	0	0	0	0	77	77	0.02%
Paraclostridium	19	0	19	12	20	32	24	75	0.02%
Belnapia	0	26	26	0	0	0	48	74	0.02%
Chloronema	59	8	67	0	0	0	5	72	0.02%
Desulfotomaculum	13	0	13	0	0	0	59	72	0.02%
EcFYyy-200	33	19	52	0	0	0	19	71	0.02%
Sphaerisporangium	0	0	0	0	30	30	41	71	0.02%
AKIW659	60	0	60	0	0	0	10	70	0.02%
Chthonomonas	0	0	0	41	25	66	4	70	0.02%
Ancalomicrobium	0	44	44	0	0	0	25	69	0.02%
Nostoc PCC-73102	49	0	49	0	0	0	19	68	0.02%
Reyranella	18	50	68	0	0	0	0	68	0.02%
Nitrobacter	0	49	49	0	18	18	0	67	0.02%
Syntrophus	22	14	36	0	0	0	31	67	0.02%
Actimicrobium	0	0	0	0	0	0	66	66	0.02%
Dactylosporangium	0	0	0	25	41	66	0	66	0.02%
Nostoc_PCC-7107	0	0	0	25	41	66	0	66	0.02%
Anaerobacter	0	0	0	0	0	0	65	65	0.02%
Kitasatospora	0	0	0	0	0	0	65	65	0.02%
Tolypothrix PCC-7601	12	0	12	53	0	53	0	65	0.02%
Achromobacter	11	53	64	0	0	0	0	64	0.02%
Arenimonas	0	0	0	26	38	64	0	64	0.02%
Lysinibacillus	63	0	63	0	0	0	0	63	0.02%
Anaerolineaceae UCG-001	54	8	62	0	0	0	0	62	0.01%
Delftia	0	0	0	0	0	0	61	61	0.01%
Devosia	0	43	43	0	0	0	18	61	0.01%
Phormidium IAM_M-71	40	21	61	0	0	0	0	61	0.01%
GCA-900066225	40	0	40	0	0	0	20	60	0.01%
Janibacter	0	0	0	59	0	59	0	59	0.01%
Ruminiclostridium	59	0	59	0	0	0	0	59	0.01%
Bdellovibrio	13	11	24	6	5	11	19	54	0.01%
Dehalobacter	27	27	54	0	0	0	0	54	0.01%

Taxa	Acetate (Ac)			Glucose (Glu)			Hay (H)		
	T1	T2	Total	T1	T2	Total	Total	Grand Total	
Propionispora	0	0	0	0	54	54	0	54	0.01%
Rhizomicrobium	0	31	31	0	0	0	23	54	0.01%
Rhodoferax	0	0	0	0	0	0	54	54	0.01%
Litorilinea	0	0	0	28	12	40	13	53	0.01%
Filimonas	0	52	52	0	0	0	0	52	0.01%
Sinomonas	52	0	52	0	0	0	0	52	0.01%
Stenotrophomonas	6	11	17	0	0	0	34	51	0.01%
Mucilaginibacter	0	28	28	0	0	0	22	50	0.01%
Nocardioides	0	0	0	15	0	15	35	50	0.01%
Pajaroellobacter	50	0	50	0	0	0	0	50	0.01%
Kineosporia	0	0	0	0	49	49	0	49	0.01%
Ruminococcaceae	28	14	42	0	7	7	0	49	0.01%
UCG-009									
Sh765B-TzT-35	28	0	28	0	0	0	21	49	0.01%
Candidatus	0	10	10	12	26	38	0	48	0.01%
Paracaedibacter									
MM1	48	0	48	0	0	0	0	48	0.01%
Terrimonas	0	0	0	31	0	31	17	48	0.01%
Actinoallomurus	47	0	47	0	0	0	0	47	0.01%
Ignavibacterium	0	15	15	0	0	0	32	47	0.01%
Trichococcus	0	0	0	0	0	0	47	47	0.01%
Kurthia	46	0	46	0	0	0	0	46	0.01%
Kaistia	0	45	45	0	0	0	0	45	0.01%
Labrys	0	0	0	13	23	36	8	44	0.01%
Pir3_lineage	0	0	0	0	20	20	24	44	0.01%
CL500	0	0	0	43	0	43	0	43	0.01%
29 marine group									
Candidatus	0	0	0	26	16	42	0	42	0.01%
Xiphinematobacter									
Geodermatophilus	0	0	0	19	23	42	0	42	0.01%
Kluyvera	42	0	42	0	0	0	0	42	0.01%
Actinomadura	0	0	0	0	41	41	0	41	0.01%
Flavobacterium	0	14	14	5	9	14	13	41	0.01%
IMCC26207	26	15	41	0	0	0	0	41	0.01%
Kribbella	0	0	0	25	16	41	0	41	0.01%
Methyloparacoccus	0	0	0	23	18	41	0	41	0.01%
Viridibacillus	41	0	41	0	0	0	0	41	0.01%
Zavarzinella	0	0	0	20	20	40	0	40	0.01%
Paludisphaera	27	12	39	0	0	0	0	39	0.01%
Psychroglaciecola	10	0	10	0	22	22	7	39	0.01%
Gloeotrichia	19	0	19	0	19	19	0	38	0.01%
SAG_32.84									
Pseudorhodoplanes	38	0	38	0	0	0	0	38	0.01%

Taxa	Acetate (Ac)			Glucose (Glu)			Hay (H)		
	T1	T2	Total	T1	T2	Total	Total	Grand Total	
alphaI cluster	0	0	0	0	0	0	37	37	0.01%
Crenothrix	0	35	35	0	0	0	0	35	0.01%
Pseudogulbenkiania	0	0	0	0	0	0	35	35	0.01%
Thermoactinomyces	0	2	2	0	0	0	33	35	0.01%
Steroidobacter	0	10	10	0	24	24	0	34	0.01%
Coxiella	19	13	32	0	0	0	0	32	0.01%
Dinghuibacter	0	0	0	0	32	32	0	32	0.01%
Burkholderia-Caballeronia-Paraburkholderia	0	31	31	0	0	0	0	31	0.01%
Adhaeribacter	24	0	24	5	0	5	0	29	0.01%
SH-PL14	0	0	0	0	23	23	6	29	0.01%
Ruminococcaceae_UCG-007	28	0	28	0	0	0	0	28	0.01%
Candidatus Endomicrobium	6	10	16	0	0	0	11	27	0.01%
Leptolyngbya PCC-6306	27	0	27	0	0	0	0	27	0.01%
Yokenella	27	0	27	0	0	0	0	27	0.01%
Aeromonas	0	0	0	0	0	0	26	26	0.01%
Desulfitibacter	0	25	25	0	0	0	0	25	0.01%
Microlunatus	0	0	0	0	25	25	0	25	0.01%
Spirosoma	0	6	6	0	0	0	19	25	0.01%
Phaselicystis	0	0	0	0	24	24	0	24	0.01%
Caldinitratiruptor	9	0	9	0	0	0	14	23	0.01%
Pedomicrobium	0	0	0	0	23	23	0	23	0.01%
Candidatus Alysiosphaera	10	0	10	12	0	12	0	22	0.01%
Cyanothece PCC_7425	9	12	21	0	0	0	0	21	0.01%
Lachnospiraceae NC2004 group	0	0	0	0	0	0	21	21	0.01%
Nostoc PCC-8976	13	0	13	0	8	8	0	21	0.01%
Edaphobacter	7	0	7	0	13	13	0	20	0.005%
Cytophaga	0	0	0	0	19	19	0	19	0.005%
Ethanoligenens	0	0	0	0	19	19	0	19	0.005%
FukuN18	0	0	0	0	0	0	19	19	0.005%
Freshwater group	0	0	0	0	0	0	19	19	0.005%
Hymenobacter	0	0	0	6	13	19	0	19	0.005%
Parafilimonas	0	0	0	7	12	19	0	19	0.005%
Parvibaculum	0	0	0	19	0	19	0	19	0.005%
Polyangium	0	0	0	0	19	19	0	19	0.005%
Sphaerobacter	0	0	0	6	9	9	4	19	0.005%
Subgroup_10	6	0	6	0	9	9	4	19	0.005%

Taxa	Acetate (Ac)			Glucose (Glu)			Hay (H)		
	T1	T2	Total	T1	T2	Total	Total	Grand Total	
1921-3	0	0	0	18	0	18	0	18	0.004%
Altererythrobacter	0	0	0	0	18	18	0	18	0.004%
Propioniciclava	0	0	0	0	0	0	18	18	0.004%
Rudaea	0	0	0	0	18	18	0	18	0.004%
Sva0081 Sediment group	0	8	8	0	0	0	10	18	0.004%
Xanthobacter	0	17	17	0	0	0	0	17	0.004%
Acetanaerobacterium	0	0	0	0	0	0	16	16	0.004%
Clostridium sensu-stricto-19	0	0	0	0	16	16	0	16	0.004%
Methylomonas	0	0	0	0	0	0	16	16	0.004%
Micromonospora	0	0	0	0	0	0	16	16	0.004%
Paracraurococcus	16	0	16	0	0	0	0	16	0.004%
FCPS473	7	0	7	0	8	8	0	15	0.004%
Ilumatobacter	0	0	0	15	0	15	0	15	0.004%
Sorangium	0	0	0	0	15	15	0	15	0.004%
Candidatus Anammoximicrobium	9	0	9	5	0	5	0	14	0.003%
Aquicella	0	8	8	5	0	5	0	13	0.003%
IS-44	13	0	13	0	0	0	0	13	0.003%
Methylomagnum	8	5	13	0	0	0	0	13	0.003%
BacC-u-018	0	0	0	6	0	6	5	11	0.003%
Deinococcus	11	0	11	0	0	0	0	11	0.003%
Dokdonella	0	0	0	0	11	11	0	11	0.003%
Harryflintia	0	11	11	0	0	0	0	11	0.003%
Luteimonas	0	0	0	0	11	11	0	11	0.003%
OM27 clade	8	0	8	0	0	0	3	11	0.003%
Snowella 0TU37S04	0	0	0	5	0	5	6	11	0.003%
Syntrophobacter	0	0	0	0	0	0	11	11	0.003%
Candidatus Omnitrophus	0	0	0	0	0	0	10	10	0.002%
Cutibacterium	10	0	10	0	0	0	0	10	0.002%
Phaeospirillum	0	0	0	0	10	10	0	10	0.002%
Possible-genus-04	0	0	0	0	10	10	0	10	0.002%
Nannocystis	0	0	0	3	6	9	0	9	0.002%
Phormidesmis ANT.L52.6	9	0	9	0	0	0	0	9	0.002%
Candidatus Jidaibacter	0	0	0	0	0	0	8	8	0.002%
JG30a-KF-32	8	0	8	0	0	0	0	8	0.002%
RuminococcaceaeUCG-011	0	8	8	0	0	0	0	8	0.002%
SWB02	8	0	8	0	0	0	0	8	0.002%

Taxa	Acetate (Ac)			Glucose (Glu)			Hay (H)		
	T1	T2	Total	T1	T2	Total	Total	Grand Total	
Desulfobulbus	0	0	0	7	0	7	0	7	0.002%
Ktedonobacter	0	0	0	7	0	7	0	7	0.002%
Luteolibacter	0	0	0	0	0	0	7	7	0.002%
Nitrosomonas	0	0	0	0	0	0	7	7	0.002%
Sphingobium	0	0	0	0	0	0	7	7	0.002%
Cyanothece PCC-7424	0	0	0	0	0	0	6	6	0.001%
Desulfoprunum	0	0	0	6	0	6	0	6	0.001%
G12-WMSP1	0	0	0	0	6	6	0	6	0.001%
Silvanigrella	6	0	6	0	0	0	0	6	0.001%
Candidatus Cardinium	5	0	5	0	0	0	0	5	0.001%
Desulfatiglans	0	5	5	0	0	0	0	5	0.001%
Desulfovibrio	0	5	5	0	0	0	0	5	0.001%
Planctopirus	5	0	5	0	0	0	0	5	0.001%
Siphonobacter	5	0	5	0	0	0	0	5	0.001%
Candidatus Odyssella	0	0	0	0	0	0	4	4	0.001%
KCM-B-112	4	0	4	0	0	0	0	4	0.001%
Fibrella	0	0	0	0	0	0	3	3	0.001%
Neochlamydia	0	3	3	0	0	0	0	3	0.001%
Taonella	0	0	0	0	0	0	2	2	0.0005%
Thermosporothrix	0	0	0	0	2	2	0	2	0.0005%
Grand Total	101738	78117	179855	34665	74334	108999	129306	418160	100.00%

Chapter 7: Concluding Remarks

The main research question for this work has been whether or OM amendments are beneficial for mitigation wetlands. I admit I remain skeptical. There are certainly drawbacks, but organic solid waste management is a pressing need worldwide (Wong et al. 2016), and to argue against a potential beneficial use is a disservice to that issue. I have made it a point to have personal discussions with wetland OM amendment advocates and I find we are rarely at odds. Some points we agree on are: topsoil use is superior to allochthonous OM, composting the OM is a beneficial (if not necessary) step, and where A-horizons have been removed for wetland construction, the nutrient-poor mineral material can (within a reasonable timeframe) develop healthy A-horizon layers in the absence of amendments (personal communication, John Galbraith, 11/10/2021). Still, more long-term studies are needed to fully assess if OM amendments are necessary.

The environment were OM amendments are used starts them off at a disadvantage: soils have been highly disturbed (Petru, Ahn, and Chescheir 2013). While there is ample evidence that SOC is a useful predictor of healthy soils (Larney and Angers 2012), my skepticism stems from findings that allochthonous OM in wetlands does not necessarily translate to SOC accumulation. Most SOC comes from below-ground inputs, even when aboveground biomass tilled-in (Mazzilli et al. 2015). One purported benefit of OM is enhanced soil water retention, but this is not necessary in saturated soils and is hardly an adequate remedy for what is inherently a hydrologic problem. The combination of soil disturbance and elevated nutrient levels of some OM amendments can help establish invasive species. Once established, invasive species are

more difficult to control (Sarri, Bonnie, and Blackburn 2016). We are obligated to weigh potential advantages with negative effects which include SOC priming (Fontaine et al. 2004), leaching of SOC-bound nutrients from soil (Rubin, Anderson, and Ballantine 2020), loss of diversity, and increased methane generation.

Methane is particularly problematic. Methane can cause wetlands to be a source, rather than sink of C (Kayranli et al. 2010). OM amendments can not only increase methane production, but also decrease the time it takes (after soil saturation) to amplify methane, and cause soil priming that releases SOC as methane. One benefit of OM is increased plant growth; however, sites with high productivity generally release more methane (Martins et al. 2017). It is common practice in rice cultivation to amend soils with OM; however, those that have investigated the methane implications have suggested stopping the practice (van der Gon and Neue 1995; Yuan et al. 2020).

OM temporarily exacerbates methane production but the cause is prolonged soil saturation. Chapter 4 identified a methane breakpoint of approximately 42 days in two different soils. After the breakpoint, total gas production increases by about 5x and methane increases by at least an order of magnitude. The breakpoint may be shortened with the addition of OM, although composting reduces this effect. Other factors such as temperature affect the breakpoint; nevertheless, there are numerous publications have reported similar breakpoint values (R Conrad 1999; Zhao et al. 2020; T. Zhang, Liu, and An 2020; RoyChowdhury et al. 2018; Weimer and Zeikus 1978; Maietta, Hondula, et al. 2020; Ramakrishnan et al. 2001). A breakpoint around 40 days appears in other contexts as well. One recent publication has showed that saturating rice field for no more than 35

days had a large impact of total methane released (Souza 2021). The breakpoint with rice straw addition was reduced to 20 days, whereas in unamended soils it was 50 days. Pester et al. (2010) saw a large shift in the rare biosphere T-RFLP (as an indicator for a rare sulfate reducing species) after about 2 months, but little change after about 2 weeks. The term "breakpoint" was introduced while intentionally avoiding a previously used term "halt phase"(Ramakrishnan et al. 2001) to call attention to the fact that methane is being generated prior to the breakpoint. In evaluating field methane rates, it may be necessary to know if the numbers represent pre- or post-breakpoint emission. The term "lag phase" was also not used to draw attention to the two quasi-steady state methane emission periods, not microbial growth dynamics (although microbial growth undoubtedly is a factor in gas production rates).

Prolonged saturation (> 40 days) in mitigation wetlands may be unnecessary. In the field study, the areas of the site that had fluctuating water levels had higher diversity and floristic quality. Hydrology is a controlling factor for methane generation, perhaps more so than redox condition (Miao et al. 2017). Natural wetlands, which usually emit less methane, are saturated less often and for shorter duration than created wetlands (Cole and Brooks 2000). Fluctuating water levels provide more opportunities for methanotrophs to oxidize methane (Yang et al. 2017). Modifying the hydrology to have water level fluctuations that include dry periods is an engineering, not ecological problem, and thus within the realm of human control. While evidence continues to suggest hydraulic variations might be used to lower methane emissions, it may result in an increase nitrous oxide (Maria E. Hernandez and Mitsch 2006). Allowing soils to

completely dry could help select for *Methanosarcina* (Kendrick and Kral 2006), largely responsible for high methane production rates (from Chapter 6). These potential adverse side effects would need to be examined.

Adding a temporal metric, like duration of saturation, may better predict wetland methane emissions (Bardgett et al. 2005; Bonetti et al. 2021). A common metric used to evaluate wetlands is water depth (C. D. Evans 2021). In a synthesis of wetland emissions for 71 wetlands worldwide, water table position was the best predictor of methane emissions, but the model was improved by considering antecedent water level (Turetsky et al. 2014). Sha et al. (2011) found that increased water depth increased methane, but their work suggested hydroperiod (saturation duration) was also a factor. The hydroperiod controls OM cycling in kettle holes (Nitzsche et al. 2017). In a study of prairie potholes, inundation extent and duration affected CH_4 (Hondula, Jones, and Palmer 2021). A high-water level position generally produces more methane, but it is also possible that, under this condition, soils have been saturated longer.

In some of my original research proposals, the hypotheses focused on finding an OM amendment that would produce more Fe and thereby suppressing methanogenesis. Data from the lab experiments showed this was not the case. Iron reduction and methanogenesis occurred together and the balance is controlled by pH (Marquart et al. 2019), which has a strong effect on anaerobic redox energetics (Jin and Kirk 2018). Under the right pH and substrate conditions, iron reduction, sulfate reduction, and methanogenesis can have the same energy conservation potentials (Kwon et al. 2016; Bethke et al. 2011). Some methanogens are facultative iron reducers: they can behave as

strict methanogens, use iron reduction to augment methanogenesis, or perform dissimilatory iron reduction (Sivan, Shusta, and Valentine 2016). In several studies, the presence of iron oxides facilitated methanogenesis (Kato, Hashimoto, and Watanabe 2012; Zhou et al. 2014; Qiu et al. 2019). The form of iron-oxides may be a factor. In one study magnetite, not ferrihydrite, stimulated methanogenesis (Tang et al. 2016). The effect is not limited to iron oxide. Increased methane production has been tied to the utilization of other electron acceptors (He et al. 2015). *Methanosarcina* utilize iron oxide reduction as part of their part of their metabolic pathway (Prakash, Chauhan, and Ferry 2019). The connection between iron reduction and methanogenesis is somewhat more complex since microbially mediated iron reduction can used in the anaerobic oxidation of methane (Egger et al. 2017). *Methanosarcina* can couple methane generation with iron reduction (Prakash, Chauhan, and Ferry 2019), so iron reducing conditions may be a poor metric to indicate the potential suppression of methanogenesis. In the lab study (Chapter 4) some trials showed that iron-oxide reduction continued during periods of high methane generation (Figure A5.4.6). *Methanosarcina* use a metabolic pathway that could explain this result. Iron reduction does not necessarily suppress methanogenesis – the relationship is much more complex.

Focusing on methanogenesis was not the original intent, but the work migrated to this topic for two primary reasons. First, methane is a currently a hot topic as even world leaders agree that controlling methane should be a priority (Abernethy et al. 2021; IPCC 2021). Second, the paradigm regarding methane generation in soils is changing. One of the stated objectives for this work was to determine if some OM amendment would

support iron-reduction and inhibit methanogenesis (Prakash, Chauhan, and Ferry 2019), which is supported by the redox ladder model. Instead, I observed methanogenesis and iron-reduction happening concurrently. Recent findings have revealed that methanogens can energetically coexist with iron reducers (Bethke et al. 2011), can utilize iron reduction to enhance metabolic pathways that lead to methane, and methanogens can coexist with iron-reducers within soil microsites (Yang et al. 2017).

Science continually evolves, and one of the most difficult steps is letting go of some of the concepts that got us where we are but are now holding us back. For wetland restorations, I argue OM amendments are not particularly beneficial and it is inappropriate to equivocate SOC and OM amendments. Adding OM to soil does not mean the material is integrated as part of the edaphic system. Consider this analogy: a car has 10 gallons of gas in the tank and two 5-gallon gerry cans of diesel are placed in the trunk. The car now has 20 gallons of fuel, but it won't go any farther.

The redox ladder is central tenet of biogeochemistry (Hansel et al. 2015). However, we need a more nuanced understanding of how microbes function and how they derive energy in order to gain a greater understanding of anaerobic biogeochemical processes. For example, one thing missing from the redox ladder framework is fermentative processes. If SOC is mostly complex polymeric organic molecules common in microbial and plant structures (Lehmann and Kleber 2015), then fermentation and hydrolysis are the key reactions that drive carbon cycling in addition to dissimilatory metal and sulfate reduction. One could argue that fermentation could be added to the ladder, and growth yields and energetics put it somewhere between iron and sulfate

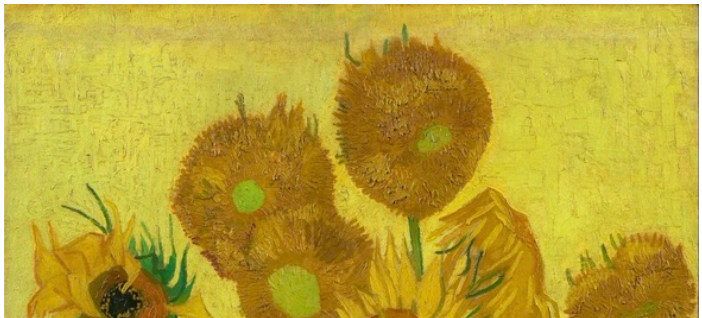
reduction (Smeaton and Van Cappellen 2018). However, with a large variety of substrates, and complex metabolic pathways that often involve multiple organisms, available energy more likely spans a large spectrum. The opening statement of R. M. Wilson et al. (2017) reads:

> *"Once inorganic acceptors are depleted, organic matter in anoxic environments decomposes by hydrolysis, fermentation and methanogenesis…"*

However, these metabolic pathways can be described another way. Hydrolysis and fermentation happen first, creating the metabolic precursors for organisms that utilize inorganic acceptors. Energetics can overlap, or co-exist within bulk soil micro-niches, and methanogenesis might not necessarily commence once higher energy acceptors are depleted. Criticism is not the intent here, but rather to serve as a reminder the overall processes are complex and intertwined.

Some of Vincent Van Gogh's most recognized paintings are of sunflowers. After plucking a blossom he would work furiously to capture its natural state, before it wilted. Yet, temporal changes were central to Van Gogh's works - each sunflower painting includes various stages of maturity. Like Van Gogh, science seeks to consolidate complexities in nature and relies on works published over time, as knowledge is evolving. The scientific precepts in each publication are important waypoints in the maturation process, even though they all inevitably whither.

> *I exaggerate, sometimes I make changes in a motif; but for all that, I do not invent the whole picture; on the contrary, I find it all ready in nature, only it must be disentangled.*



Sunflowers

- Vincent Van Gogh.

Appendices

Appendix 1: A Brief History of Wetlands

> *Hope and the future … are not in … the cultivated fields, not in the towns and cities, but in the impervious and quaking swamps.* - Henry David Thoreau

Marshes, bogs and swamps were the wellsprings of civilization (Edward Maltby 1986). Cultivation of cereal grains is often held up as the basis for the growth of city centers. However, cities began in river valleys: the fertile crescent, Egypt's Nile, and the Indus, all with reliable access to fresh water. Cereal-based cultivation became widespread since these grains are adaptable to a variety of soils and climates (Jared Diamond 1999). Still, many of the world's earliest known population centers were centered around marsh agriculture (Figure A1.1). Rice, a staple marsh crop, is the second largest grain produced worldwide, after maize. Maize, grown primarily on raised marsh hedgerows, was a central part of Mesoamerican wetland agriculture.

Food crops are not the only foundation for civilization. Equally important are plant materials used to make things. Marsh reeds have historically been used to make a variety of essential products (Coles and Coles 1989), as shown in Photo A1.1.

Utilization of wetland areas by pre-historic cultures does not mean the areas were unaltered. On the contrary, humans throughout history utilized many of the same wetland adaptations in use today, including drainage and dredging and filling. However, until mechanization became available, all work needed to be done by hand so impacts were much smaller (Kiviat 2014).

Bogs and swamps were central to the advancement of civilization as a source of iron during the iron age. Prior to the industrial revolution and the invention of modern blast furnaces, bog iron was the primary ore source (P. W. Scott, Ealey, and Rollinson 2011; Heite 1974; Thelemann et al. 2017). Bog iron can be smelted at comparatively low temperatures. Bogs and swamps provided not only iron but was also the source of charcoal from timber (Badger 2007), used to smelt iron and for heating.

Early European colonists in America, at least the ones that survived, sought out marshes and swamps (Vileisis 1997). Marshes provided hay for cattle, plentiful fish, and beaver pelts. Swamps were a seemingly inexhaustible source of timber. Wood from cypress trees were naturally water-resistant and made good shingles and foundation material. The honeymoon was short-lived as swamps were impediments to travel and development, disease vectors, and refuges for native and African Americans fleeing oppression and slavery (Vileisis 1997). As a result, private landowners began to prefer draining waterlogged areas despite their recognized benefits.

Some have claimed that the preference to drain swamps meant they were considered *waste*lands (Shaw and Fredline 1956; Stine 2008). However, this is an inaccurate perception that, unfortunately, has been carried forward by scientists - a self-defeating practice contrary to the desire to protect these land forms (Mitra, Wassmann, and Vlek 2005; Meyer 1994; Kettenring and Tarsa 2020). In the United States, swamps have historically been central to Southern regional identity and character. Despite the desire of many in the South to protect swamps, the area was overrun after the Civil War (1965) by Northern logging interests who “left a wasteland in [their] wake” (A. Wilson 2006). The

Great Dismal Swamp in Virginia was vitally important to both Native Americans and later European settlers (Sawyer 2010). Even though marshes, swamps, and bogs were commonly drained to take advantage of their agricultural value (Prince 1997; Margaret Jones Bolsterli 2008), and later their value as inexpensive residential property (Vuic 2021), they also held cultural value, inspiring enduring poetry and art, such as Henry Wadsworth Longfellow's most well know poem "Evangeline" and Joseph Rusling Meeker's "The Land of Evangeline" (Photo A1.2) (Miller 1989).

The fate of swamps, marshes, and bogs in the United States changed dramatically in 1849 due to a confluence of circumstances. The federal Swamp Land Act was passed, granting overflowed land to the State of Louisiana. Other states soon followed. States were allowed to sell the land to developers provided the proceeds would go toward constructing levees and drains for farmland conversion. The ill-fated intent was to help control Mississippi River flooding, but upstream swamp drainage made flooding progressively worse (Christopher Morris 2012). By 1849 terra cotta tile drains, introduced by John Johnston, increased crop production fourfold (Marion M. Weaver 1964) and drainage quickly became ubiquitous. Also completed in 1849: the Illinois River Canal and the Galena & Chicago Union Railroad (Figure A1.2). These opened corridors for crops and related goods to be quickly transported from the Midwest up and down the Mississippi river and to the east coast. Inexpensive, rapid methods of moving commodities buoyed crop values and the cost of installing tile drains was within reach for most farmers (William Cronon 1991).

By the early 1900's, the effect of drainage for farm conversion was becoming apparent to bird hunters and strict laws were enacted (R.K. Sawyer 2013). American sportsmen such as President Theodore Roosevelt, well-known for National Parks and forest conservation, helped set aside a large network of wildlife refuges, most of which were for migratory birds (John F. Reiger 1975). Unfortunately, Roosevelt (and his contemporaries) were unaware of landscape ecology and connectivity (Jean Paul Metzger and Pedro H.S. Brancalion 2016) and were strong advocates of draining swamps.

The effects of draining swamps and marshes eventually became apparent: increased Mississippi river flooding (Christopher Morris 2012), giant peat fires (Marjory Stoneman Douglas 1947), hurricane damage (Vileisis 1997), and the dust bowl. Although these were ongoing problems in the first half of the 1900s, the Federal government was ill equipped to address environmental concerns in the midst of a great depression and two world wars. Economic development following World War II caused widespread environmental damage to support homesteads for an emerging middle class, which had a particularly strong impact on swamps in Florida (Vuic 2021).

Alongside swamp drainage, the need for conservation grew and got a much-needed kick-start after Ducks Unlimited was formed in 1937. The attention to migratory birds eventually led to the 1956 publication "Wetlands of the United States, Their Extent and Value to Waterfowl and Other Wildlife" (Shaw and Fredline 1956). It was here that the term "wetland" was coined, and a broad spectrum of wetland values were cataloged. This prompted further wetland study, and in so doing the values and services that they provide to our society have been found to be expansive (Woodward and Wui 2001).

Wetlands have gone from being valued as bird habitat to be an integral part of our national water management scheme (Thorslund et al. 2017; David Moreno-Mateos and Margaret A. Palmer 2016), and even the Supreme Court weighs in on just how far we will go to protect them (Arthur et al. 2014). The United Nations has declared the 2020's the decade of restoration, and a new US initiative by President Biden calls for 30% of our land to be restored by 2030 (Executive Order 14008, Section 216). Mitigation wetlands are likely to be a large part of those initiatives.

Figures

Figure A1.1 – Known ancient wetland agricultural centers (dark blue shading).

Fertile crescent and Indus Valley areas, traditionally thought to be the areas with the oldest examples of large scale, organized agriculture, are shown in light blue. However, wetland agricultural centers are comparable in age.

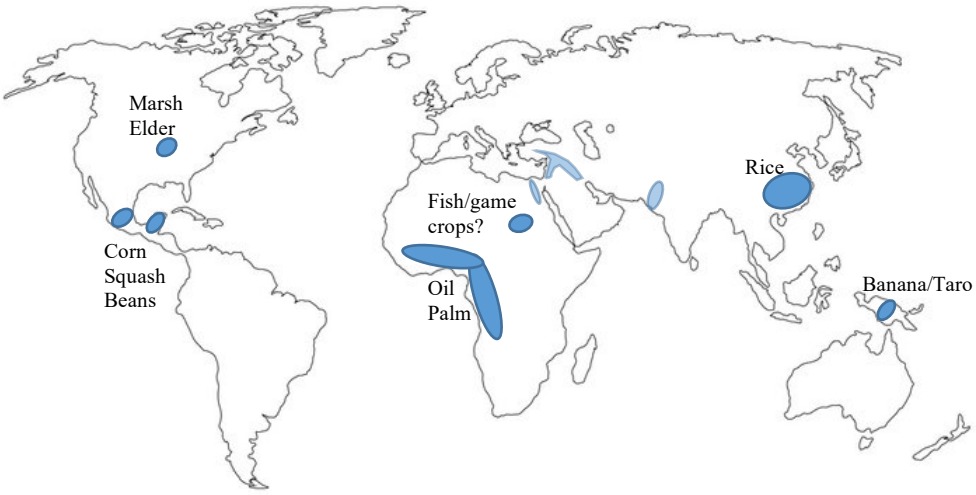
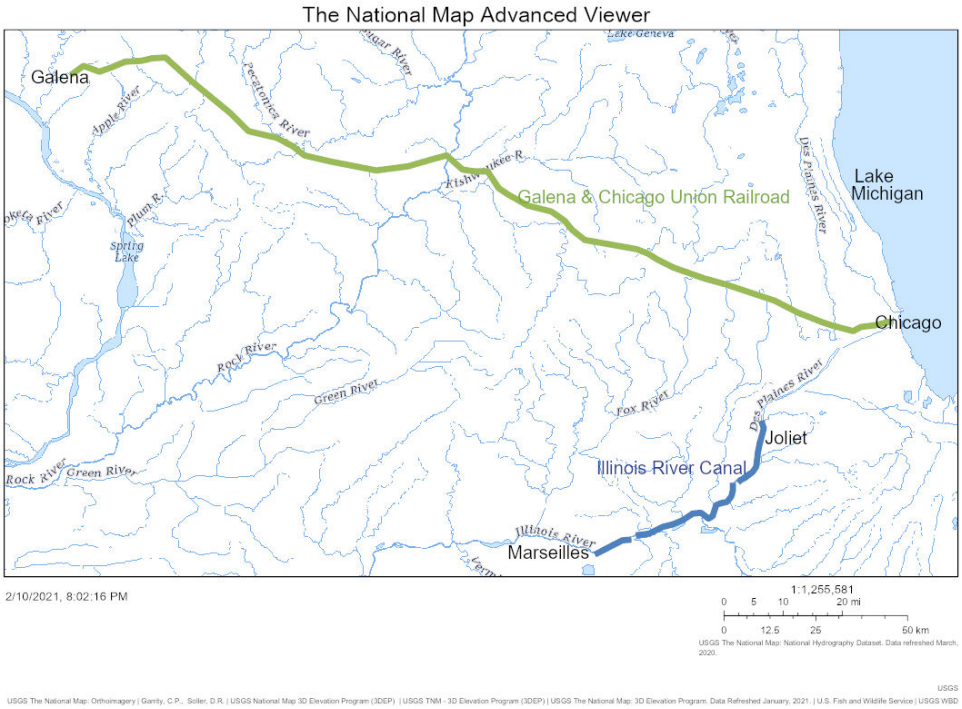


Figure A1.2 – Key Transportation Corridors Completed in 1849.



Photos

Photo A1.1 – Cattail is one of many wetland plants that can be used for a variety of purposes and was a basis for some civilizations.

Photo A1.2 – The Land of Evangeline – Joseph Rusling Meeker

Over their heads the towering and tenebrous boughs of the cypress

Met in a dusky arch, and trailing mosses in mid-air

Waved like banners that hang on the walls of cathedrals.

Excerpt from “Evangeline” by Henry Wadsworth Longfellow

Appendix 2: Additional Data and Discussion

Methods & Statistics

The experimental and statistical methods used throughout this body of work have been carefully selected, either through foresight or through failure. For example, the initial trials for laboratory soil incubation studies used plastic containers. The plastic was oxygen gas permeable at a rate that was clearly a factor allowing for the re-oxidation of reduced iron. The interior of the plastic cups were painted with iron and manganese oxides (Photo A2.1) to test if their reduction could be used as a non-invasive indicator of iron reduction in the system. However, it was apparent in those cases that the iron-oxide coating reduced oxygen permeability. Those failures, months in the making, led to the use of glass jars. Soil for the lab experiments was removed from the field site, dried and sifted, destroying ped structures. While intact cores were given serious consideration, in a mitigation wetland the earthwork used to prepare the site is comparable to drying and sieving. As in any experiment, more replication could have been used and greater attention could have been given to experimental unit homogeneity. However, there is a disadvantage in too much replication which limits Type I statistical errors (false positives). If we can't see the effect of a treatment through the noise of a messy experiment, it is unlikely we would see an effect though the noise of the inherent heterogeneity of a natural system. One way to increase statistical validity is progressive replication. There is a need for replicate studies in ecology, but still they are rare (Fraser et al. 2020). Progressive replication here means similar conditions in the lab studies in the

field. In general, my statistical methods have evolved as I became more comfortable understanding the appropriate statistical methods and what they mean.

Statistical analyses require an understanding of the null hypothesis. I struggled for years with null hypothesis testing, believing it was the product of merciless statisticians purposely twisting our brain in knots. Fortunately, the drama of the 2020 presidential election clarified the need for null testing. Peter Navarro, in his Immaculate Deception report, makes the "scientific" argument that election fraud led to Trump's loss. Navarro's report includes colorful tables (Figure A2.1) that he claims are "evidence". His logical progression is: 1) there was massive fraud, as shown in his tables; 2) Trump lost; 3) since Trump lost, fraud must be the cause. While it is obvious that Navarro's logic is flawed and colorful tables aren't evidence, there is an even greater flaw of sidestepping a null hypothesis. A null hypothesis would start by assuming that some large societal perturbation (e.g. Covid-19) resulted in greater access to mail-in and absentee ballots, but this had no effect on the election outcome. Then, data would be examined to see if there was any evidence to suggest the possibility that the null hypothesis (no change in the election outcome) was wrong. This step was completely ignored and Navarro's argument began with the alternative hypothesis: Covid (or something else, the story keeps changing) allowed fraud to change the outcome. The null hypothesis is the legal equivalent to innocent until proven guilty, and that must be the starting point. I read the 2020 election legal challenges and court findings, and this is why all the lawsuits failed. The lack of evidence was never considered because there was a dereliction of process (Fisher 1971).

ANOVA is one of the principal statistical tools for scientists. I find statistical principles difficult to comprehend, but in the case of ANOVA the acronym is partly to blame. The method is not, as the name suggests, an Analysis of Variances. Instead, it is an analysis based on pooled variances. If we measure tree heights and girths several times, we get averages and standard deviations. The averages can't be combined because they are different measurements, but it's safe to assume the deviations in both measurements are similar, so those may be combined to get a better estimate of the amount of experimental error. In my own effort to understand this concept you may hear me use the term analysis based on pooled variances in place of ANOVA.

Multidimensional analysis is a useful tool for discovering correlations as this 3-D rendering illustrates. Figure A2.2 shows the same data from 3 perspectives. The first image appears to be a scatterplot with no meaningful relationships, but as the image is rotated a clear correlation emerges, particularly in the last image where the data is sloped along the z-axis. I often use multidimensional analyses as a visual tool but then only discuss the ensuing correlations.

Chapter 2 presents charts based on a numerical scoring system, where scientific studies showing a positive effect of a treatment received one point, no effect zero points, and a negative effect negative one point. This scoring system resulted in values ranging from +1 to -1. In the published version, the values were converted to percentages and displayed them on the charts. This choice still troubles me, particularly as my understanding of statistics has improved, because it implies that the numerical values have intrinsic meaning. Ultimately, the decision was made to use numerical scores

following the example set in a previous publication by Dr. Palmer (Margaret A. Palmer and Hondula 2014). The numbers are more correctly numerical categorical values represented by numbers. In hindsight it may have been better to remove the numbers to emphasize the qualitative nature of the scoring system (Figure A2.3).

Chapter 4 reports a model of methane gas production in saturated soils that followed a bi-modal segmented linear pattern, but some in some trials the pattern was sigmoidal. The first sigmoidal growth model was introduced by Pierre-François Verhulst and used the concept of ecological carrying capacity (K) (Vaclav Smil 2019), where the system grows to some maximum value, hence the equation:

$$N(t) = \frac{K}{1 + \left(\frac{K - N(0)}{N(0)}\right)\exp(-at)}$$

However, the Gompertz function was used here.

$$N(t) = K \exp\left(\log\left(\frac{N(0)}{K}\right)\right)\exp\left(-at\right)$$

The difference in the functions is more easily discernible from the curves. Gompertz is skewed, with a larger percentage of the growth (~ 63 %) prior to the inflection (Figure A2.4). A discriminatory test between these functions was not performed, nor was consideration given to a myriad of other sigmoidal function possibilities, but instead reliance was placed on the judgement of Vaclav Smil, that many organisms reproduce faster during their initial growth phases (Vaclav Smil 2019). When observing growth of microorganisms in soil, one is actually observing many overlapping growth curves, so there is no reason to expect that the combined curves would take on any particular shape. Still, sigmoidal growth has historically proven consistent across many complex systems.

Chapter 5 uses an ex post facto blocking variable, Water, from data collected during the experiment. The goal of the field study was to evaluate the effect of OM amendments: however, hydrology had a much stronger effect on the results and needed to be blocked out. The study was designed to group replicate plots in four hydraulically similar blocks. Block areas were chosen based on similar surface elevations (Appendix 4). The equivalency only held as long as blocks were inundated. The percolation rates were not the same at all blocks, and as a result Block D, with a high percolation rate, would dry out sooner than other blocks and Block C, with a low percolation rate, almost never dried out (see SHA report for further discussion). Some parameters such as E_H may have a greater response to quickly changing soil saturation conditions (Water), whereas other parameters such as root growth is more represented by persistent soil conditions represented (Block). Therefore, the nuisance variable (Meehl 1970) "Water" was introduced, by recording whether the ground surface was 0 = dry, 1 = wet, 2 saturated (see Photo 5.3). In some cases, there was a larger response to Water than Block. For example, the E_H during dryer periods (when not all plots were inundated) was more impacted by Water ($p < 0.001$) than Block ($p = 0.003$)(Table A2.1). Type III sums of squares analyses were used because in SAS this method ignores the order, so Block and Water are considered equally. Statistical calculations were conducted using both blocking variables and, in all cases, where blocking by Water was significant ($p < 0.05$), blocking by Block was also significant, so there is no loss of statistical information using only Block.

Multidimensional plots were sometimes utilized to help discern some of the variable relations ships in the data. Many of the correlations discussed in Chapter 5 came from reviewing a multidimensional plot of the data (Figure A2.5). The figure shows that soil Db was inversely correlated with root mass, likely because low density roots displace mineral soils to reduce the Db. This is in contrast to longer term bulk density reductions, where organic matter and fine soil particles create pedological structures (Rabot et al. 2018). Roots also appear to correlate strongly with shallow OM. Root inputs are the principal source of OM in soils (Mazzilli et al. 2015),(Dijkstra, Zhu, and Cheng 2021). Deep OM (> 15cm, below the amended layer) was inversely correlated with the sub soil sand content. Sand provides little surface area to retain leached OM (Amendola et al. 2018). Phosphate appeared to be correlated with shoot (but not root) biomass and cattail percent cover. Since cattail appears physically larger and more dense than other vegetation (Photo A2.2) it might seem phosphate preferentially increased cattail biomass production. However, given the same percent coverage, there was at most 20% higher biomass with cattail, which is within the range of the Peet classification ranges. Phosphorous, then, seems to stimulate the growth of all plants (unlike N, which preferentially stimulated cattail growth).

from Chapter 2: The role of organic amendments in wetland restorations

The idea for this publication originated from a conversation between myself and William Buettner of the Maryland State Highway Administration. It was he who questioned the efficacy and value of organic matter (OM) amendments used for wetland restorations. Examples of OM amendment benefits are plentiful (Getahun et al. 2020; Larney and Angers 2012; Ozores-Hampton 2021) in unsaturated soil, leading to an expectation that OM addition will always improve soil function. Scientists often laud the multitude of benefits of OM (e.g. Photo A2.3), even when their research does not bear it out. The idea that OM will improve wetland establishment and function seems to have originated from Kentula and Hairston (Kentula et al. 1992), who suggested using OM in wetland restorations to provide opportunities for study. Several years later (but prior to such studies), Stauffer and Brooks (Stauffer and Brooks 1997) cited Kentula's book, stating: "Organic matter soil amendments may be the best known method to accelerate the development of a functional wetland at created sites (Kentula et al. 1992, Bishel 1994)." Clearly the Kentula reference was not evidentiary, nor was Bishel's (Bishel-Machung et al. 1996). Bishel observed that SOC was higher in natural wetlands but acknowledged that factors other than SOC (e.g. soil texture) could also explain their observations. Stauffer and Brooks has since been widely cited as evidence of OM's efficacy. This may introduce an inappropriate scientific bias (Hobbs et al. 2011), leading to quotes such as this, which I shared in my exit seminar:

> *"We recommend adding organic amendments to sandy created wetland soils if the initial OM [SOC], … or hydrology are limiting. However, in this sandy*

wetland with nearly ideal hydrology (inundated twice per day) and non-limiting bulk density, compost amendment at these rates had little to no effect on soil morphology and redox features in the long term." (E. T. Ott et al. 2020)

It may seem I make too much of OM amendments. However, in Maryland's mitigation guidance document (Walbeck, Clearwater, and Neff 2011) OM amendments are the only item emphasized for mitigation planning (Figure A2.6). This gives the impression OM amendments are the most important factor, but the benefits are not well documented and there can be adverse effects. Emphasizing amendment use distracts from other factors that would have a greater impact, like establishing proper hydrology.

In "The role of organic amendments in wetland restorations" we used a numerical scoring system as an attempt at limiting bias. We reviewed published research where OM had been used as a soil amendment in a wetland restoration. Wherever OM had a beneficial or desired effect +1 point was assigned and if there was no effect, +0 points. In some cases, OM had a negative or undesired effect (for example, excess methane generation): -1 point. There are many parameters that have been reported, so they were grouped into categories and the combined points were averaged to generate a score. Even though the objective was an unbiased evaluation, the interpretation of a score can also be biased. An aggregate score of +1 means OM was measurably beneficial every time in every category, so this, and a score of -1, is unambiguous. However, an aggregate score of +0.3 does not necessarily mean it was beneficial 30% of the time, or 30% of the parameters in that category improved. There is no clear frame of reference to determine what score would constitute a meaningful OM contribution. The clearest distinction was

that the use of topsoil outperformed other types of OM (Figure 2.1), with a score of 0.53 (Topsoil) versus 0.22 (OM), and these differences tested significant using a Wilcoxon rank sum test. Another important find, contrary to expectations, was that OM added to soils that were low in SOC did not have higher scores (0.31 vs. 0.30).

Scoring systems can be superficial. In many cases, positive scores were of no genuine value. For example, one parameter affected by the addition of OM amendments is water retention. OM increases soil water retention in unsaturated soils. Although I did not find a publication that verified this scientifically, the longstanding widespread use of OM in this context seems sufficient evidence. However, as scientists we should re-evaluate the value of this parameter in the context of wetlands. OM amendments retain low matric potential water, easily lost, and is not an effective buffer if water inputs are uncontrolled. Soil texture is a better predictor of soil water retention. A well-cited publication that concludes SOC increases water retention (Rawls et al. 2003). The publication shows that soil texture is a good predictor of moisture retention, and adding the variable SOC increases the accuracy of the prediction by 10 – 15% in some soils. However, the study also found higher SOC in fine grained soils *decreases* water holding capacity. The article considers SOC, not added OM. If soils are saturated, a requirement for wetland soil function, then soil water retention is not meaningful. Adding OM to retain soil moisture may be an effective short-term band-aid when soils are dry, but it cannot correct an underlying hydrologic problem. Wetlands have been lost historically through intentional drainage (Appendix 1), and the higher priority for wetland restoration should be restoring appropriate hydrology (Zedler 2000).

from Chapter 3: Macro and Microscopic Visual Imaging Tools to Investigate Metal Reducing Bacteria in Soils

Wetland soils are characteristically hydric, or anaerobic, when saturated. It is the hydricity that alters the biogeochemistry of the soil allowing it to store nutrients and carbon by switching to oxidation-reduction process that are slower and more enzymatically restrictive. Therefore, having a simple, effective method of testing the soil hydricity is useful tool. One such device is an Indicator of Reduction in Soils (IRIS) sleeve, made out of PVC pipe or flat film. Under anaerobic conditions, metal reducing microorganisms alter and remove metal-oxides that are painted onto the IRIS. The reaction is slow even under ideal conditions, removing about 4% of the metal (iron) oxides per day (key finding from Chapter 3). The observed reaction is an integration over several days and according to the accepted protocol (B. L. Vasilas et al. 2013) and average removal should be greater than 1% per day measured over a 30-day period.

The clear-IRIS method was recently expanded upon by LeFevre, who used an automated camera to collect images with fine temporal (hourly) and photogenic resolution (LeFevre et al. 2021). They also recorded comparable paint removal rates. The minimum removal rate in this study, in saturated soil, was 4% day-1 (iron-oxide) and LeFevre recorded rates of 13% and 8% day-1.

During the course these experiments, all three standard tests for were used anaerobic soil condition (Berkowitz et al. 2021), including IRIS. These tests are measures of anaerobic conditions in the soil, not necessarily a test of soils being hydric, which requires that the anaerobic conditions are sustained. These tests may occasionally

(incorrectly) be identified as hydric soils tests. In this experiment IRIS test results were interpreted based on a 30-day period; however, the NTCHS method (2015) either omits the time interval, or specifically clarifies there is no standard interval (Berkowitz et al. 2021).

The interpretation of IRIS, as well as other anaerobic soils tests, are supposed to be done in the context of rainfall data, which was not discussed in Chapter 5. Here's why. If rainfall periods are wetter than normal based on WETS data, then the anaerobic soil tests may not be used for compliance testing to show soils are hydric. The field experiments did occur during an unusually heavy rainfall period. Construction was delayed in 2018 and again in February, March, May and June of 2019 due to heavy rains. Then, during the field data collection period, 6 of the 11 months had higher than normal rainfall (Figure A2.7). As this work was not intended to show compliance with hydric soil tests, there was any loss of scientific value.

Chapter 6 refers to rice cultivation research as a useful analog to wetland studies. The crossover of disciplines extends to IRIS film. Recently, IRIS has been suggested as a water management tool for rice farmers (A. E. Evans, Limmer, and Seyfferth 2021), and the same group has developed an imaging tool to aid in quantification of IRIS removal (Limmer, Evans, and Seyfferth 2021).

from Chapter 4: Quantification of potential methane emissions following oxic soil inundation with organic matter amendments

Chapter 4 is a manuscript submitted to Biogeochemistry journal, which summarizes results from the lab studies. The manuscript focuses on methane generation in saturated soils and how OM amendments affect methane generation. The lab study covered a much broader range of topics; however, the findings were not suitable for publication but may be the basis for future research. Key points are briefly summarized here.

One of the initial challenges is the lab studies was the use of plastic containers. The original intent was to follow the methods used in previous experiments (Updegraff et al. 1995); however, because the focus was iron reduction, the oxygen-permeable plastic interfered with the results. Oxygen, permeating the plastic walls, precipitated ferrous iron. In some trials we painted the interior of the plastic cups with iron or manganese oxides (Photo A2.1). We hypothesized these coatings may serve as an indicator of iron reduction, without affecting the results. However, this was not the case. The total gas production rate in the unpainted plastic cups was consistently higher, presumably because oxygen permeate was available and added to carbon metabolism (Figure A2.8). Microbial activity creates an oxygen barrier in saturated soils (Brune 2000). Similarly, radial oxygen loss (in roots) can create iron-oxide coatings that reduce oxygen permeability (Møller and Sand-Jensen 2008). Based on observations with iron-oxide plastic cups we hypothesize that ferrous iron oxidation at the soil surface in wetlands may contribute to the thin anoxic layer at the soil surface.

With glass jars, a set of trials was included to compare results of a headspace filled with air (20% oxygen) versus 100% nitrogen. In these cases, the total gas produced was consistently lower (34% on average) with air in the headspace (Figure A2.9). Apparently, O_2 was not stimulating aerobic processes, but suppressing other anaerobic processes. For example, there was an 83% reduction in CH_4 with air headspace. Over the course of the incubation oxygen in the headspace was slowly displaced by biogenic gases (mostly CO_2). However, the rate at which oxygen levels fell were much faster than the predicted rate based on dilution alone. Therefore, other pathways must have been involved, such as the abiotic (or biotic) oxidation of ferrous iron. The hay amended trials (with air headspace) depleted oxygen very rapidly, and as a result CH_4 production decreased only slightly (19%), much less than other amendments.

A series of trials were conducted a source of iron-oxide (ferrihydrite or goethite). Ferrihydrite and goethite was prepared as described in Schwertmann and Fechter (1994). Results from these trials were consistent, but ultimately not publishable since duplicate samples (n=2) were insufficient to show differences at $p < 0.05$. We hypothesized that adding the metal-oxides would increase soluble metal (Fe^{2+}) production and increase CO_2 production due to increase respiration from metal-reducing bacteria. However, Fe^{2+} and CO_2 decreased (Figures A2.10 and A2.11). One possible explanation for this effect is that the added metal oxides provide reactive surface area that stabilizes organic carbon (Wagai and Mayer 2007; Lalonde et al. 2012).

Another important parameter is carbon use efficiency (cue), which is defined as: Equation 5.1 (R. L. Sinsabaugh et al. 2013):

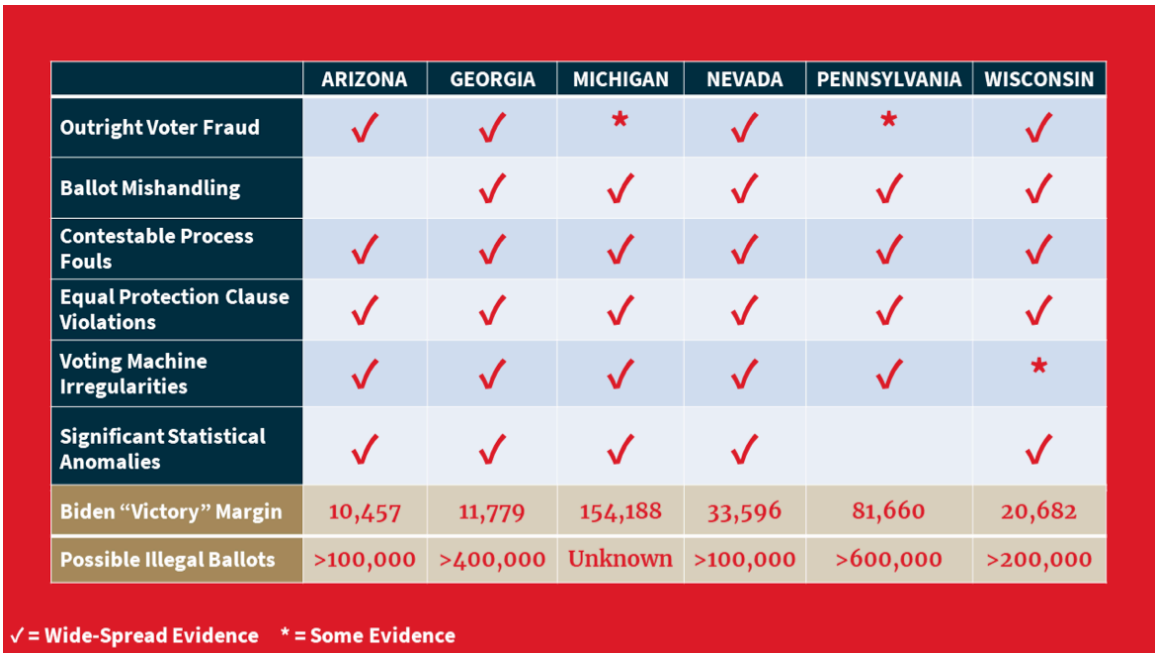
$$\frac{microbial\ C}{microbial\ C\ +\ respired\ C}$$

Microbial C was determined using chloroform fumigation (D. S. Jenkinson and Powlson 1976; Oren et al. 2018) adapted for saturated soils but the analyses yielded nonsensical results. In some trials the delta microbial carbon value would be negative. The cause of the negative values was likely microbial carbon associated with the viable cells in the added OM. Fresh hay contains viable living cells which would lyse upon anaerobic incubation and release cytoplasmic material measured by this method (Tate, Ross, and Feltham 1988). The increase in microbial carbon from anaerobic catabolism may be small in comparison. Attempting to correct for this was challenging because of the inherent sample heterogeneity. In some cases the cue values were much higher than the expected maximum of 0.55 (R. S. Sinsabaugh 1994). This is likely because the headspace gas was purged with nitrogen. It was not possible to completely purge trapped O_2 in the soil, so the headspace was vented for the 24 hours, purging the Birch effect gases (Birch 1958). As a result, there was a large quantity of respired C missing from equation 5.1.

I am often asked if Carbon:Nitrogen (C:N) ratios were considered in this work. The C:N ratio of the soil carbon is particularly important in determining if a given system is nitrogen limited based on the C:N ratio of the organisms (plants or microbes) living in that system. There were several reasons C:N ratio was not useful in the experiments. There was not as much separation in the C:N ratios of the OM substrates (Table A2.2).

Only biosolids differed appreciably due to the elevated levels of ammonia in the samples. This was not consistent across Bloom samples since composting removes most of the ammonia through the activity of ammonia oxidizing bacteria. Also, the loading rates used added so much OM that only a small fraction (< 1%) was used during the 60-day incubations. With so little of the OM stock depleted, N limitation could not be detected.

Figures



	ARIZONA	GEORGIA	MICHIGAN	NEVADA	PENNSYLVANIA	WISCONSIN
Outright Voter Fraud	✓	✓	*	✓	*	✓
Ballot Mishandling		✓	✓	✓	✓	✓
Contestable Process Fouls	✓	✓	✓	✓	✓	✓
Equal Protection Clause Violations	✓	✓	✓	✓	✓	✓
Voting Machine Irregularities	✓	✓	✓	✓	✓	*
Significant Statistical Anomalies	✓	✓	✓	✓		✓
Biden "Victory" Margin	10,457	11,779	154,188	33,596	81,660	20,682
Possible Illegal Ballots	>100,000	>400,000	Unknown	>100,000	>600,000	>200,000

✓ = Wide-Spread Evidence * = Some Evidence

Figure A2.1 – Colorful table from Peter Navarro's Immaculate Deception Report. Each checkmark is cited as evidence of a hypothesis there were election irregularities. There was no null hypothesis testing – that election errors did not affect the outcome.

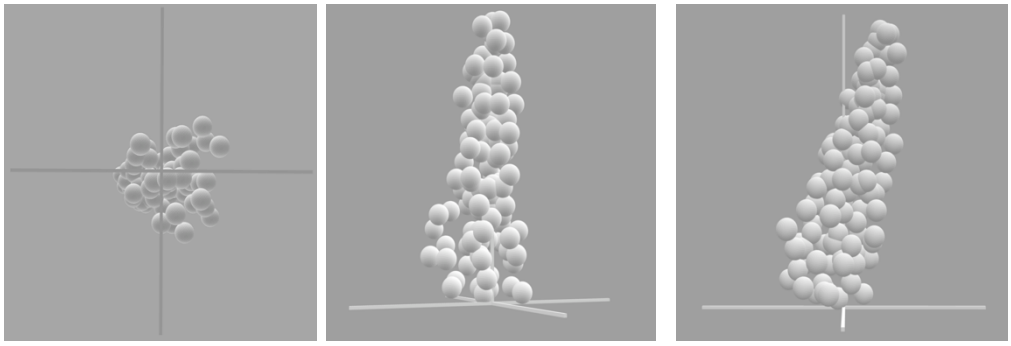


Figure A2.2 – 3-D rendering that illustrates how data that is apparently random can show a distinct correlation when viewed from another perspective.

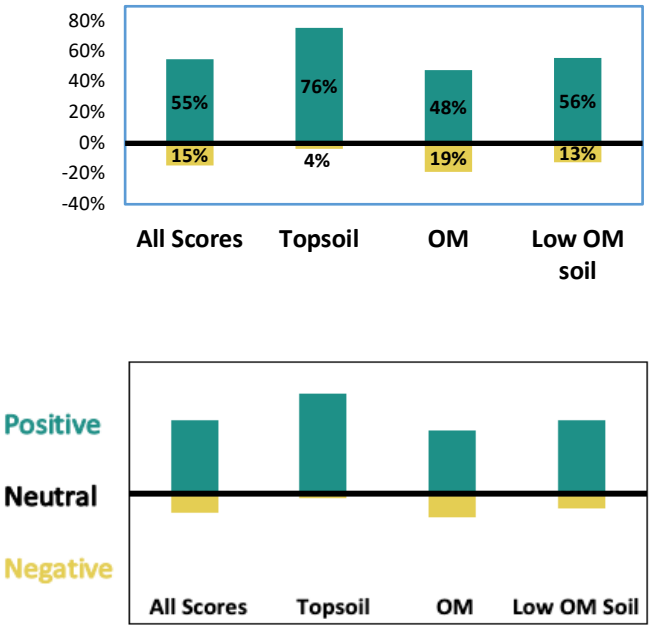


Figure A2.3 – Bar chart with percentage labeled values removed to emphasize qualitative nature of the data.

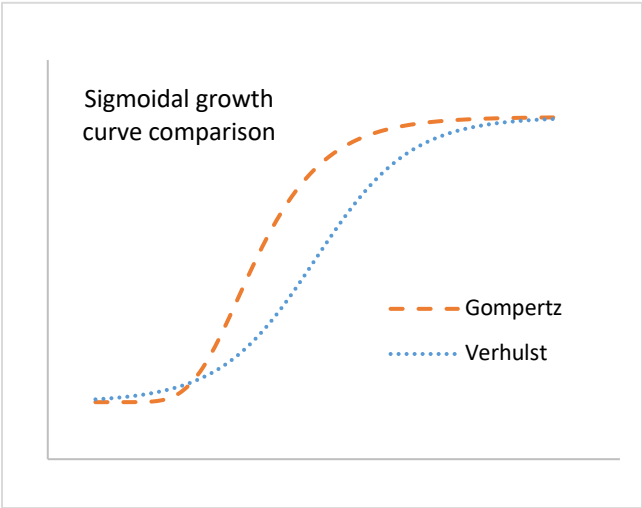


Figure A2.4 – Sigmoidal growth curves.

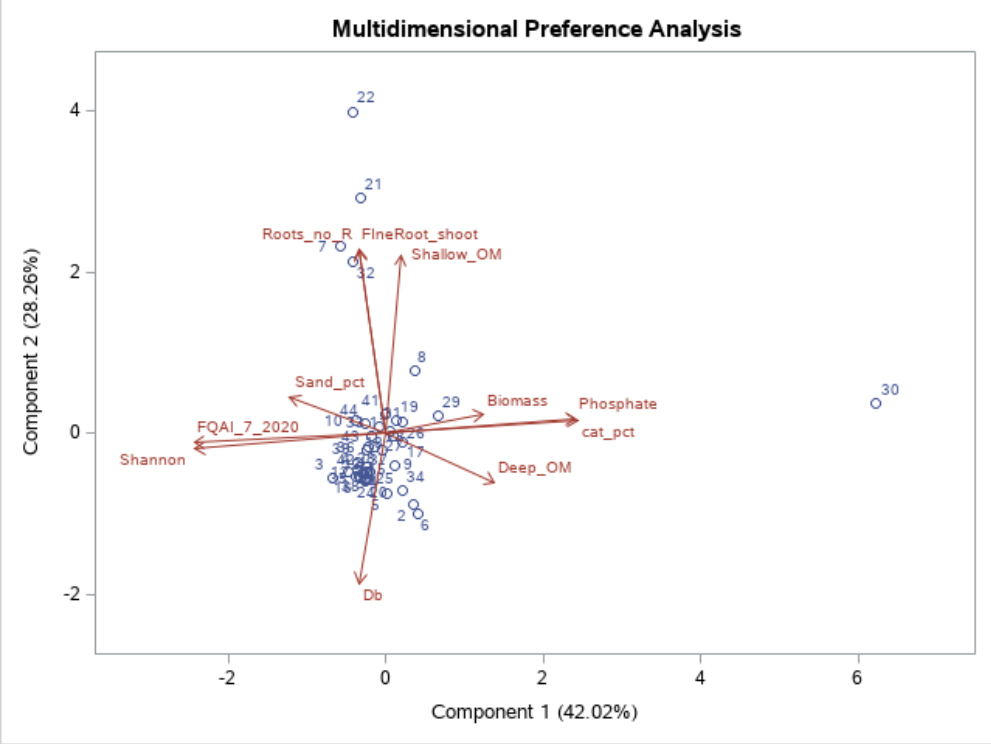


Figure A2.5 – Multidimensional Plot from Chapter 5 data.

☐ Soil and substrate amendments needed to meet hydric soil characteristics and maintain the specified plant species. * A minimum of 60 cubic yards of organic matter per acre is required. The addition of supplemental large woody debris may also be recommended.

Figure A2.6 – OM amendments * emphasized for wetland mitigation planning.

AgACIS for Caroline County

AgACIS — Copy | CSV | PDF | Print

Monthly Total Precipitation for GREENSBORO 1.4 ENE, MD (CoCoRaHS)

Click column heading to sort ascending, click again to sort descending.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
2019	3.00	4.22	4.41	4.16	5.65	5.68	1.97	4.86	0.42	3.13	2.05	3.90	45.45
2020	3.33	3.47	4.30	4.42	4.00	3.16	7.31	9.95	10.10	6.46	5.03	7.87	69.40
2021	2.03	5.77	5.57	3.36	3.54	4.09	4.26	M	M	M	M	M	M
Mean	**2.79**	**4.49**	**4.76**	**3.98**	**4.40**	**4.31**	**4.51**	**7.40**	**5.26**	**5.80**	**3.54**	**5.89**	**57.43**

AgACIS — Format for export | Print

WETS Station: BALTIMORE-WASHINGTON INTERNATIONAL AIRPORT, MD

Requested years: 2000 - 2020

Month	Temperature (°F)			Precipitation (inches)				
	Avg daily max	Avg daily min	Avg daily mean	Avg	30% chance will have less than	30% chance will have more than	Avg number of days with 0.10 inch or more	Average total snowfall
Jan	42.4	25.4	33.9	2.73	2.21	3.12	6	6.5
Feb	45.8	27.0	36.4	2.92	1.74	3.54	5	8.3
Mar	54.8	34.5	44.6	3.48	2.32	4.16	7	2.3
Apr	66.2	44.2	55.2	3.74	2.64	4.43	6	0.0
May	75.0	54.2	64.6	4.17	2.74	5.00	7	0.0
Jun	83.9	63.4	73.7	4.57	3.10	5.46	8	0.0
Jul	88.0	68.0	78.0	5.02	3.37	6.00	8	0.0
Aug	86.0	66.7	76.3	4.45	2.88	5.35	7	0.0
Sep	79.3	59.4	69.4	4.42	2.00	5.40	5	0.0
Oct	68.0	47.6	57.8	4.28	2.10	5.23	6	0.0
Nov	57.3	37.1	47.2	3.19	1.97	3.85	5	0.1
Dec	47.0	29.8	38.4	3.83	2.75	4.52	7	3.0
Annual:					41.59	51.12		
Average	66.1	46.4	56.3	-	-	-	-	-
Total	-	-	-	46.79			76	20.2

Figure A2.7 – WETS tables for field study site.

Precipitation data with a square outline exceeded the WETS value, which delayed construction (2019) and affect most of the study period (2020). Plots were constructed in September 2019, during an unusually low rainfall period.

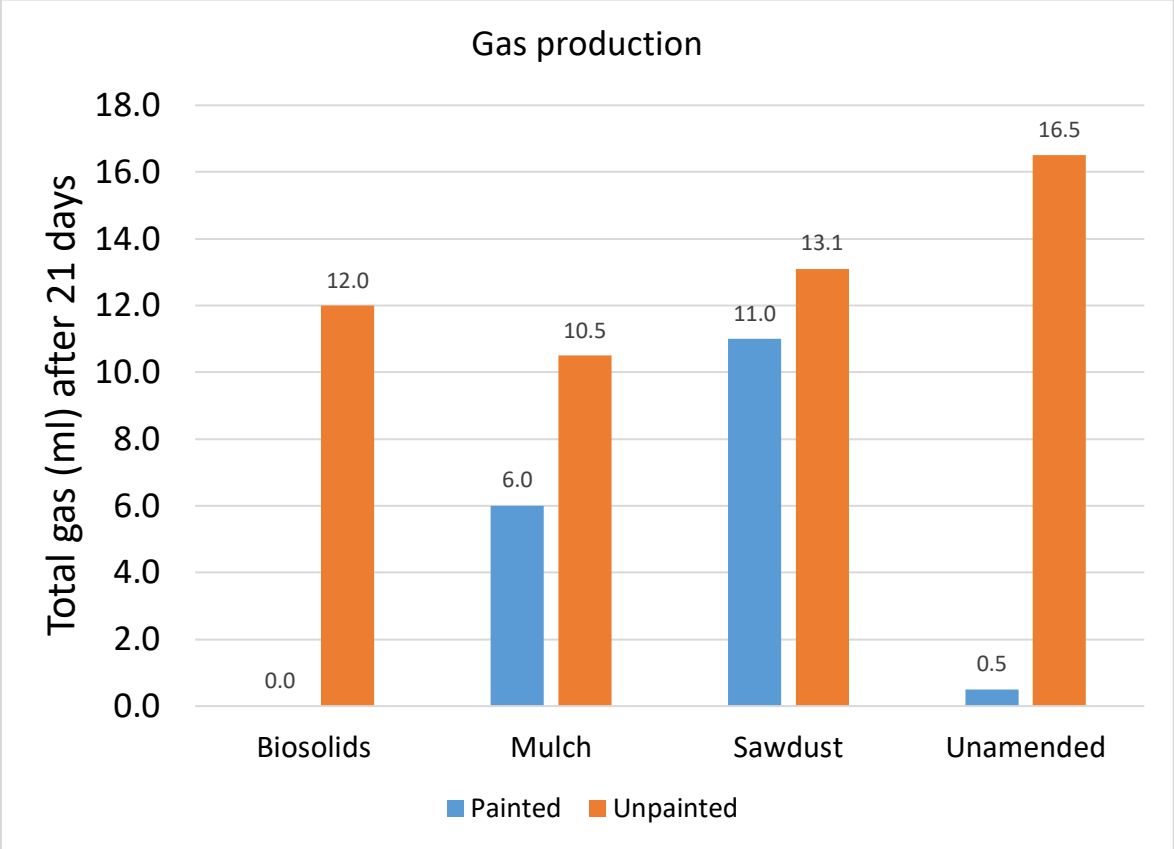


Figure A2.8 – Gas production from plastic containers painted with iron-oxide.

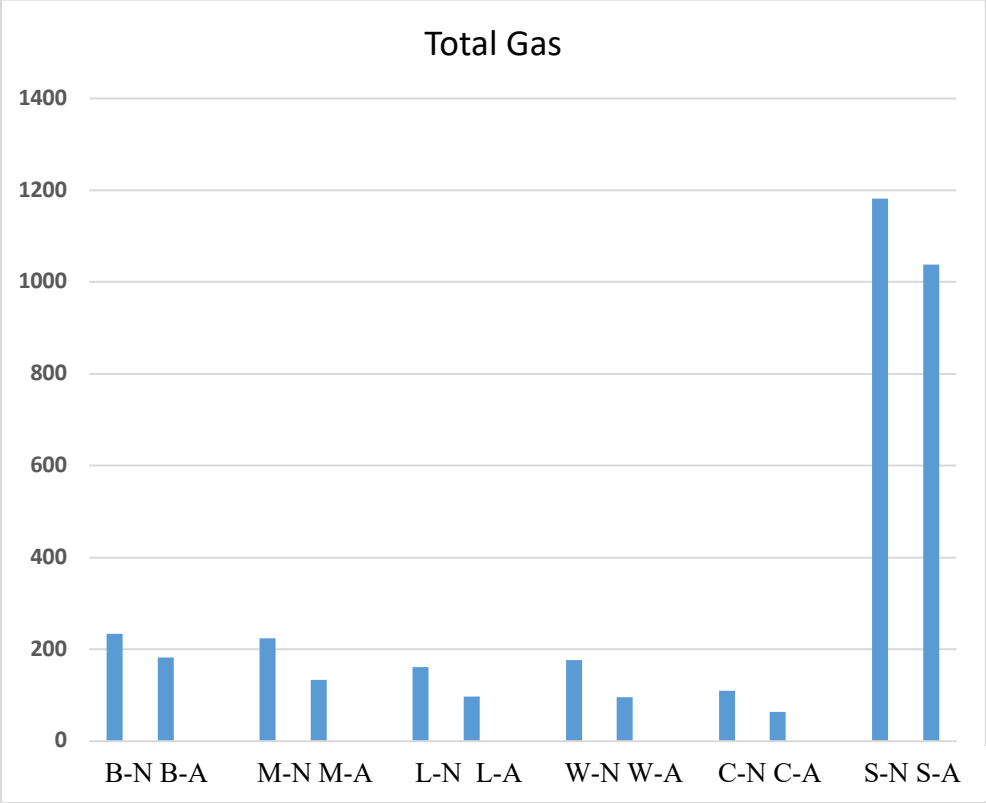


Figure A2.9 – Total gas production in microcosm incubations with nitrogen headspace (-N) and air headspace (-A).

Letter represent OM amendments: B – biosolids; M – (composted) manure; L – LeafGro (composted leaves and plant matter); W – (wood) mulch; C – Control (unamended); S – straw. Air headspace consistently reduced total gas production.

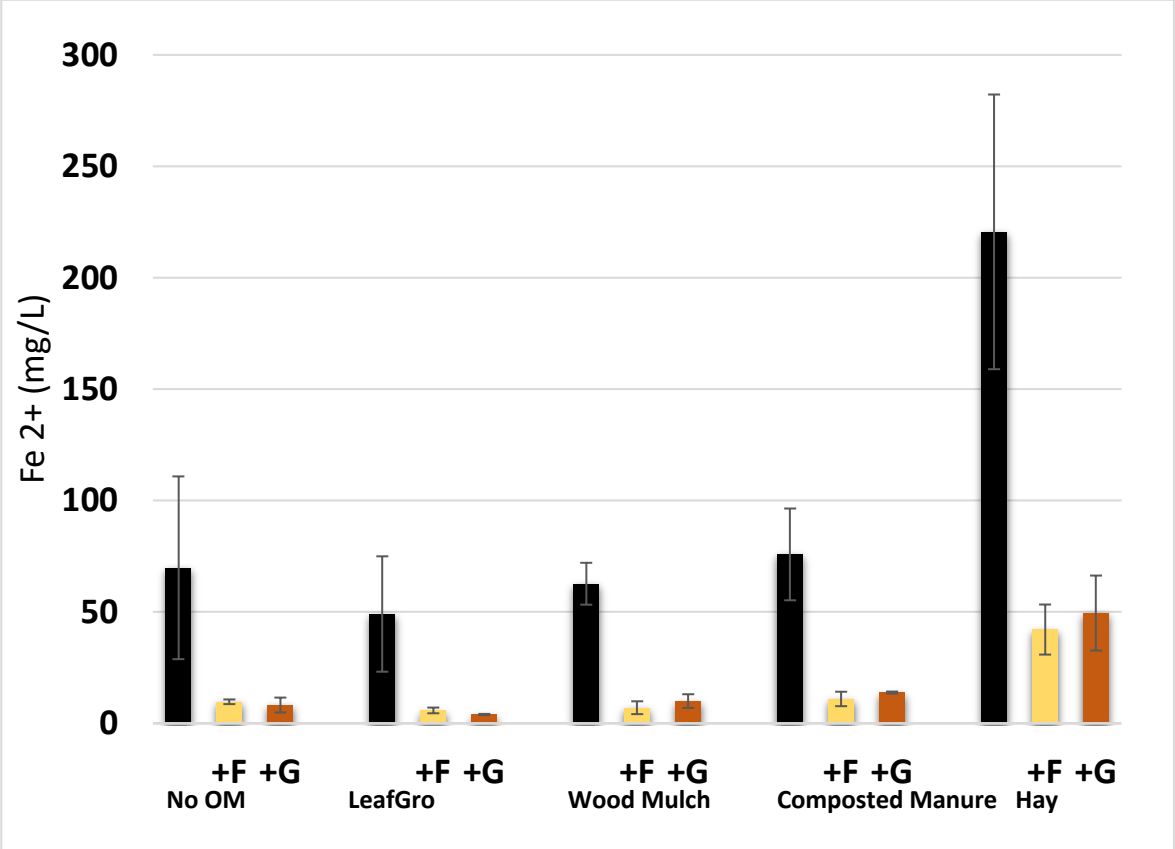


Figure A2.10 – Ferrous iron concentrations with ferrihydrite (+F) and goethite (+G) amendments.

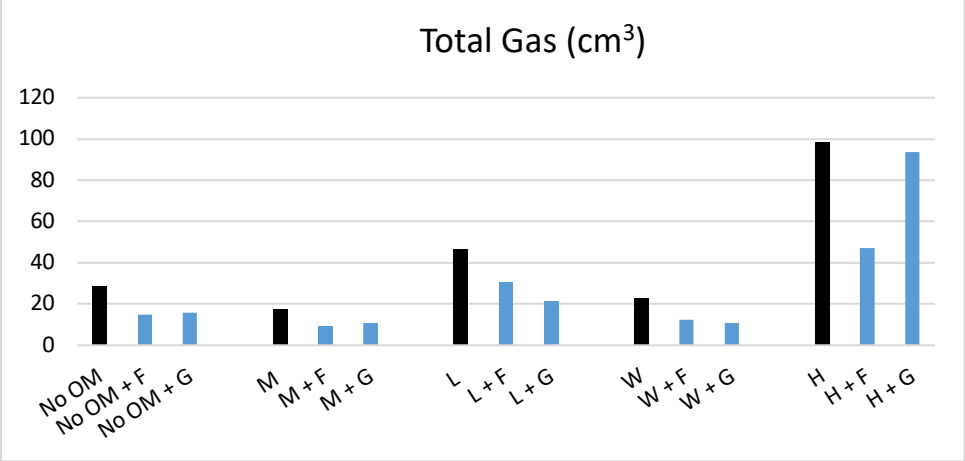


Figure A2.11– Total gas with ferrihydrite (+F) and goethite (+G) amendments. Letter represent OM amendments: M – (composted) manure; L – LeafGro (composted leaves and plant matter); M – (wood) mulch; H – hay.

Tables

Table A2.1– Example MANOVA analysis results showing Blocking variable “Water”.

Water is more sensitive than “Block”, but both are low enough that using only “Block” does not result in loss of statistical information.

MANOVA Analysis					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	3	542743	180914	4.85	0.003
Water	4	5219655	1304914	34.98	< 0.001
Plot	10	252220	25222	0.68	0.7454

Table A2.2 – Average Carbon:Nitrogen (C:N) ratio of OM amendments used in the lab and field studies.

Biosolids were low due to the presence of ammonia in un-composted samples.

Source	C:N ratio
Biosolids	6.2
Composted manure	17.1
Wood mulch	27.8
Hay	24.3

Photos

Photo A2.1 – Manganese and Iron oxide painted plastic containers.

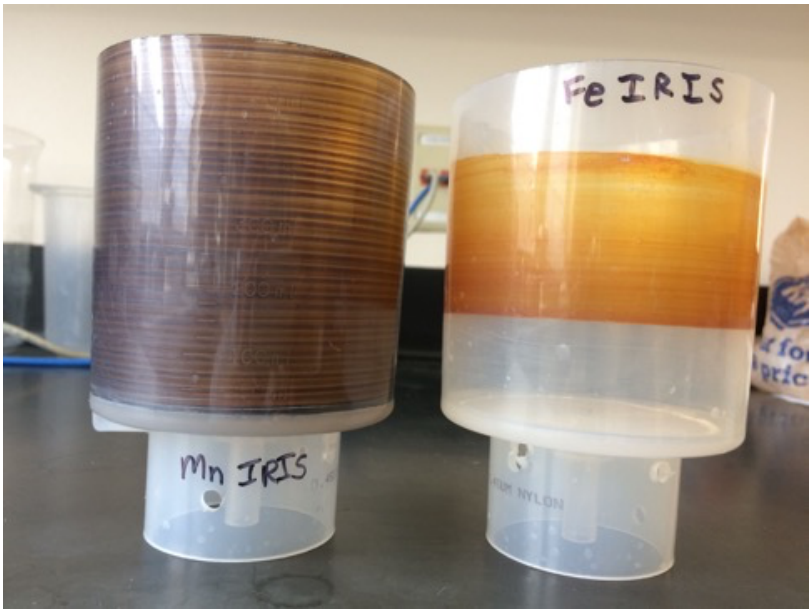
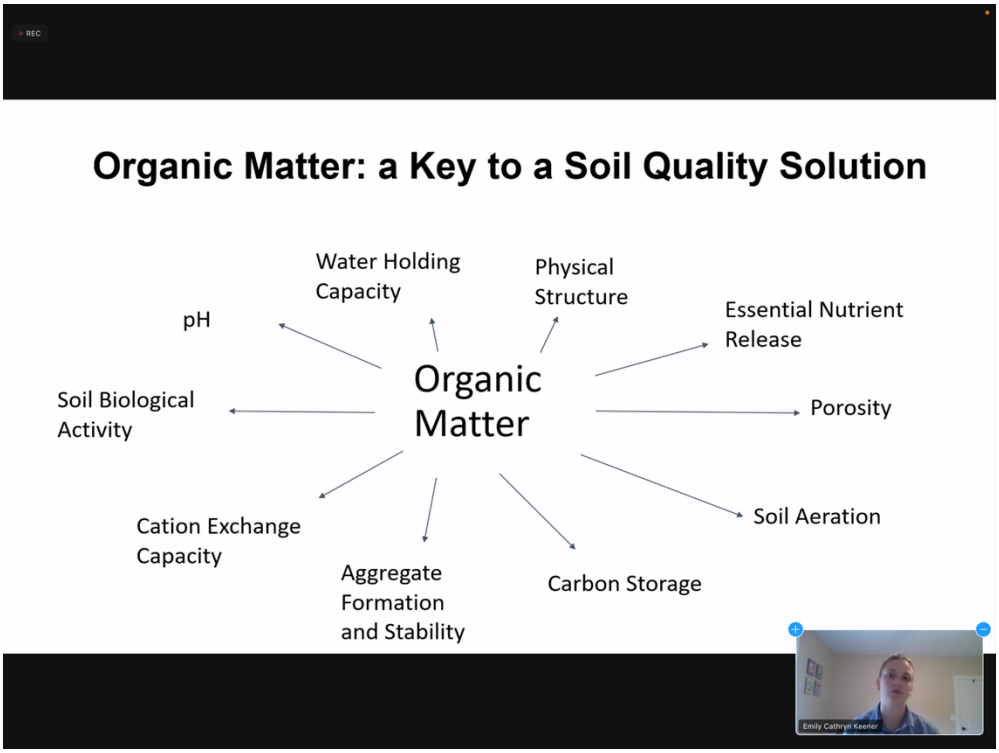


Photo A2.2 – Cattail.

Cattail appear to have a richer green color, are taller, and denser.

Photo A2.3 – Benefits of Organic Matter Amendments
from Emily Keener's Master's thesis defense.



Appendix 3: Future Work

Throughout the course of studies, I have worked to stay focused on a singular Thesis topic: the effect of OM amendments on wetland restoration. There were also several ideas worthy of study that did not fit the topic and/or there was not time to formally pursue and may serve as templates for future proposals.

Seed Sprouting

The cost of controlling undesired plant species is high. Undesired plants cause hundreds of billions of dollars in damages annually, and hundreds of millions are spent on control measures . It is a worldwide problem (Hoffmann and Broadhurst 2016). Much of the financial burden for undesired species control in the USA is borne by individual States (Hiatt et al. 2019), which many can ill afford. Available control strategies are costly (Weidlich et al. 2020) and often ineffective (Kettenring and Tarsa 2020; Elsey-Quirk and Leck 2021). Conservationists herald the coming Decade of Restoration (promoted by the United Nations), and a new US initiative led by President Biden that calls for 30% of land in the USA to be restored by 2030 (Executive Order 14008, Section 216). However, without more cost-effective undesired species control strategies, such efforts could saddle coming generations with untenable maintenance costs. Many invasive species control strategies require counterproductive disturbance (Grime 1977), (Jones et al. 2018), (Lang et al. 2015). The most common controls are chemical herbicides or non-chemical (i.e., fire or cutting). Herbicides can be effective, but are only

accessible in developed countries, require repeated application, are indiscriminate (also harm native species), and efficacy is not assured (Elsey-Quirk and Leck 2021; Hazelton et al. 2018). Non-chemical methods, such as physical removal, are more generally available but labor intensive. One of the main drawbacks of physical removal is disturbance, which is often the leading contributor to invasion. Cutting undesired species may also lead to inadvertent removal of native species (Weidlich et al. 2020). Undesired species removal is often insufficient and the even more labor intensive removal of litter is also needed (Elsey-Quirk and Leck 2021; Lishawa et al. 2019). Thus, there continues to be an urgent need to develop cost-effective strategies for undesired species control (Kettenring and Tarsa 2020; Weidlich et al. 2020)

The availability of light is one of the main reasons wetlands are susceptible to invasion . Shaded conditions reduce seed sprouting efficiency . One of the traits that allow some species are able to invade an area is phenotypic plasticity to tolerate low light environments (Perry and Galatowitsch 2004; Gruntman, Segev, and Tielbörger 2020; Martin, Canham, and Marks 2009). Once established, undesired species shade-out native plants and decrease the soil temperature, further decreasing native plants' abilities to germinate and sprout (Winikoff et al. 2020; Keyport et al. 2019).

In a recent greenhouse study using seed of 12 native species, we observed seedlings that were able to sprout in low light conditions. The study was to be conducted in a greenhouse with controlled lighting and temperature; however, Covid-19 restrictions led us to move the experiment into a basement with poor lighting (3 - 5 PPFD[3]), and

[3] Photosynthetic Photon Flux Density

limited temperature control. Nevertheless, we observed that several native seeds were able to germinate. Crimson eyed rose mallow, and to a lesser degree swamp sunflower, seed box and fox sedge were able to germinate with limited light (Figure A3.1).

For the preliminary seed sprouting study, we used the same seed mix used in the field study (Chapter 5). Due to hydraulic conditions at the site (prolonged, deep inundation), none of seeds from the mix sprouted in the experimental field plots despite $80,000 spent on seeds site wide and $500 on re-seeding the experimental plots. Nevertheless, we observed three native plant species that were present in the legacy seed bank with relatively high floristic quality that were able to grow under a cattail, barnyard grass umbrella (Figure A3.2). The response varied by hydraulic condition. A jointleaf rush (an unidentified subspecies of *Juncus articulatus*) grew under both wet (inundated) and dry conditions. Bog bullrush grew under moderate hydrology, and *Elocharis Palustris* was able to grow prolifically under all but persistently inundated conditions.

Based on these findings there may be native wetland species able to sprout and grow under unfavorable (low light) conditions in a variety of hydraulic conditions which may lead to an alternative undesired species control strategy. A greenhouse study in collaboration with a group at the university to Utah is being planned to identify native wetland species that can germinate in low light, similar to a recent study in Annals of Botany (Rosbakh, Phartyal, and Poschlod 2020), which cataloged seed germination by light intensity, temperature fluctuations and oxygen availability in five wetland community types. In a follow-up study, intensive seed application at several invaded sites would be evaluated to identify the best performing species.

Many studies consider a variety for germination characteristics (Hayasaka et al. 2020), or are focused on a desire to rapidly promote diversity (Price, Spyreas, and Matthews 2019; Russell and Beauchamp 2017). This proposed study would look exclusively at priority native species (as suggested by Hess, Mesléard, and Buisson (2019)) able to propagate in invaded habitats and create soil legacy effects in the midst of undesired species, building a native species seed bank, priming the soil for later sere species. Seed based restoration approaches have the potential to be cost effective (Kettenring and Tarsa 2020), and avoid unnecessary (counterproductive) site disturbance (Weidlich et al. 2020; Hazelton et al. 2018).

Mitigation Wetland Greenhouse Gas Model

Neubauer 2014; Neubauer and Megonigal 2015) created a model that estimates the return period after which methane emitting wetlands begin to have a net global cooling effect. The calculation is based on the CO_2:CH_4 balance in gases emitted. In wetlands with high methane emissions the return period may be on the order of hundreds of years. An alternative model can be created using CH_4 emissions and the amount of carbon (CO_2) sequestered in the soil and plants (DOE 1998) (CO_2 respiration is considered carbon neutral). To estimate sequestration rates I considered data from the field site as well as some general resources that estimate C sequestration, over time, in soils (Bernal and Mitsch 2012) and plants (Schöngart et al. 2011) estimated tree growth rates and density in mitigation wetlands using the planting guidance set forth by the MDE (Walbeck, Clearwater, and Neff 2011). Preliminary results (Figure A3.3) showed a return period similar to Neubauer's model.

I would like to formalize this model and publish the findings using either published data and/or collect additional data to fill some data holes.

Soil Potentiation

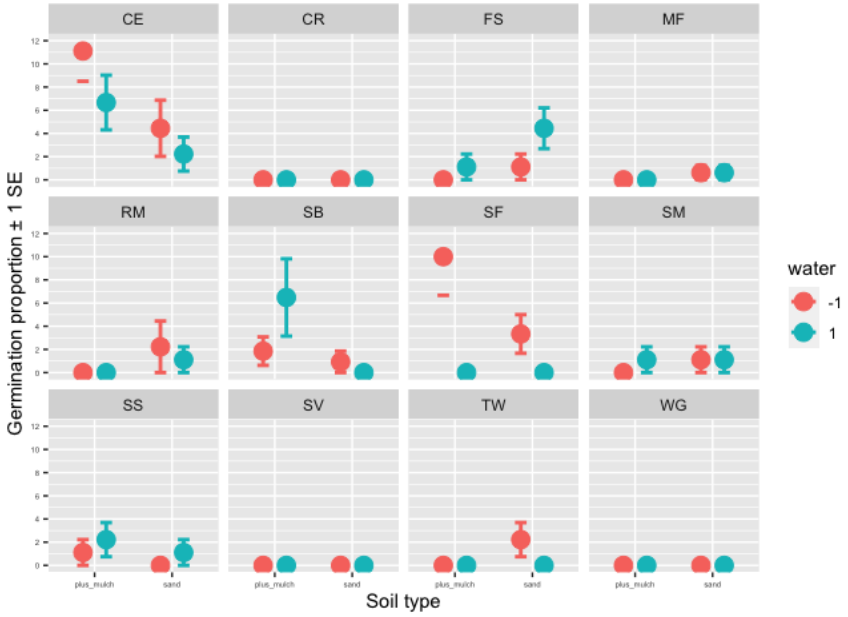
Throughout this work I have used a parameter "RedoxE". Redox potential is a common test that provides some information about the electron density in soils that governs biogeochemical reactions. Alone, this measurement is of limited value without knowing the soil's hydrogen density (pH). An example of the pitfall of the redox value is in K. Yu and Patrick (2004), who reported a redox window that minimized greenhouse gases. They claim that below -150mV methanogenesis begins. However, they neglected to give sufficient weight to a corresponding increase in pH, which can also limit methane (Z. P. Wang et al. 1993). Redox results are two dimensional variables, so it makes direct comparisons of just one dimension (redox or pH) insufficient. I am not aware of, or do not have the skill, to analyze two dimensional variables. RedoxE is a one-dimensional variable that permits the use of simple statistical tools. A future goal would be to formalize this concept and propose it as an alternate way to evaluate redox data.

Redox measures the electrical potential of soils. However, it does not reveal the micro electrical current (Armstrong 2000). The use of an operational amplification circuit measures redox potential (M. C. Rabenhorst 2009) (Photo A3.1). Soil microbes are often surrounded by a conductive extracellular polymeric substances (XPSs) which allows them to transfer electrical potential between cells, within the XPSs, and to terminal electron acceptors such as iron-oxide (Xiao et al. 2018). If these substances are removed it changes microbial behavior. XPS may retain moisture and retain conductivity as soils are drying. Similarly, there may be a lag after soils dry out before cells can regenerate their XPS. This may be one reason we observed a lag in E_H response (compared to

dipyridyl) when soils began to dry out in the field study. I hypothesize that measuring redox potential is not indicative of a microbe's ability to utilize XPS, whereas a measure of micro-electric current may.

This idea is the least fleshed-out of potential post-doctoral pursuits, but I have had very encouraging conversations with both Dr. Wilmoth and Arron Thompson (Georgia Tech) about this potential research topic.

Figures



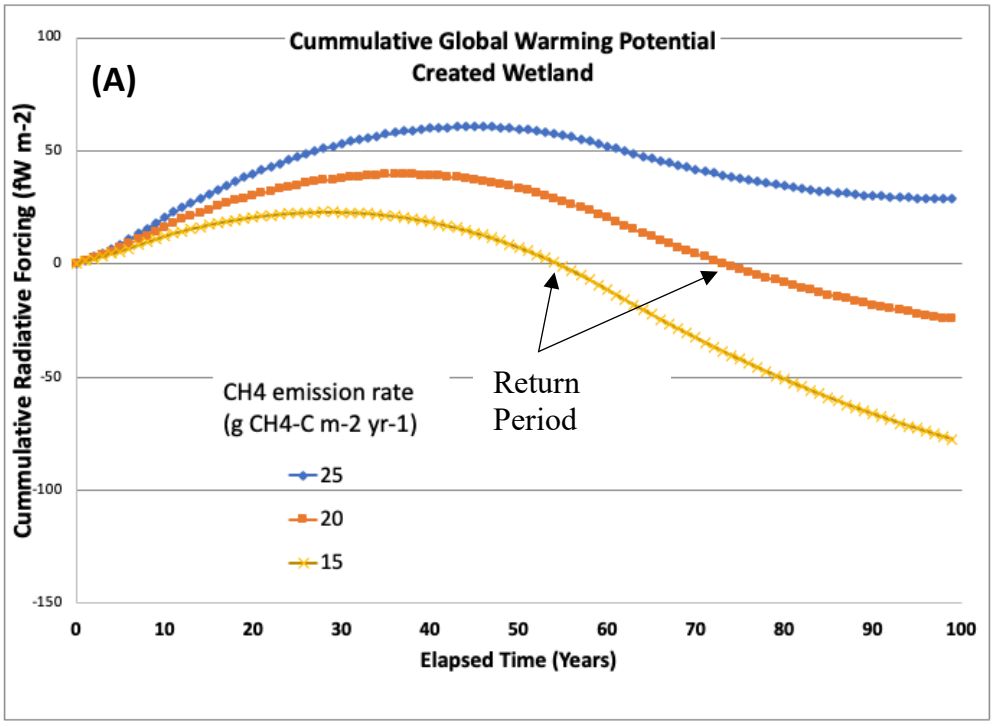
CE – Crimson eyed rose mallow
CR – Common Rush
FS – Fox Sedge
MF – Alleghany Monkeyflower
RM - Rattlesnake Mana grass
SB – Seedbox
SF – Swamp Sunflower
SM – Swamp Milkweed
SS – Shallow Sedge
SV – Swamp Verbena
TW – Trumpetweed
WG – Woolgrass

Figure A3.1 – Wetland seed germination. All seeds germinated in low light conditions.

The chart also shows differences in sprouting based on water level and OM amendment.

Figure A3.2 – Table with percent cover (based on Peet cover class) of undesired (brown shading) and desired (green shading) species.
Intensity of color also shows cover class. Values in row 1 are coefficients of conservation. Cattail grew well in wet (nearly continuously flooded) and moderate (fluctuating water levels) locations. In contrast, barnyard grass grew best under dry conditions, where cattail did poorly.
Three species, bog bulrush, jointleaf rush, and spike rush, grew well in areas shaded by cattail and barnyard grass. Bog bullrush preferred moderate hydrology and jointleaf rush grew best in both dry and wet locations. Spike rush was adapted to all hydraulic conditions at the site.

Block	Plot	Hydrology	Cattail	Barnyard Grass	Bog Bullrush	Jointleaf Rush Juncus articulatus	Elocharis Palustris Common spkie-rush
			1	2	9	6	5
			Class				
A	B	Moderate	4	9	4	0	8
A	C	Moderate	4	8	0	5	8
A	H1	Moderate	0	6	6	0	8
A	H3	Moderate	8	7	4	4	7
A	H6	Moderate	4	6	0	4	9
A	M1	Moderate	2	8	3	1	7
A	M3	Moderate	6	7	5	0	8
A	M6	Moderate	10	9	0	0	9
A	W1	Moderate	9	6	0	5	6
A	W3	Moderate	5	7	7	2	6
A	W6	Moderate	2	8	5	1	9
B	B	Moderate	4	3	4	0	0
B	C	Moderate	7	2	6	5	8
B	H1	Moderate	8	0	3	2	8
B	H3	Moderate	2	6	6	4	9
B	H6	Moderate	5	6	4	5	9
B	M1	Moderate	8	5	0	1	8
B	M3	Moderate	9	0	3	0	8
B	M6	Moderate	7	9	6	0	9
B	W1	Moderate	6	4	0	3	9
B	W3	Moderate	9	0	4	4	6
B	W6	Moderate	6	6	6	0	8
C	B	Wet	7	0	0	4	8
C	C	Wet	7	0	0	7	6
C	H1	Wet	7	8	0	5	5
C	H3	Wet	8	5	0	7	7
C	H6	Wet	9	0	0	8	8
C	M1	Wet	9	1	0	3	4
C	M3	Wet	10	0	0	3	2
C	M6	Wet	10	0	0	0	0
C	W1	Wet	8	5	0	7	7
C	W3	Wet	4	3	0	8	0
C	W6	Wet	6	0	0	7	5
D	B	Dry	0	9	0	0	7
D	C	Dry	0	8	0	6	9
D	H1	Dry	0	8	0	6	7
D	H3	Dry	5	6	1	7	0
D	H6	Dry	6	6	0	6	8
D	M1	Dry	2	8	1	0	9
D	M3	Dry	8	7	0	1	9
D	M6	Dry	10	10	0	0	9
D	W1	Dry	0	6	1	7	9
D	W3	Dry	0	7	0	8	8
D	W6	Dry	0	7	0	8	8
		Average	5.5	5.1	1.8	3.5	6.9
	Average (moderate hydrology)		5.7	5.5	3.5	2.1	7.6
	Average (wet hydrology)		7.7	2.0	0.0	5.4	4.7
	Average (dry hydrology)		2.8	7.5	0.3	4.5	7.5



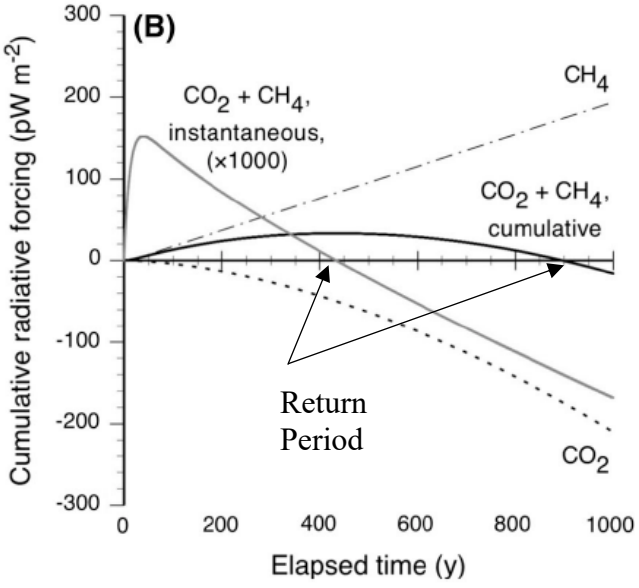
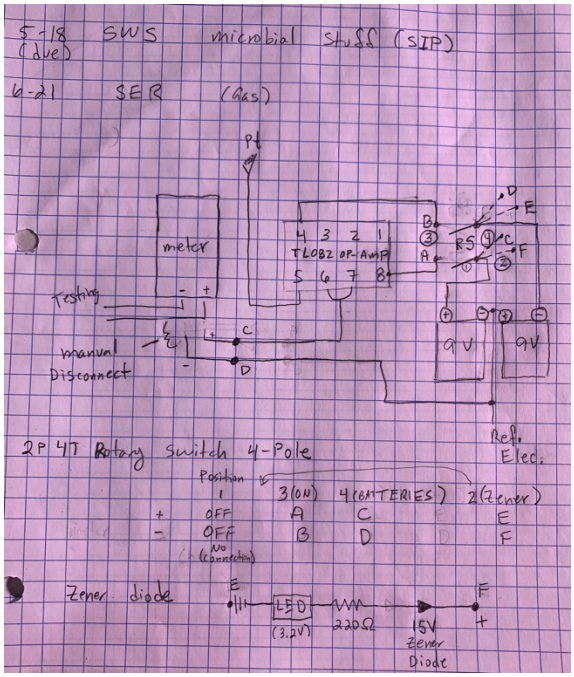


Figure A3.3 – Scott (a) and Neubauer (b) models showing return period, after which wetlands become global warming syncs.

Photos

Photo A3.1 – Redox measurement device and circuit diagram.



Appendix 4: Source of atmospheric carbon dioxide verification

What is the best way to communicate science – especially to a skeptical audience? I often speak to climate change skeptics and this is my response. The most import tool we have is credibility. I tell people I didn't believe fossil fuels were contributing to global warming - until I did the calculations myself. The response has been unanimously favorable.

The accepted benchmark for atmospheric carbon dioxide is the National Oceanic and Atmospheric Administration's (NOAA) observatory at NOAA reports total carbon dioxide and annual increases in ppm/year. Values can be obtained here. I trust these number because, throughout my 40+ years of science, I have personally collected and analyzed air samples that match Muana Loa CO_2 numbers. I've seen the numbers increase first hand.

Fossil fuels are a primary world commodity, and their production is carefully monitored. One source of fossil fuel production is the US Energy Information Administration. Production numbers from this site for Oil, natural gas, and coal are summarized in the table below.

	Given units		lbs carbon		liters CO_2	
Fuel	2017	2018	2017	2018	2017	2018
Coal (mst)	8,344,220	8,672,045	5.84E15	6.07E15	1.35E15	1.41E15
Oil (mb/d)	98,119	100,818	9.26E15	9.52E15	2.14E15	2.20E15
Natural Gas (bcf)	130,895	137,785	--	--	3.67E15	3.86E15
				Total	7.16E15	7.47E15

Mst = thousands of short tons (2000 lbs)
mb/d = thousands of barrels per day
bcf = billions of cubic feet

Converting these numbers to volume of carbon dioxide:

Coal: Assume coal is 35%C based on the average chemical formula of $C_{13}H_9O_9NS$.
Oil: Assume oil is 84%C. The specific gravity of crude oil is 0.88 kg/liter and an oil barrel is 159 liters.
10^3 barrel/day * 365 days * 159 liters/barrel * 0.88 kg/L * 2.2lb /kg * 0.84 gC/g
Convert lbs carbon to liters CO_2:
1 lbs * 1kg/2.2lb * 10^3 g/kg * 1 mol/44g * 22.4 l/mol
Natural Gas: 1cf methane → 1cf CO_2 * 28 l/cubic foot

The atmosphere is 4.2E9 cubic km. I looked this number up, but I also calculated it myself. The calculation is fairly complex and omitted here for simplicity. There are 1E12 L in 1 km^3. Therefore, the atmosphere is ~4.2E21 liters.

liters CO_2 Emitted		Atmosphere volume	Theoretical annual increase from coal, oil, and gas	
2017	2018		2017	2018
7.16E15	7.47E15	4.21E21	1.7E-6	1.8E-6
Annual increase at Muana Loa			1.9E-6	2.9E-6

The annual increase in CO_2 observed at Muana Loa is very similar to the values calculated based on the amount of carbon dioxide release by burning coal, oil and natural

gas. The number should be, and is, slightly lower since hydrocarbons are not the only CO_2 sources.

Appendix 5: Supplemental Figures and Tables from Publications

Note: Second digit denotes chapter number.

e.g. Figure A5.2.1

A5 – Appendix 5

.2. – Chapter 2

1 – Figure number

Figures

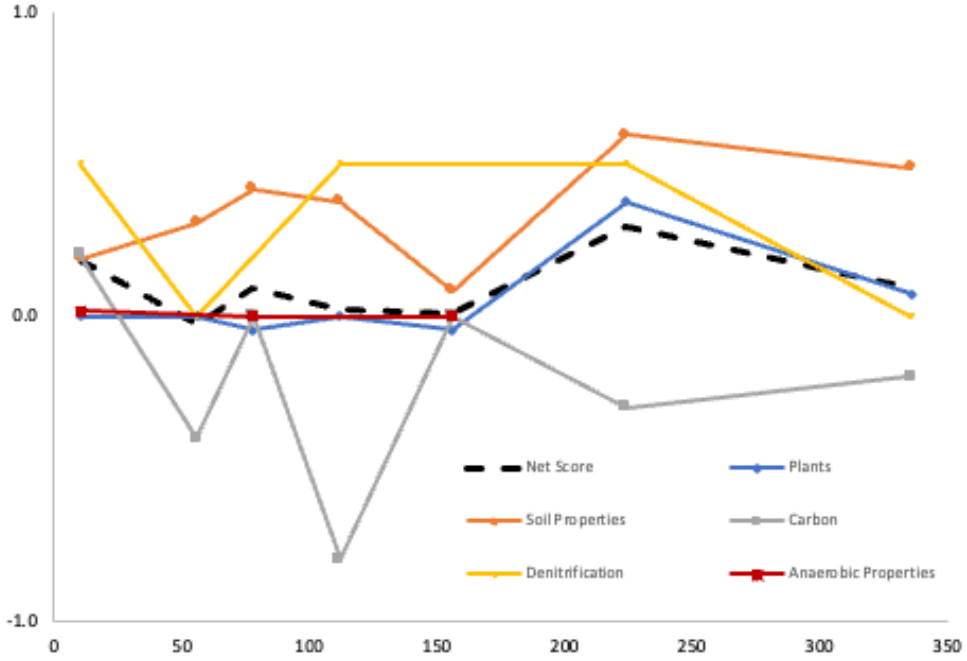


Figure A5.2. 1– Average score of response metrics from studies that varied the organic amendment dose.

We show the net score and the score for each of the 5 categories. The lowest dose values were not reported in Mg/Ha but using the bulk density provided for the amendment we were able to estimate that the dose would have been much less than the lowest reported value of 56 Mg/Ha, so all values are shown as 10 Mg/Ha.

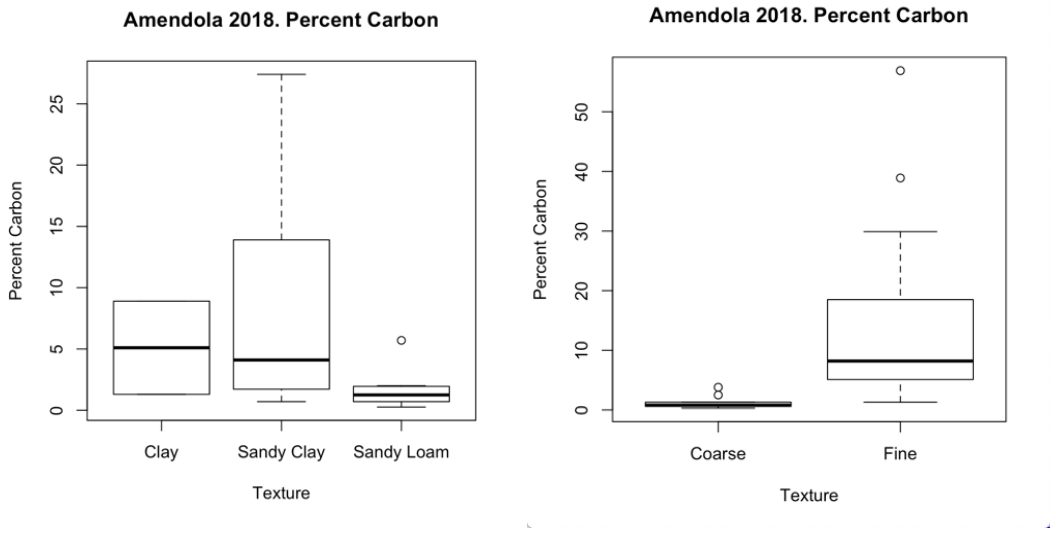


Figure A5.2. 2 – Soil percent carbon versus soil texture.

Data from Amendola et al. (2018). Figure 2a uses the texture groups (low medium and high clay) in Amendola and Figure 2b groups soil as coarse (sandy modifier) versus fine.

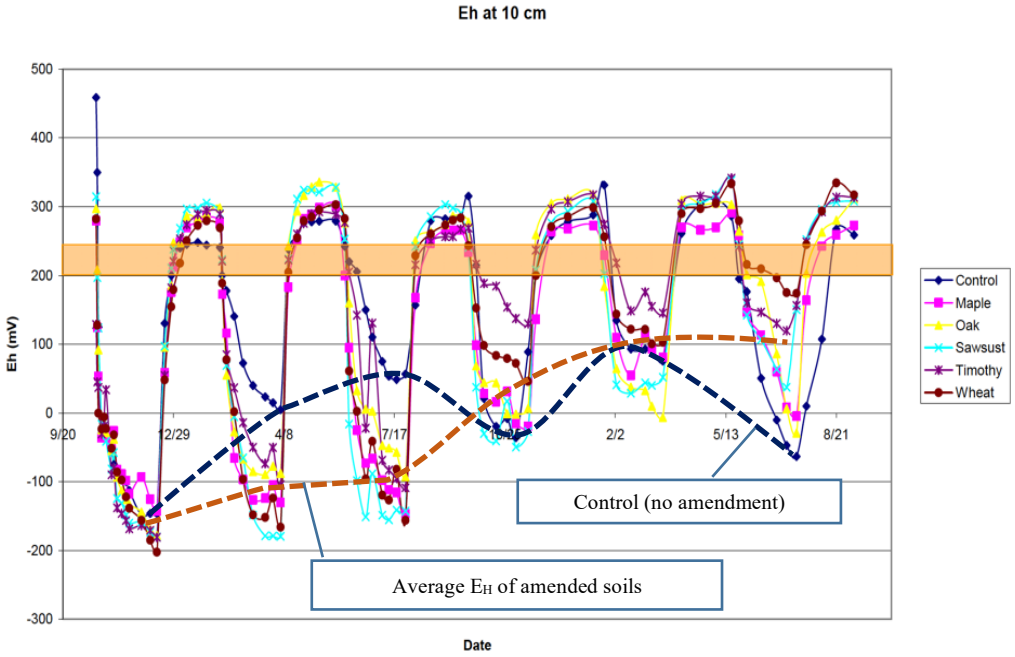


Figure A5.2. 3 – The effect of several types of organic amendments on E_H values. Experiment was done in soil columns. Columns were periodically drained to cycle between oxidizing and reducing conditions. Orange banded area represents NRCS Technical Standard for hydric soils. From Gray (2010).

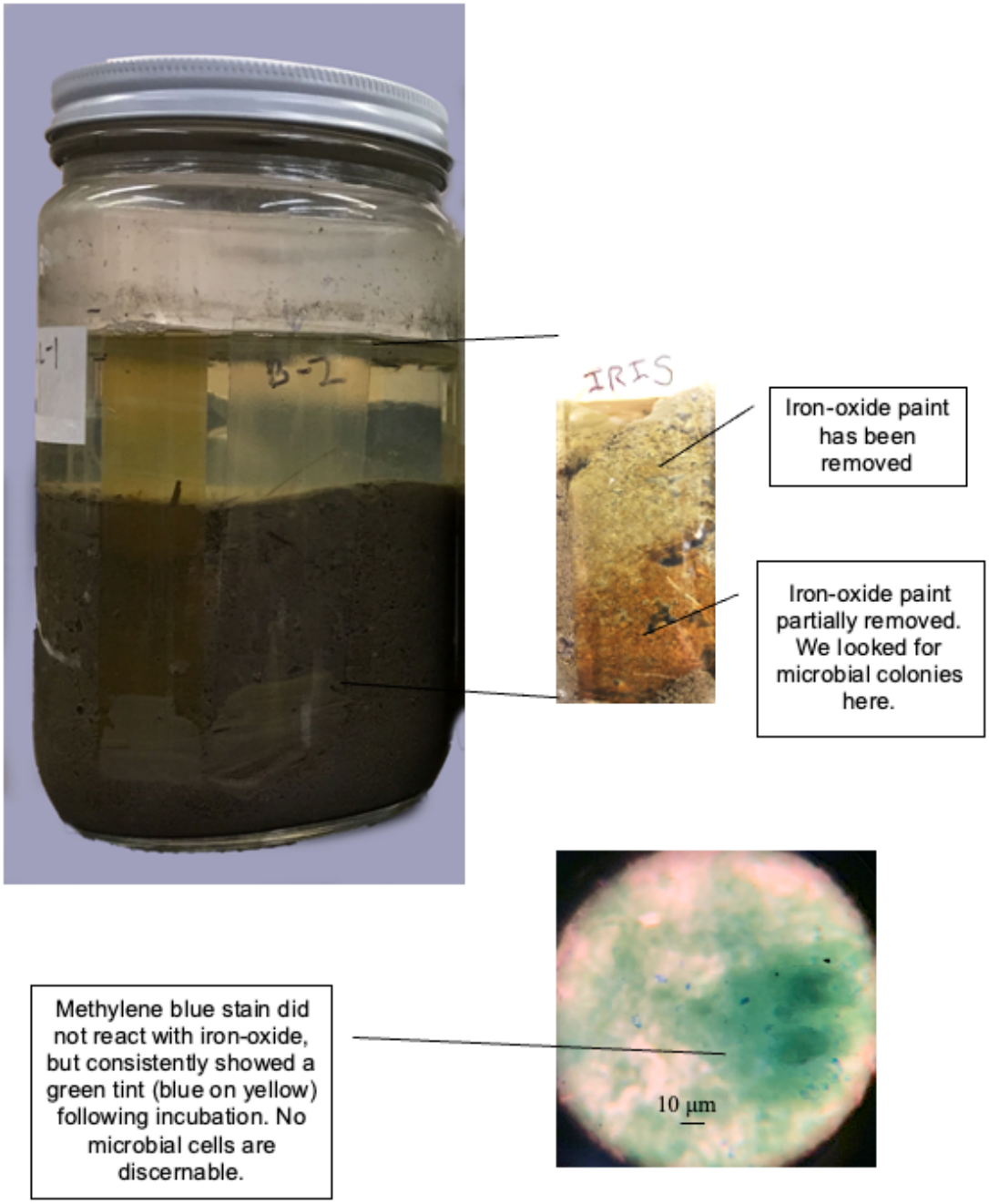


Figure A5.3. 1 – Glass jar incubation.

Glass microscope slide has been painted with metal-oxide paint (iron-oxide shown).

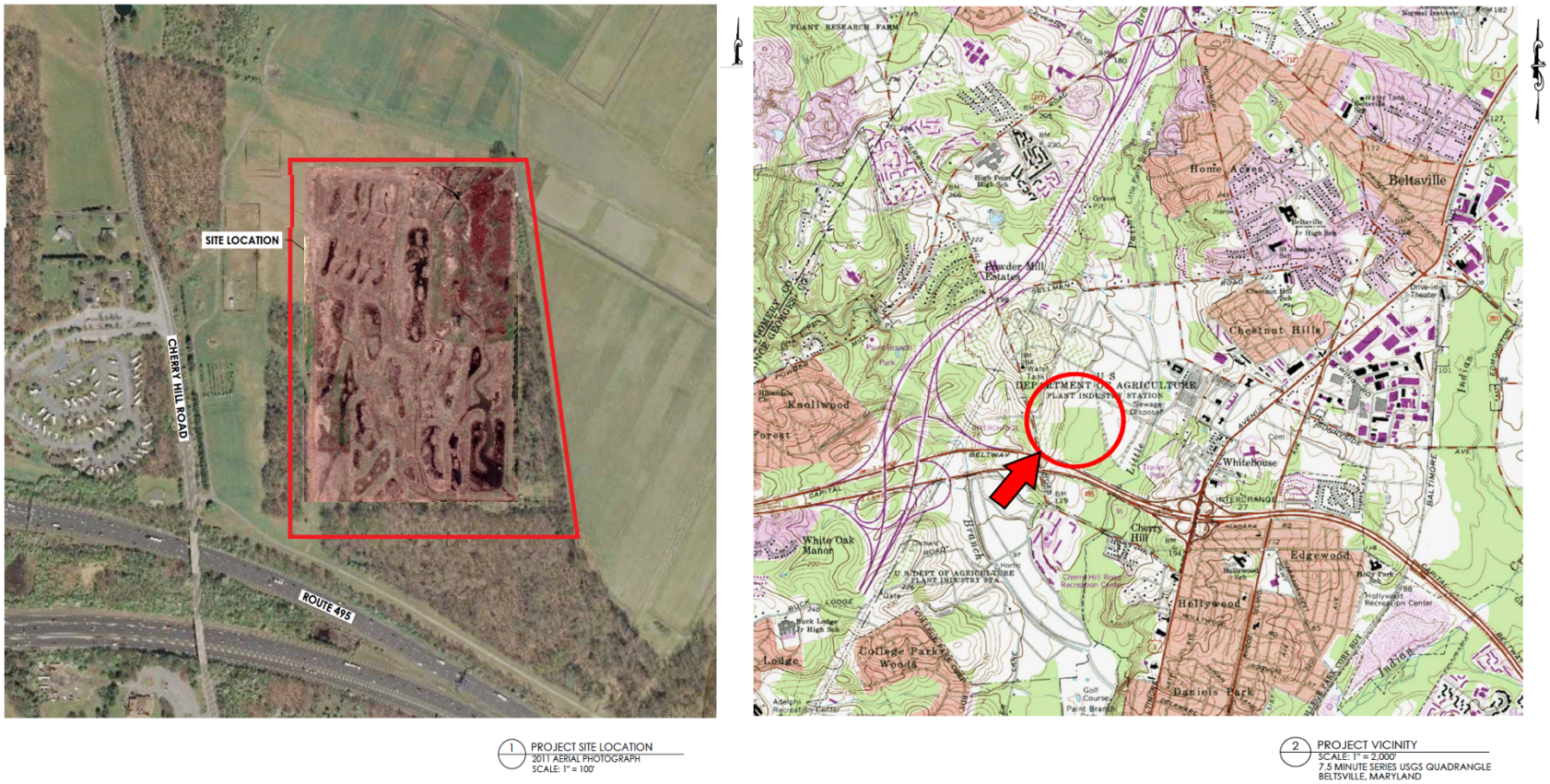


Figure A5.3. 2a – Field study area in Beltsville, MD.

The aerial image (left), from site construction documents, has been overlain with the mitigation wetland post-construction.

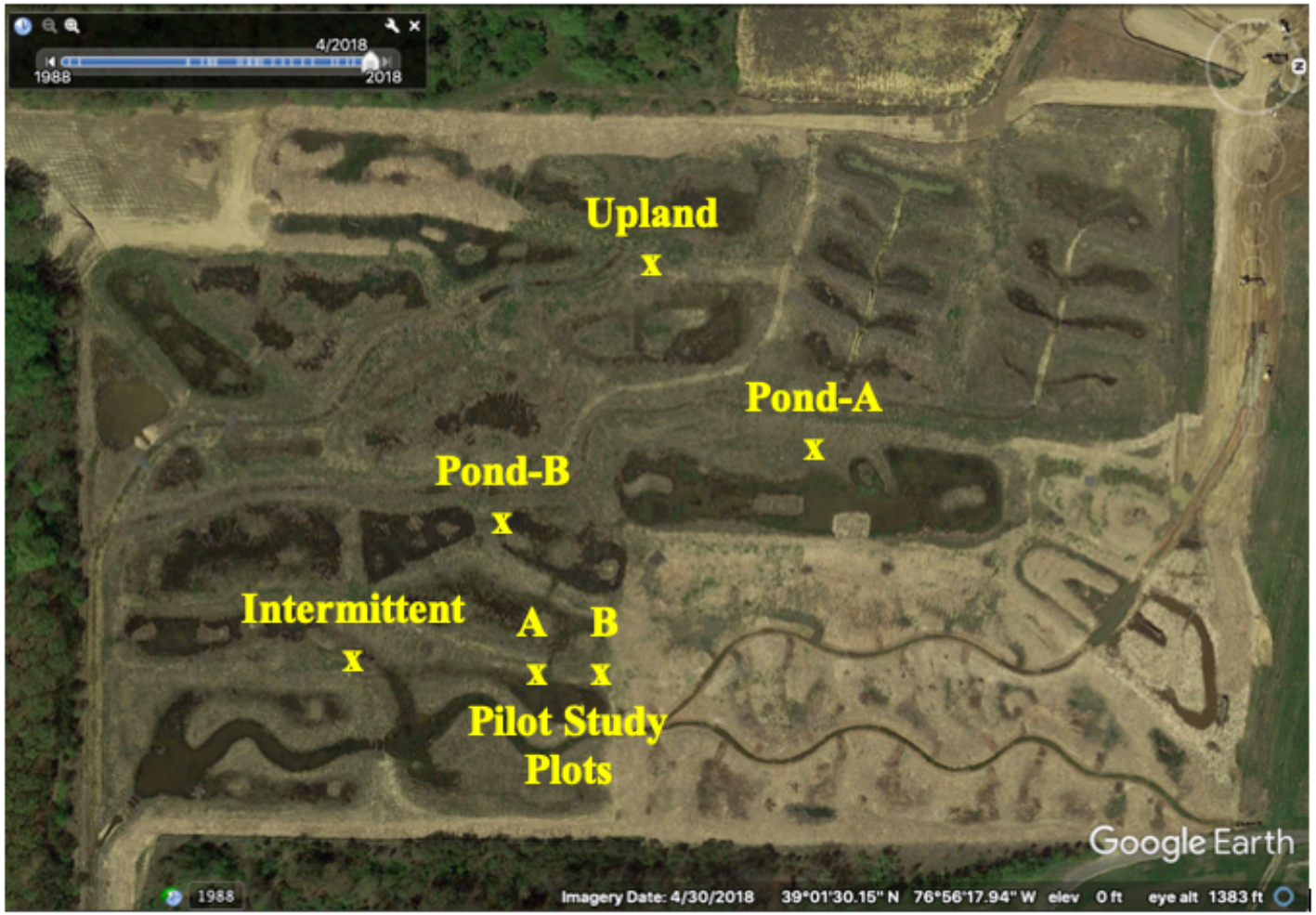


Figure A5.3.2b – IRIS locations.

Emphasizing two locations adjacent to ponds (Pond-A & Pond-B), an intermittent stream Intermittent) and in an area not adjacent to standing water (Upland).

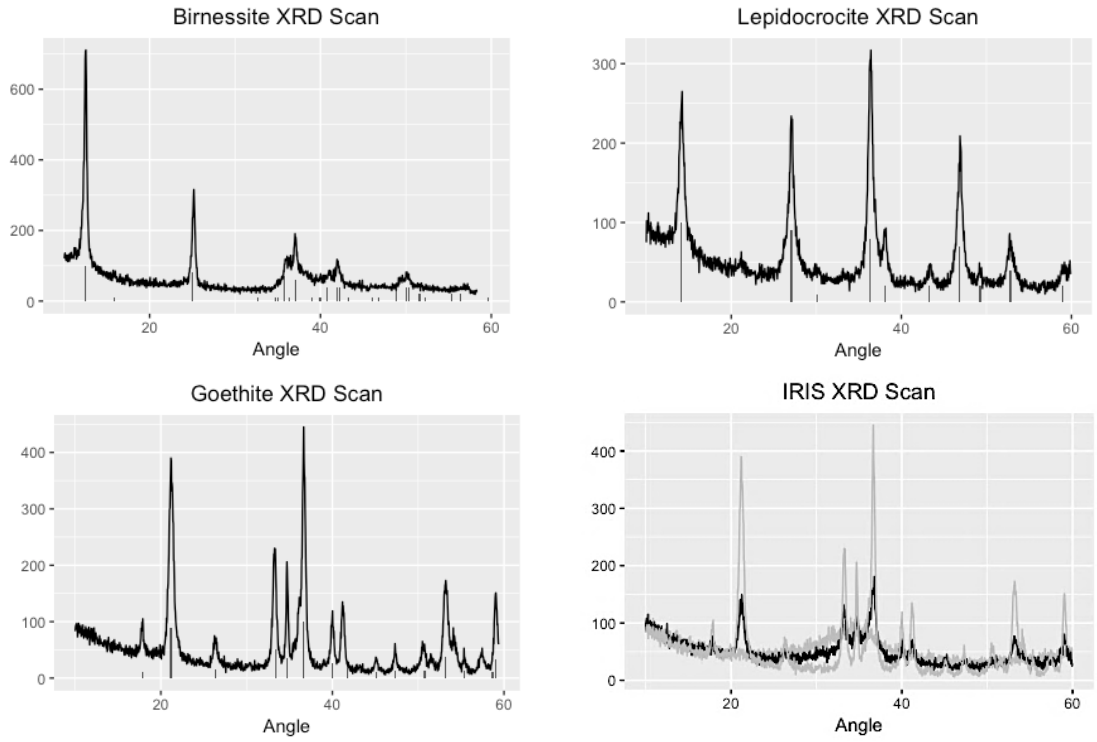


Figure A5.3. 3 – Graphs showing metal oxide X-Ray Diffraction scans.
Including 2a (birnessite) and 2b (lepidocrocite). Bars on baseline show representative peaks. IRIS paint (2d) is a mixture of goethite (2c) and ferrihydrite (Figure 2d, grey scan). Ferrihydrite has one broad peak centered at about 33°: Based on the relative peak heights, our IRIS sample was about 30% - 40% goethite.

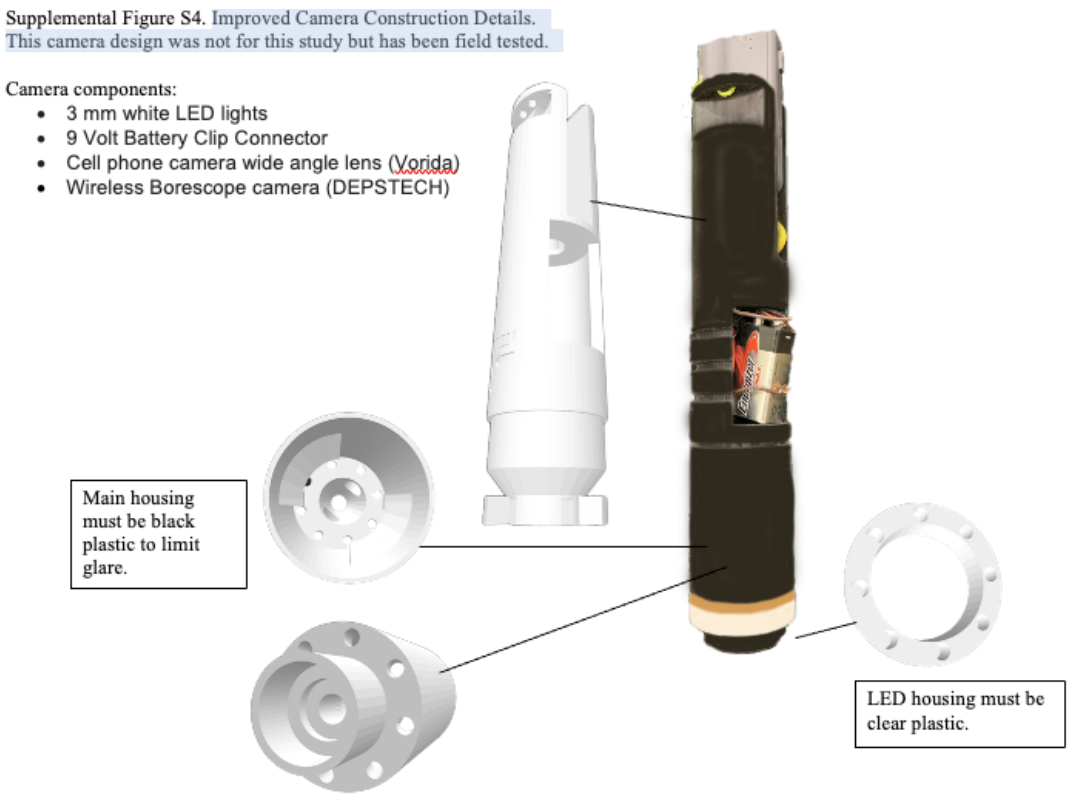


Figure A5.3. 4 – Improved Camera Construction Details.

This camera design was not for this study but has been field tested.

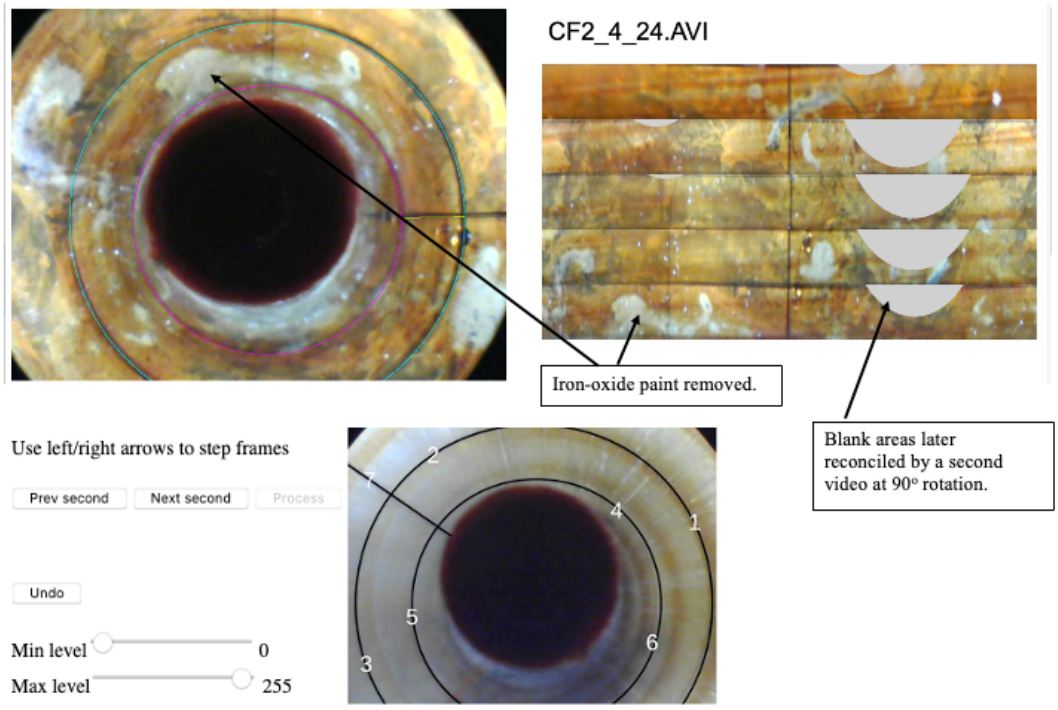


Figure A5.3. 5 – Output from online tool for translating video to single, flat image. Iron-oxide painted CIRIS tube. Rings represent 2 cm increments – top 10 cm of tube is shown. CF2 = Plot C; Iron-oxide tube (F); Tube 2 of 2.

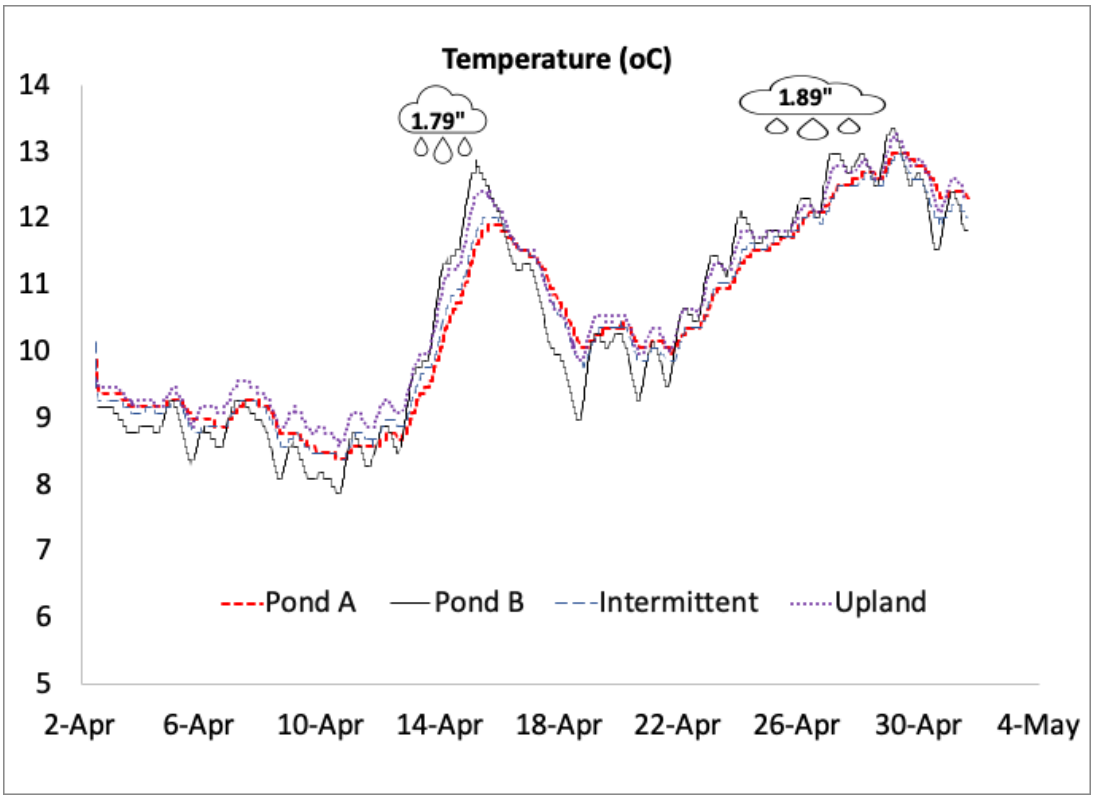


Figure A5.3. 6 – Subsurface temperatures varied in response to rainfall events. Results not statistically different ($p > 0.99$).

Figure A5.4. 1 – Typical sampling setup (All Experiments).
A gas tight syringe was inserted first, relieving the excess pressure and recording the volume of biogenic gas produced. With the pressure was equalized to 1 atmosphere, a separate syringe was used for gas and liquid sampling. If desired, an inert gas (e.g. nitrogen) may be injected into the headspace, up to the gas tight syringe volume, prior to sampling. The experiment was performed in 1-liter jars (left) and we used the same gas and liquid sampling technique shown here with in 40 mL VOA vials (right). The liquid height in the jars was sufficient for liquid sampling with a 3” syringe.

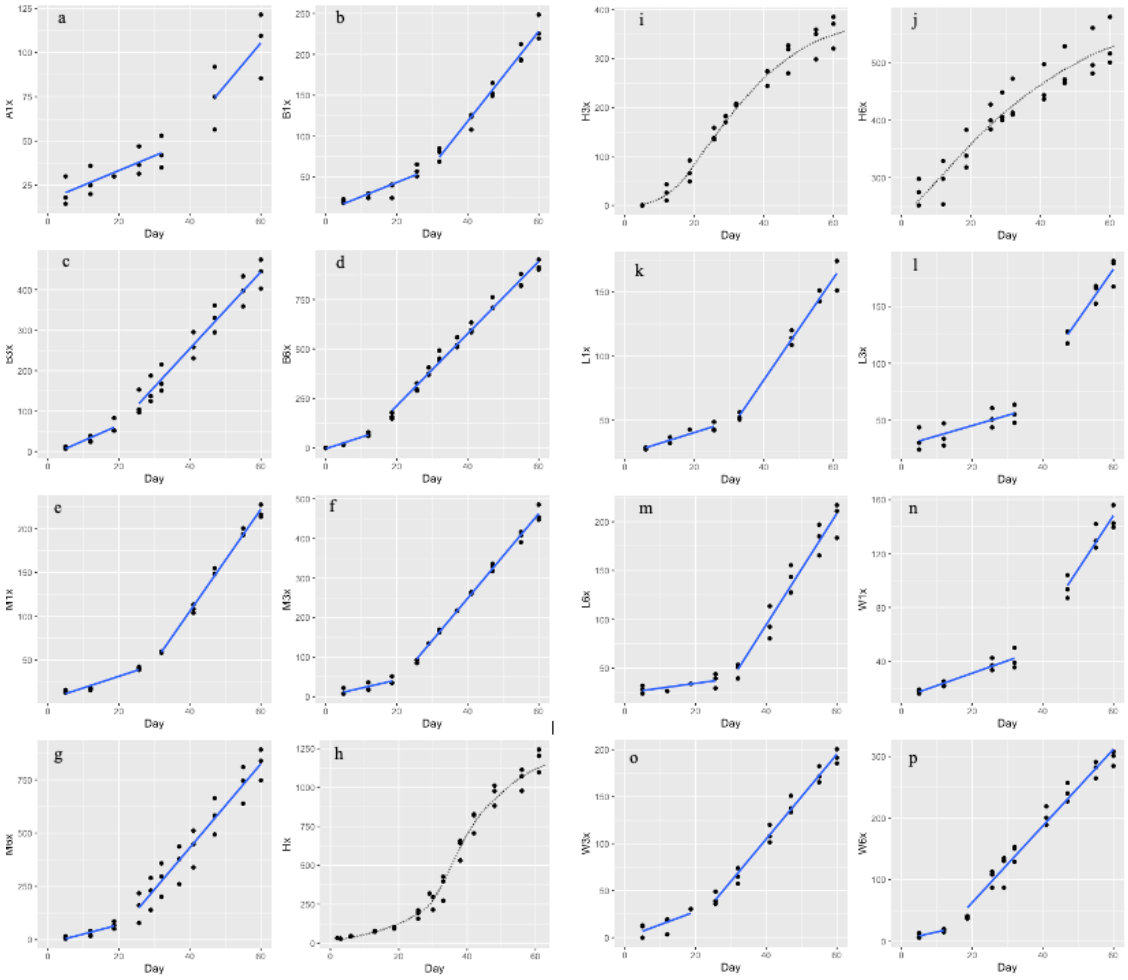
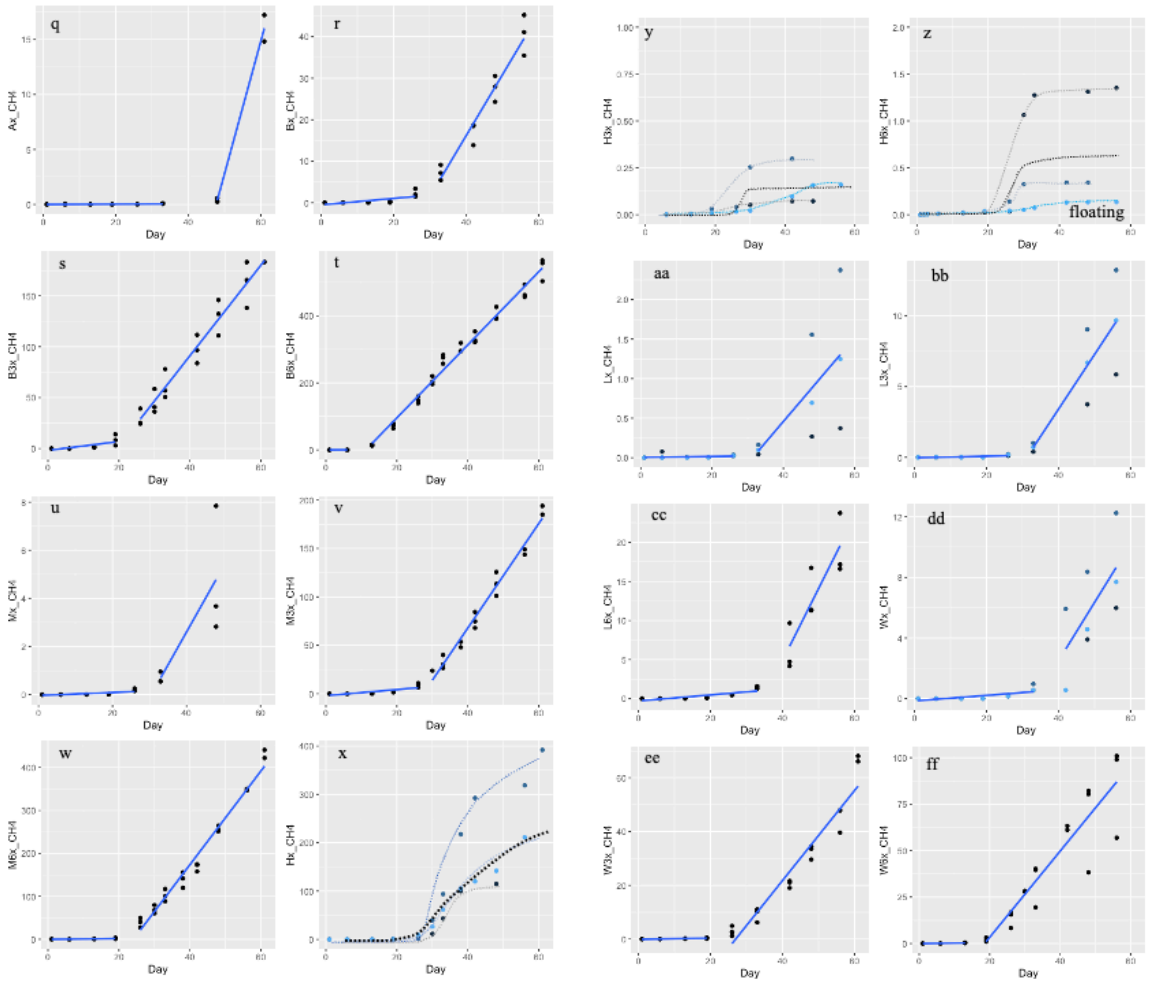


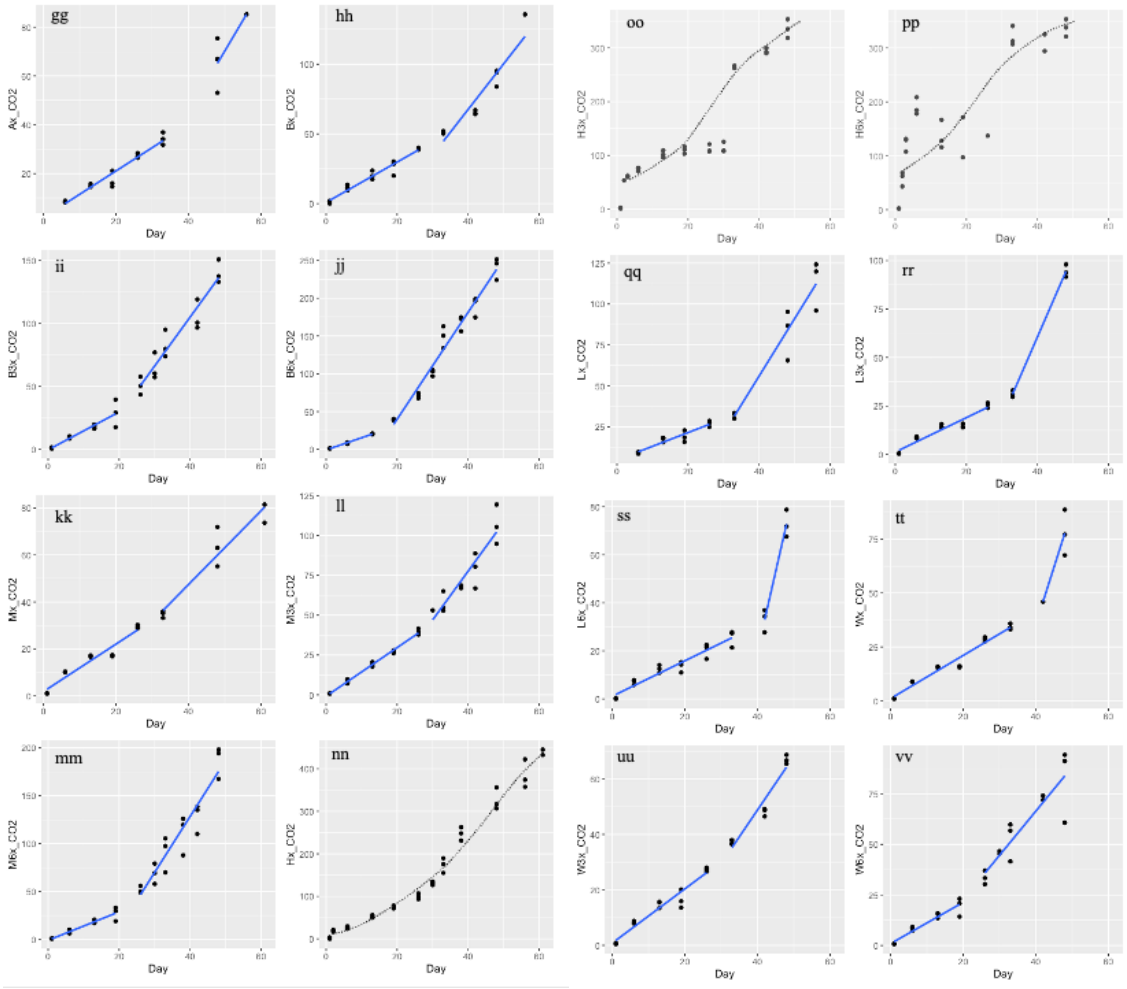
Figure A5.4. 2&3 – Experiment 1. Microcosm incubations gas production with time.

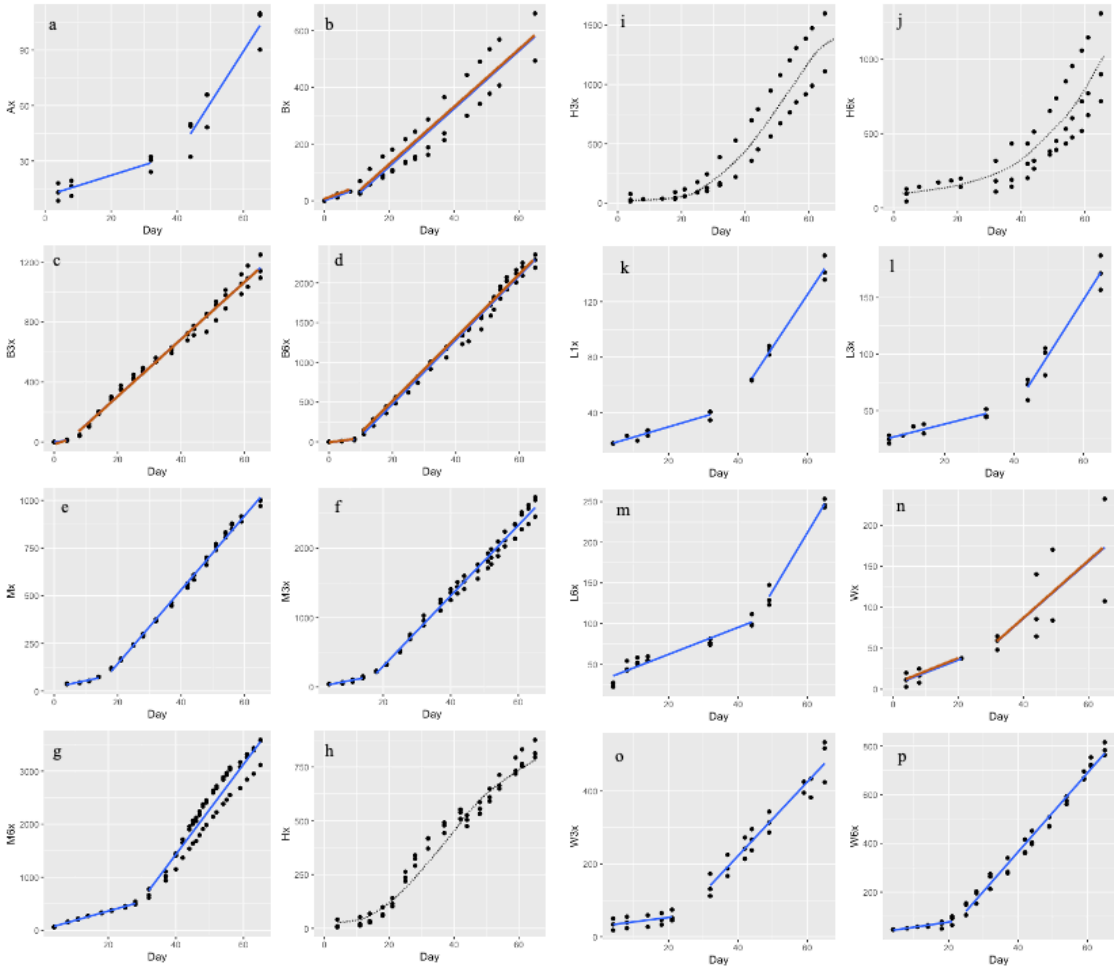
Figure A5.4.2 is in Sandy Loam and A5.4.3 in Sandy Clay Loam. The vertical axes represent cumulative total gas produced in cubic centimeters. Lines represent linear functions generated with the Segmented R function, except hay (H#x) which used sigmoidal curves generated with the SSgompertz R function. Graphs with orange (not blue) lines indicate instances where Segmented was not able to assign dual linear functions. In those cases Segmented breakpoints were based on total gas curves, except Figures A5.4.4b, h, & n, where methane curves (Figures A5.4.4r, x, & dd) were used.

Sandy Clay Loam



Sandy Clay Loam

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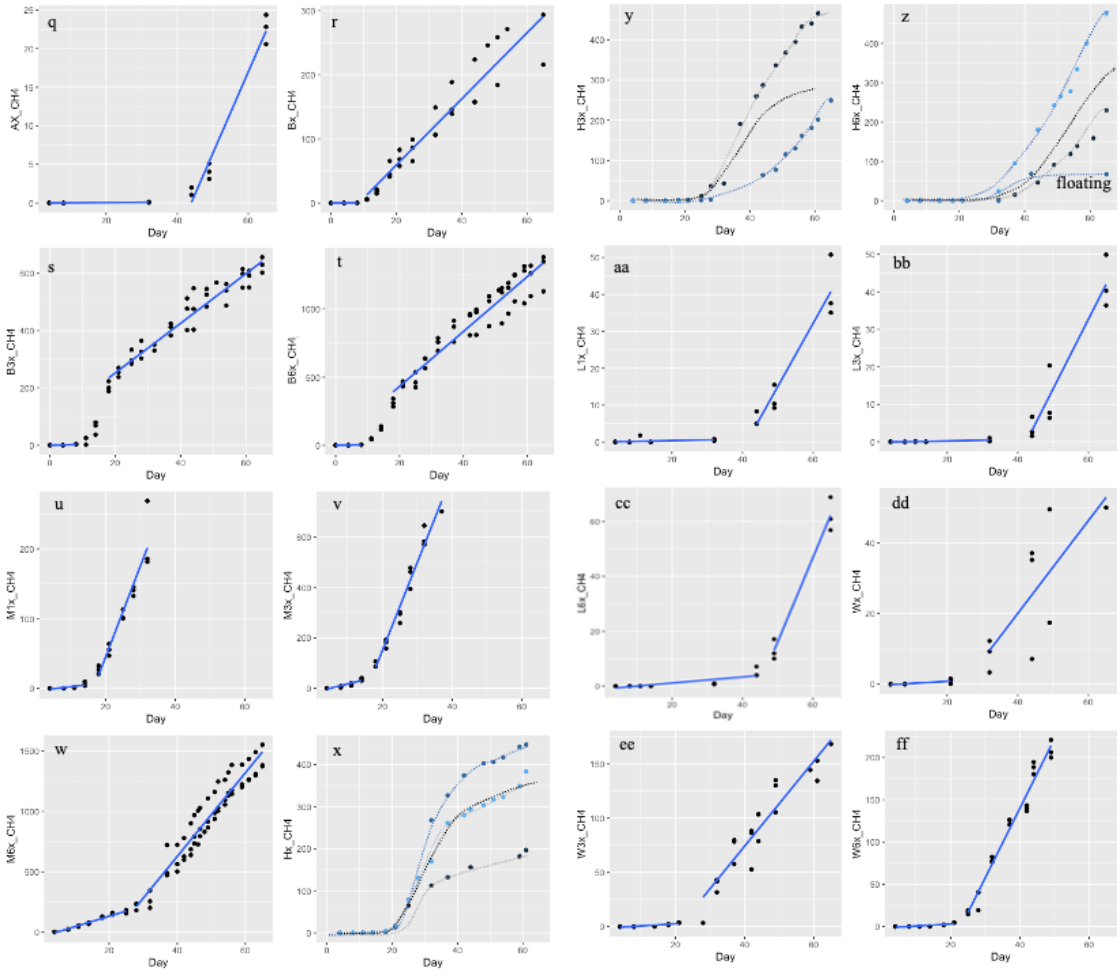
Sandy Loam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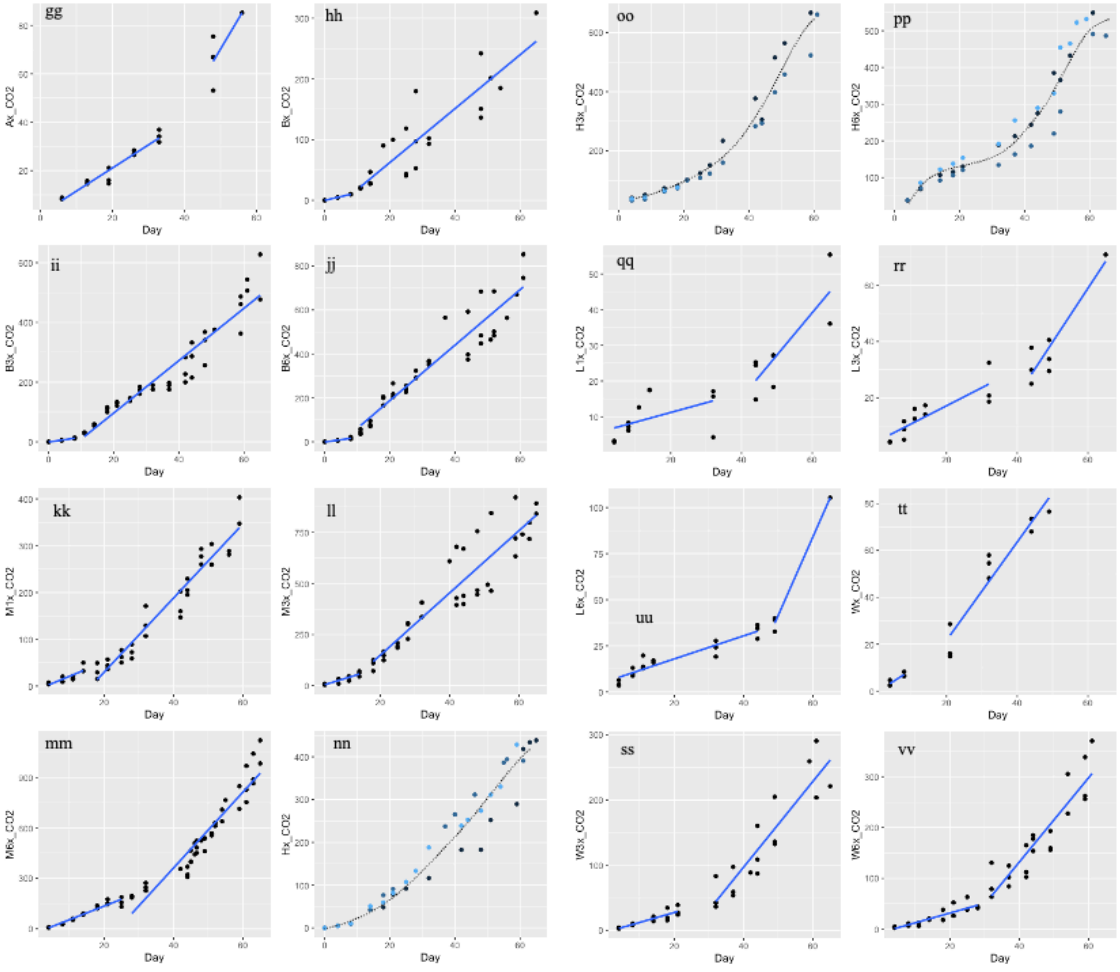
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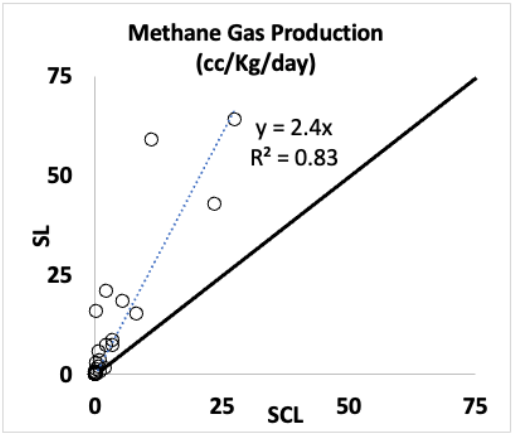


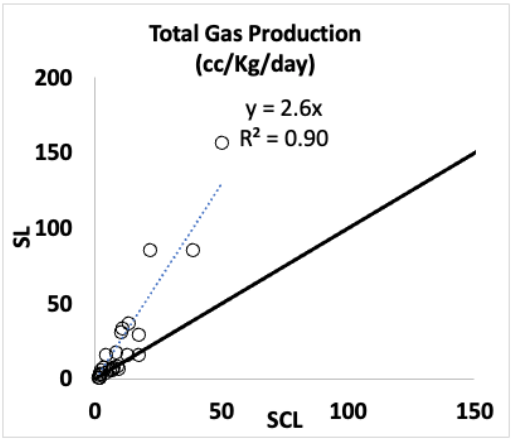


Figure A5.4. 4 – Experiment 1. Biogenic methane and total gas production rate in the SL soil versus the SCL mesocosms.

The SL mesocosms had, on average, 2.4 times higher methane gas production than the SCL. The SL mesocosms had, on average, 2.6 times higher total gas production than the SCL.

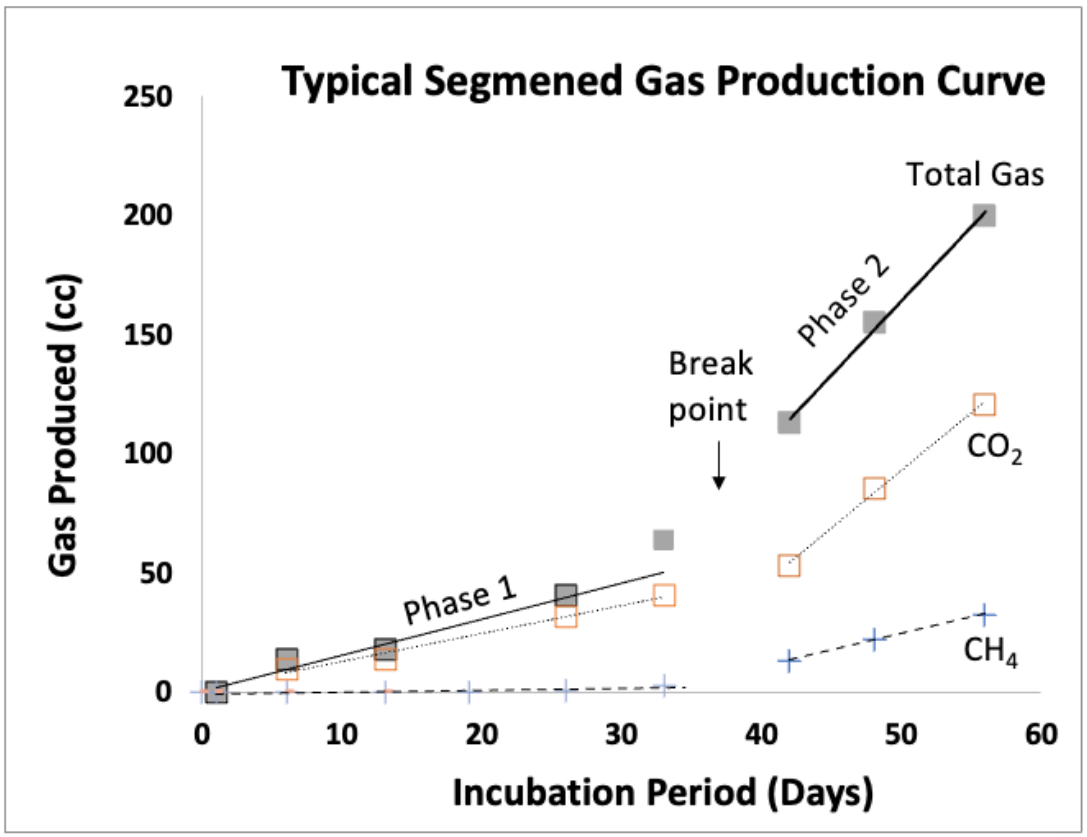


Figure A5.4. 5 – Typical gas saturated soils amended with organic matter (All Experiments).

Gases were best modeled using a segmented linear function. After a breakpoint the average total gas production increases by a factor of 5 whereas there is a sharp increase in methane production. Note that hay amended trials exhibited a typical sinusoidal pattern.

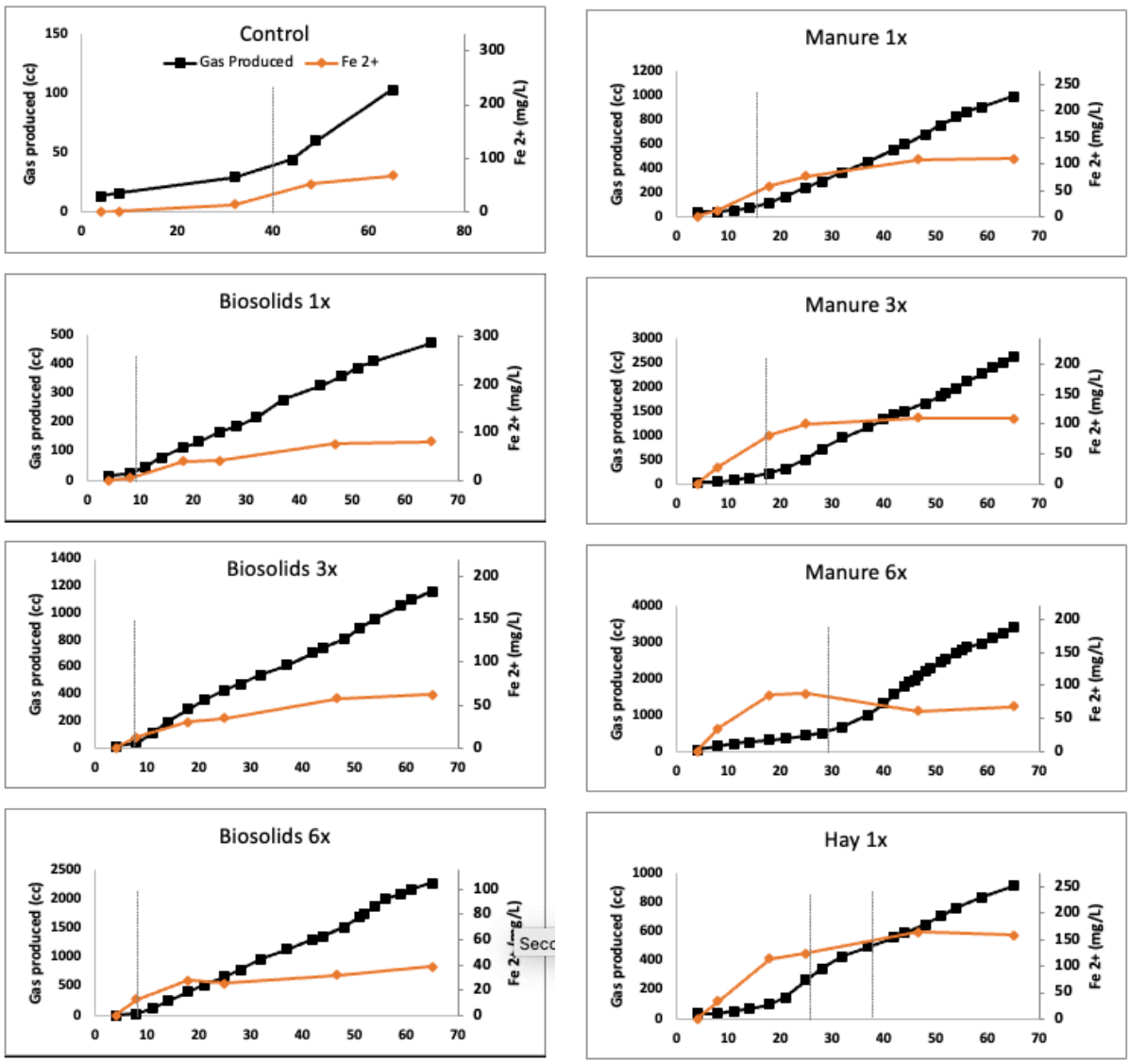
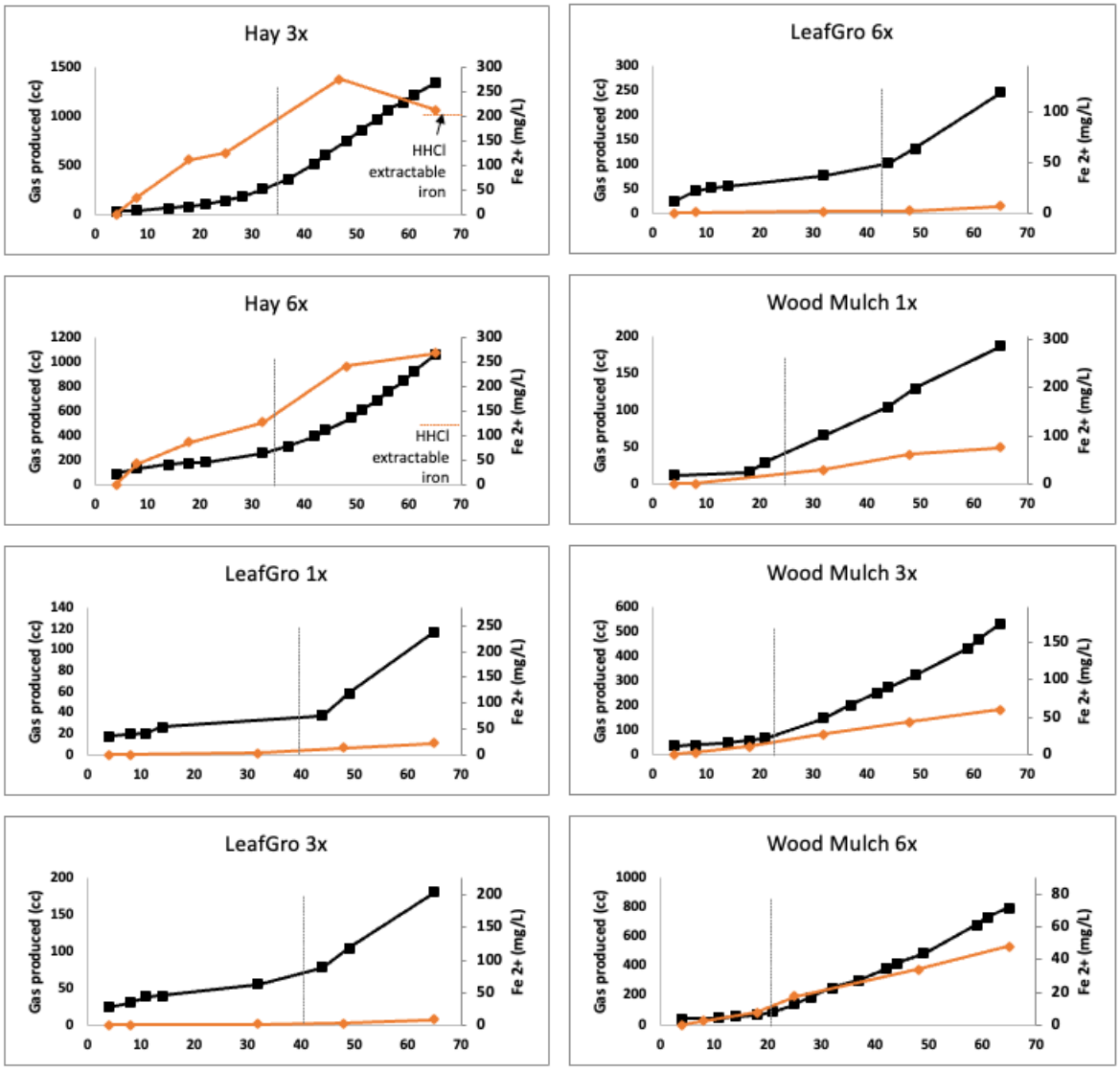


Figure A5.4. 6 – Experiment 1. Biogenic gas and ferrous iron versus time.

x-axis – incubation time (days). Soil – Sandy Loam

The maximum y axis value represents the theoretical maximum based on hydroxyl amine hydrochloride extractable iron oxides in the soil.

The vertical dashed line represents the breakpoint.


Hay 3x
Gas produced (cc)
Fe 2+ (mg/L)
HHCl extractable iron
LeafGro 6x
Hay 6x
HHCl extractable iron
Wood Mulch 1x
LeafGro 1x
Wood Mulch 3x
LeafGro 3x
Wood Mulch 6x

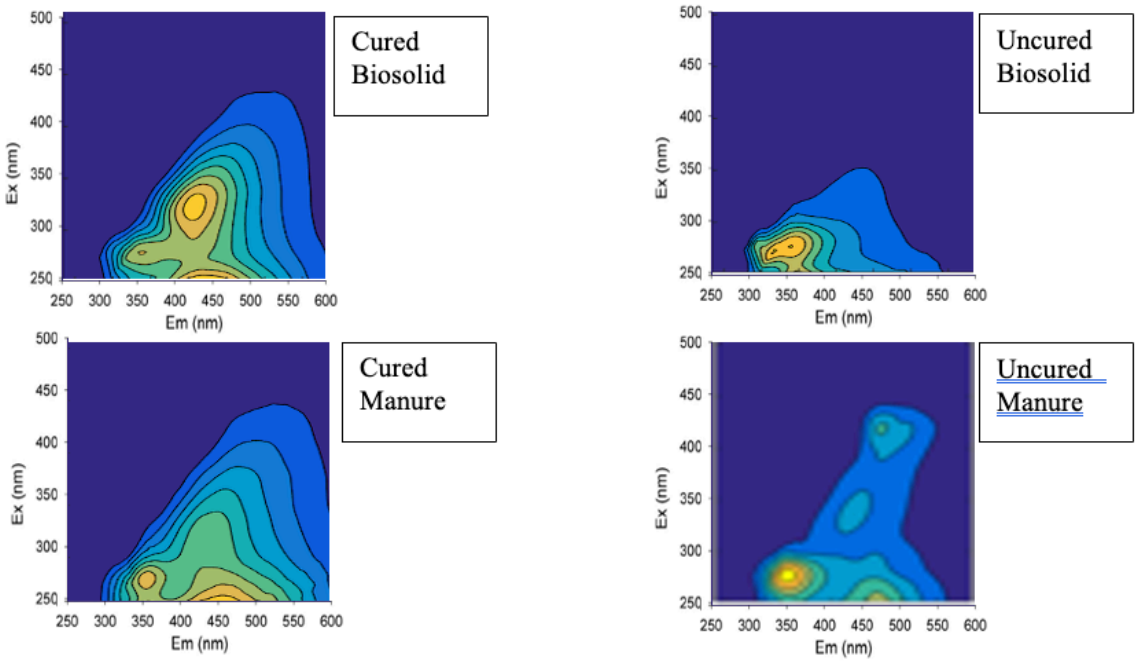


Figure A5.4. 7 – Experiment 2. Fluorescent spectral scans of amendments of organic amendments.

Scans used dissolved organic carbon from water extractions. The vertical axis is emission wavelength and the horizontal axis is the excitation wavelength. Colors represent emission intensity (blue = low, yellow = high). Dissolved organic carbon that is red-shifted, or stretched upward, is considered to have higher levels of organic acids and a lower nominal oxygen state and lower chemical energy.

For comparison figures that show typical fluorescent spectral pattern, see Yu et al. 2010 .

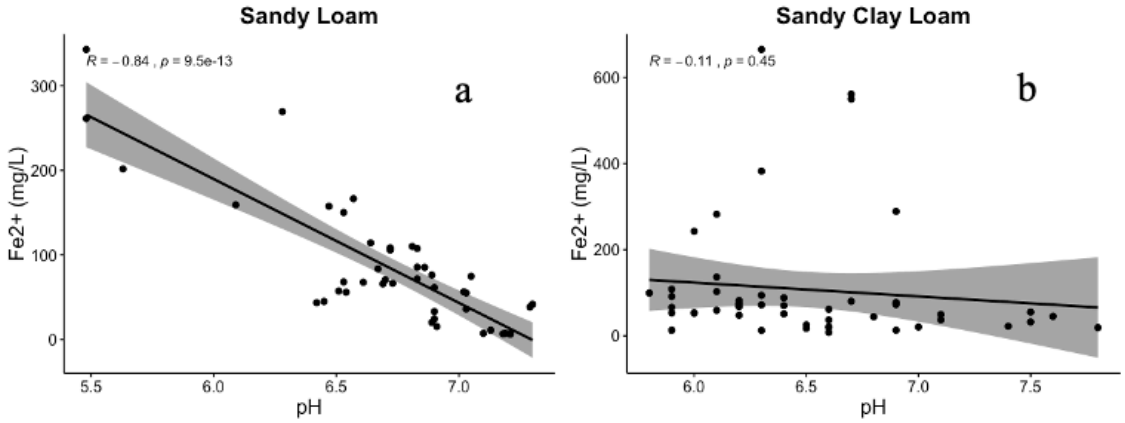


Figure A5.4. 8 – Experiment 1. Ferrous iron (Fe^{2+}) concentration versus pH. Values taken after 60 days of incubation of saturated soils with various organic matter amendments.

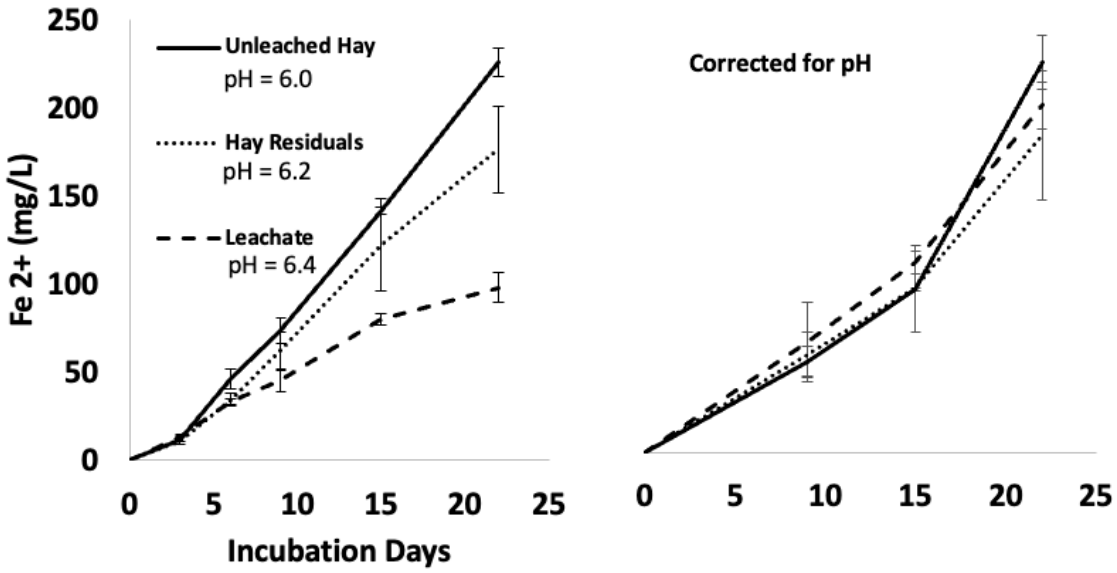


Figure A5.4. 9 – Experiment 4. Ferrous iron concentrations with hay leachate and residuals mathematically corrected for the pH

Observed pH and Fe^{2+} values for day 22 of the incubation are summarized in the table below: A linear regression of these values yields:
Fe = pH * (-115.7) + 815.5 r^2 = 0.73, p = 0.003
We corrected Fe values using a reference value of pH value, which was /an intermediate pH in preceding experiments.
$Fe_{(corrected)} = Fe_{(observed)} * ((6.2 - pH) * (-115.7))$
ANOVA analysis shows the $Fe_{(observed)}$ were different ($p < 0.05$), but $Fe_{(corrected)}$ were not ($p = 0.61$).
The pH correction would only be valid around the average Fe value of ~100 mg/L, so the correction factor was scaled based on the average Fe value at the time interval, or -115.7 * $Fe_{(observed\_average)}/100$.

pH	$Fe_{(observed)}$	$Fe_{(corrected)}$
6.08	132.8	120.34
5.96	121.6	95.26
5.96	112.8	86.46
6.16	100.8	97.59
6.18	102.4	101.50
6.24	108.8	114.84
6.38	73.6	95.83
6.38	79.2	101.43
6.35	67.2	85.96

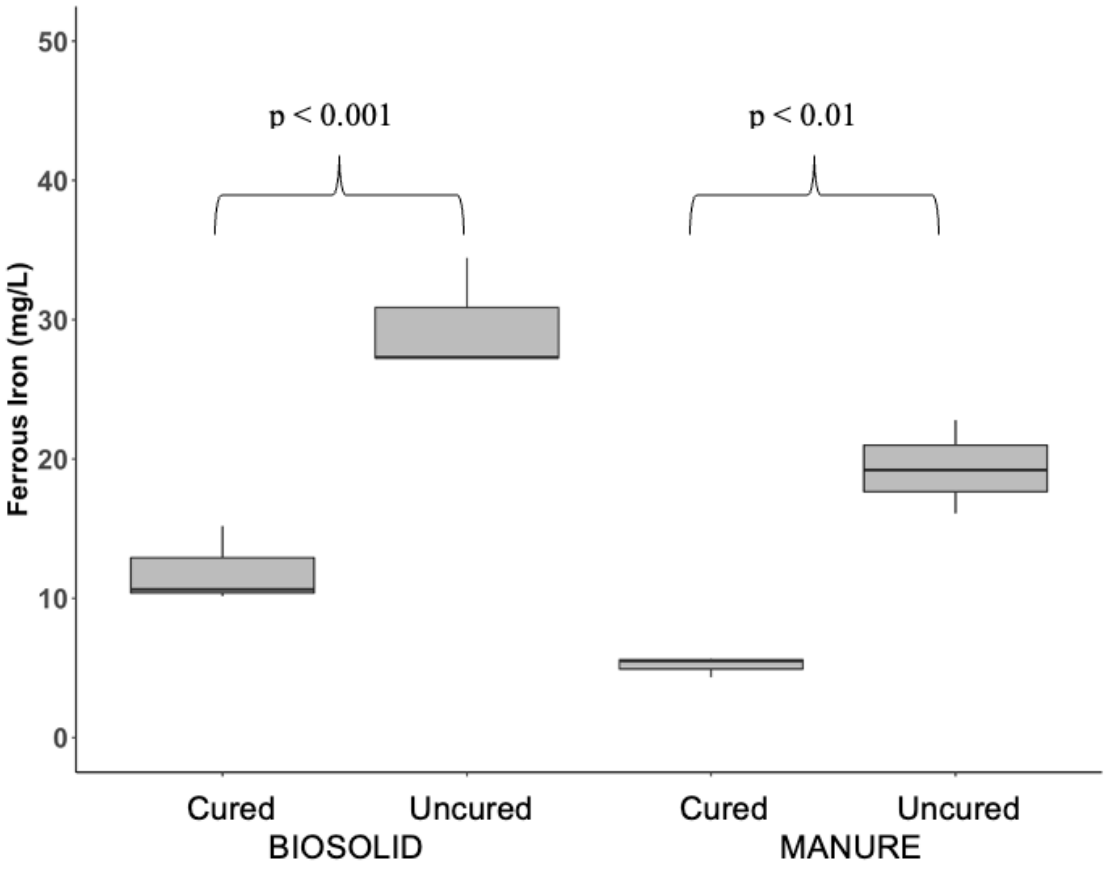


Figure A5.4. 10 – Experiment 2. Ferrous iron concentration in the liquid phase at the end of the incubation period (13 days).

Incubation was carried out with SL soil. The aged organic materials were from the same source but had been aged for at least 3 months. This Figure represents the same data shown in Figure 4.2, but were mathematically corrected for the pH relationship as described in Figure A5.4.9.

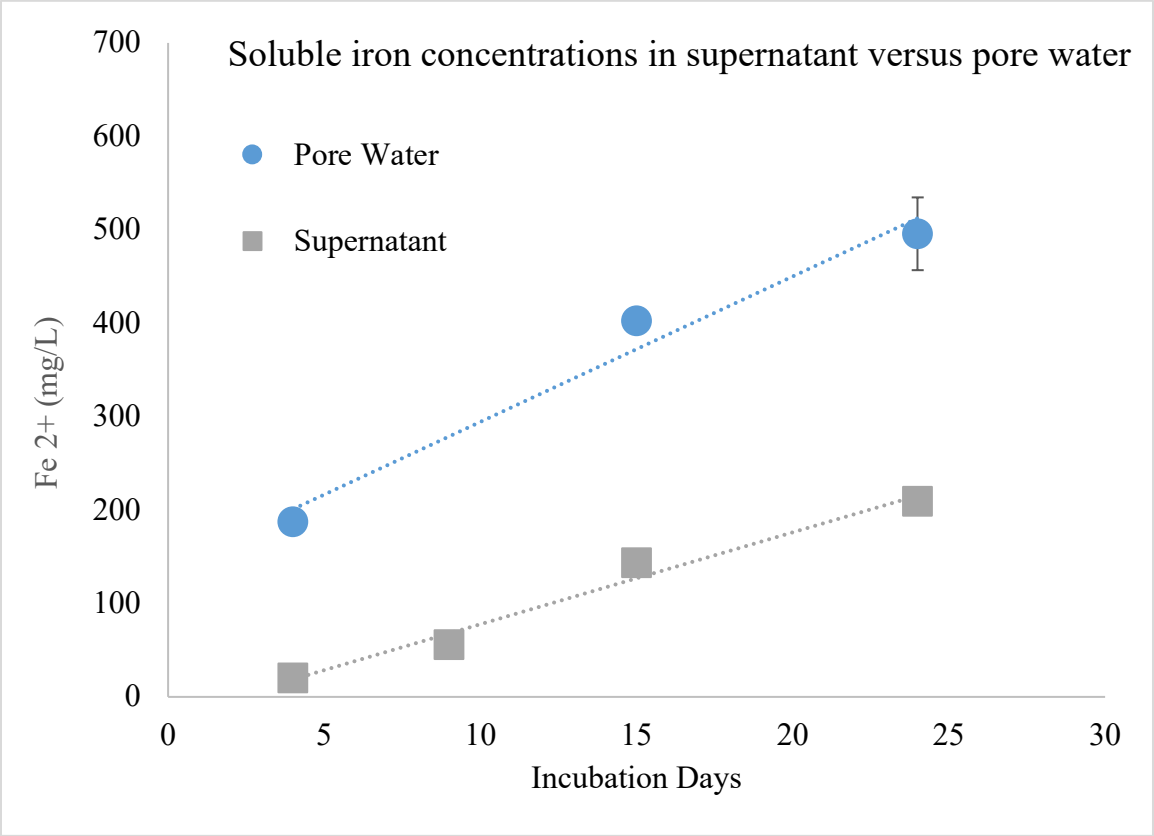


Figure A5.4. 11 – Concentration of Fe^{2+} in pore water versus supernatant.

Tables

Table A5.2. 1 – Studies used for the synthesis including type of amendment, amount and depth of amendment, and temporal sequence of adding amendment and taking subsequent measurements.

Publication	Amendment & Setting	Mixture Depth	Dose	Study Period
Hanson 1977	Sewage sludge Salt Marsh	15 cm	200g/m^2 2x per month 12 months	Start: January 1975 Sample: December 1975
Craft 1988	Topsoil	10cm	Unknown	1908 - 1984
Handa & Jefferies 2000	Composted O horizon material Salt Marsh	surface applied	5 mm	Start: June 1996 Sample: July 1996 - August 1997
O'Brien & Zedler 2006	Kelp compost Salt Marsh	30 cm	40l per 2.24 m^2	Start: December 2000 Sample: April - August 2002
*Pietrzykowski et al. 2015	Ground wood waste compost Tidal Freshwater swamp	15-25 cm	78 & 156 Mg/Ha (compost)	Start: April 2004 Sample: April 2007
*Pietrzykowski et al. 2015	Upland Topsoil (not SWT) + Ground wood waste compost Tidal Freshwater swamp	15-25 cm	78 & 156 Mg/Ha (compost)	Start: April 2004 Sample: April 2007
*Dickinson 2007	Ground wood waste compost Tidal Freshwater swamp	15 cm	78 & 156 Mg/Ha (compost)	Start: March - April 2004 Sample: September 2005 - July 2006
*Dickinson 2007	Upland Topsoil (not SWT) + Ground wood waste compost Tidal Freshwater swamp	15 cm	78 & 156 Mg/Ha (compost)	Start: March - April 2004 Sample: September 2005 - July 2006
*Ott 2018 Ch 2, 3. & 5	Composted yard waste Tidal Freshwater swamp	15 cm	78 & 156 Mg/Ha (compost)	Start: March - April 2004 Sample: 2016
*Ott 2018 Ch. 3 & 5	Upland Topsoil (not SWT) + Ground wood waste compost	15 cm	78 & 156 Mg/Ha (compost)	Start: March - April 2004 Sample: 2016

Publication	Amendment & Setting	Mixture Depth	Dose	Study Period
	Tidal Freshwater swamp			
Alsfeld et al. 2009	Unspecified OM amendments Freshwater palustrine	Not specified	Not specified	Start: 1989 - 2002 Sample: 2004 - 2005
Anderson and Cowell 2004	Wetland salvaged topsoil Freshwater palustrine	Surface applied	15-30 cm (specified in construction plans, not experimentally verified)	Start: 1994 or before Sample: November 1999 & June 2000
*Bruland et al. 2009	Wood compost Freshwater palustrine	10 cm	56, 112, 224, 336 Mg/Ha	Start: July 2002 Sample: July 2003
*Bergschneider 2005	Wood and yard waste compost Freshwater palustrine	15 cm	56, 112, 224, 336 Mg/Ha	Start: July 2002 Sample: January 2003 - October 2004
*Bailey et al. 2007	Wood and yard waste compost Freshwater palustrine	15 cm	56, 112, 224, 336 Mg/Ha	Start: July 2002 Sample: April - October 2005
*Winton and Richardson 2015	Wood and yard waste Freshwater palustrine	15 cm	56, 112, 224, 336 Mg/Ha	Start: July 2002 Sample: September 2011
*Ott 2018 Ch. 4 & 5	Wood and yard waste compost	15 cm	56, 112, 224, 336 Mg/Ha	Start: 2003 Sample: 2016
Ballantine et al. 2012 Ballantine et al. 2015	Straw Freshwater palustrine	10 cm	8 Kg C per 4 m^2 plot 441 g / Kg C ~9 Mg/Ha	Start: 2007 Sample: 2010
Ballantine et al. 2012 Ballantine et al. 2015	Topsoil Freshwater palustrine	10 cm	8 Kg C per 4 m^2 plot 6 - 198 g / Kg C No equivalent dose	Start: 2007 Sample: 2010
Ballantine et al. 2012 Ballantine et al. 2015	Biochar Freshwater palustrine	10 cm	8 Kg C per 4 m^2 plot 614 g / Kg C ~12 Mg/Ha	Start: 2007 Sample: 2010
Yao et al. 2018	Straw Freshwater palustrine	10 cm	8 Kg C per 4 m^2 plot 6 - 198 g / Kg C ~9 Mg/Ha	Start: July 2007 Sample: May 2013
Yao et al. 2018	Topsoil Freshwater palustrine	10 cm	8 Kg C per 4 m^2 plot 614 g / Kg C No equivalent dose	Start: July 2007 Sample: May 2013
Yao et al. 2018	Biochar Freshwater palustrine	10 cm	8 Kg C per 4 m^2 plot 441 g / Kg C	Start: July 2007 Sample: May 2013

Publication	Amendment & Setting	Mixture Depth	Dose	Study Period
			~12 Mg/Ha	
Brown and Bedford 1997	Wetland salvaged topsoil (seed bank) Freshwater marshes	15 cm	15 cm	Start: 1992 Sample: 1994 & 1995
Bruland and Richardson 2004	Upland Topsoil Freshwater riverine and palustrine	15 cm	15 cm	Start: varied - site ages 4 to 16 years Sample: 2002
Bruland and Richardson 2004	Leaf compost Freshwater riverine and palustrine	30 cm	30 cm	Start: varied - site ages 4 to 16 years Sample: 2002
Bruland and Richardson 2004	Sawdust Freshwater riverine and palustrine	30 cm	30 cm	Start: varied - site ages 4 to 16 years Sample: 2002
Doherty & Zedler 2015	Woodchips Freshwater Marsh	16 cm mound	50/50 mix soil + woodchips	Start: April 2012 & 2013 Sample: June - August 2013
Gabor et al. 1994	Alfalfa meal Freshwater lacustrine	Surface applied	2L leached to 6,200 mg/L Dissolved Organic N	Start: June 1989 Sample: August 1990
Gibson et al. 1994	Alfalfa, straw Salt Marsh	15 cm	3 Kg/m^2 ~15 Mg/Ha	Start: February 1990 Sample: September 1990 and October 1991
Morrissey & Franklin 2015	Plant Litter Tidal freshwater	Litterbags buried 5 - 15 cm	OM % increased from 8% to 16%	Start: February 1990 Sample: September 1990 and October 1991
Morrissey & Franklin 2015	(undefined) Compost Tidal freshwater	Litterbags buried 5 - 15 cm	OM % increased from 8% to 16%	Start: January 2011 Sample: January 2012
*Stauffer & Books 1997	Leaf Litter (Compost) Freshwater plaustrine	15 cm	10 - 15 cm	Start: 1991 Sample: July-August, 1991 & 1992
*Stauffer & Books 1997	Salvaged Marsh Soil (SMS) Freshwater plaustrine	15 cm	15 cm	Start: 1991 Sample: July-August, 1991 & 1992
Stolt 1998	Maple leaves Freshwater palustrine	15 cm	26 g C / Kg soil in buried leaf bags	Start: 1993 Sample: May 1994 -June 1995

Publication	Amendment & Setting	Mixture Depth	Dose	Study Period
Hossler and Bouchard 2010	Preserved wetland soil Freshwater palustrine	not specified	not specified	Varied Sites were 3 - 8 years old
Sutton-Grier et al. 2009	woodchips + biosolids Freshwater riverine	20 cm	Varied: final SOM % 5.9 - 24.9%	Start: July 2004 Sample: September 2004, 2005, 2006

Table A5.2. 2 – Metrics used for scoring.

Positive results were in comparison to an unamended control and were statistically significant by ANOVA or showed an increasing trend.

Criteria	Metric for positive score
Plants	
Biomass	Higher biomass (root or shoot, scored separately).
Plant size	Larger plant size.
Growth	Higher biomass or relative growth rate.
Basal area	Larger basal area or basal trunk swelling.
Tree height	Taller trees.
Diameter	Larger trunk diameter, may be measure at breast height or crown diameter.
Leaf length	Longer leaf length.
Branch count	Higher branch count.
Roots	Higher total mean root length, average root length, root diameter or root count.
Survivorship	More plants survived.
Stem Density	Higher stem density.
Percent cover	Higher percent cover or stem density.
Prevalence	Higher prevalence index.
Flowering	More flowers produced.
Shannon (diversity) index	Higher diversity as measured by the Shannon index.
Richness	Higher species richness or number of species.
Evenness	Higher evenness value.
Total species	More total species.
Soil Properties	
Nitrogen fixation	Increase in measured N fixation or N mineralization rate.
Nitrogen (as nutrient)	Increase in total nitrogen, TKN or ammonia (each scored separately)
Bulk Density	Decrease in bulk density to a value below rooting threshold.
Moisture	Increase in soil moisture or water holding capacity.
Cation Exchange Capacity	Increase in CEC.
Major nutrients	Higher levels of phosphorous, calcium, magnesium, potassium, iron. manganese (each scored separately).
Minor nutrients	Higher micronutrients. All micronutrients grouped together and averaged.
P sorption index	Lower PSI
Carbon	
Soil organic carbon	Total carbon (as C or OM) compared to control. Where possible, positive scores were only assigned if we could verify a long-term increase in soil organic carbon or increasing trend.
Nitrogen	
Denitrification	Increase in denitrification enzyme assay or denitrification potential Decrease in nitrate concentration. Increase in denitrifier abundance.
Anaerobic parameters	
Redox potential	Lower redox potential.
Microbial biomass	Higher microbial biomass carbon (assumed to be anaerobic organisms). Higher microbial nitrogen.
Methane	Lower methane emissions.
Hydric Soil Indicators	Developed redoximorphic soil features recognized by the Technical Standard. All Technical Standard criteria equal.
Soil Color	Increase in Munsell value, decrease in chroma.

Table A5.2. 3 – Studies used for the synthesis including details for assigned values by category.

Table abbreviations (in order of appearance): N – Nitrogen: OM – organic matter (percent): Db – bulk density: TKN – Total Kjeldahl Nitrogen: P – phosphorous: K – potassium: Ca – calcium: Mg – magnesium: Fe – iron: C – carbon (percent): SOC – soil organic carbon: Mg/ha – megagram per hectare: DEA – denitrification enzyme assay: NH4 – ammonia: Mn - manganese

Publication	Explanation	Plants	Soil Properties	Carbon	Denitri-fication	Anaerobic Parameters
Hanson 1977	Soil properties: N fixation rates (+1), total N (+1)		1.00			
Craft 1988	Carbon: Total C and C accumulation rate (+1) Soil Factors: Total N and N accumulation rate (+1); Total P and P accumulation rate (+1); Db (+0)		0.6667	1		
Handa & Jefferies 2000	Plant response: basal area of Puccinellia phryganodes (+1), biomass of Puccinellia phryganodes (+0), biomass of Carex subspathacea (+0) Anaerobic properties: redox potential (+0).	0.33				0.00
O'Brien & Zedler 2006	Kelp compost compared to rototilled plots. Plants: plant size (+0), growth (+1/5 - Batis maritima only improved); survivorship (+0) Carbon: percent OM (+0) Soil properties: Db 0-5cm (+1), Db 5-8cm (+1), moisture 0-5cm (+0), moisture 5-8cm (+1), TKN (+1)	0.07	0.80	0.00		
*Pietrzykowski et al. 2015	Plants: tree height (+0), diameter (+0), diameter at breast height (+0), basal trunk swelling (+0).	0.00				
*Pietrzykowski et al. 2015	Plants: tree height (+0), diameter (+0), diameter at breast height (+0), basal trunk swelling (+0).	0.00				
*Dickinson 2007	2 loading rates for some parameters Plants: trunk diameter (+0, +0), crown diameter (+0), branch count (+0), evenness (+0, +0), Shannon index(+0, +0), Total Species (+0, +0), richness (-1, -1), total mean root length (+0), average root length (+0), root diameter (+0), root count (+0) Soil factors: P (+0), K(+0), Ca (+0.5), Mg (+0.5), Fe(+0.5), micronutrients (+0.5)	-0.14	0.33			

Publication	Explanation	Plants	Soil Properties	Carbon	Denitri-fication	Anaerobic Parameters
*Dickinson 2007	Plants: trunk diameter (+1), crown diameter (+0), branch count (+0), diversity/richness (-1), total mean root length (+0), average root length (+0), root diameter (+0), root count (+0) Soil factors: P (-1), K(+1), Ca (+1), Mg (+1), Fe(+1), micronutrients (+0.5)	0.00	0.58			
*Ott 2018 Ch 2, 3. & 5	Anaerobic properties: Soil color (Table 2.3) (+0), Hydric soil indicator (Table 2.4) 2 of 5 locations in the control met hydric soil indicators, 1 of 4 amended locations met hydric soil indicators at both doses (-1, -1) Soil factors (Table 3.3 and Figure 3.2): Db (+0, +0), P (+0), K (+0), Ca (+1), Mg (+1), Fe (+0), micronutrients (Table 3.2) (+0), %N (+0) Carbon (Table 5.4): % C (+0)		0.11	0.00		-0.33
*Ott 2018 Ch. 2, 3 & 5	Anaerobic properties: Soil color (Table 2.3) increase in value decrease in chroma (+1), Hydric soil indicator (Table 2.4) 2 of 5 locations in the control met hydric soil indicators, 4 of 4 amended locations met hydric soil indicators (+1) Soil factors (Table 3.3 and Figure 3.2): Db (+1), P (+0), K (+0), Ca (+1), Mg (+1), Fe (+0), micronutrients (Table 3.2) (+0), %N (+0) Carbon (Table 5.4): % C (+1)		0.38	1.00		1.00
Alsfeld et al. 2009	Plants: comparison of 20 sites, 12 with OM and 8 w/out OM; multiple richness and cover % metrics not significant (+0) Carbon: % OM across 20 sites (+0)	0.00		0.00		
Anderson and Cowell 2004	Plants: percent coverage (+0), prevalence (+1); richness (+0); evenness (+0), Shannon index (+0); biomass (+0) Soil properties: P (+0), Mg (+1), Ca (+1), K (+1) Carbon: "highly significant difference (t-test, p , 0.001) was found between the mean SOC in mulched (5.9 ± 0.5%) and non-mulched (2.6 ± 0.3%)"	0.17	0.75	1.00		
*Bruland et al. 2009	Soil properties: Db (+1), P (+1) Anaerobic properties: Microbial biomass carbon - 4 loading rates, 2 sites (+0, +0, +0.5, +0) Carbon: SOC (+0, +0, +0, +1, +0, +0, +1, +1) - "the control treatments had lower SOC than the 336 Mg/ha treatments in both zones and the 224 Mg/ha treatment in the wetter zone" Nitrogen: DEA - 4 loading rates, 2 sites (+0, +1, +0, +0; +0,+0,+1,+0)		1.00	0.38	0.25	0.13

Publication	Explanation	Plants	Soil Properties	Carbon	Denitri-fication	Anaerobic Parameters
*Bergschneider 2005	Carbon: total C - initial increase followed by decline in 4 loading rates whereas control showed steady increase (-1, -1, -1, -1)			-1.00		
*Bailey et al. 2007	Plants: average across 4 treatment levels: Richness (-1/4), Evenness (+0), Shannon Index (-1/4), biomass (+0). Soil properties: P (+0.5) - 2 of 4 treatment levels ; total N 4 loading rates (+1, +1, +1, +1) Carbon: total C compared to Bergschneider 2005 - 4 levels (-1, -1, +1, +1)	-0.13	0.75	0.00		
*Winton and Richardson 2015	Carbon: organic C (-1) Compared to Bailey 2005 Anaerobic properties: methane (+0)			-1.00		1.00
*Ott 2018 Ch. 4 & 5	Carbon: Soil Mass C (Table 4.9) - Compared C ratios compared to those observed by Winton and Richardson (2015) - (+1, -1, -1, -1) Anaerobic properties: (Table 4.5) Treated and untreated samples tested positive for hydric soil metric (+0) Soil Properties (Figure 4.5): Db (+1, +1 +1, +1, +1, +1, +0, +1) Soil properties (Table 4.11): P (+0.25), Mg (+0.5), Ca (+0.75), K (+0), Fe (+0.5), micronutrients (+0.32) Soil properties (Table 4.12): P (+0.25), Mg (+0), Ca (+0.5), K (+0), Fe (+0.75), micronutrients (+0.08) Plants (Figure 5.5): (+0, +0, +1, +1).	0.50	0.53	-0.50		0.00
Ballantine et al. 2012 Ballantine et al. 2015	Plants: biomass (+0) Anaerobic properties: microbial biomass (+1), methane (-1) Carbon: total C (+0) control lost C with time, treatment also decreased with time Soil properties: Db -4 sites (+0, +0, +0, +0), moisture (+1), CEC (+0), increased in total N (+0.5).	0.00	0.29	0.00		0.00
Ballantine et al. 2012 Ballantine et al. 2015	Plants: biomass (+0) Anaerobic properties: microbial biomass (+1), methane (-1) Carbon: total C (+1) control lost C with time, treatment increased with time Soil properties: Db -4 sites (+0, +0, +0, +1), moisture (+1), CEC (+0); increased in total N (+0.5).	0.00	0.43	1.00		0.00

Publication	Explanation	Plants	Soil Properties	Carbon	Denitri-fication	Anaerobic Parameters
Ballantine et al. 2012 Ballantine et al. 2015	Plants: biomass (+0) Anaerobic properties: microbial biomass (+0), methane (-1) Carbon: total C (+0) control lost C with time, treatment also decreased with time Soil properties: Db -4 sites (+0, +0, +0, +1), moisture (+0.5), CEC (+0); increased in total N (+0.5).	0.00	0.36	0.00		-0.50
Yao et al. 2018	Nitrogen: denitrification potential (+1) Carbon: total C (+0) Soil factors: ammonia (+1), nitrate (+0), N-mineralization (+0), potential nitrification (+0). Anaerobic properties: soil microbial carbon (+0), soil microbial nitrogen (+0)		0.25	0.00	1.00	0.00
Yao et al. 2018	Nitrogen: denitrification potential increased, but 50x below natural wetlands (+0.5) Carbon: total C (+1) Soil factors: ammonia (+0), nitrate (+0), N-mineralization (+0), potential nitrification (+0) Anaerobic properties: soil microbial carbon (+0), soil microbial nitrogen (+1)		0.00	1.00	1.00	0.50
Yao et al. 2018	Nitrogen: denitrification potential increased, but 50x below natural wetlands (+0.5) Carbon: total C (+1) Soil factors: ammonia (+1), nitrate (+0), N-mineralization (-1), potential nitrification (+0) Anaerobic properties: soil microbial carbon (+0), soil microbial nitrogen (+0)		0.00	1.00	1.00	0.00
Brown and Bedford 1997	Plants: number of species (+1); percent cover (+1)	1.00				
Bruland and Richardson 2004	Soil properties: Moisture (-1), Db (+0), WHC (+1), P sorption index (+1) Carbon: SOC (-1) Anaerobic properties: Microbial biomass carbon (+0).		0.25	-1.00		1.00

Publication	Explanation	Plants	Soil Properties	Carbon	Denitri-fication	Anaerobic Parameters
Bruland and Richardson 2004	Results from Table 4. Compared to TS amended plots. BoBi (site name) + leaf compost Soil properties: Moisture (+1), Db (+1), WHC (+1), P sorption index (-1) Carbon: SOC (+1) Anaerobic properties: Microbial biomass carbon (+1).		0.38	1.00		1.00
Bruland and Richardson 2004	Results from Table 4. Compared to TS amended plots. StCr (site name) + sawdust Soil properties: Moisture (+1), Db (+0), WHC (-1), P sorption index (+1) Carbon: SOC (+1) Anaerobic properties: Microbial biomass carbon (+1).		0.40	1.00		1.00
Doherty & Zedler 2015	Woodchips were compared to medium soil mounds (of the same height). Soil factors: moisture (+0) Plants: survival (+0), flowering (-1), percent cover (-1); shoot biomass (+0), root biomass (+0), leaf length (+0), relative growth rate (+0)	-0.29	0.00			
Gabor et al. 1994	Soil factors: P (+0), N (+0) Nitrogen: dissolved N (+0)		0.00		0.00	
Gibson et al. 1994	Carbon: SOC (+0) Soil factors: TKN (+0)		0.00	0.00		
Morrissey & Franklin 2015	Nitrogen: denitrification potential (+0), denitrifier abundance (+0) Carbon: SOC (-1) - decreasing trend with time (Figure 1a) Soil factors: moisture (+0), ammonia (+0), nitrate (+0), N-mineralization (+0) Anaerobic properties: Redox (+0)		0.00	-1.00	0.00	1.00
Morrissey & Franklin 2015	Nitrogen: denitrification potential (+0), denitrifier abundance (+1) Carbon: SOC (-1) - decreasing trend with time (Figure 1a) Soil factors: moisture (-1), ammonia (+0), nitrate (+0) Anaerobic properties: Redox (+0)		-0.33	-1.00	0.50	1.00
*Stauffer & Books 1997	Plants: survival rate (+1) Carbon: %OM (+1) Soil factors: P (+0), K (+0), Ca (+0), Mg (+0), %N (+0), NH4 (+1)	1.00	0.17	1.00		

Publication	Explanation	Plants	Soil Properties	Carbon	Denitri-fication	Anaerobic Parameters
*Stauffer & Books 1997	Plants: stem density (+0), % coverage (+1), prevalence index (+0); Shannon Index (+0), richness (+1) Carbon: %OM (+1) Soil factors: P (+0), K (+0), Ca (+0), Mg (+1), %N (+0), NH4 (+0)	0.40	0.17	1.00		
Stolt 1998	Plants: roots (+0) - circumstantial evidence of Increased root growth. Carbon: organic C: (-1) Unamended peds increase C: amended peds lost C Soil factors: Fe (-1), Mn (-1) Anaerobic properties: redoximorphic features (+1)	0.00	-1.00	-1.00		1.00
Hossler and Bouchard 2010	Carbon: organic C: (+1) Site with wetland topsoil had significantly higher SOC, comparable to natural wetlands Anaerobic properties: microbial biomass (+0) Soil properties: Db (+0.5)		1.00	1.00		0.00
Sutton-Grier et al. 2009	Plants: leaf %N (+0), biomass (+0), richness (-1) Nitrogen: denitrification potential (+0.47). Of 19 values reported, 2 were at the control value and 4 were below (13*1 + 2*0 + 4*-1 = 9/19) Anaerobic properties: microbial biomass (+1)	-0.33	1.00		0.47	1.00

Table A5.2.3 (continued). Average scores were used for Figures 2.1 & 2.3.

		All Categories	Plants	Soil Properties	Carbon	Denitri-fication	Anaerobic Parameters
	Number of studies with positive average metric values		7	21	12	6	11
	Number of studies with negative average metric values		4	2	7	0	2
	Number of studies with 0 as the average metric value		8	6	8	2	7
	Total number of values	103	19	29	27	8	20
	Sum of all positive values		3.47	11	11	4.22	9.63
	Sum of all negative values		-0.89	-1.33	-6.50		-0.83
	Sum of all values	30.6	2.58	10.13	4.88	4.22	8.79
	Average of positive values		0.50	0.55	0.95	0.70	0.88
	Average of negative values		-0.22	-0.67	-0.93		-0.42
Scores (Sum of all values divided by total number of values)							
All categories		0.30	0.14	0.35	0.18	0.53	0.44
Supplemental Table 1 lists amendments (TS* or alOM**) for all studies							
Studies using TS		0.53	0.26	0.47	0.75	1.00	0.50
Studies using alOM		0.22	0.14	0.30	-0.06	0.46	0.42
Studies with initial SOC < 2.5%***		0.31	0.15	0.39	0.30	0.81	0.16

* TS - topsoil
** alOM – allochthonous organic matter
*** SOC – soil organic carbon

Table A5.2. 4 – Plants scores divided into subcategories of biomass, survival and diversity.

Publication	Overall Plants	Survival	Diversity	Biomass	Plant Response by sub-category
Handa & Jefferies 2000	0.33			0.33	Biomass: basal area of Puccinellia phryganodes (+1), biomass of P. p. (+0), biomass of Carex subspathacea (+0)
O'Brien & Zedler 2006	0.07	0.00		0.10	Biomass: plant size (+0), growth (+1/5 - Batis maritima only improved) Survivorship (+0)
*Pietrzykowski et al. 2015	0.00			0.00	Biomass: tree height (+0), diameter (+0), diameter at breast height (+0), basal trunk swelling (+0).
*Pietrzykowski et al. 2015	0.00			0.00	Biomass: tree height (+0), diameter (+0), diameter at breast height (+0), basal trunk swelling (+0).
*Dickinson 2007	-0.14		-0.25	0.00	Biomass: trunk diameter (+0, +0), crown diameter (+0), branch count (+0), total mean root length (+0), average root length (+0), root diameter (+0), root count (+0) Diversity: evenness (+0, +0), Shannon index(+0, +0), Total Species (+0, +0), richness (-1, -1)
*Dickinson 2007	0.00		-1.0	0.14	Biomass: trunk diameter (+1), crown diameter (+0), branch count (+0), total mean root length (+0), average root length (+0), root diameter (+0), root count (+0) Diversity: diversity/richness (-1)
Alsfeld et al. 2009	0.0		0.00		Diversity: comparison of 20 sites, 12 with OM and 8 w/out OM; multiple richness metrics not significant (+0) Survival: cover % metrics not significant (+0)
Anderson and Cowell 2004	0.17	0.00	0.25	0.00	Survival: percent coverage (+0) Diversity: richness (+0); evenness (+0), Shannon index (+0), prevalence (+1) Biomass: (+0)
*Bailey et al. 2007	-0.13		-0.17	0.00	Diversity: average across 4 treatment levels: Richness (-1/4), Evenness (+0), Shannon Index (-1/4) Biomass: (+0).
*Ott 2018 Ch. 4 & 5	0.5			0.50	Biomass: (Figure 5.5) (+0, +0, +1, +1)
Ballantine et al. 2012 Ballantine et al. 2015	0.00			0.00	Biomass: (+0)

Publication	Overall Plants	Survival	Diversity	Biomass	Plant Response by sub-category
Ballantine et al. 2012 Ballantine et al. 2015	0.00			0.00	Biomass: (+0)
Ballantine et al. 2012 Ballantine et al. 2015	0.00			0.00	Biomass: (+0)
Brown and Bedford 1997	1.00	1.00	1.00		Survival: percent cover (+1) Diversity: number of species (+1)
Doherty & Zedler 2015	-0.29	-0.67		0.00	Survival: Survival (+0), flowering (-1), percent cover (-1) Biomass: shoot biomass (+0), root biomass (+0), leaf length (+0), relative growth rate (+0)
*Stauffer & Books 1997	1.00	1.00			Survival: survival rate (+1)
*Stauffer & Books 1997	0.40	1.00	0.33	0.00	Biomass: stem density (+0) Survival: % coverage (+1) Diversity: prevalence index (+0); Shannon Index (+0), richness (+1)
Stolt 1998	0.00			0.00	Biomass: roots (+0) - circumstantial evidence of Increased root growth.
Sutton-Grier et al. 2009	-0.33		-1.00	0.00	Biomass: (+0) Diversity: richness (-1)
Total of values	2.58	2.33	-0.83	1.08	
Number of values	19	6	8	16	
Scores	**0.14**	**0.39**	**-0.10**	**0.07**	

Table A5.2. 5 – Denitrification Rates.

Study	Denitrification Rate (as reported)	Denitrification converted to ng N / g / hour	Reference Rate ng N / g / hour
Bruland et al. 2009 Wood Compost	350 – 850 ng N_2O cm^3 $hour^{-1}$	170 - 420[1]	170 – 200 (Unamended Control)
Morrisey & Franklin 2015 Plant Litter	90 pmol N g^{-1} $hour^{-1}$	1.3[2]	1.2 (Unamended Control)
Morrisey & Franklin 2015 Compost	280 pmol N g^{-1} $hour^{-1}$	4[2]	
Sutton-Grier et al. 2009 Topsoil/Wood/Biosolids	0 - 630 ng N g^{-1} $hour^{-1}$	0 - 630	10^5 (Unamended Control?)
Yao et al. 2018 Straw	18 µg N Kg^{-1} $hour^{-1}$	18[3]	5.5 (Unamended Control)
Yao et al. 2018 Topsoil	45 µg N Kg^{-1} $hour^{-1}$	45[3]	
Yao et al. 2018 Biochar	62 µg N Kg^{-1} $hour^{-1}$	62[3]	3,370 (Natural Site)
Selected studies including restored and natural sites (to estimate expected range of denitrification)			
Bruland et al. 2006 RoBr site	10 (23)[4] ng N_2O cm^3 $hour^{-1}$	6	30
Bruland et al. 2006 GrLa site	10 (40) ng N_2O cm^3 $hour^{-1}$	4.5	44
Bruland et al. 2006 ABC site	75 (120) ng N_2O cm^3 $hour^{-1}$	40	150
Bruland et al. 2006 DiSw site	95 (50) ng N_2O cm^3 $hour^{-1}$	112	265
Hunter & Faulkner 2001	65 (350) ng N_2O g^{-1} $hour^{-1}$	41	223
Selected studies providing denitrification rates in reference wetlands			
Gutknecht et al. 2006	16 (ave, n=7) mgN g^{-1} day^{-1}	--	673[6]
Groffman et al. 1996	2,150 (ave, n=12) µg N Kg^{-1} $hour^{-1}$	--	2,150
D'Angelo & Reddy 1999 (organic soils)	2.24 µmol N g^{-1} day^{-1}	--	1,306[7]
D'Angelo & Reddy 1999 (mineral soils)	0.86 µmol N g^{-1} day^{-1}	--	502

[1] 28g N_2 / 44 g N_2O, 1cm^3 / 1.3g

[2] 0.014 ng / pmol
[3] Units Equivalent
[4] Numbers in parenthesis natural sites. 28g N_2 / 44 g N_2O, Bulk densities (g / cm^3): RoBr 1.0 (0.53); GrLa 1.4 (0.58); 1.2 (0.5); 0.54 (0.12)
[5] Estimated based on sample with lowest %OM.
[6] Excluding Groffman et al. 1996 and D'Angelo & Reddy 1999, tabulated separately.
[7] 14,000 ng / μmol, 1 day / 24 hour

Table A5.3. 1 – Soil profiles descriptions.

The wetland mitigation site was constructed by stripping and stockpile existing topsoil and re-grading to create the desired contours. Then the entire site was mulched with blended combination of topsoil from the site, salvaged topsoil from a former wetland, and organic matter (composted wood chips). This blend created a sandy clay loam A horizon with Munsell soil color 10YR 3/2.

Plot Location	Mulch depth (cm)	Subsoil (immediately below mulch)	Most likely original soil series
Pond-A	6	Silty clay loam 2.5YR 5/1 2.5YR 4/6 concentrations	Elkton Btg
Pond-B	3	Sandy clay 2.5Y 4/1 5Y 4/6 concentrations	Fallsington Btg
Intermittent	17	Silty clay 2.5YR 5/1 2.5YR 4/6 concentrations	Lenni Btg
Upland	14	Silt loam 10YR 5/5	Christiana BE

Profile photos (to 30 cm)

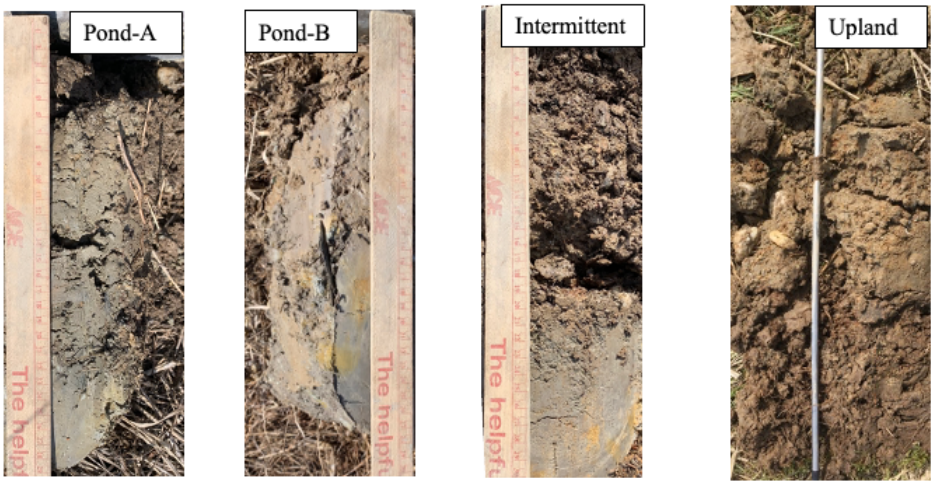


Table A5.3. 2 – Image adjustments.

In order to evaluate images on a computer and make the images clear in printed media, we found image adjustments helpful.

Tube Type	Brightness	Contrast	Temperature	Transparency (%)*
FIRIS	-20	+90	6,500	35
FIRIS	+40	+80	6,500	35
MCIRIS Video**	+50	+40	6,500	35
MIRIS	-20	+75	6,500	35
MCIRIS	+40	+40	6,500	40
FCIRIS Video**	0	+20	6,500	35

* Transparency adjustment necessary to assign iron-oxide removal values.

** Average setting. Values varied.

Modifying image color and contrast settings may appear different to different users, so our settings should only be considered guidelines. In cases where the electronic image was not clear, comparison to the actual tube was used to remove the ambiguity.

Table A5.4. 1 – Soil analyses for incubation microcosms.

Soil	Sand	Silt	Clay	Fe (hydroxylamine hydrochloride) mg/g	Fe (dithionite) mg/g	Fe (oxalate) mg/g	% C	% moisture
SCL	60	15	24	0.39 ± 0.02	2.2 ± 0.28	2.5 ± 0.10	2.08 ± 0.01	18.081 ± 0.002
SL	74	11	15	0.18 ± 0.01	0.29 ± 0.04	0.79 ± 0.03	0.61 ± 0.02	2.721 ± 0.001

Table A5.4. 2 – Percent of hydroxylamine hydrochloride extractable iron. Fe_{HHCl} measured in the liquid phase at the end of the incubation period (Experiment 1).

	SCL				SL			
Treatment	**Fe^{2+} (mg/L)**	**stdev**	**% Fe_{HHCl} used**	**stdev**	**Fe^{2+} (mg/L)**	**stdev**	**% Fe_{HHCl} used**	**stdev**
Control	69.8	50.2	1.7%	1.2%	67.6	2.6	20%	0.8%
B1	45.5	30.6	1.5%	1.0%	80.8	8.0	27%	2.0%
B3	40.6	7.2	1.9%	0.3%	62.1	11.0	28%	4.6%
B6	32.1	19.9	3.3%	2.5%	38.8	2.8	34%	9.7%
M1	75.8	25.2	2.1%	0.6%	109.5	4.3	40%	2.2%
M3	85.4	7.5	3.1%	0.3%	95.6	22.9	50%	3.0%
M6	62.4	22.1	19%	13%	54.0	26.7	61%	11%
L1	49.1	31.7	9.9%	6.5%	22.9	9.2	8%	2.9%
L3	17.9	9.0	4.1%	1.9%	8.3	2.3	4%	1.1%
L6	26.0	9.5	8.6%	3.0%	6.9	0.6	5%	0.4%
W1	62.7	11.5	1.7%	0.4%	75.6	11.1	25%	2.7%
W3	63.8	9.2	2.3%	0.4%	60.5	6.7	28%	4.0%
W6	38.7	18.6	2.3%	1.0%	44.8	1.1	42%	8.4%
H1	220.6	75.5	43%	14%	158.1	8.2	58%	5.1%
H3	523.8	199.8	79%	74%	214.4	77.9	109%	39%
H6	466.8	154.1	155%	47%	268.6	70.9	236%	53%

Table A5.4. 3 – MANOVA summary table for Fe^{2+} production.
Experiment 1 - considering organic amendment type and dose (OM), pH (as continuous variable), and soil type (SL and SCL).

Factor	DF	Exp. DF	F Value	p
OM	28	63	17.44	< 0.0001
pH	1	63	1.09	0.30
Soil	1	63	0.06	0.81

Bibliography

Abernethy, S., F. M. O'Connor, C. D. Jones, and R. B. Jackson. 2021. "Methane Removal and the Proportional Reductions in Surface Temperature and Ozone." *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* 379 (2210): 20210104. https://doi.org/10.1098/rsta.2021.0104.

Abiven, Samuel, Safya Menasseri, and Claire Chenu. 2009. "The Effects of Organic Inputs over Time on Soil Aggregate Stability – A Literature Analysis." *Soil Biology and Biochemistry* 41 (1): 1–12. https://doi.org/10.1016/j.soilbio.2008.09.015.

Alam, Md.Zahangir, A. Fakhru'l-Razi, and Abul H. Molla. 2003. "Biosolids Accumulation and Biodegradation of Domestic Wastewater Treatment Plant Sludge by Developed Liquid State Bioconversion Process Using a Batch Fermenter." *Water Research* 37 (15): 3569–78. https://doi.org/10.1016/S0043-1354(03)00260-4.

Alsfeld, Amy J., Jacob L. Bowman, and Amy Deller-Jacobs. 2009. "Effects of Woody Debris, Microtopography, and Organic Matter Amendments on the Biotic Community of Constructed Depressional Wetlands." *Biological Conservation* 142 (2): 247–55. https://doi.org/10.1016/j.biocon.2008.10.017.

Altor, Anne E., and William J. Mitsch. 2006. "Methane Flux from Created Riparian Marshes: Relationship to Intermittent versus Continuous Inundation and Emergent Macrophytes." *Ecological Engineering* 28 (3): 224–34. https://doi.org/10.1016/j.ecoleng.2006.06.006.

Amendola, D., M. Mutema, V. Rosolen, and V. Chaplot. 2018. "Soil Hydromorphy and Soil Carbon: A Global Data Analysis." *Geoderma* 324 (August): 9–17. https://doi.org/10.1016/j.geoderma.2018.03.005.

Anderson, Christopher J., and Bruce C. Cowell. 2004. "Mulching Effects on the Seasonally Flooded Zone of West-Central Florida, USA Wetlands." *Wetlands* 24 (4): 811–19. https://doi.org/10.1672/0277-5212(2004)024[0811:MEOTSF]2.0.CO;2.

Andreas, Barbara K, John J Mack, and James S McCormac. 2004. "Floristic Quality Assessment Index (FQAI) for Vascular Plants and Mosses for the State of Ohio." *Ohio Environmental Protection Agency, Division of Surface Water, Wetland Ecology Group, Columbus, Ohio*, 217.

Ann, Y., K.R. Reddy, and J.J. Delfino. 1999. "Influence of Chemical Amendments on Phosphorus Immobilization in Soils from a Constructed Wetland." *Ecological Engineering* 14 (1–2): 157–67. https://doi.org/10.1016/S0925-8574(99)00026-9.

Armstrong, W. 2000. "Oxygen Distribution in Wetland Plant Roots and Permeability Barriers to Gas-Exchange with the Rhizosphere: A Microelectrode and Modelling Study with Phragmites Australis." *Annals of Botany* 86 (3): 687–703. https://doi.org/10.1006/anbo.2000.1236.

Arthur, J. Daniel, Jon Seekins, David Winter, and David Alleman. 2014. "Waters of the United States: Understanding Definitions and Jurisdictional Determinations Specific to Shale Resource Development." In *Shale Energy*

Engineering 2014, 512–19. Pittsburgh, Pennsylvania, United States: American Society of Civil Engineers. https://doi.org/10.1061/9780784413654.054.
Badger, Curtis J. 2007. *A Natural History of Quiet Waters*. University of Virginia Press.
Bailey, David E., James E. Perry, and W. Lee Daniels. 2007. "Vegetation Dynamics in Response to Organic Matter Loading Rates in a Created Freshwater Wetland in Southeastern Virginia." *Wetlands* 27 (4): 936–50. https://doi.org/10.1672/0277-5212(2007)27[936:VDIRTO]2.0.CO;2.
Baldwin, Andrew H., Michael S. Egnotovich, and Ernest Clarke. 2001. "Hydrologic Change and Vegetation of Tidal Freshwater Marshes: Field, Greenhouse, and Seed-Bank Experiments." *Wetlands* 21 (4): 519–31. https://doi.org/10.1672/0277-5212(2001)021[0519:HCAVOT]2.0.CO;2.
Ballantine, Katherine A., Johannes Lehmann, Rebecca L. Schneider, and Peter M. Groffman. 2015. "Trade-Offs between Soil-Based Functions in Wetlands Restored with Soil Amendments of Differing Lability." *Ecological Applications* 25 (1): 215–25. https://doi.org/10.1890/13-1409.1.
Ballantine, Katherine, and Rebecca Schneider. 2009. "Fifty-Five Years of Soil Development in Restored Freshwater Depressional Wetlands." *Ecological Applications* 19 (6): 1467–80. https://doi.org/10.1890/07-0588.1.
Bantilan-Smith, Meris, Gregory L. Bruland, Richard A. MacKenzie, Adonia R. Henry, and Christina R. Ryder. 2009. "A Comparison of the Vegetation and Soils of Natural, Restored, and Created Coastal Lowland Wetlands in Hawai'i." *Wetlands* 29 (3): 1023–35. https://doi.org/10.1672/08-127.1.
Bardgett, R, W Bowman, R Kaufmann, and S Schmidt. 2005. "A Temporal Approach to Linking Aboveground and Belowground Ecology." *Trends in Ecology & Evolution* 20 (11): 634–41. https://doi.org/10.1016/j.tree.2005.08.005.
Batty, L. C. 2003. "Effects of External Iron Concentration upon Seedling Growth and Uptake of Fe and Phosphate by the Common Reed, Phragmites Australis (Cav.) Trin Ex. Steudel." *Annals of Botany* 92 (6): 801–6. https://doi.org/10.1093/aob/mcg205.
Bedford, Barbara L, Mark R Walbridge, and Allison Aldous'. 1999. "Patterns in Nutrient Availability and Plant Diversity of Temperate North American Wetlands." *Ecology* 80 (7): 20.
Bergschneider, Cara. 2005. "Determining an Appropriate Organic Matter Loading Rate For a Created Coastal Plain Forested Wetland." Master's thesis, Virginia Polytechnic Institute.
Berkowitz, Jacob F., Sanderson Page, and Chris V. Noble. 2014. "Potential Disconnect between Observations of Hydrophytic Vegetation, Wetland Hydrology Indicators, and Hydric Soils in Unique Pitcher Plant Bog Habitats of the Southern Gulf Coast." *Southeastern Naturalist* 13 (4): 721. https://doi.org/10.1656/058.013.0410.
Berkowitz, Jacob F., and James Barrett Sallee. 2011. "Investigating Problematic Hydric Soils Using Hydrology, IRIS Tubes, Chemistry, and the Hydric Soils Technical Standard." *Soil Science Society of America Journal* 75 (6): 2379–85. https://doi.org/10.2136/sssaj2011.0040.

Berkowitz, Jacob F., Michael J. Vepraskas, Karen L. Vaughan, and Lenore M. Vasilas. 2021. “Development and Application of the Hydric Soil Technical Standard.” *Soil Science Society of America Journal* 85 (3): 469–87. https://doi.org/10.1002/saj2.20202.

Bernal, Blanca, J. Patrick Megonigal, and Thomas J. Mozdzer. 2017. “An Invasive Wetland Grass Primes Deep Soil Carbon Pools.” *Global Change Biology* 23 (5): 2104–16. https://doi.org/10.1111/gcb.13539.

Bernal, Blanca, and William J. Mitsch. 2012. “Comparing Carbon Sequestration in Temperate Freshwater Wetland Communities.” *Global Change Biology* 18 (5): 1636–47. https://doi.org/10.1111/j.1365-2486.2011.02619.x.

Bethke, C. M., R. A. Sanford, M. F. Kirk, Q. Jin, and T. M. Flynn. 2011. “The Thermodynamic Ladder in Geomicrobiology.” *American Journal of Science* 311 (3): 183–210. https://doi.org/10.2475/03.2011.01.

Birch, H. F. 1958. “The Effect of Soil Drying on Humus Decomposition and Nitrogen Availability.” *Plant and Soil* 10 (1): 9–31. https://doi.org/10.1007/BF01343734.

Bishel-Machung, Laurie, Robert P. Brooks, Sharon S. Yates, and Kevin L. Hoover. 1996. “Soil Properties of Reference Wetlands and Wetland Creation Projects in Pennsylvania.” *Wetlands* 16 (4): 532–41. https://doi.org/10.1007/BF03161343.

Blagodatsky, Sergey, Evgenia Blagodatskaya, Tatyana Yuyukina, and Yakov Kuzyakov. 2010. “Model of Apparent and Real Priming Effects: Linking Microbial Activity with Soil Organic Matter Decomposition.” *Soil Biology and Biochemistry* 42 (8): 1275–83. https://doi.org/10.1016/j.soilbio.2010.04.005.

Bonetti, Giuditta, Stacey M. Trevathan-Tackett, Paul E. Carnell, Sarah Treby, and Peter I. Macreadie. 2021. “Local Vegetation and Hydroperiod Influence Spatial and Temporal Patterns of Carbon and Microbe Response to Wetland Rehabilitation.” *Applied Soil Ecology* 163 (July): 103917. https://doi.org/10.1016/j.apsoil.2021.103917.

Bornette, Gudrun, and Sara Puijalon. 2011. “Response of Aquatic Plants to Abiotic Factors: A Review.” *Aquatic Sciences* 73 (1): 1–14. https://doi.org/10.1007/s00027-010-0162-7.

Bräuer, S. L., J. B. Yavitt, and S. H. Zinder. 2004. “Methanogenesis in McLean Bog, an Acidic Peat Bog in Upstate New York: Stimulation by H_2 /CO_2 in the Presence of Rifampicin, or by Low Concentrations of Acetate.” *Geomicrobiology Journal* 21 (7): 433–43. https://doi.org/10.1080/01490450490505400.

Bridgham, Scott D., Hinsby Cadillo-Quiroz, Jason K. Keller, and Qianlai Zhuang. 2013. “Methane Emissions from Wetlands: Biogeochemical, Microbial, and Modeling Perspectives from Local to Global Scales.” *Global Change Biology* 19 (5): 1325–46. https://doi.org/10.1111/gcb.12131.

Brown, Jordan, and Mark D. Norris. 2018. “Detecting Soil and Plant Community Changes in Restored Wetlands Using a Chronosequence Approach.” *Wetlands Ecology and Management* 26 (3): 299–314. https://doi.org/10.1007/s11273-017-9574-7.

Brown, Stephen C., and Barbara L. Bedford. 1997. “Restoration of Wetland Vegetation with Transplanted Wetland Soil: An Experimental Study.” *Wetlands* 17 (3): 424–37. https://doi.org/10.1007/BF03161432.

Bruland, Gregory L., Curtis J. Richardson, and W. Lee Daniels. 2009. “Microbial and Geochemical Responses to Organic Matter Amendments in a Created Wetland.” *Wetlands* 29 (4): 1153–65. https://doi.org/10.1672/08-201.1.

Bruland, Gregory L., Curtis J. Richardson, and Stephen C. Whalen. 2006. “Spatial Variability of Denitrification Potential and Related Soil Properties in Created, Restored, and Paired Natural Wetlands.” *Wetlands* 26 (4): 1042–56. https://doi.org/10.1672/0277-5212(2006)26[1042:SVODPA]2.0.CO;2.

Brune, A. 2000. “Life at the Oxic–Anoxic Interface: Microbial Activities and Adaptations.” *FEMS Microbiology Reviews* 24 (5): 691–710. https://doi.org/10.1016/S0168-6445(00)00054-1.

Bryant, Kelly. 2010. “Indicator of Reduction in Soils (IRIS) for Wetland Identification in Queensland.” *19th World Congress of Soil Science, Soil Solutions for a Changing World*, 4.

Bubier, Jill, and Tim R. Moore. 1994. “An Ecological Perspective on Methane Emissions from Northern Wetlands.” *Tree* 9 (12).

Budelsky, Rachel A., and Susan M. Galatowitsch. 1999. “Effects of Moisture, Temperature, and Time on Seed Germination of Five Wetland *Carices* : Implications for Restoration.” *Restoration Ecology* 7 (1): 86–97. https://doi.org/10.1046/j.1526-100X.1999.07110.x.

Bünemann, Else K., Giulia Bongiorno, Zhanguo Bai, Rachel E. Creamer, Gerlinde De Deyn, Ron de Goede, Luuk Fleskens, et al. 2018. “Soil Quality – A Critical Review.” *Soil Biology and Biochemistry* 120 (May): 105–25. https://doi.org/10.1016/j.soilbio.2018.01.030.

Burchell, Michael R., R. Wayne Skaggs, Charles R. Lee, Steven Broome, George M. Chescheir, and Jason Osborne. 2007. “Substrate Organic Matter to Improve Nitrate Removal in Surface-Flow Constructed Wetlands.” *Journal of Environment Quality* 36 (1): 194. https://doi.org/10.2134/jeq2006.0022.

Burgin, Shelley. 2009. “‘Mitigation Banks’ for Wetland Conservation: A Major Success or an Unmitigated Disaster?” *Wetlands Ecology and Management* 18 (1): 49–55. https://doi.org/10.1007/s11273-009-9147-5.

Burke, David J. 1997. “Donor Wetland Soil Promotes Revegetation in Wetland Trials.” *Restoration & Management Notes* 15 (2): 168–72.

Cabin, Robert J. 2007. “Science-Driven Restoration: A Square Grid on a Round Earth?” *Restoration Ecology* 15 (1): 1–7. https://doi.org/10.1111/j.1526-100X.2006.00183.x.

Calabrese, Salvatore, Alicia Garcia, Jared L Wilmoth, Xinning Zhang, and Amilcare Porporato. 2021. “Critical Inundation Level for Methane Emissions from Wetlands.” *Environmental Research Letters* 16 (4): 044038. https://doi.org/10.1088/1748-9326/abedea.

Callahan, BJ, PJ McMurdie, MJ Rosen, AW Han, AA Johnson, and SP Holmes. 2016. “DADA2: High-Resolution Sample Inference from Illumina Amplicon Data.” *Nature Methods* 13: 581–83. https://doi.org/10.1038/nmeth.

Campbell, Deborah A, Charles Andrew Cole, and Robert P Brooks. 2002. "A Comparison of Created and Natural Wetlands in Pennsylvania, USA." *Wetlands Ecology and Management*, no. 10: 41–49.

Cardinale, Bradley J. 2011. "Biodiversity Improves Water Quality through Niche Partitioning." *Nature* 472 (7341): 86–89. https://doi.org/10.1038/nature09904.

Carpenter, Stephen R. 1996. "Microcosm Experiments Have Limited Relevance for Community and Ecosystem Ecology." *Ecology* 77 (3): 677–80. https://doi.org/10.2307/2265490.

Castañeda, C., J Herrero, and JA Conesa. 2013. "Distribution, Morphology and Habitats of Saline Wetlands: A Case Study from Monegros, Spain." *Geologica Acta* 11 (4). https://doi.org/10.1344/105.000002055.

Castañeda, C., E. Luna, and M. Rabenhorst. 2017. "Reducing Conditions in Soil of Gallocanta Lake, Northeast Spain: Reducing Conditions in Soil." *European Journal of Soil Science* 68 (2): 249–58. https://doi.org/10.1111/ejss.12407.

Castenson, Karen L., and Martin C. Rabenhorst. 2006. "Indicator of Reduction in Soil (IRIS)." *Soil Science Society of America Journal* 70 (4): 1222. https://doi.org/10.2136/sssaj2005.0130.

Catrouillet, Charlotte, Mélanie Davranche, Aline Dia, Martine Bouhnik-Le Coz, Rémi Marsac, Olivier Pourret, and Gérard Gruau. 2014. "Geochemical Modeling of Fe(II) Binding to Humic and Fulvic Acids." *Chemical Geology* 372 (April): 109–18. https://doi.org/10.1016/j.chemgeo.2014.02.019.

Chaudhari, Pravin R., Dodha V. Ahire, Vidya D. Ahire, Manab Chkravarty, and Saroj Maity. 2013. "Soil Bulk Density as Related to Soil Texture, Organic Matter Content and Available Total Nutrients of Coimbatore Soil." *International Journal of Scientific and Research Publications* 3 (2): 1–8.

Chen, Xi, Yujun Xu, Hong-jian Gao, Jingdong Mao, Wenying Chu, and Michael L. Thompson. 2018. "Biochemical Stabilization of Soil Organic Matter in Straw-Amended, Anaerobic and Aerobic Soils." *Science of The Total Environment* 625 (June): 1065–73. https://doi.org/10.1016/j.scitotenv.2017.12.293.

Cheng, Liang, Ralf Cord-Ruwisch, and Mohamed A. Shahin. 2013. "Cementation of Sand Soil by Microbially Induced Calcite Precipitation at Various Degrees of Saturation." *Canadian Geotechnical Journal* 50 (1): 81–90. https://doi.org/10.1139/cgj-2012-0023.

Cherry, K.A., M. Shepherd, P.J.A. Withers, and S.J. Mooney. 2008. "Assessing the Effectiveness of Actions to Mitigate Nutrient Loss from Agriculture: A Review of Methods." *Science of The Total Environment* 406 (1–2): 1–23. https://doi.org/10.1016/j.scitotenv.2008.07.015.

Chowdhury, Taniya Roy, and Richard P. Dick. 2013. "Ecology of Aerobic Methanotrophs in Controlling Methane Fluxes from Wetlands." *Applied Soil Ecology* 65 (March): 8–22. https://doi.org/10.1016/j.apsoil.2012.12.014.

Christopher Morris. 2012. *The Big Muddy: An Environmental History of the Mississippi and Its Peoples, from Hernando De Soto to Hurricane Katrina.* New York, NY: Oxford University Press.

Clarkson, Beverley R, Anne-Gaelle E Ausseil, and Philippe Gerbeaux. 2013. "Wetland Ecosystem Services." In *Ecosystem Services in New Zealand –*

Conditions and Trends, edited by Dymonnd JR, 11. Manaaki Whenua Press, Lincoln, New Zealand.
Clewell, Andre F. 1981. "Vegetational Restoration Techniques on Reclaimed Phosphate Strip Mines in Florida." *Wetlands* 1 (1): 158–70. https://doi.org/10.1007/BF03160461.
Cole, Charles Andrew, and Robert P Brooks. 2000. "A Comparison of the Hydrologic Characteristics of Natural and Created Mainstem Floodplain Wetlands in Pennsylvania." *Ecological Engineering* 14 (3): 221–31. https://doi.org/10.1016/S0925-8574(99)00004-X.
Coles, Bryony., and J. M. Coles. 1989. *People of the Wetlands : Bogs, Bodies, and Lake-Dwellers*. Ancient Peoples and Places ; v. 106; Ancient Peoples and Places (Thames and Hudson) ; v. 106. New York, N.Y. : Thames & Hudson,.
Conrad, R. 1999. "Contribution of Hydrogen to Methane Production and Control of Hydrogen Concentrations in Methanogenic Soils and Sediments." *FEMS Microbiology Ecology* 28 (3): 193–202. https://doi.org/10.1111/j.1574-6941.1999.tb00575.x.
Conrad, R, and M Klose. 2006. "Dynamics of the Methanogenic Archaeal Community in Anoxic Rice Soil upon Addition of Straw." *European Journal of Soil Science*, 9.
Conrad, Ralf. 2020. "Importance of Hydrogenotrophic, Aceticlastic and Methylotrophic Methanogenesis for Methane Production in Terrestrial, Aquatic and Other Anoxic Environments: A Mini Review." *Pedosphere* 30 (1): 25–39. https://doi.org/10.1016/S1002-0160(18)60052-9.
Cordell, Susan, Rebecca Ostertag, Jené Michaud, and Laura Warman. 2016. "Quandaries of a Decade-Long Restoration Experiment Trying to Reduce Invasive Species: Beat Them, Join Them, Give up, or Start over?: Quandaries of a Decade-Long Restoration Experiment." *Restoration Ecology* 24 (2): 139–44. https://doi.org/10.1111/rec.12321.
Dahl, T.E. 2011. "Status and Trends of Wetlands in the Conterminous United States 2004 to 2009." *U.S. Department of the Interior; Fish and Wildlife, Washington D.C. 108 Pp.* https://www.fws.gov/wetlands/Status-And-Trends-2009/index.html.
Darrah, Sarah E., Yara Shennan-Farpón, Jonathan Loh, Nick C. Davidson, C. Max Finlayson, Royal C. Gardner, and Matt J. Walpole. 2019. "Improvements to the Wetland Extent Trends (WET) Index as a Tool for Monitoring Natural and Human-Made Wetlands." *Ecological Indicators* 99 (April): 294–98. https://doi.org/10.1016/j.ecolind.2018.12.032.
David Moreno-Mateos and Margaret A. Palmer. 2016. "Watershed Processes as Drivers for Aquatic Ecosystem Restoration." In *Foundations of Restoration Ecology*, 395–423. Island Press, Washington, DC, USA.
Davidson, Nick C. 2014. "How Much Wetland Has the World Lost? Long-Term and Recent Trends in Global Wetland Area." *Marine and Freshwater Research* 65 (10): 934. https://doi.org/10.1071/MF14173.
Day, S. D., J. R. Seiler, and N. Persaud. 2000. "A Comparison of Root Growth Dynamics of Silver Maple and Flowering Dogwood in Compacted Soil at

Differing Soil Water Contents." *Tree Physiology* 20 (4): 257–63. https://doi.org/10.1093/treephys/20.4.257.
Deng, H., Z.H. Ye, and M.H. Wong. 2009. "Lead, Zinc and Iron (Fe2+) Tolerances in Wetland Plants and Relation to Root Anatomy and Spatial Pattern of ROL." *Environmental and Experimental Botany* 65 (2–3): 353–62. https://doi.org/10.1016/j.envexpbot.2008.10.005.
Dexter, A R. 2004. "Soil Physical Quality Part I. Theory, Effects of Soil Texture, Density, and Organic Matter, and Effects on Root Growth," 14.
Dickinson, Sarah Beth. 2007. "Influences of Soil Amendments and Microtopography on Vegetation at a Created Tidal Freshwater Swamp in Southeastern Virginia." Master's Thesis, Virginia Polytechnic Institute and State University.
Dijkstra, Feike A., Biao Zhu, and Weixin Cheng. 2021. "Root Effects on Soil Organic Carbon: A Double-edged Sword." *New Phytologist* 230 (1): 60–65. https://doi.org/10.1111/nph.17082.
DOE, US. 1998. "Method for Calculating Carbon Sequestration by Trees in Urban and Suburban Settings." Voluntary Reporting of Greenhouse Gases, US Department of Engergy, Energy Information Adminstration.
Doherty, James M., Jeffrey F. Miller, Stephanie G. Prellwitz, Anita M. Thompson, Steven P. Loheide, and Joy B. Zedler. 2014. "Hydrologic Regimes Revealed Bundles and Tradeoffs Among Six Wetland Services." *Ecosystems* 17 (6): 1026–39. https://doi.org/10.1007/s10021-014-9775-3.
Doherty, James M., and Joy B. Zedler. 2015. "Increasing Substrate Heterogeneity as a Bet-Hedging Strategy for Restoring Wetland Vegetation." *Restoration Ecology* 23 (1): 15–25. https://doi.org/10.1111/rec.12154.
Dorau, Kristof, Michael Eickmeier, and Tim Mansfeldt. 2016. "Comparison of Manganese and Iron Oxide-Coated Redox Bars for Characterization of the Redox Status in Wetland Soils." *Wetlands* 36 (1): 133–41. https://doi.org/10.1007/s13157-015-0724-5.
Dorau, Kristof, Samuel Papenfuß, and Tim Mansfeldt. 2018. "Temperature-Dependent Oxide Removal from Manganese- and Iron Oxide-Coated Soil Redox Bars." *Journal of Soils and Sediments* 18 (3): 680–87. https://doi.org/10.1007/s11368-017-1812-3.
Downie, Helen F., Joel P. Standerwick, Letitia Burgess, Louise S. Natrajan, and Jonathan R. Lloyd. 2018. "Imaging Redox Activity and Fe(II) at the Microbe-Mineral Interface during Fe(III) Reduction." *Research in Microbiology*, June. https://doi.org/10.1016/j.resmic.2018.05.012.
Drake, Harold L., Marcus A. Horn, and Pia K. Wüst. 2009. "Intermediary Ecosystem Metabolism as a Main Driver of Methanogenesis in Acidic Wetland Soil." *Environmental Microbiology Reports* 1 (5): 307–18. https://doi.org/10.1111/j.1758-2229.2009.00050.x.
Dronova, Iryna. 2015. "Object-Based Image Analysis in Wetland Research: A Review." *Remote Sensing* 7 (5): 6380–6413. https://doi.org/10.3390/rs70506380.
Edward Maltby. 1986. *Waterlogged Wealth: Why Waste the World's Wet Places?* International Institute for Environment and Development.

Egger, Matthias, Mathilde Hagens, Célia J. Sapart, Nikki Dijkstra, Niels A.G.M. van Helmond, José M. Mogollón, Nils Risgaard-Petersen, et al. 2017. "Iron Oxide Reduction in Methane-Rich Deep Baltic Sea Sediments." *Geochimica et Cosmochimica Acta* 207 (June): 256–76. https://doi.org/10.1016/j.gca.2017.03.019.

Eickhorst, Thilo, and Rolf Tippkötter. 2008. "Improved Detection of Soil Microorganisms Using Fluorescence in Situ Hybridization (FISH) and Catalyzed Reporter Deposition (CARD-FISH)." *Soil Biology and Biochemistry* 40 (7): 1883–91. https://doi.org/10.1016/j.soilbio.2008.03.024.

Elliott, E.T., C.A. Palm, D.E. Reuss, and C.A. Monz. 1991. "Organic Matter Contained in Soil Aggregates from a Tropical Chronosequence: Correction for Sand and Light Fraction." *Agriculture, Ecosystems & Environment* 34 (1–4): 443–51. https://doi.org/10.1016/0167-8809(91)90127-J.

Elsey-Quirk, Tracy, and Mary Allessio Leck. 2021. "High Reinvasion Potential of Phragmites Australis in a Delaware River (USA) Tidal Freshwater Marsh Following Chemical Treatment: The Role of the Seedbank." *Wetlands* 41 (1): 12. https://doi.org/10.1007/s13157-021-01398-6.

Erwin, Kevin L., G. Ronnie Best, W. J. Dunn, and P. M. Wallace. 1984. "Marsh and Forested Wetland Reclamation of a Central Florida Phosphate Mine." *Wetlands* 4 (1): 87–104. https://doi.org/10.1007/BF03160489.

Erwin, Kevin L., and G. Ronnie Best. 1985. "Marsh Community Development in a Central Florida Phosphate Surface-Mined Reclaimed Wetland." *Wetlands* 5 (1): 155–66. https://doi.org/10.1007/BF03160794.

Evans, Abby E., Matt A. Limmer, and Angelia L. Seyfferth. 2021. "Indicator of Redox in Soil (IRIS) Films as a Water Management Tool for Rice Farmers." *Journal of Environmental Management* 294 (September): 112920. https://doi.org/10.1016/j.jenvman.2021.112920.

Evans, C D. 2021. "Overriding Water Table Control on Managed Peatland Greenhouse Gas Emissions." *Nature* Under Accelerated Review: 22.

Falk, Donald A., Adam C. Watts, and Andrea E. Thode. 2019. "Scaling Ecological Resilience." *Frontiers in Ecology and Evolution* 7 (July): 275. https://doi.org/10.3389/fevo.2019.00275.

Fanelli, Daniele. 2012. "Negative Results Are Disappearing from Most Disciplines and Countries." *Scientometrics* 90 (3): 891–904. https://doi.org/10.1007/s11192-011-0494-7.

Fennessy, M. S., J. J. Mack, A. Rokosch, M. Knapp, and M. Micacchion. 2004. "Biogeochemical and Hydrological Investigations of Natural and Mitigation Wetlands." Ohio EPA Technical Report WET/2004-5. Ohio Environmental Protection Agency, Wetlands Ecology Unit, Division of Surface Water, Columbus, OH.

Fenstermacher, D. E., M. C. Rabenhorst, M. W. Lang, G. W. McCarty, and B. A. Needelman. 2016. "Carbon in Natural, Cultivated, and Restored Depressional Wetlands in the Mid-Atlantic Coastal Plain." *Journal of Environment Quality* 45 (2): 743. https://doi.org/10.2134/jeq2015.04.0186.

Ferry, J G. 1992. "Methane from Acetate." *Journal of Bacteriology* 174 (17): 5489–95. https://doi.org/10.1128/JB.174.17.5489-5495.1992.

Ferry, James G. 2020. “Methanosarcina Acetivorans: A Model for Mechanistic Understanding of Aceticlastic and Reverse Methanogenesis.” *Frontiers in Microbiology* 11: 1806. https://doi.org/10.3389/fmicb.2020.01806.
Fetzer, Silke, Friedhelm Bak, and Ralf Conrad. 1993. “Sensitivity of Methanogenic Bacteria from Paddy Soil to Oxygen and Desiccation.” *FEMS Microbiology Ecology* 12: 107–15.
Fierer, Noah. 2017. “Embracing the Unknown: Disentangling the Complexities of the Soil Microbiome.” *Nature Reviews Microbiology* 15 (10): 579–90. https://doi.org/10.1038/nrmicro.2017.87.
Fisher, Ronald A. 1971. *The Design of Experiments (9th Ed.)*. Macmillan.
Fontaine, Sébastien, Gérard Bardoux, Danielle Benest, Bruno Verdier, André Mariotti, and Luc Abbadie. 2004. “Mechanisms of the Priming Effect in a Savannah Soil Amended with Cellulose.” *Soil Science Society of America Journal* 68 (1): 125–31. https://doi.org/10.2136/sssaj2004.1250.
Fraser, Hannah, Ashley Barnett, Timothy H. Parker, and Fiona Fidler. 2020. “The Role of Replication Studies in Ecology.” *Ecology and Evolution* 10 (12): 5197–5207. https://doi.org/10.1002/ece3.6330.
Gabor, T S, H R Murkin, M P Stainton, J A Boughen, and R D Titman. 1994. “Nutrient Additions to Wetlands in the Interlake Region of Manitoba, Canada: Effects of a Single Pulse Addition in Spring.” *Hydrobiologia*, 14.
German, Donovan P., Stephany S. Chacon, and Steven D. Allison. 2011. “Substrate Concentration and Enzyme Allocation Can Affect Rates of Microbial Decomposition.” *Ecology* 92 (7): 1471–80. https://doi.org/10.1890/10-2028.1.
Gersberg, R, B Elkins, and C Goldman. 1983. “Nitrogen Removal in Artificial Wetlands.” *Water Research* 17 (9): 1009–14. https://doi.org/10.1016/0043-1354(83)90041-6.
Getahun, Alemayehu, Diriba Muleta, Fassil Assefa, Solomon Kiros, and Mariangela Hungria. 2020. “Biochar and Other Organic Amendments Improve the Physicochemical Properties of Soil in Highly Degraded Habitat.” *European Journal of Engineering Research and Science* 5 (3): 331–38. https://doi.org/10.24018/ejers.2020.5.3.1735.
Glissmann, Kristin, and Ralf Conrad. 2002. “Saccharolytic Activity and Its Role as a Limiting Step in Methane Formation during the Anaerobic Degradation of Rice Straw in Rice Paddy Soil.” *Biology and Fertility of Soils* 35 (1): 62–67. https://doi.org/10.1007/s00374-002-0442-z.
Gon, H. A. C. Denier van der, and H. U. Neue. 1995. “Influence of Organic Matter Incorporation on the Methane Emission from a Wetland Rice Field.” *Global Biogeochemical Cycles* 9 (1): 11–22. https://doi.org/10.1029/94GB03197.
Gray, Adam Lincoln. 2010. “Redoximorphic Features Induced by Organic Amendments and Simulated Wetland Hydrology.” Master’s Thesis, Univrersity of Maryland.
Green, Helen Elise. 2014. “Use of Theoretical and Conceptual Frameworks in Qualitative Research.” *Nurse Researcher* 21 (6): 34–38.
Green, Laura, and Marlyse Duguid. 2020. “Vascular Plant Diversity of Forested Wetlands in Southern New England.” *Rhodora* 122 (990). https://doi.org/10.3119/19-21.

Grime, J. P. 1977. “Evidence for the Existence of Three Primary Strategies in Plants and Its Relevance to Ecological and Evolutionary Theory.” *The American Naturalist* 111 (982): 1169–94. https://doi.org/10.1086/283244.

Groffman, Peter M., Gay C. Hanson, Erik Kiviat, and Gretchen Stevens. 1996. “Variation in Microbial Biomass and Activity in Four Different Wetland Types.” *Soil Science Society of America Journal* 60 (2): 622. https://doi.org/10.2136/sssaj1996.03615995006000020041x.

Gruntman, M, U Segev, and K Tielbörger. 2020. “Shade-induced Plasticity in Invasive *Impatiens Glandulifera* Populations.” Edited by Alan Gange. *Weed Research* 60 (1): 16–25. https://doi.org/10.1111/wre.12394.

Guo, Xiao-xia, Hong-tao Liu, and Shu-biao Wu. 2019. “Humic Substances Developed during Organic Waste Composting: Formation Mechanisms, Structural Properties, and Agronomic Functions.” *Science of The Total Environment* 662 (April): 501–10. https://doi.org/10.1016/j.scitotenv.2019.01.137.

Gutknecht, Jessica L. M., Robert M. Goodman, and Teri C. Balser. 2006. “Linking Soil Process and Microbial Ecology in Freshwater Wetland Ecosystems.” *Plant and Soil* 289 (1–2): 17–34. https://doi.org/10.1007/s11104-006-9105-4.

Hansel, Colleen M, Chris J Lentini, Yuanzhi Tang, David T Johnston, Scott D Wankel, and Philip M Jardine. 2015. “Dominance of Sulfur-Fueled Iron Oxide Reduction in Low-Sulfate Freshwater Sediments.” *The ISME Journal* 9 (11): 2400–2412. https://doi.org/10.1038/ismej.2015.50.

Havens, Kirk J., Lyle M. Varnell, and Julie G. Bradshaw. 1995. “An Assessment of Ecological Conditions in a Constructed Tidal Marsh and Two Natural Reference Tidal Marshes in Coastal Virginia.” *Ecological Engineering* 4 (2): 117–41. https://doi.org/10.1016/0925-8574(94)00051-6.

Hayasaka, Daisuke, Moe Nakagawa, Yu Maebara, Tomohiro Kurazono, and Koya Hashimoto. 2020. “Seed Germination Characteristics of Invasive Spartina Alterniflora Loisel in Japan: Implications for Its Effective Management.” *Scientific Reports* 10 (1): 2116. https://doi.org/10.1038/s41598-020-58879-7.

Haynes, R J, and R Naidu. 1998. “Influence of Lime, Fertilizer and Manure Applications on Soil Organic Matter Content and Soil Physical Conditions: A Review.” *Nutrient Cycling in Agroecosystems* 15.

Hazelton, Eric L. G., Rebekah Downard, Karin M. Kettenring, Melissa K. McCormick, and Dennis F. Whigham. 2018. “Spatial and Temporal Variation in Brackish Wetland Seedbanks: Implications for Wetland Restoration Following Phragmites Control.” *Estuaries and Coasts* 41 (S1): 68–84. https://doi.org/10.1007/s12237-017-0289-z.

He, Shaomei, Stephanie A. Malfatti, Jack W. McFarland, Frank E. Anderson, Amrita Pati, Marcel Huntemann, Julien Tremblay, et al. 2015. “Patterns in Wetland Microbial Community Composition and Functional Gene Repertoire Associated with Methane Emissions.” *MBio* 6 (3): e00066-15. https://doi.org/10.1128/mBio.00066-15.

Heite, Edward F. 1974. “The Delmarva Bog Iron Industry.” *Northeast Historical Archaeology:* 3 (3): 18–34.

Hendrick, Ronald L., and Kurt S. Pregitzer. 1996. “Applications of Minirhizotrons to Understand Root Function in Forests and Other Natural Ecosystems.” *Plant and Soil* 185 (2): 293–304. https://doi.org/10.1007/BF02257535.

Hernandez, Maria E., and William J. Mitsch. 2006. “Influence of Hydrologic Pulses, Flooding Frequency, and Vegetation on Nitrous Oxide Emissions from Created Riparian Marshes.” *Wetlands* 26 (3): 862–77. https://doi.org/10.1672/0277-5212(2006)26[862:IOHPFF]2.0.CO;2.

Hernandez, M.E., and D.K. Newman. 2001. “Extracellular Electron Transfer:” *Cellular and Molecular Life Sciences* 58 (11): 1562–71. https://doi.org/10.1007/PL00000796.

Hess, Manon C.M., François Mesléard, and Elise Buisson. 2019. “Priority Effects: Emerging Principles for Invasive Plant Species Management.” *Ecological Engineering* 127 (February): 48–57. https://doi.org/10.1016/j.ecoleng.2018.11.011.

Hiatt, Drew, Kristina Serbesoff-King, Deah Lieurance, Doria R. Gordon, and S. Luke Flory. 2019. “Allocation of Invasive Plant Management Expenditures for Conservation: Lessons from Florida, USA.” *Conservation Science and Practice* 1 (7). https://doi.org/10.1111/csp2.51.

Hobbs, Richard J., Lauren M. Hallett, Paul R. Ehrlich, and Harold A. Mooney. 2011. “Intervention Ecology: Applying Ecological Science in the Twenty-First Century.” *BioScience* 61 (6): 442–50. https://doi.org/10.1525/bio.2011.61.6.6.

Hodges, C., E. King, J. Pett-Ridge, and A. Thompson. 2018. “Potential for Iron Reduction Increases with Rainfall in Montane Basaltic Soils of Hawaii.” *Soil Science Society of America Journal* 82 (1): 176. https://doi.org/10.2136/sssaj2017.06.0193.

Hoeltje, S M, and C A Cole. 2007. “Losing Function Through Wetland Mitigation in Central Pennsylvania, USA.” *Environ Manage*, 18.

Hoffmann, Benjamin D., and Linda M. Broadhurst. 2016. “The Economic Cost of Managing Invasive Species in Australia.” *NeoBiota* 31 (September): 1–18. https://doi.org/10.3897/neobiota.31.6960.

Hondula, Kelly L, C Nathan Jones, and Margaret A Palmer. 2021. “Effects of Seasonal Inundation on Methane Fluxes from Forested Freshwater Wetlands.” *Environmental Research Letters* 16 (8): 084016. https://doi.org/10.1088/1748-9326/ac1193.

Hossler, Katie, and Virginie Bouchard. 2010. “Soil Development and Establishment of Carbon-based Properties in Created Freshwater Marshes.” *Ecological Applications* 20 (2): 539–53. https://doi.org/10.1890/08-1330.1.

Hossler, Katie, Virginie Bouchard, M. Siobhan Fennessy, Serita D. Frey, Evelyn Anemaet, and Ellen Herbert. 2011. “No-net-loss Not Met for Nutrient Function in Freshwater Marshes: Recommendations for Wetland Mitigation Policies.” *Ecosphere* 2 (7): 1–36. https://doi.org/10.1890/ES11-00009.1.

Howard, J, S Hoyt, K Isensee, M Telszewski, and E Pidgeon. 2014. “Coastal Blue Carbon: Methods for Assessing Carbon Stocks and Emissions Factors in Mangroves, Tidal Salt Marshes, and Seagrasses.” Conservation International, Intergovernmental Oceanographic Commission of UNESCO, International Union for Conservation of Nature. Arlington, Virginia, USA.

Hu, Zhenhu, Yuanlu Liu, Guowei Chen, Xianyang Gui, Tianhu Chen, and Xinmin Zhan. 2011. "Characterization of Organic Matter Degradation during Composting of Manure–Straw Mixtures Spiked with Tetracyclines." *Bioresource Technology* 102 (15): 7329–34. https://doi.org/10.1016/j.biortech.2011.05.003.

Huang, Bin, Kewei Yu, and Robert P. Gambrell. 2009. "Effects of Ferric Iron Reduction and Regeneration on Nitrous Oxide and Methane Emissions in a Rice Soil." *Chemosphere* 74 (4): 481–86. https://doi.org/10.1016/j.chemosphere.2008.10.015.

Hubbe, Martin A, Mousa Nazhad, and Carmen Sánchez. 2010. "Composting as a Way to Convert Cellulosic Biomass and Organic Waste into High-Value Soil Amendments: A Review." *BioResources* 5 (4): 47.

Hungate, Bruce A., Rebecca L. Mau, Egbert Schwartz, J. Gregory Caporaso, Paul Dijkstra, Natasja van Gestel, Benjamin J. Koch, et al. 2015. "Quantitative Microbial Ecology through Stable Isotope Probing." Edited by P. D. Schloss. *Applied and Environmental Microbiology* 81 (21): 7570–81. https://doi.org/10.1128/AEM.02280-15.

Hunter, Rachael G., and Stephen P. Faulkner. 2001. "Denitrification Potentials in Restored and Natural Bottomland Hardwood Wetlands." *Soil Science Society of America Journal* 65 (6): 1865. https://doi.org/10.2136/sssaj2001.1865.

Hussain, Masroor, Dongmei Chen, Angela Cheng, Hui Wei, and David Stanley. 2013. "Change Detection from Remotely Sensed Images: From Pixel-Based to Object-Based Approaches." *ISPRS Journal of Photogrammetry and Remote Sensing* 80 (June): 91–106. https://doi.org/10.1016/j.isprsjprs.2013.03.006.

Ingersoll, Todd L., and Lawrence A. Baker. 1998. "NItrate Removal in Wetland Microcosms." *Water Research* 32 (3).

IPCC. 2021. "Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (Eds.)]." Cambridge University Press. In Press.

Iversen, C. M., M. T. Murphy, M. F. Allen, J. Childs, D. M. Eissenstat, E. A. Lilleskov, T. M. Sarjala, V. L. Sloan, and P. F. Sullivan. 2012. "Advancing the Use of Minirhizotrons in Wetlands." *Plant and Soil* 352 (1–2): 23–39. https://doi.org/10.1007/s11104-011-0953-1.

Jabłoński, Sławomir, Paweł Rodowicz, and Marcin Łukaszewicz. 2015. "Methanogenic Archaea Database Containing Physiological and Biochemical Characteristics." *International Journal of Systematic and Evolutionary Microbiology* 65 (Pt_4): 1360–68. https://doi.org/10.1099/ijs.0.000065.

Jacoby, Karl. 2014. *Crimes against Nature*. 1st ed. University of California Press. http://www.jstor.org/stable/10.1525/j.ctt5vjzwv.

Jared Diamond. 1999. *Guns, Germs and Steel*. W.W. Norton.

Jean Paul Metzger and Pedro H.S. Brancalion. 2016. “Landscape Ecology and Restoration Processes.” In *Foundations of Restoration Ecology*, 90–120. Island Press, Washington, DC, USA.

Jenkinson, B. J., and D. P. Franzmeier. 2006. “Development and Evaluation of Iron-Coated Tubes That Indicate Reduction in Soils.” *Soil Science Society of America Journal* 70 (1): 183. https://doi.org/10.2136/sssaj2004.0323.

Jenkinson, D.S., and D.S. Powlson. 1976. “The Effects of Biocidal Treatments on Metabolism in Soil—V.” *Soil Biology and Biochemistry* 8 (3): 209–13. https://doi.org/10.1016/0038-0717(76)90005-5.

Jetten, Mike S.M., Alfons J.M. Stams, and Alexander J.B. Zehnder. 1990. “Acetate Threshold Values and Acetate Activating Enzymes in Methanogenic Bacteria.” *FEMS Microbiology Letters* 73 (4): 339–44. https://doi.org/10.1111/j.1574-6968.1990.tb03958.x.

Jin, Qusheng, and Matthew F. Kirk. 2018. “PH as a Primary Control in Environmental Microbiology: 1. Thermodynamic Perspective.” *Frontiers in Environmental Science* 6 (May): 21. https://doi.org/10.3389/fenvs.2018.00021.

John F. Reiger. 1975. *American Sportsmen And the Origins of Conservation*. New York: Winchester Press.

Johnson, Taylor B. 2016. “Influence of Urban Disturbances, Soil Properties, and Other Abiotic and Biotic Factors an L. Maackii Invasions of Remnant Urban Riparian Hardwood Forests.” Augusuna College.

Jones, Holly P., Peter C. Jones, Edward B. Barbier, Ryan C. Blackburn, Jose M. Rey Benayas, Karen D. Holl, Michelle McCrackin, Paula Meli, Daniel Montoya, and David Moreno Mateos. 2018. “Restoration and Repair of Earth’s Damaged Ecosystems.” *Proceedings of the Royal Society B: Biological Sciences* 285 (1873): 20172577. https://doi.org/10.1098/rspb.2017.2577.

Kato, Souichiro, Kazuhito Hashimoto, and Kazuya Watanabe. 2012. “Methanogenesis Facilitated by Electric Syntrophy via (Semi)Conductive Iron-Oxide Minerals: Methanogenesis via (Semi)Conductive Minerals.” *Environmental Microbiology* 14 (7): 1646–54. https://doi.org/10.1111/j.1462-2920.2011.02611.x.

Kaushal, Sujay S., and Kenneth T. Belt. 2012. “The Urban Watershed Continuum: Evolving Spatial and Temporal Dimensions.” *Urban Ecosystems* 15 (2): 409–35. https://doi.org/10.1007/s11252-012-0226-7.

Kayranli, Birol, Miklas Scholz, Atif Mustafa, and Åsa Hedmark. 2010. “Carbon Storage and Fluxes within Freshwater Wetlands: A Critical Review.” *Wetlands* 30 (1): 111–24. https://doi.org/10.1007/s13157-009-0003-4.

Keiluweit, Marco, Tom Wanzek, Markus Kleber, Peter Nico, and Scott Fendorf. 2017. “Anaerobic Microsites Have an Unaccounted Role in Soil Carbon Stabilization.” *Nature Communications* 8 (1): 1771. https://doi.org/10.1038/s41467-017-01406-6.

Kendrick, Michael G., and Timothy A. Kral. 2006. “Survival of Methanogens During Desiccation: Implications for Life on Mars.” *Astrobiology* 6 (4): 546–51. https://doi.org/10.1089/ast.2006.6.546.

Kentula. 1997. “A Step Toward a Landscape Approach in Riparian Restoration.” *Restoration Ecology* 5 (4S).
Kentula, M.E., R.P. Brooks, S.E. Gwin, C.C. Hollands, A.D. Sherman, and J.C. Sifneos. 1992. *Wetlands: An Approach to Improving Decision Making in Wetland Restoration and Creation.* Island Press, Washington, DC, USA.
Kestha, Amr. 2019. “Hydrology, Soil Redox, and Pore-Water Iron Regulate Carbon Cycling in Natural and Restored Tidal Freshwater Wetlands in the Chesapeake Bay, Maryland, USA.” University of Maryland.
Kettenring, Karin M., and Emily E. Tarsa. 2020. “Need to Seed? Ecological, Genetic, and Evolutionary Keys to Seed-Based Wetland Restoration.” *Frontiers in Environmental Science* 8 (August): 109. https://doi.org/10.3389/fenvs.2020.00109.
Kettenring, Karin M., and Dennis F. Whigham. 2018. “The Role of Propagule Type, Resource Availability, and Seed Source in Phragmites Invasion in Chesapeake Bay Wetlands.” *Wetlands* 38 (6): 1259–68. https://doi.org/10.1007/s13157-018-1034-5.
Keyport, Samantha, Brendan D. Carson, Olivia Johnson, Beth A. Lawrence, Shane C. Lishawa, Nancy C. Tuchman, and John J. Kelly. 2019. “Effects of Experimental Harvesting of an Invasive Hybrid Cattail on Wetland Structure and Function: Cattail Harvest Affects Wetland Properties.” *Restoration Ecology* 27 (2): 389–98. https://doi.org/10.1111/rec.12859.
Kim, Su–Hun, Jae–Han Lee, Ji–Sun Lim, Yoshiyuki Shinog, Chang–Hoon Lee, and Taek–Keun Oh. 2018. “Comparison of Methane Emissions on Soil Texture in Korean Paddy Fields.” *Journal of the Faculty of Agriculture, Kyushu University* 63 (2): 393–97. https://doi.org/10.5109/1955660.
Kirk, Guy. 2004. *The Biogeochemistry of Submerged Soils*. 1st ed. Wiley. https://doi.org/10.1002/047086303X.
Kiviat, Erik. 2014. “Adaptation of Human Cultures to Wetland Environments.” *2nd International Conference - Water Resources and Wetlands*, 12.
Kotsyurbenko, O. R., M. V. Glagolev, A. Y. Merkel, A. F. Sabrekov, and I. E. Terentieva. 2019. “Methanogenesis in Soils, Wetlands, and Peat.” In *Biogenesis of Hydrocarbons*, edited by Alfons J.M. Stams and Diana Sousa, 1–18. Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-53114-4_9-1.
Kotsyurbenko, O.R. 2005. “Trophic Interactions in the Methanogenic Microbial Community of Low-Temperature Terrestrial Ecosystems.” *FEMS Microbiology Ecology* 53 (1): 3–13. https://doi.org/10.1016/j.femsec.2004.12.009.
Krause, Lars, Danh Biesgen, Aaron Treder, Steffen A. Schweizer, Erwin Klumpp, Claudia Knief, and Nina Siebers. 2019. “Initial Microaggregate Formation: Association of Microorganisms to Montmorillonite-Goethite Aggregates under Wetting and Drying Cycles.” *Geoderma* 351 (October): 250–60. https://doi.org/10.1016/j.geoderma.2019.05.001.
Kuehn, K. A., B. M. Ohsowski, S. N. Francoeur, and R. K. Neely. 2011. “Contributions of Fungi to Carbon Flow and Nutrient Cycling from Standing Dead Typha Angustifolia Leaf Litter in a Temperate Freshwater Marsh.”

Limnology and Oceanography 56 (2): 529–39. https://doi.org/10.4319/lo.2011.56.2.0529.

Kwon, Man Jae, Edward J. O'Loughlin, Maxim I. Boyanov, Jennifer M. Brulc, Eric R. Johnston, Kenneth M. Kemner, and Dionysios A. Antonopoulos. 2016. "Impact of Organic Carbon Electron Donors on Microbial Community Development under Iron-and Sulfate-Reducing Conditions." *PloS One* 11 (1): e0146689.

Lalonde, Karine, Alfonso Mucci, Alexandre Ouellet, and Yves Gélinas. 2012. "Preservation of Organic Matter in Sediments Promoted by Iron." *Nature* 483 (7388): 198–200. https://doi.org/10.1038/nature10855.

Lang, Megan, Greg McCarty, Thomas Ducey, P. Hunt, J. Miller, M. Rabenhorst, Andrew H Baldwin, et al. 2015. "Effects and Effectiveness of USDA Wetland Conservation Practices in the Mid-Atlantic Region: A Report on the Conservation Effects Assessment Project Mid-Atlantic Regional Wetland Assessment 2008 - 2015." Available at. https://www.nrcs.usda. gov/Internet/FSE_DOCUMENTS/nrcseprd1097415.pdf.

Larney, Francis J., and Denis A. Angers. 2012. "The Role of Organic Amendments in Soil Reclamation: A Review." *Canadian Journal of Soil Science* 92 (1): 19–38. https://doi.org/10.4141/cjss2010-064.

Lawrence, Beth A., Shane C. Lishawa, Nia Hurst, Buck T. Castillo, and Nancy C. Tuchman. 2017. "Wetland Invasion by Typha×glauca Increases Soil Methane Emissions." *Aquatic Botany* 137 (January): 80–87. https://doi.org/10.1016/j.aquabot.2016.11.012.

LeFevre, Olivia Victoria, Thorsten Knappenberger, Joey Nathan Shaw, and Yaniv Olshansky. 2021. "Camera Illustration of Indicator of Reduction in Soils (IRIS) Reduction Dynamics." *Agricultural & Environmental Letters* 6 (3). https://doi.org/10.1002/ael2.20051.

Lehmann, Johannes, and Markus Kleber. 2015. "The Contentious Nature of Soil Organic Matter." *Nature*, November. https://doi.org/10.1038/nature16069.

Lentini, Christopher J., Scott D. Wankel, and Colleen M. Hansel. 2012. "Enriched Iron (III)-Reducing Bacterial Communities Are Shaped by Carbon Substrate and Iron Oxide Mineralogy." *Frontiers in Microbiology* 3. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3541049/.

Leprince, F., and H. Quiquampoix. 1996. "Extracellular Enzyme Activity in Soil: Effect of PH and Ionic Strength on the Interaction with Montmorillonite of Two Acid Phosphatases Secreted by the Ectomycorrhizal Fungus Hebeloma Cylindrosporum." *European Journal of Soil Science* 47 (4): 511–22. https://doi.org/10.1111/j.1365-2389.1996.tb01851.x.

Limmer, Matt A., Abby E. Evans, and Angelia L. Seyfferth. 2021. "The IRIS Imager: A Freeware Program for Quantification of Paint Removal on Iris Films." *Soil Science Society of America Journal*.

Lin, Ying-Feng, Shuh-Ren Jing, Tze-Wen Wang, and Der-Yuan Lee. 2002. "Effects of Macrophytes and External Carbon Sources on Nitrate Removal from Groundwater in Constructed Wetlands." *Environmental Pollution* 119 (3): 413–20. https://doi.org/10.1016/S0269-7491(01)00299-8.

Lishawa, Shane C., Beth A. Lawrence, Dennis A. Albert, Daniel J. Larkin, and Nancy C. Tuchman. 2019. “Invasive Species Removal Increases Species and Phylogenetic Diversity of Wetland Plant Communities.” *Ecology and Evolution*, April, ece3.5188. https://doi.org/10.1002/ece3.5188.

Lou, Yunsheng, Lixuan Ren, Zhongpei Li, Taolin Zhang, and Kazuyuki Inubushi. 2007. “Effect of Rice Residues on Carbon Dioxide and Nitrous Oxide Emissions from a Paddy Soil of Subtropical China.” *Water, Air, and Soil Pollution* 178 (1–4): 157–68. https://doi.org/10.1007/s11270-006-9187-x.

Louca, Stilianos, Michael Doebeli, and Laura Wegener Parfrey. 2018. “Correcting for 16S RRNA Gene Copy Numbers in Microbiome Surveys Remains an Unsolved Problem.” *Microbiome* 6 (1): 41. https://doi.org/10.1186/s40168-018-0420-9.

Lu, Jia-Hsun, Chuan Chen, Chihpin Huang, and Duu-Jong Lee. 2020. “Glucose Fermentation with Biochar Amended Consortium Microbial Consortium Shift.” *Bioengineered* 11 (1): 272–80. https://doi.org/10.1080/21655979.2020.1735668.

Luo, Zhongxin, Shengjie Li, Xianfang Zhu, and Guodong Ji. 2018. “Carbon Source Effects on Nitrogen Transformation Processes and the Quantitative Molecular Mechanism in Long-Term Flooded Constructed Wetlands.” *Ecological Engineering* 123 (November): 19–29. https://doi.org/10.1016/j.ecoleng.2018.08.011.

Maietta, Christine E., Zachary A. Bernstein, Joshua R. Gaimaro, Jeffrey S. Buyer, Martin C. Rabenhorst, Victoria L. Monsaint-Queeney, Andrew H. Baldwin, and Stephanie A. Yarwood. 2019. “Aggregation but Not Organo-Metal Complexes Contributed to C Storage in Tidal Freshwater Wetland Soils.” *Soil Science Society of America Journal* 83 (1): 252–65. https://doi.org/10.2136/sssaj2018.05.0199.

Maietta, Christine E., Kelly L. Hondula, C. Nathan Jones, and Margaret A. Palmer. 2020. “Hydrological Conditions Influence Soil and Methane-Cycling Microbial Populations in Seasonally Saturated Wetlands.” *Frontiers in Environmental Science* 8 (November): 593942. https://doi.org/10.3389/fenvs.2020.593942.

Maietta, Christine E., Victoria Monsaint-Queeney, Lindsay Wood, Andrew H. Baldwin, and Stephanie A. Yarwood. 2020. “Plant Litter Amendments in Restored Wetland Soils Altered Microbial Communities More than Clay Additions.” *Soil Biology and Biochemistry* 147 (August): 107846. https://doi.org/10.1016/j.soilbio.2020.107846.

Mander, Ülo, Ain Kull, Evelyn Uuemaa, Kiira Mõisja, Mart Külvik, Tambet Kikas, Janar Raet, Julien Tournebize, and Kalev Sepp. 2018. “Green and Brown Infrastructures Support a Landscape-Level Implementation of Ecological Engineering.” *Ecological Engineering* 120 (September): 23–35. https://doi.org/10.1016/j.ecoleng.2018.05.019.

Mander, Ülo, Martin Maddison, Kaido Soosaar, and Kristjan Karabelnik. 2011. “The Impact of Pulsing Hydrology and Fluctuating Water Table on Greenhouse Gas Emissions from Constructed Wetlands.” *Wetlands* 31 (6): 1023–32. https://doi.org/10.1007/s13157-011-0218-z.

Margaret Jones Bolsterli. 2008. *During Wind and Rain: The Jones Family Farm in the Arkansas Delta, 1848-2006.* Fayetteville, AK: University of Arkansas Press.

Marion M. Weaver. 1964. *History of Tile Drainage in America Prior to 1900*. New York: Waterloo Press.

Marjory Stoneman Douglas. 1947. *The Everglades: River of Grass.* Rinehart.

Markelova, Ekaterina, Christopher T. Parsons, Raoul-Marie Couture, Christina M. Smeaton, Benoit Madé, Laurent Charlet, and Philippe Van Cappellen. 2017. "Deconstructing the Redox Cascade: What Role Do Microbial Exudates (Flavins) Play?" *Environmental Chemistry* 14 (8): 515. https://doi.org/10.1071/EN17158.

Marquart, Kyle A., Ben R. Haller, Janet M. Paper, Theodore M. Flynn, Maxim I. Boyanov, Ganiyat Shodunke, Colleen Gura, Qusheng Jin, and Matthew F. Kirk. 2019. "Influence of PH on the Balance between Methanogenesis and Iron Reduction." *Geobiology* 17 (2): 185–98. https://doi.org/10.1111/gbi.12320.

Martin, Patrick H, Charles D Canham, and Peter L Marks. 2009. "Why Forests Appear Resistant to Exotic Plant Invasions: Intentional Introductions, Stand Dynamics, and the Role of Shade Tolerance." *Frontiers in Ecology and the Environment* 7 (3): 142–49. https://doi.org/10.1890/070096.

Martins, Paula Dalcin, David W. Hoyt, Sheel Bansal, Christopher T. Mills, Malak Tfaily, Brian A. Tangen, Raymond G. Finocchiaro, et al. 2017. "Abundant Carbon Substrates Drive Extremely High Sulfate Reduction Rates and Methane Fluxes in Prairie Pothole Wetlands." *Global Change Biology* 23 (8): 3107–20. https://doi.org/10.1111/gcb.13633.

Matzek, Virginia, Shawn Warren, and Colleen Fisher. 2016. "Incomplete Recovery of Ecosystem Processes after Two Decades of Riparian Forest Restoration: Incomplete Recovery of Ecosystem Processes." *Restoration Ecology* 24 (5): 637–45. https://doi.org/10.1111/rec.12361.

Mazzilli, Sebastián R., Armen R. Kemanian, Oswaldo R. Ernst, Robert B. Jackson, and Gervasio Piñeiro. 2015. "Greater Humification of Belowground than Aboveground Biomass Carbon into Particulate Soil Organic Matter in No-till Corn and Soybean Crops." *Soil Biology and Biochemistry* 85 (June): 22–30. https://doi.org/10.1016/j.soilbio.2015.02.014.

McDonald, Tein, George D Gann, Justin Jonson, and Kingsley W Dixon. 2016. "International Standards for the Practice of Ecological Restoration - Including Principles and Key Concepts," 48.

McGrady-Steed, Jill, Patricia M. Harris, and Peter J. Morin. 1997. "Biodiversity Regulates Ecosystem Predictability." *Nature* 390 (6656): 162–65.

McInerney, Michael J, Jessica R Sieber, and Robert P Gunsalus. 2009. "Syntrophy in Anaerobic Global Carbon Cycles." *Current Opinion in Biotechnology* 20 (6): 623–32. https://doi.org/10.1016/j.copbio.2009.10.001.

McMahon, Shawna K., Mark A. Williams, Peter J. Bottomley, and David D. Myrold. 2005. "Dynamics of Microbial Communities during Decomposition of Carbon-13 Labeled Ryegrass Fractions in Soil." *Soil Science Society of America Journal* 69 (4): 1238. https://doi.org/10.2136/sssaj2004.0289.

McNicol, Gavin, Cove S. Sturtevant, Sara H. Knox, Iryna Dronova, Dennis D. Baldocchi, and Whendee L. Silver. 2017. "Effects of Seasonality, Transport Pathway, and Spatial Structure on Greenhouse Gas Fluxes in a Restored Wetland." *Global Change Biology* 23 (7): 2768–82. https://doi.org/10.1111/gcb.13580.

Meehl, Paul E. 1970. "Nuisance Variables and the Ex Post Facto Design." In *Studies in the Philosophy of Science*, 373–402. IV. University of Minnesota.

Megonigal, J. P., M. E. Hines, and P. T. Visscher. 2004. "Anaerobic Metabolism: Linkages to Trace Gases and Aerobic Processes." In *Biogeochemistry*, 317–424. Elsevier-Pergamon, Oxford, UK.

Megonigal, J Patrick, and Frank P Day. 1988. "Organic Matter Dynamics in Four Seasonally Flooded Forest Communities of the Dismal Swamp" 75 (9): 11.

Melton, Emily D., Elizabeth D. Swanner, Sebastian Behrens, Caroline Schmidt, and Andreas Kappler. 2014. "The Interplay of Microbially Mediated and Abiotic Reactions in the Biogeochemical Fe Cycle." *Nature Reviews Microbiology* 12 (12): 797–808. https://doi.org/10.1038/nrmicro3347.

Melvin, Norman C. 2003. "Wetland Restoration, Enhancement, and Management." United States Department of Agriculture Natural Resources Conservation Service. https://www.nrcs.usda.gov/wps/portal/nrcs/detail/national/water/wetlands/restore/?cid=nrcs143_010912.

Meruane, Gabriel, and Tomás Vargas. 2003. "Bacterial Oxidation of Ferrous Iron by Acidithiobacillus Ferrooxidans in the PH Range 2.5–7.0." *Hydrometallurgy* 71 (1–2): 149–58. https://doi.org/10.1016/S0304-386X(03)00151-8.

Meyer, William B. 1994. "When Dismal Swamps Became Priceless Wetlands." *American Heritage* 45 (3): 6.

Miao, Guofang, Asko Noormets, Jean-Christophe Domec, Montserrat Fuentes, Carl C. Trettin, Ge Sun, Steve G. McNulty, and John S. King. 2017. "Hydrology and Microtopography Control Carbon Dynamics in Wetlands: Implications in Partitioning Ecosystem Respiration in a Coastal Plain Forested Wetland." *Agricultural and Forest Meteorology* 247 (December): 343–55. https://doi.org/10.1016/j.agrformet.2017.08.022.

Mikutta, Robert, Markus Kleber, Margaret S. Torn, and Reinhold Jahn. 2006. "Stabilization of Soil Organic Matter: Association with Minerals or Chemical Recalcitrance?" *Biogeochemistry* 77 (1): 25–56. https://doi.org/10.1007/s10533-005-0712-6.

Miller, D. C. 1989. *Dark Eden: The Swamp in Nineteenth-Century American Culture.* Vol. Cambridge studies in American literature and culture. Cambridge England: Cambridge University Press.

Mills, Aaron L., Janet S. Herman, George M. Hornberger, and Todd H. DeJesús. 1994. "Effect of Solution Ionic Strength and Iron Coatings on Mineral Grains on the Sorption of Bacterial Cells to Quartz Sand." *Applied and Environmental Microbiology* 60 (9): 3300–3306. https://doi.org/10.1128/AEM.60.9.3300-3306.1994.

Minasny, B., and A. B. McBratney. 2018. "Rejoinder to the Comment on: B. Minasny & A.B. McBratney. 2018. Limited Effect of Organic Matter on Soil

Available Water Capacity: Letter to the Editor." *European Journal of Soil Science* 69 (1): 155–57. https://doi.org/10.1111/ejss.12526.
Mirreh, H. F., and J. W. Ketcheson. 1972. "Influence of Soil Bulk Density and Matric Pressure on Soil Resistance to Penetration." *Canadian Journal of Soil Science* 52 (3): 477–83. https://doi.org/10.4141/cjss72-059.
Mitra, Sudip, Reiner Wassmann, and Paul LG Vlek. 2005. "An Appraisal of Global Wetland Area and Its Organic Carbon Stock." *Current Science* 88 (1): 25.
Mitsch, William J., Blanca Bernal, and Maria E. Hernandez. 2015. "Ecosystem Services of Wetlands." *International Journal of Biodiversity Science, Ecosystem Services & Management* 11 (1): 1–4. https://doi.org/10.1080/21513732.2015.1006250.
Mitsch, William J., Blanca Bernal, Amanda M. Nahlik, Ülo Mander, Li Zhang, Christopher J. Anderson, Sven E. Jørgensen, and Hans Brix. 2013. "Wetlands, Carbon, and Climate Change." *Landscape Ecology* 28 (4): 583–97. https://doi.org/10.1007/s10980-012-9758-8.
Mitsch, William J., and Renee F. Wilson. 1996. "Improving the Success of Wetland Creation and Restoration with Know-How, Time, and Self-Design." *Ecological Applications* 6 (1): 77–83. https://doi.org/10.2307/2269554.
Møller, Claus Lindskov, and Kaj Sand-Jensen. 2008. "Iron Plaques Improve the Oxygen Supply to Root Meristems of the Freshwater Plant, *Lobelia Dortmanna*." *New Phytologist* 179 (3): 848–56. https://doi.org/10.1111/j.1469-8137.2008.02506.x.
Montero, Pablo, and José A. Vilar. 2014. "TSclust: An R Package for Time Series Clustering." *Journal of Statistical Software* 62 (1). https://doi.org/10.18637/jss.v062.i01.
Montzka, S. A., E. J. Dlugokencky, and J. H. Butler. 2011. "Non-CO2 Greenhouse Gases and Climate Change." *Nature* 476 (7358): 43–50. https://doi.org/10.1038/nature10322.
Moore, Dwayne R. J., and Paul A. Keddy. 1988. "The Relationship between Species Richness and Standing Crop in Wetlands: The Importance of Scale." *Vegetatio* 79 (1–2): 99–106. https://doi.org/10.1007/BF00044853.
Moreno-Mateos, David, Mary E. Power, Francisco A. Comín, and Roxana Yockteng. 2012. "Structural and Functional Loss in Restored Wetland Ecosystems." Edited by Michel Loreau. *PLoS Biology* 10 (1): e1001247. https://doi.org/10.1371/journal.pbio.1001247.
Morrissey, Ember M., and Rima B. Franklin. 2015. "Resource Effects on Denitrification Are Mediated by Community Composition in Tidal Freshwater Wetlands Soils: Resource and Community Effects on Denitrification." *Environmental Microbiology* 17 (5): 1520–32. https://doi.org/10.1111/1462-2920.12575.
Moser, Kurt, Changwoo Ahn, and Gregory Noe. 2007. "Characterization of Microtopography and Its Influence on Vegetation Patterns in Created Wetlands." *Wetlands* 27 (4): 1081–97. https://doi.org/10.1672/0277-5212(2007)27[1081:COMAII]2.0.CO;2.
Moser, Kurt F., Changwoo Ahn, and Gregory B. Noe. 2009. "The Influence of Microtopography on Soil Nutrients in Created Mitigation Wetlands."

Restoration Ecology 17 (5): 641–51. https://doi.org/10.1111/j.1526-100X.2008.00393.x.

Muggeo, Vito M R. 2008. "Segmented: An R Package to Fit Regression Models with Broken-Line Relationships" 8: 7.

Müller, Caroline, Kacilda Naomi Kuki, Daniel Teixeira Pinheiro, Laíse Rosado de Souza, Advânio Inácio Siqueira Silva, Marcelo Ehlers Loureiro, Marco Antonio Oliva, and Andréa Miyasaka Almeida. 2015. "Differential Physiological Responses in Rice upon Exposure to Excess Distinct Iron Forms." *Plant Soil*, 16.

Mutyala, Sakuntala, Changman Kim, Young Eun Song, Himanshu Khandelwal, Jiyun Baek, Eunhee Seol, You-kwan Oh, and Jung Rae Kim. 2021. "Enabling Anoxic Acetate Assimilation by Electrode-Driven Respiration in the Obligate Aerobe, Pseudomonas Putida." *Bioelectrochemistry* 138 (April): 107690. https://doi.org/10.1016/j.bioelechem.2020.107690.

Myers, C. R., and K. H. Nealson. 1988. "Bacterial Manganese Reduction and Growth with Manganese Oxide as the Sole Electron Acceptor." *Science* 240 (4857): 1319–21. https://doi.org/10.1126/science.240.4857.1319.

Nahlik, Amanda M., and William J. Mitsch. 2010. "Methane Emissions From Created Riverine Wetlands." *Wetlands* 30 (4): 783–93. https://doi.org/10.1007/s13157-010-0038-6.

Narrowe, Adrienne B., Jordan C. Angle, Rebecca A. Daly, Kay C. Stefanik, Kelly C. Wrighton, and Christopher S. Miller. 2017. "High-Resolution Sequencing Reveals Unexplored Archaeal Diversity in Freshwater Wetland Soils: Freshwater Wetland Archaeal Diversity." *Environmental Microbiology* 19 (6): 2192–2209. https://doi.org/10.1111/1462-2920.13703.

National Technical Committee for Hydric Soils (NTCHS). 2015. "Hydric Soils Technical Note 11: Hydric Soils Technical Standard and Data Submission Requirements for Field Indicators of Hydric Soils." Washington, DC: United States Department of Agriculture (USDA), NRCS.

Neubauer, Scott C. 2014. "On the Challenges of Modeling the Net Radiative Forcing of Wetlands: Reconsidering Mitsch et al. 2013." *Landscape Ecology* 29 (4): 571–77. https://doi.org/10.1007/s10980-014-9986-1.

Neubauer, Scott C., and J. Patrick Megonigal. 2015. "Moving Beyond Global Warming Potentials to Quantify the Climatic Role of Ecosystems." *Ecosystems* 18 (6): 1000–1013. https://doi.org/10.1007/s10021-015-9879-4.

Neubauer, Scott C., and Jos T.A. Verhoeven. 2019. "Wetland Effects on Global Climate: Mechanisms, Impacts, and Management Recommendations." In *Wetlands: Ecosystem Services, Restoration and Wise Use*, 39–62. Springer.

Neufeld, Josh D., Marc G. Dumont, Jyotsna Vohra, and J. Colin Murrell. 2007. "Methodological Considerations for the Use of Stable Isotope Probing in Microbial Ecology." *Microbial Ecology* 53 (3): 435–42. https://doi.org/10.1007/s00248-006-9125-x.

Nitzsche, Kai Nils, Thomas Kalettka, Katrin Premke, Gunnar Lischeid, Arthur Gessler, and Zachary Eric Kayler. 2017. "Land-Use and Hydroperiod Affect Kettle Hole Sediment Carbon and Nitrogen Biogeochemistry." *Science of The*

Total Environment 574 (January): 46–56. https://doi.org/10.1016/j.scitotenv.2016.09.003.
Noble, R., and S. J. Roberts. 2004. "Eradication of Plant Pathogens and Nematodes during Composting: A Review." *Plant Pathology* 53 (5): 548–68. https://doi.org/10.1111/j.0032-0862.2004.01059.x.
Noll, Aaron, Courtney Mobilian, and Christopher Craft. 2019. "Five Decades of Wetland Soil Development of a Constructed Tidal Salt Marsh, North Carolina, USA." *Ecological Restoration* 37 (3): 163–70. https://doi.org/10.3368/er.37.3.163.
Nottingham, Andrew T., Howard Griffiths, Paul M. Chamberlain, Andrew W. Stott, and Edmund V.J. Tanner. 2009. "Soil Priming by Sugar and Leaf-Litter Substrates: A Link to Microbial Groups." *Applied Soil Ecology* 42 (3): 183–90. https://doi.org/10.1016/j.apsoil.2009.03.003.
NRCS. 2010. "Wetland Restoration Code 657," 5.
Obalum, S.E., G.U. Chibuike, S. Peth, and Y. Ouyang. 2017. "Soil Organic Matter as Sole Indicator of Soil Degradation." *Environmental Monitoring and Assessment* 189 (4): 176. https://doi.org/10.1007/s10661-017-5881-y.
O'Brien, Erin L., and Joy B. Zedler. 2006. "Accelerating the Restoration of Vegetation in a Southern California Salt Marsh." *Wetlands Ecology and Management* 14 (3): 269–86. https://doi.org/10.1007/s11273-005-1480-8.
Ohno, Tsutomu, and Bradley S. Crannell. 1996. "Green and Animal Manure-Derived Dissolved Organic Matter Effects on Phosphorus Sorption." *Journal of Environment Quality* 25 (5): 1137. https://doi.org/10.2134/jeq1996.00472425002500050029x.
Oren, Adi, Nativ Rotbart, Mikhail Borisover, and Asher Bar-Tal. 2018. "Chloroform Fumigation Extraction for Measuring Soil Microbial Biomass: The Validity of Using Samples Approaching Water Saturation." *Geoderma* 319 (June): 204–7. https://doi.org/10.1016/j.geoderma.2018.01.007.
Ott, Emily. 2018. "Soil Genesis and Vegetation Response to Amendments and Microtopography in Two Virginia Coastal Plain Created Wetlands." Thesis, Virginia Polytechnic Institute and State University.
Ott, Emily T., John M. Galbraith, W. Lee Daniels, and W. Michael Aust. 2020. "Effects of Amendments and Microtopography on Created Tidal Freshwater Wetland Soil Morphology and Carbon." *Soil Science Society of America Journal* 84 (2): 638–52. https://doi.org/10.1002/saj2.20057.
Ozores-Hampton, Monica. 2021. *"Impact of Compost on Soil Health." In Compost Utilization in Production of Horticultural Crops*. CRC Press.
Pal, Levin, Blaž Stres, Tjaša Danevčič, Simona Leskovec, and Ines Mandic-Mulec. 2010. "Transformations of Mineral Nitrogen Applied to Peat Soil during Sequential Oxic/Anoxic Cycling." *Soil Biology and Biochemistry* 42 (8): 1338–46. https://doi.org/10.1016/j.soilbio.2010.03.013.
Palmer, M.A., E.S. Bernhardt, J. D. Allan, P.S. Lake, G. Alexander, S. Brooks, J. Carr, et al. 2005. "Standards for Ecologically Successful River Restoration: Ecological Success in River Restoration." *Journal of Applied Ecology* 42 (2): 208–17. https://doi.org/10.1111/j.1365-2664.2005.01004.x.

Palmer, Margaret A., and Kelly L. Hondula. 2014. “Restoration As Mitigation: Analysis of Stream Mitigation for Coal Mining Impacts in Southern Appalachia.” *Environmental Science & Technology* 48 (18): 10552–60. https://doi.org/10.1021/es503052f.

Palmer, Margaret A., Kelly L. Hondula, and Benjamin J. Koch. 2014. “Ecological Restoration of Streams and Rivers: Shifting Strategies and Shifting Goals.” *Annual Review of Ecology, Evolution, and Systematics* 45 (1): 247–69. https://doi.org/10.1146/annurev-ecolsys-120213-091935.

Paustian, Keith, Johannes Lehmann, Stephen Ogle, David Reay, G. Philip Robertson, and Pete Smith. 2016. “Climate-Smart Soils.” *Nature* 532 (7597): 49–57. https://doi.org/10.1038/nature17174.

Peet, Robert K., Thomas R. Wentworth, and Peter S. White. 1998. “A Flexible, Multipurpose Method for Recording Vegetation Composition and Structure.” *Castanea* 63 (3): 262–74.

Pereira, Eduardo Gusmão, Marco Antonio Oliva, Advânio Inacio Siqueira-Silva, Laíse Rosado-Souza, Daniel Teixeira Pinheiro, and Andréa Miyasaka Almeida. 2014. “Tropical Rice Cultivars from Lowland and Upland Cropping Systems Differ in Iron Plaque Formation.” *Journal of Plant Nutrition* 37 (9): 1373–94. https://doi.org/10.1080/01904167.2014.888744.

Perry, Laura G., and Susan M. Galatowitsch. 2004. “The Influence of Light Availability on Competition between Phalaris Arundinacea and a Native Wetland Sedge.” *Plant Ecology (Formerly Vegetatio)* 170 (1): 73–81. https://doi.org/10.1023/B:VEGE.0000019019.78909.f0.

Pester, Michael, Norbert Bittner, Pinsurang Deevong, Michael Wagner, and Alexander Loy. 2010. “A ‘Rare Biosphere’ Microorganism Contributes to Sulfate Reduction in a Peatland.” *The ISME Journal* 4 (12): 1591–1602. https://doi.org/10.1038/ismej.2010.75.

Petru, Bradley J., Changwoo Ahn, and George Chescheir. 2013. “Alteration of Soil Hydraulic Properties during the Construction of Mitigation Wetlands in the Virginia Piedmont.” *Ecological Engineering* 51 (February): 140–50. https://doi.org/10.1016/j.ecoleng.2012.12.073.

Pickering, Catherine, and Jason Byrne. 2014. “The Benefits of Publishing Systematic Quantitative Literature Reviews for PhD Candidates and Other Early-Career Researchers.” *Higher Education Research & Development* 33 (3): 534–48. https://doi.org/10.1080/07294360.2013.841651.

Pietrzykowski, Marcin, W. Lee Daniels, and Sara C. Koropchak. 2015. “Microtopographic Effects on Growth of Young Bald Cypress (Taxodium Distichum L.) in a Created Freshwater Forested Wetland in Southeastern Virginia.” *Ecological Engineering* 83 (October): 135–43. https://doi.org/10.1016/j.ecoleng.2015.06.024.

Pimentel, David, Rodolfo Zuniga, and Doug Morrison. 2005. “Update on the Environmental and Economic Costs Associated with Alien-Invasive Species in the United States.” *Ecological Economics* 52 (3): 273–88. https://doi.org/10.1016/j.ecolecon.2004.10.002.

Poggenburg, Christine, Robert Mikutta, Axel Schippers, Reiner Dohrmann, and Georg Guggenberger. 2018. “Impact of Natural Organic Matter Coatings on

the Microbial Reduction of Iron Oxides." *Geochimica et Cosmochimica Acta* 224 (March): 223–48. https://doi.org/10.1016/j.gca.2018.01.004.
Poulton, Simon W., and Donald E. Canfield. 2005. "Development of a Sequential Extraction Procedure for Iron: Implications for Iron Partitioning in Continentally Derived Particulates." *Chemical Geology* 214 (3–4): 209–21. https://doi.org/10.1016/j.chemgeo.2004.09.003.
Prakash, Divya, Shikha S. Chauhan, and James G. Ferry. 2019. "Life on the Thermodynamic Edge: Respiratory Growth of an Acetotrophic Methanogen." *Science Advances* 5 (8): eaaw9059. https://doi.org/10.1126/sciadv.aaw9059.
Prather, Michael J., Christopher D. Holmes, and Juno Hsu. 2012. "Reactive Greenhouse Gas Scenarios: Systematic Exploration of Uncertainties and the Role of Atmospheric Chemistry: ATMOSPHERIC CHEMISTRY AND GREENHOUSE GASES." *Geophysical Research Letters* 39 (9): n/a-n/a. https://doi.org/10.1029/2012GL051440.
Price, Edward P. F., Greg Spyreas, and Jeffrey W. Matthews. 2019. "Wetland Compensation and Its Impacts on β-Diversity." *Ecological Applications* 29 (1): e01827. https://doi.org/10.1002/eap.1827.
Prince, Hugh C. 1997. *Wetlands of the American Midwest : A Historical Geography of Changing Attitudes*. University of Chicago Geography Research Paper ; No. 241; University of Chicago Geography Research Paper ; No. 241. Chicago : The University of Chicago Press,. Table of contents http://catdir.loc.gov/catdir/toc/uchi052/97022226.html.
Qiu, Cunpu, Youzhi Feng, Meng Wu, Ming Liu, Weitao Li, and Zhongpei Li. 2019. "NanoFe3O4 Accelerates Methanogenic Straw Degradation by Improving Energy Metabolism." *Bioresource Technology* 292 (November): 121930. https://doi.org/10.1016/j.biortech.2019.121930.
Quintero, R. I., M. J. Galotto, F. Rodriguez, and A. Guarda. 2014. "Preparation and Characterization of Cellulose Acetate Butyrate/Organoclay Nanocomposites Produced by Extrusion: ANTIMICROBIAL CELLULOSE ACETATE BUTYRATE NANOCOMPOSITES." *Packaging Technology and Science* 27 (6): 495–507. https://doi.org/10.1002/pts.2043.
Rabenhorst, M. C. 2005. "Biologic Zero: A Soil Temperature Concept." *Wetlands* 25 (3): 616–21. https://doi.org/10.1672/0277-5212(2005)025[0616:BZASTC]2.0.CO;2.
———. 2009. "Making Soil Oxidation-Reduction Potential Measurements Using Multimeters." *Soil Science Society of America Journal* 73 (6): 2198–2201. https://doi.org/10.2136/sssaj2009.0040.
Rabenhorst, M. C., and Kristine A. Persing. 2017. "A Synthesized Manganese Oxide for Easily Making Durable Manganese-Coated IRIS Tubes." *Soil Science Society of America Journal* 81 (1): 233. https://doi.org/10.2136/sssaj2016.10.0348n.
Rabenhorst, Martin C. 2018. "A System for Making and Deploying Oxide-Coated Plastic Films for Environmental Assessment of Soils." *Soil Science Society of America Journal* 82 (5): 1301. https://doi.org/10.2136/sssaj2018.05.0178.

Rabenhorst, Martin C., and S. N. Burch. 2006. “Synthetic Iron Oxides as an Indicator of Reduction in Soils (IRIS).” *Soil Science Society of America Journal* 70 (4): 1227. https://doi.org/10.2136/sssaj2005.0354.

Rabot, E., M. Wiesmeier, S. Schlüter, and H.-J. Vogel. 2018. “Soil Structure as an Indicator of Soil Functions: A Review.” *Geoderma* 314 (March): 122–37. https://doi.org/10.1016/j.geoderma.2017.11.009.

Rahlff, Janina, Christian Stolle, Helge-Ansgar Giebel, Thorsten Brinkhoff, Mariana Ribas-Ribas, Dorothee Hodapp, and Oliver Wurl. 2017. “High Wind Speeds Prevent Formation of a Distinct Bacterioneuston Community in the Sea-Surface Microlayer.” *FEMS Microbiology Ecology* 93 (5). https://doi.org/10.1093/femsec/fix041.

Ramakrishnan, Balasubramanian, Tillmann Lueders, Peter F Dun, and Michael W Friedrich. 2001. “Archaeal Community Structures in Rice Soils from Different Geographical Regions before and after Initiation of Methane Production.” *FEMS Microbiology Ecology* 37: 12.

Rawls, W.J., Y.A. Pachepsky, J.C. Ritchie, T.M. Sobecki, and H. Bloodworth. 2003. “Effect of Soil Organic Carbon on Soil Water Retention.” *Quantifying Agricultural Management Effects on Soil Properties and Processes* 116 (1): 61–76. https://doi.org/10.1016/S0016-7061(03)00094-6.

Rice, James A. 2001. “Humin.” *Soil Science* 166 (11): 848–57.

Richardson, Curtis J., Gregory L. Bruland, Matthew F. Hanchey, and Ariana E. Sutton-Grier. 2016. “Soil Restoration: The Foundation of Successful Wetland Reestablishment.” In *Soil Restoration: The Foundation of Successful Wetland Reestablishment*, 2nd ed., Chapter 19:469–93. CRC Press.

Rigby, M., R. G. Prinn, P. J. Fraser, P. G. Simmonds, R. L. Langenfelds, J. Huang, D. M. Cunnold, et al. 2008. “Renewed Growth of Atmospheric Methane.” *Geophysical Research Letters* 35 (22): L22805. https://doi.org/10.1029/2008GL036037.

Rivenshield, Angela, and Nina L. Bassuk. 2007. “Using Organic Amendments to Decrease Bulk Density and Increase Macroporosity in Compacted Soils.” *Arboriculture and Urban Forestry* 33 (2): 140.

R.K. Sawyer. 2013. *Texas Market Hunting*. College Station, TX: Texas A&M University Press.

Rodhe, Henning. 1990. “A Comparison of the Contribution of Various Gases to the Greenhouse Effect.” *Science* 248 (4960): 1217–19.

Rosbakh, Sergey, Shyam S Phartyal, and Peter Poschlod. 2020. “Seed Germination Traits Shape Community Assembly along a Hydroperiod Gradient.” *Annals of Botany* 125 (1): 67–78. https://doi.org/10.1093/aob/mcz139.

RoyChowdhury, Taniya, Lisa Bramer, David W. Hoyt, Young-Mo Kim, Thomas O. Metz, Lee Ann McCue, Heida L. Diefenderfer, Janet K. Jansson, and Vanessa Bailey. 2018. “Temporal Dynamics of CO 2 and CH 4 Loss Potentials in Response to Rapid Hydrological Shifts in Tidal Freshwater Wetland Soils.” *Ecological Engineering* 114 (April): 104–14. https://doi.org/10.1016/j.ecoleng.2017.06.041.

Rubin, Rachel L., Todd R. Anderson, and Kate A. Ballantine. 2020. “Biochar Simultaneously Reduces Nutrient Leaching and Greenhouse Gas Emissions in

Restored Wetland Soils." *Wetlands* 40 (6): 1981–91. https://doi.org/10.1007/s13157-020-01380-8.
Ruddiman, William F., Zhengtang Guo, Xin Zhou, Hanbin Wu, and Yanyan Yu. 2008. "Early Rice Farming and Anomalous Methane Trends." *Quaternary Science Reviews* 27 (13–14): 1291–95. https://doi.org/10.1016/j.quascirev.2008.03.007.
Russell, Kimberley N., and Vanessa B. Beauchamp. 2017. "Plant Species Diversity in Restored and Created Delmarva Bay Wetlands." *Wetlands* 37 (6): 1119–33. https://doi.org/10.1007/s13157-017-0945-x.
Saaltink, Rémon M., Stefan C. Dekker, Maarten B. Eppinga, Jasper Griffioen, and Martin J. Wassen. 2017. "Plant-Specific Effects of Iron-Toxicity in Wetlands." *Plant and Soil* 416 (1–2): 83–96. https://doi.org/10.1007/s11104-017-3190-4.
Saha, Goutam Kumar. 2016. *Wetland: Crisis and Options*. 1st ed. New Dehli: Daya Publishing House.
Saidpullicino, D, F Erriquens, and G Gigliotti. 2007. "Changes in the Chemical Characteristics of Water-Extractable Organic Matter during Composting and Their Influence on Compost Stability and Maturity." *Bioresource Technology* 98 (9): 1822–31. https://doi.org/10.1016/j.biortech.2006.06.018.
Sanderman, Jonathan, Tomislav Hengl, and Gregory J. Fiske. 2017. "Soil Carbon Debt of 12,000 Years of Human Land Use." *Proceedings of the National Academy of Sciences* 114 (36): 9575–80. https://doi.org/10.1073/pnas.1706103114.
Sarri, Kristen J, Robert Bonnie, and Christine Blackburn. 2016. "2016-2018 NISC Management Plan," 50.
Sawyer, Roy T. 2010. *America's Wetland: An Environmental and Cultural History of Tidewater Virginia and North Carolina.* Charlottesville, Va.: University of Virginia Press.
Schöngart, J., J. Arieira, C. Felfili Fortes, E. Cezarine de Arruda, and C. Nunes da Cunha. 2011. "Age-Related and Stand-Wise Estimates of Carbon Stocks and Sequestration in the Aboveground Coarse Wood Biomass of Wetland Forests in the Northern Pantanal, Brazil." *Biogeosciences* 8 (11): 3407–21. https://doi.org/10.5194/bg-8-3407-2011.
Schwertmann, U., and H. Fechter. 1994. "The Formation of Green Rust and Its Transformation to Lepidocrocite." *Clay Minerals* 29 (01): 87–92. https://doi.org/10.1180/claymin.1994.029.1.10.
Scott, Brian, Andrew H. Baldwin, Kate Ballantine, Margaret Palmer, and Stephanie Yarwood. 2020. "The Role of Organic Amendments in Wetland Restorations." *Restoration Ecology* 28 (4): 776–84. https://doi.org/10.1111/rec.13179.
Scott, P W, P J Ealey, and G K Rollinson. 2011. "Bog Iron Ore from Lowland Point, St Keverne, Lizard, Cornwall." *Geoscience in South-West England* 12: 9.
Seabloom, Eric W., A. G. van der Valk, and Kirk A. Moloney. 1998. "The Role of Water Depth and Soil Temperature in Determining Initial Composition of Prairie Wetland Coenoclines." *Plant Ecology* 138.

Seitzinger, S., J. A. Harrison, J. K. Böhlke, A. F. Bouwman, R. Lowrance, B. Peterson, C. Tobias, and G. Van Drecht. 2006. "Denitrification Across Landscapes and Waterscapes: A Synthesis." *Ecological Applications* 16 (6): 2064–90. https://doi.org/10.1890/1051-0761(2006)016[2064:DALAWA]2.0.CO;2.

Sha, Chenyan, William J. Mitsch, Ülo Mander, Jianjian Lu, Jackie Batson, Li Zhang, and Wenshan He. 2011. "Methane Emissions from Freshwater Riverine Wetlands." *Ecological Engineering* 37 (1): 16–24. https://doi.org/10.1016/j.ecoleng.2010.07.022.

Shaw, Samuel P., and C. Gordon Fredline. 1956. "Wetlands of the United States: Their Extand and Their Value to Waterfowl and Other Wildlife." Fish and Wildlife Service.

Shimizu, Masayuki, Jihai Zhou, Christian Schröder, Martin Obst, Andreas Kappler, and Thomas Borch. 2013. "Dissimilatory Reduction and Transformation of Ferrihydrite-Humic Acid Coprecipitates." *Environmental Science & Technology* 47 (23): 13375–84. https://doi.org/10.1021/es402812j.

Sinsabaugh, R. S. 1994. "Enzymic Analysis of Microbial Pattern and Process." *Biology and Fertility of Soils* 17 (1): 69–74. https://doi.org/10.1007/BF00418675.

Sinsabaugh, Robert L., Stefano Manzoni, Daryl L. Moorhead, and Andreas Richter. 2013. "Carbon Use Efficiency of Microbial Communities: Stoichiometry, Methodology and Modelling." Edited by James Elser. *Ecology Letters* 16 (7): 930–39. https://doi.org/10.1111/ele.12113.

Sivan, O., S. S. Shusta, and D. L. Valentine. 2016. "Methanogens Rapidly Transition from Methane Production to Iron Reduction." *Geobiology* 14 (2): 190–203. https://doi.org/10.1111/gbi.12172.

Sleeper, B. E., and R. L. Ficklin. 2016. "Edaphic and Vegetative Responses to Forested Wetland Restoration with Created Microtopography in Arkansas." *Ecological Restoration* 34 (2): 117–23. https://doi.org/10.3368/er.34.2.117.

Smeaton, Christina M., and Philippe Van Cappellen. 2018. "Gibbs Energy Dynamic Yield Method (GEDYM): Predicting Microbial Growth Yields under Energy-Limiting Conditions." *Geochimica et Cosmochimica Acta* 241 (November): 1–16. https://doi.org/10.1016/j.gca.2018.08.023.

Souza, Rodolfo. 2021. "Optimal Drainage Timing for Mitigating Methane Emissions from Rice Paddy Fields." *Geoderma*, no. 394: 9.

State Highway Administration. 2018. "Standard Specifications for Construction and Materials." Maryland Department of Transportation.

Stauffer, Aura L., and Robert P. Brooks. 1997. "Plant and Soil Responses to Salvaged Marsh Surface and Organic Matter Amendments at a Created Wetland in Central Pennsylvania." *Wetlands* 17 (1): 90–105. https://doi.org/10.1007/BF03160721.

Steinbachová-Vojtíšková, Lenka, Edita Tylová, Aleš Soukup, Hana Novická, Olga Votrubová, Helena Lipavská, and Hana Čížková. 2006. "Influence of Nutrient Supply on Growth, Carbohydrate, and Nitrogen Metabolic Relations in Typha Angustifolia." *Environmental and Experimental Botany* 57 (3): 246–57. https://doi.org/10.1016/j.envexpbot.2005.06.003.

Stelk, M.J., J Christie, R. Weber, R.R.III Lewis, J. Zedler, M. Micacchion, and J. Merritt. 2017. "Wetland Restoration - Contemporary Issues & Lessons Learned." *Association of State Wetland Managers, Windham, Maine. Pp. 153*, 2017.

Stewart, Ryan D., Jinshi Jian, Ayush J. Gyawali, Wade E. Thomason, Brian D. Badgley, Mark S. Reiter, and Michael S. Strickland. 2018. "What We Talk about When We Talk about Soil Health." *Ael* 3 (1): 0. https://doi.org/10.2134/ael2018.06.0033.

Stine, Jeffrey K. 2008. *America's Forested Wetlands: From Wasteland to Valued Resource*. 1st ed. Forest History Society Issues Series. Durham, N.C: Forest History Society.

Straub, Kristina L., Marcus Benz, and Bernhard Schink. 2001. "Iron Metabolism in Anoxic Environments at near Neutral PH." *FEMS Microbiology Ecology* 34 (3): 181–86. https://doi.org/10.1111/j.1574-6941.2001.tb00768.x.

Strycharz-Glaven, Sarah M., Rachel M. Snider, Anthony Guiseppi-Elie, and Leonard M. Tender. 2011. "On the Electrical Conductivity of Microbial Nanowires and Biofilms." *Energy & Environmental Science* 4 (11): 4366. https://doi.org/10.1039/c1ee01753e.

Stumm, Werner, and Barbara Sulzberger. 1992. "The Cycling of Iron in Natural Environments: Considerations Based on Laboratory Studies of Heterogeneous Redox Processes." *Geochimica et Cosmochimica Acta* 56 (8): 3233–57. https://doi.org/10.1016/0016-7037(92)90301-X.

Suding, K., E. Higgs, M. Palmer, J. B. Callicott, C. B. Anderson, M. Baker, J. J. Gutrich, et al. 2015. "Committing to Ecological Restoration." *Science* 348 (6235): 638–40. https://doi.org/10.1126/science.aaa4216.

Suding, Katharine N., Scott L. Collins, Laura Gough, Christopher Clark, Elsa E. Cleland, Katherine L. Gross, Daniel G. Milchunas, and Steven Pennings. 2005. "Functional-and Abundance-Based Mechanisms Explain Diversity Loss Due to N Fertilization." *Proceedings of the National Academy of Sciences of the United States of America* 102 (12): 4387–92.

Sun, Li, Phillip B. Pope, Vincent G. H. Eijsink, and Anna Schnürer. 2015. "Characterization of Microbial Community Structure during Continuous Anaerobic Digestion of Straw and Cow Manure: Community Structure during Anaerobic Digestion." *Microbial Biotechnology* 8 (5): 815–27. https://doi.org/10.1111/1751-7915.12298.

Sutton-Grier, Ariana E., Mengchi Ho, and Curtis J. Richardson. 2009. "Organic Amendments Improve Soil Conditions and Denitrification in a Restored Riparian Wetland." *Wetlands* 29 (1): 343–52.

Tang, Jia, Li Zhuang, Jinlian Ma, Ziyang Tang, Zhen Yu, and Shungui Zhou. 2016. "Secondary Mineralization of Ferrihydrite Affects Microbial Methanogenesis in Geobacter-Methanosarcina Cocultures." Edited by G. Voordouw. *Applied and Environmental Microbiology* 82 (19): 5869–77. https://doi.org/10.1128/AEM.01517-16.

Tate, K.R., D.J. Ross, and C.W. Feltham. 1988. "A Direct Extraction Method to Estimate Soil Microbial c: Effects of Experimental Variables and Some

Different Calibration Procedures." *Soil Biology and Biochemistry* 20 (3): 329–35. https://doi.org/10.1016/0038-0717(88)90013-2.
Teh, Yit Arn, Whendee L Silver, and Mark E. Conrad. 2005. "Teh, Yit Arn, Whendee L. Silver, and Mark E. Conrad. 'Oxygen Effects on Methane Production and Oxidation in Humid Tropical Forest Soils.' 11, No. 8 (2005): ." *Global Change Biology* 11 (8): 1283–97.
Thelemann, Michael, Wiebke Bebermeier, Philipp Hoelzmann, and Enrico Lehnhardt. 2017. "Bog Iron Ore as a Resource for Prehistoric Iron Production in Central Europe — A Case Study of the Widawa Catchment Area in Eastern Silesia, Poland." *CATENA* 149 (February): 474–90. https://doi.org/10.1016/j.catena.2016.04.002.
Thorslund, Josefin, Jerker Jarsjo, Fernando Jaramillo, James W. Jawitz, Stefano Manzoni, Nandita B. Basu, Sergey R. Chalov, et al. 2017. "Wetlands as Large-Scale Nature-Based Solutions: Status and Challenges for Research, Engineering and Management." *Ecological Engineering* 108 (November): 489–97. https://doi.org/10.1016/j.ecoleng.2017.07.012.
Tiquia, S.M, and N.F.Y Tam. 2000. "Fate of Nitrogen during Composting of Chicken Litter." *Environmental Pollution* 110 (3): 535–41. https://doi.org/10.1016/S0269-7491(99)00319-X.
Titus, Jonathan Harold. 1990. "Microtopography and Woody Plant Regeneration in a Hardwood Floodplain Swamp in Florida." *Bulletin of the Torrey Botanical Club* 117 (4): 429. https://doi.org/10.2307/2996840.
Trettin, Carl, and Martin Jurgensen. 2002. "Carbon Cycling in Wetland Forest Soils." In *The Potential of U.S. Forest Soils to Sequester Carbon and Mitigate the Greenhouse Effect*, edited by J Kimble, R Lal, Richard Birdsey, and Linda Heath, 311–31. CRC Press. https://doi.org/10.1201/9781420032277.ch19.
Turetsky, Merritt R., Agnieszka Kotowska, Jill Bubier, Nancy B. Dise, Patrick Crill, Ed R. C. Hornibrook, Kari Minkkinen, et al. 2014. "A Synthesis of Methane Emissions from 71 Northern, Temperate, and Subtropical Wetlands." *Global Change Biology* 20 (7): 2183–97. https://doi.org/10.1111/gcb.12580.
Uchimiya, Minori, and Alan T. Stone. 2009. "Reversible Redox Chemistry of Quinones: Impact on Biogeochemical Cycles." *Chemosphere* 77 (4): 451–58. https://doi.org/10.1016/j.chemosphere.2009.07.025.
Updegraff, Karen, John Pastor, Scott D. Bridgham, and Carol A. Johnston. 1995. "Environmental and Substrate Controls over Carbon and Nitrogen Mineralization in Northern Wetlands." *Ecological Applications* 5 (1): 151–63. https://doi.org/10.2307/1942060.
Usher, K.M., A.H. Kaksonen, I. Cole, and D. Marney. 2014. "Critical Review: Microbially Influenced Corrosion of Buried Carbon Steel Pipes." *International Biodeterioration & Biodegradation* 93 (September): 84–106. https://doi.org/10.1016/j.ibiod.2014.05.007.
Vaccaro, Lynn E., Barbara L. Bedford, and Carol A. Johnston. 2009. "Litter Accumulation Promotes Dominance of Invasive Species of Cattails (Typha Spp.) in Lake Ontario Wetlands." *Wetlands* 29 (3): 1036–48. https://doi.org/10.1672/08-28.1.
Vaclav Smil. 2019. *Growth*. Cambridge, Massachusetts: MIT Press.

Vasilas, Bruce L., Martin Rabenhorst, Jeffry Fuhrmann, Anastasia Chirnside, and Shreeam Inamdar. 2013. "Wetland Biogeochemistry Techniques," 355–442. https://doi.org/10.1007/978-94-007-6860-4_7.

Vasilas, L.M., and B.L. Vasilas. 2011. *Mid-Atlantic Hydric Soils Committee. A Guide to Hydric Soils in the Mid-Atlantic Region, Ver. 2.0.* USDA, NRCS, Morgantown, WV. pp. 136.

Vaughan, Karen L., Florence Miller, Nico Navarro, and Christopher Appel. 2016. "Visual Assessment of Sulfate Reduction to Identify Hydric Soils." *Soil Science Society of America Journal* 80 (4): 1114–19. https://doi.org/10.2136/sssaj2016.02.0035.

Venterink, Olde H., M. J. Wassen, A. W. M. Verkroost, and P. C. De Ruiter. 2003. "Species Richness - Productivity Patterns Differ Between N-, P-, and K-Limited Wetlands." *Ecology* 84 (8): 2191–99. https://doi.org/10.1890/01-0639.

Vileisis, Ann. 1997. *Discovering the Unknown Landscape : A History of America's Wetlands*. Washington, D.C. : Island Press,.

Vincent, Christopher, Diane Rowland, Chaein Na, and Bruce Schaffer. 2017. "A High-Throughput Method to Quantify Root Hair Area in Digital Images Taken in Situ." *Plant and Soil* 412 (1–2): 61–80. https://doi.org/10.1007/s11104-016-3016-9.

Vuic, Jason. 2021. *The Swamp Peddlers: How Lot Sellers, Land Scammers, and Retirees Built Modern Florida and Transformed the American Dream*. CHAPEL HILL: University of North Carolina Press. http://www.jstor.org/stable/10.5149/9781469663173_vuic.

Wagai, Rota, and Lawrence M. Mayer. 2007. "Sorptive Stabilization of Organic Matter in Soils by Hydrous Iron Oxides." *Geochimica et Cosmochimica Acta* 71 (1): 25–35. https://doi.org/10.1016/j.gca.2006.08.047.

Wakeley, James S., R. W. Livchar, and C. V. Noble. 2010. "Regional Supplement to the Corps of Engineers Wetland Delineation Manual: Atlantic and Gulf Coastal Plain Region. Version 2.0." ERDC/EL TR-10-20. U.S. Army Corps of Engineers pp. 180.

Walbeck, David, Denise Clearwater, and Kelly P. Neff. 2011. "Maryland Nontidal Wetland Mitigation Guidance." Maryland Department of Environment Nontidal Wetlands and Waterways Division.

Wang, Bobo, Yanfen Wang, Xiaoyong Cui, Yiming Zhang, and Zhisheng Yu. 2019. "Bioconversion of Coal to Methane by Microbial Communities from Soil and from an Opencast Mine in the Xilingol Grassland of Northeast China." *Biotechnology for Biofuels* 12 (1): 236. https://doi.org/10.1186/s13068-019-1572-y.

Wang, Z. P., R. D. DeLaune, W. H. Patrick, and P. H. Masscheleyn. 1993. "Soil Redox and PH Effects on Methane Production in a Flooded Rice Soil." *Soil Science Society of America Journal* 57 (2): 382. https://doi.org/10.2136/sssaj1993.03615995005700020016x.

Watanabe, Kohtaro, Shunsaku Nishiuchi, Konstantin Kulichikhin, and Mikio Nakazono. 2013. "Does Suberin Accumulation in Plant Roots Contribute to

Waterlogging Tolerance?" *Frontiers in Plant Science* 4. https://doi.org/10.3389/fpls.2013.00178.
Weidlich, Emanuela W. A., Flávia G. Flórido, Taísi B. Sorrini, and Pedro H. S. Brancalion. 2020. "Controlling Invasive Plant Species in Ecological Restoration: A Global Review." Edited by Guadalupe Peralta. *Journal of Applied Ecology* 57 (9): 1806–17. https://doi.org/10.1111/1365-2664.13656.
Weimer, P. J., and J. G. Zeikus. 1978. "Acetate Metabolism InMethanosarcina Barkeri." *Archives of Microbiology* 119 (2): 175–82. https://doi.org/10.1007/BF00964270.
William Cronon. 1991. *Nature's Metropolis*. New York: W.W. Norton & Co.
Willson, Kevin G., Angela N. Perantoni, Zachary C. Berry, Matthew I. Eicholtz, Yvette B. Tamukong, Stephanie A. Yarwood, and Andrew H. Baldwin. 2017. "Influences of Reduced Iron and Magnesium on Growth and Photosynthetic Performance of Phragmites Australis Subsp. Americanus (North American Common Reed)." *Aquatic Botany* 137 (January): 30–38. https://doi.org/10.1016/j.aquabot.2016.11.005.
Wilson, Anthony. 2006. *Shadow and Shelter: The Swamp in Southern Culture.* Vol. 2006. Mississippi: Jackson University Press.
Wilson, Rachel M., Malak M. Tfaily, Virginia I. Rich, Jason K. Keller, Scott D. Bridgham, Cassandra Medvedeff Zalman, Laura Meredith, et al. 2017. "Hydrogenation of Organic Matter as a Terminal Electron Sink Sustains High CO 2 :CH 4 Production Ratios during Anaerobic Decomposition." *Organic Geochemistry* 112 (October): 22–32. https://doi.org/10.1016/j.orggeochem.2017.06.011.
Winikoff, Sarah G., Daniel J. Larkin, Shana L. Meier, and Jacques C. Finlay. 2020. "Vegetation Trajectories of Restored Agricultural Wetlands Following Sediment Removal." *Restoration Ecology* 28 (3): 612–22. https://doi.org/10.1111/rec.13128.
Wintle, Brendan A., Heini Kujala, Amy Whitehead, Alison Cameron, Sam Veloz, Aija Kukkala, Atte Moilanen, et al. 2019. "Global Synthesis of Conservation Studies Reveals the Importance of Small Habitat Patches for Biodiversity." *Proceedings of the National Academy of Sciences* 116 (3): 909–14. https://doi.org/10.1073/pnas.1813051115.
Winton, R. Scott, and Curtis J. Richardson. 2015. "The Effects of Organic Matter Amendments on Greenhouse Gas Emissions from a Mitigation Wetland in Virginia's Coastal Plain." *Wetlands* 35 (5): 969–79. https://doi.org/10.1007/s13157-015-0674-y.
Wohl, Ellen, Stuart N. Lane, and Andrew C. Wilcox. 2015. "The Science and Practice of River Restoration." *Water Resources Research* 51 (8): 5974–97. https://doi.org/10.1002/2014WR016874.
Wolf, Evan C., Eliška Rejmánková, and David J. Cooper. 2019. "Wood Chip Soil Amendments in Restored Wetlands Affect Plant Growth by Reducing Compaction and Increasing Dissolved Phenolics." *Restoration Ecology* 27 (5): 1128–36. https://doi.org/10.1111/rec.12942.

Wolf, Kristin L., Changwoo Ahn, and Gregory B. Noe. 2011. “Development of Soil Properties and Nitrogen Cycling in Created Wetlands.” *Wetlands* 31 (4): 699–712. https://doi.org/10.1007/s13157-011-0185-4.

Wong, Jonathan W. C., Rao Y. Surampalli, Tian C. Zhang, Rajeshwar D. Tyagi, and Ammaiyappan Selvam, eds. 2016. *Sustainable Solid Waste Management*. Reston, VA: American Society of Civil Engineers. https://doi.org/10.1061/9780784414101.

Woodward, Richard T., and Yong-Suhk Wui. 2001. “The Economic Value of Wetland Services: A Meta-Analysis.” *Ecological Economics* 37 (2): 257–70. https://doi.org/10.1016/S0921-8009(00)00276-7.

Wu, Shubiao, Peter Kuschk, Hans Brix, Jan Vymazal, and Renjie Dong. 2014. “Development of Constructed Wetlands in Performance Intensifications for Wastewater Treatment: A Nitrogen and Organic Matter Targeted Review.” *Water Research* 57 (June): 40–55. https://doi.org/10.1016/j.watres.2014.03.020.

Xiao, Wei, Adele M. Jones, Richard N. Collins, and T. David Waite. 2018. “Investigating the Effect of Ascorbate on the Fe(II)-Catalyzed Transformation of the Poorly Crystalline Iron Mineral Ferrihydrite.” *Biochimica et Biophysica Acta (BBA) - General Subjects* 1862 (8): 1760–69. https://doi.org/10.1016/j.bbagen.2018.05.005.

Xu, Shangqi, Xia Liu, Xiujun Li, and Chunjie Tian. 2019. “Soil Organic Carbon Changes Following Wetland Restoration: A Global Meta-Analysis.” *Geoderma* 353 (November): 89–96. https://doi.org/10.1016/j.geoderma.2019.06.027.

Yagi, Kazuyuki, and Katsuyuki Minami. 1990. “Effect of Organic Matter Application on Methane Emission from Some Japanese Paddy Fields.” *Soil Science and Plant Nutrition* 36 (4): 599–610. https://doi.org/10.1080/00380768.1990.10416797.

Yang, Wendy H., Gavin McNicol, Yit Arn Teh, Katerina Estera-Molina, Tana E. Wood, and Whendee L. Silver. 2017. “Evaluating the Classical Versus an Emerging Conceptual Model of Peatland Methane Dynamics: Peatland Methane Dynamics.” *Global Biogeochemical Cycles* 31 (9): 1435–53. https://doi.org/10.1002/2017GB005622.

Yao, Heng, and Ralf Conrad. 1999. “Thermodynamics of Methane Production in Different Rice Paddy Soils from China, the Philippines and Italy.” *Soil Biology and Biochemistry*, 11.

Yarwood, Stephanie A. 2018. “The Role of Wetland Microorganisms in Plant-Litter Decomposition and Soil Organic Matter Formation: A Critical Review.” *FEMS Microbiology Ecology* 94 (11). https://doi.org/10.1093/femsec/fiy175.

Ye, Rongzhong, and William R. Horwath. 2017. “Influence of Rice Straw on Priming of Soil C for Dissolved Organic C and CH4 Production.” *Plant and Soil* 417 (1–2): 231–41. https://doi.org/10.1007/s11104-017-3254-5.

Ye, Rongzhong, Qusheng Jin, Brendan Bohannan, Jason K. Keller, Steven A. McAllister, and Scott D. Bridgham. 2012. “PH Controls over Anaerobic Carbon Mineralization, the Efficiency of Methane Production, and Methanogenic Pathways in Peatlands across an Ombrotrophic–Minerotrophic

Gradient." *Soil Biology and Biochemistry* 54 (November): 36–47. https://doi.org/10.1016/j.soilbio.2012.05.015.

Yu, Guang-Hui, Yi-Hong Luo, Min-Jie Wu, Zhu Tang, Dong-Yang Liu, Xing-Ming Yang, and Qi-Rong Shen. 2010. "PARAFAC Modeling of Fluorescence Excitation−emission Spectra for Rapid Assessment of Compost Maturity." *Bioresource Technology* 101 (21): 8244–51. https://doi.org/10.1016/j.biortech.2010.06.007.

Yu, Kewei, and William H. Patrick. 2004. "Redox Window with Minimum Global Warming Potential Contribution from Rice Soils." *Soil Science Society of America Journal* 68 (6): 2086–91. https://doi.org/10.2136/sssaj2004.2086.

Yu, Lingfei, Yao Huang, Feifei Sun, and Wenjuan Sun. 2017. "A Synthesis of Soil Carbon and Nitrogen Recovery after Wetland Restoration and Creation in the United States." *Scientific Reports* 7 (1): 7966. https://doi.org/10.1038/s41598-017-08511-y.

Yuan, Quan, Xiaozhen Huang, Junpeng Rui, Shaojun Qiu, and Ralf Conrad. 2020. "Methane Production from Rice Straw Carbon in Five Different Methanogenic Rice Soils: Rates, Quantities and Microbial Communities." *Acta Geochimica* 39 (2): 181–91. https://doi.org/10.1007/s11631-019-00391-5.

Yvon-Durocher, Gabriel, Andrew P. Allen, David Bastviken, Ralf Conrad, Cristian Gudasz, Annick St-Pierre, Nguyen Thanh-Duc, and Paul A. del Giorgio. 2014. "Methane Fluxes Show Consistent Temperature Dependence across Microbial to Ecosystem Scales." *Nature* 507 (7493): 488–91. https://doi.org/10.1038/nature13164.

Zedler, Joy B. 2000. "Progress in Wetland Restoration Ecology." *Trends in Ecology & Evolution* 15 (10): 402–7.

Zedler, Joy B., and Suzanne Kercher. 2004. "Causes and Consequences of Invasive Plants in Wetlands: Opportunities, Opportunists, and Outcomes." *Critical Reviews in Plant Sciences* 23 (5): 431–52. https://doi.org/10.1080/07352680490514673.

Zeng, Ning. 2008. "Carbon Sequestration via Wood Burial." *Carbon Balance and Management* 3 (1): 1. https://doi.org/10.1186/1750-0680-3-1.

Zhalnina, Kateryna, Katherine B. Louie, Zhao Hao, Nasim Mansoori, Ulisses Nunes da Rocha, Shengjing Shi, Heejung Cho, et al. 2018. "Dynamic Root Exudate Chemistry and Microbial Substrate Preferences Drive Patterns in Rhizosphere Microbial Community Assembly." *Nature Microbiology* 3 (4): 470–80.

Zhang, Tianbao, Xiaohui Liu, and Yu An. 2020. "Fluctuating Water Level Effects on Soil Greenhouse Gas Emissions of Returning Farmland to Wetland." *Journal of Soils and Sediments* 20 (11): 3857–66. https://doi.org/10.1007/s11368-020-02730-z.

Zhang, Yuechao, Leilei Xiao, Qinqin Hao, Xin Li, and Fanghua Liu. 2020. "Ferrihydrite Reduction Exclusively Stimulated Hydrogen Production by *Clostridium* with Community Metabolic Pathway Bifurcation." *ACS Sustainable Chemistry & Engineering* 8 (20): 7574–80. https://doi.org/10.1021/acssuschemeng.9b07702.

Zhao, Mingliang, Guangxuan Han, Juanyong Li, Weimin Song, Wendi Qu, Franziska Eller, Jianping Wang, and Changsheng Jiang. 2020. "Responses of Soil CO2 and CH4 Emissions to Changing Water Table Level in a Coastal Wetland." *Journal of Cleaner Production* 269 (October): 122316. https://doi.org/10.1016/j.jclepro.2020.122316.

Zhi, Wei, and Guodong Ji. 2012. "Constructed Wetlands, 1991–2011: A Review of Research Development, Current Trends, and Future Directions." *Science of The Total Environment* 441 (December): 19–27. https://doi.org/10.1016/j.scitotenv.2012.09.064.

Zhou, Shungui, Jielong Xu, Guiqin Yang, and Li Zhuang. 2014. "Methanogenesis Affected by the Co-Occurrence of Iron(III) Oxides and Humic Substances." *FEMS Microbiology Ecology* 88 (1): 107–20. https://doi.org/10.1111/1574-6941.12274.

Zhuang, Li, Jielong Xu, Jia Tang, and Shungui Zhou. 2015. "Effect of Ferrihydrite Biomineralization on Methanogenesis in an Anaerobic Incubation from Paddy Soil: Iron Mineralization and Methanogenesis." *Journal of Geophysical Research: Biogeosciences* 120 (5): 876–86. https://doi.org/10.1002/2014JG002893.