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Forested Wetland Mitigation: Developing Techniques to Restore Northern White-Cedar on Clay Settling Areas in Northern Michigan

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FORESTED WETLAND MITIGATION: DEVELOPING TECHNIQUES TO RESTORE NORTHERN WHITE-CEDAR ON CLAY SETTLING AREAS IN NORTHERN MICHIGAN

By

Sean R. Westley

A THESIS

Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

In Applied Ecology

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College of Forest Resources and Environmental Science

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List of Abbreviations

Abstract

When permitted activities degrade or destroy wetlands, mitigation is required by both state and federal laws (Michigan Environment, Great Lakes & Energy (EGLE) and Section 404 of the Clean Water Act). Forested wetlands are considered keystone ecosystems, but restoration of these systems is often limited by the environmental complexities and the slow growth of the long-lived tree species. Using a combination of greenhouse and field experiments, my research goal was to develop techniques to create northern white-cedar (Thuja occidentalis L.) forested wetlands in reclaimed/abandoned mining quarries. There are numerous environmental stressors in the inorganic sediments of reclaimed quarries including high moisture retention, low porosity, and lack of nutrients. I tested using soil amendments in full factorial treatments using: fertilizer (F), arbuscular mycorrhizae (AM), and organic matter. In addition to the soil amendment experiments, I also tested how seedling survival was influenced by soil moisture. Our results show that soil moisture had the greatest influence on survival in both the greenhouse and field trials. The addition of organic matter and AM improved seedling survival and growth while fertilizer decreased survival. My research indicates that the addition of organic matter and planting at the right water table levels are the best techniques to create forested wetlands in quarry sediments.

1 Introduction

Forested wetlands cover $\sim 6\%$ of the global land area, roughly 5.5% of the 48 contiguous United States, and are considered critical keystone ecosystems (Royal et al. 2018). Forested wetlands provide many ecosystem services, such as providing high-quality and diverse habitats, improving water quality, sequestering a large amount of carbon, and providing cold water to streams (Ott et al. 2016, Varin et al. 2019). Forested wetlands also offer significant economic value from wood products to habitats for hunting. Despite the importance of these habitats, forested wetlands are declining nationally in the U.S. at 2.4% annually, which is roughly 1.2 million acres lost per year (Dahl et al. 2004). In the Great Lakes region, Northern white-cedar (NWC: *Thuja occidentalis* L.) forested wetlands (swamps) are the most common and perhaps the most important wetland type. Many terrestrial species utilize cedar swamps in northern Michigan, such as white-tailed deer (Odocoileus virginianus), as NWC provides the primary browse source during the long, harsh winters (Parikh 2019). However, many NWC wetlands are degraded from anthropogenic alterations like harvesting, urbanization, road construction, and mining. When wetlands are lost from permitted activities, the Michigan Department of Environment, Great Lakes & Energy (EGLE) requires mitigation to replace these habitats. Despite the wetland restoration requirement, many forested wetlands are not mitigated/created due to the complexity and slow growth of trees. This is why many wetland restoration projects end in herbaceous-dominated marshes (Dahl et al., 2004).

Excavation for limestone and other mining operations globally cause great ecological impacts (Naja et al., 2011). The bedrock geology of Gulliver, Michigan, is a sedimentary limestone rock that belongs to the Manistique Group within the Niagara series. Formed

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during the early Silurian, this bedrock formation is part of the Michigan Basin that composes a large portion of the southern coast of Michigan's Upper Peninsula (UP). This limestone is rich in calcium, which is being mined by Carmeuse Lime & Stone – Port Inland Operation and is an open-pit calcite mine operation. To excavate the limestone, vegetation and topsoil are removed to access the limestone formation. The cap-rock is removed and sent through the primary crusher that macerates the limestone to roughly 17 cm in size. The crushed limestone is sent through a series of conveyor belts that transports the rock to several milling machines. Carmeuse produces eight different products that are milled to various sizes. The grain sizes once milled range from 0.3 cm to 12 cm. During this process, the limestone is treated and washed, becoming a slurry. This residual limestone slurry is then backfilled into the open pit, which is referred to as a clay settling area (CSA).

Forested restoration research is complicated due to the many factors that affect the survival and regeneration of a forest. The sediment created in the CSA will likely be nutrient deficient, with high pH, high soil moisture retention, and dense clay soil. Very fine-grained soils can potentially influence cation and anion exchange rates which could hinder the bioavailability of nutrients in the already high pH clay soil (Hofmeyer et al., 2009, Duval et al., 2010, Crowley et al., 2011, Bi et al., 2020). Grain proximity also increases a soil's ability to retain moisture and limit gas exchange—potentially leading to an anoxic soil. In NWC swamps, it has been shown that hummock-hollows (topography) play a key role in growth and survival (Chimner et al., 1996, Kangas et al., 2016). Hummock-hollows offer a reprieve to the often-anaerobic wetland soil conditions. Other calcareous wetland creation studies indicated that pH ranges could affect the efficiency of

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the vegetation establishment by impacting flora establishment and survival. Specifically, NWC regeneration studies have shown that the desired pH ranges are between 5.5 and 7.0. Either end of this spectrum showed to have a detriment to the germination/regeneration of NWC (Hofmeyer et al. 2009). In nutrient-limited soils, evidence suggests that the addition of arbuscular mycorrhizal fungi (AM) to NWC root structure will aid in survival and growth (Anwar 2016). Many plant species share the mutualistic interaction with AM, as the fungi aid in mining nutrients.

Our overarching goal is to develop techniques to create forested wetlands in post-mining landscapes that will be counted towards mitigation credits. We hypothesize that: 1) with the CSA sediment being extremely nutrient-poor, soil amendments (fertilizer and AM) will optimize NWC growth and survival; 2) the planted NWC will be sensitive to small changes in water table levels due to the fine texture of the CSA.

2 Materials and Methods

2.1 Greenhouse Methods

Due to the variety of difficulties that field experiments present and the uncontrolled environmental conditions, I conducted three greenhouse experiments to complement the field portion of our research. The first two experiments tested soil amendments (fertilizer, arbuscular mycorrhizae (AM), and organic matter and the third experiment tested the influence of soil moisture on seedling survival. These greenhouse experiments were conducted at the College of Forest Resources and Environmental Science at Michigan Technological University.

All three greenhouse experiments were conducted under similar conditions. The greenhouse had grow lights on for 12 hours a day. Watering for our two soil amendment experiments utilized drip irrigation that watered the seedlings for \sim 1 minute in the winter and ~2 minutes in the summer every 12 hours.

All seedlings were purchased from Vans Pines Nursery (Holland, Mi. USA.). The seedlings averaged 17 cm in height (base of the seedling to the top of the seedling) and were shipped in an organic soil plug (Jiffyplug) to prevent the seedlings from drying out.

The CSA sediments were collected at the Carmuse mining site and then transported back to the greenhouse sealed in 38 L buckets. The organic matter (OM) used was a 1:1 ratio of peat from Vans Pines Nursery and dried sphagnum moss (Mosser Lee's Long Fibered Sphagnum Moss, Mosser Lee Company, Millston, Wi. USA.). I used a commercialgrade 50 species arbuscular mycorrhizae (AM) (BioOrganics Micronized

Endomycorrhizal, 2799 Creamery Rd. New Hope, PA. 18938) for our mycorrhizal treatments.

The fertilizer (F) mixings were purchased locally at Erikson Feed and Seed (Houghton, MI. USA.), and I combined them to create one complete fertilizer. To do this, I took a 5 gal bucket and mixed: 1) 3 cups of DI water, 2) a bag of Hi-Yield Hydrated Lime (2.27 kg) (ferti-lome Bonham, TX. USA), 3) a bag of Hi-Yield Copperas (1.8 kg), 4) a bag of Hi-Yield Iron Plus (1.8 kg), 5) 9 kg bag of Quality Fertilizer (Ray's Feed Mill) at 19/19/19 proportion. The fertilizer was mixed into a homogenous slurry.

2.1.1 Edaphic Greenhouse Experiments Planting Methods

Our two soil amendment experiments were planted the week of August $10th$, 2019 (Tables 1 and 2). Both of these experiments began with the same planting process. Every 2 L plastic planting pot received a garden mesh liner (a polypropylene landscape fabric liner) placed at the bottom to cover the drain holes (roughly cut to size). The liner was used to help prevent sediment loss while still allowing the excess moisture to escape. Each pot was labeled and assigned a number for individual monitoring $(N = 10)$.

The first full factorial greenhouse experiment tested CSA soil with the additions of fertilizer and AM. To prepare the seedlings, I washed off the organic plugs (Jiffyplugs) the seedlings were shipped in by dipping the seedlings into water until the roots were bare. The root structures would then be pointed downward, held by the trunk, and then filled with the CSA sediment around the seedling making sure the top of the pot was level with the base of the seedling to ensure that each seedling was planted vertically in the

pot. This same planting process was repeated for each seedling. However, when fertilizer was added, the pot was first approximately filled to 1/3 full with CSA, then 3 g of fertilizer was added. This way the fertilizer was not directly touching the root structure. I added 3 g of AM amendment by holding the seedling over the pot it would be planted in so that if any AM did not stick to the roots it would still be in the soil. Once a treatment type was completely planted we would clean everything used to try and reduce any contamination.

The second greenhouse experiment used the CSA soil mixed in a 1:1 ratio with the created organic matter amendment and tested with a full factorial experiment. The steps above were repeated as closely as possible for each seedling planted. After each treatment was planted I sterilized all tools used. This reduced any chance of contamination between planted repetitions.

Treatment	Fert.	AM	Planted
CSA Control	No	N ₀	10
$CSA + AM$	No	Yes	10
$CSA + F$	Yes	N ₀	10
$CSA + AM + F$	Yes	Yes	10

Table 1. Summary planting table for greenhouse edaphic experiment for CSA soils.

CSA= Clay-settling-area soil, AM= Arbuscular mycorrhizae, F= Fertilizer.

Treatment	Fert.	AM	Planted
Peat Control	N ₀	N ₀	10
$CSA + OM + AM + F$	Yes	Yes	10
$CSA + OM + F$	Yes	N ₀	10
$CSA + OM + AM$	N ₀	Yes	10
$CSA + OM$	No	N ₀	10

Table 2. Summary planting table for the greenhouse organic soil experiment. CSA and organic mixed 1:1.

 $CSA = \text{clay-settling-area soil}, AM = \text{Arbuscular mycorrhizae}, F = \text{Fertilizer}, P = \text{beat}.$

2.1.2 Soil Moisture Experiment

The third greenhouse experiment spanned from 8/10/2019 to 1/20/2021 to test the effects of water table levels on NWC seedling survival and growth. This was done by using different heights of clear polyethylene terephthalate glycol (PETG) tubing (Giddings Machine Company, Windsor, Co. USA.) filled with CSA sediment placed in a tub of water. I filled the tubes with CSA sediment to the top and allowed them to sit vertically for two weeks in a depot holder (Stuewe & Sons, Tangent Oregon). During this period, I watered the CSA to help it settle. This process had to be repeated several times for many of the taller tubes.

The CSA sediment was amended with 1 g of fertilizer and AM before planting bare root seedlings (same as previous experiments). After planting, a small amount of organic matter was added to the top of the tubes to help reduce the stress of being planted in the CSA. Once the seedlings were planted into the tubes, they were placed into the depot

holders within aquaponic deep trays (Sustainable Hydroponics & Garden Supply) allowing the manipulation of the water levels. The final average water table levels were 21 cm 30 cm, 38 cm, 45 cm, and 61 cm below the soil surface ($N = 10$ of each level).

2.1.3 Greenhouse Monitoring Methods

To monitor the survival and growth of the greenhouse experiments I made daily sediment moisture checks. In both of the sediment experiments, I used drip lines that were checked frequently to ensure they were intact and provided the correct amount of water. Water table levels were also checked daily in the soil moisture experiment and water was added every few days. Each month I conducted seedling height and diameter measurements. Height was measured by gently pulling on the crown and using a soft measuring tape to measure the seedling at its tallest height. Seedling diameters were measured by using digital calipers (155 mm) at the base of the seedling as close to the ground as possible.

During the week of January 20, 2021, root and shoot biomass was measured from both of the CSA sediment experiments. Each seedling was pulled from the pots and washed to remove all soil from the root structures. Seedlings were then cut at the root shoot interface and oven-dried for 12 hours at 60° C temperature. I measured the dry below and above-ground biomass. After measuring biomass, all of the foliar tissue was collected from each CSA experiment. After being collected, the 80 greenhouse samples were shipped to the Northern Research Station, Forestry Sciences Laboratory (1831 Hwy 169 E. Grand Rapids, MN. 55744) for further analysis.

2.1.4 Statistical Analysis of the Greenhouse Study

To perform statistical analysis for the greenhouse experiments, I used Minitab 19 software and R Studio (Minitab 19 software, Minitab, LLC, R Studio). For each of the greenhouse experiments, I used the analysis of variance (ANOVA) test to test if survival is influenced by treatment.

2.2 Field Experiment Methods

The field study was conducted on a 60-ha abandoned quarry section at Carmeuse Lime & Stone, Inc. property located near Gulliver, Michigan (45°58'18.75"N, 85°54'18.28"W: Figure 1). Eleven permanent plots were created in 2019 that spanned a gradient of soil wetness (Figure 1). Similar to the greenhouse experiments, the same created fertilizer and AM amendments were used for each plot.

Figure 1. Google Earth image of the study site and plot locations.

2.2.1 Field Experiment Planting Process

Seedlings were planted in early June 2019. Each of the four treatment types was planted in a separate row spaced ~1.5 m apart at each plot and were 1 m apart in the rows (Table 3). The first row planted was the Jiffyplug control (JPC), and the second row was the Jiffyplug inoculated (JPI). The same row order was executed for the non-organic matter (bare-root control) (BRC) and the bare-root inoculated (BRI). The soil amendments used to create our inoculation treatment type were: the complete fertilizer and AM commercial inoculum. I applied 15 g of fertilizer and 5 g of AM to seedlings in the amendment treatments.

Table 3. Summary treatments for field study.

Treatment	Row	Fert.	AM	Planted
Jiffyplug Control		N ₀	N ₀	10
Jiffyplug + $F + AM$	2	Yes	Yes	10
Bare-Root	3	N ₀	N ₀	10
$Bare-Root + F + AM$	4	Yes	Yes	10

Jiffyplug capsules = the organic capsule around the root structure, $F =$ fertilizer, $AM =$ arbuscular mycorrhizae

2.2.2 Field Experiment Monitoring Methods

Initial height and diameter measurements were conducted 2 weeks after planting to allow the CSA sediment to settle. Seedling survival and growth were measured at the end of the growing season. Diameters were measured using digital calipers at the base of each seedling as close to the ground as possible. Seedling height was measured by gently pulling on the crown and measuring from the base to the tallest vertical point of the seedling.

2.2.3 Statistical Analysis for the Field Study

Statistical analysis was done using Minitab 19 software (Minitab 19 software, Minitab, LLC). To test the relationships between treatment type vs seedling survival we used the analysis of variance test (ANOVA). In addition to the ANOVA, I used the Tukey posthoc test to analyze the means of each analysis against one another. The ANCOVA method will be used to test plot location, plot pH, treatment type, average water table, minimum water table, and maximum water table against survival.

2.2.4 Field Experiment Environmental Monitoring

Groundwater levels were monitored at our site using monitoring wells. One groundwater monitoring well was installed at each plot during the spring of 2019 and was constructed using 6.35 cm diameter PVC (polyvinyl chloride) tubing to 154 cm lengths. Thin cuts were made in each tube to allow water to enter and the tube was wrapped in polypropylene garden fabric liner to keep sediment out. The bottom of the well was capped with a PVC (polyvinyl chloride) 6.35 cm cap. Monitoring wells were installed into hand-auger holes.

A Solonist pressure transducer (Solonist Canada Ltd. Georgetown, ON L7G 4R8) was installed in each well to continuously monitor groundwater levels. In addition, hand measurements were conducted every 2 weeks by hand measurement. We also handmeasured the pH at each well using a pH meter (Bluelab Combo Meter, Bluelab USA). Precipitation data for Gulliver, Mi. was obtained from the National Oceanic and Atmospheric Administration (NOAA).

2.3 CSA Sediment Chemistry Methods

We used in situ resins to quantify soil chemical conditions. Plant-Root Simulator (PRS) probes (Western AG Saskatoon, SK, Canada s7N 2G6) were used to measure cation and anion exchanges within the CSA sediment (e.g., Total N, NO3, NH4, Ca, Mg, K, P, Fe, Mn, Cu, Zn, B, S, Pb, Al, and Cd). These resins come as color-coded plastic encasements that are 15 cm x 3 cm x 0.5 cm. In the center of the probe encasement is a membrane made of networked polystyrene and cross-linked with divinylbenzene. The orange probe

denotes anion exchange, and the purple probe denotes cation exchange. To ensure the membranes remain charged, polystyrene and $SO₃$ charge the cation probes. In contrast, the anion probes are fixed with NH_4+ . With the membranes having fixed charges, the PRS probes act as a general-purpose ion exchanger, and the ions exchange under constant electrostatic attraction.

To use the PRS resin probes, we inserted the pointed tip into the ground \sim 14 cm making sure to bury the entire membrane casing. We left our probes in-situ for three weeks with five probes at each plot. We placed probes along several sites (no-fill zones 1-3, 5-7, and 20-23) to cover the range of moisture levels within the CSA soil. The probes were located near the monitoring.

Probes were installed in early July and retrieved 3 weeks later. Western AG recommends leaving the probes for an extended period to allow maximum ion exchange (Hartsock et al., 2018). The recommended probe retrieval process was to spray the probe with deionized (DI) water immediately on removal to remove all sediment stuck to the membrane. We used DI water to remove any sediment from the membranes after probes were pulled from the CSA soil. Western AG also shared their wetland soil data to use for comparison $(N > 10,000)$ to our resin data. In addition to these soil tests, CSA sediment samples were sent to the Northern Research Station, Forestry Sciences Laboratory (1831 Hwy 169 E. Grand Rapids, MN. 55744) for chemical analysis.

2.3.1 Foliar Analysis

Foliar samples were collected and analyzed for elemental composition from both field and greenhouse experiments to better quantify the nutrient conditions of the seedlings. All leaf tissue was collected during the destructive analysis of both greenhouse sediment experiments. When the foliar samples were collected from seedlings in the field experiment they were pooled to reduce the amount needed from each seedling. To reduce the stress on the surviving field seedlings, foliar samples were pooled. The greenhouse and field samples collected were then dried in paper bags before being shipped to the Northern Research Station, Forestry Sciences Laboratory (1831 Hwy 169 E. Grand Rapids, MN. 55744).

Native foliar samples of cedar from across the region were collected and analyzed to compare to values from the greenhouse and field I sampled from 50 sites across the UP and subdivided by major soil types utilizing the Soilweb database by the University of California, Davis (casoilresource.lawr.ucdavis.edu). At each location, we cut and pooled samples from 10 trees/seedlings (N=10 per site). Foliar tissue was collected from the south side of each tree/seedling (Van den Driessche. 1974, Hockman, 1989, Wang et al., 1997) at my designated heights. The height categories that I collected, were trees taller than 3 m and trees under 3 m. Canopy cover was recorded at each foliar sample taken. If seedlings were present, I also collected foliar tissues from seedlings no greater than 0.3 m in height. All foliar samples from native seedlings were cut from roughly \sim 2 years of age and younger by identifying the newest growth on the leaves before cutting (Boulfroy et al., 2012). Samples were immediately bagged for transport back to Michigan

Technological University and were stored in a dry paper bag to begin the drying process until samples were shipped.

Dried foliar samples (oven-dried for 24 hours at 70 C) were shipped to the Northern Research Station, Forestry Sciences Laboratory (1831 Hwy 169 E. Grand Rapids, MN. 55744). Samples were digested using a microwave (CEM MARS6) and an acid bath $(HNO₃ + HCL + H₂O₂)$ and then analyzed by a Thermo-Fisher iCAP 7600 Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) and Elemental Analyzer (Costech Analytical Technologies Inc., Valencia Ca, USA). Along with our collected CSA soil sample. We measured full elemental spectroscopy: P, K, S, Mo, Mn, Fe, Ni, Cu, Zn, B, Na, Sr, Ba, Ti, Al, Si, V, Cr, Co, Cd, Pb, As, Se, Mg, Ca.

2.3.2 Chemical Analysis Statistical Methods

To test if our seedlings were elementally different from native cedar elemental composition, we first used the Soilweb database by the University of California, Davis (casoilresource.lawr.ucdavis.edu) to select locations. I created categorical groupings for native seedlings by clustering them by major soil type. GPS points were used in the field to correspond to Soilweb locations and the major soil types. This allowed me to collect foliar samples from various soil and nutrient types. The goal of our tests is to better understand the full elemental composition of the planted seedlings. I will do this by using the Shapiro Test for Normality will test our samples for normality, allowing me to use the Elbow method. The elbow method will determine the point where data does not change in cluster membership. This test will aid our understanding of the number of expected categories or clusters. Following the Elbow test, I will then run a Cluster Analysis Test

that will determine the number of clusters created by the elemental composition of all samples collected.

Elements	Reference				
$(ug/10cm^{-2})$	Wetlands	NF3	NF7	NF20	NF23
Total N	43.75	15.74	12.28	13.68	16.8
$NO3-N$	40	9.28	5.08	8.4	8.36
NH ₄	3.75	6.46	7.2	5.28	8.44
Ca	1622.5	2505.7	2154.6	2653.3	2655.9
Mg	355.5	857.57	344.16	322.48	281.89
K	69.25	22.63	9.32	6.87	33.55
${\bf P}$	9	0.49	0.35	0.88	0.65
Fe	103.75	14.04	0.68	3.1	16.87
Mn	12.25	0.25	θ	0.06	0.12
Cu	0.225	0.68	0.36	0.27	0.25
Zn	0.875	1.06	1.27	1.03	3.54
B	0.525	0.38	0.04	0.07	0.1
S	109.75	1876.2	1285.9	1556.6	1453.3
Pb	0.01	0.16	0.05	0.07	0.04
Al	$\overline{0}$	10.21	6.92	8.1	7.65
C _d	$\overline{0}$	$\overline{0}$	0.01	$\overline{0}$	$\overline{0}$

Table 4. Elemental composition of reference wetlands and our field CSA soils.

3 Results

3.1 Greenhouse Experiments Results 3.1.1 Edaphic Soil Survival

CSA Soil Experiment # 1

Average seedling survival across the treatments was 55%, with significant differences seen between the treatments (Table 5). The lowest survival was seen with the two treatments that had fertilizer addition. The highest survival was seen in the CSA sediment with the addition of AM (100%). The second-largest survival was seen in the control (90%) (Table 5). There was little interaction between adding fertilizer and AM additions (Figure 2).

Seedling biomass across the treatments ranged between 11 and 19 g and averaged 15.8 g (Table 5). The largest average total biomass was seen in both AM treatment types (CSA+AM, CSA+AM+F), however, there was large within-treatment variability that lead to no significant differences seen between the treatments (P=0.30). The root-to-shoot ratio was lower in the CSA control and CSA+AM compared to the two fertilizer treatments. The average root-to-shoot ratio was 1.46, while on average, the root biomass was about 1.5 times more than the above-ground biomass (Table 5).

Table 5. Average edaphic soil experiment biomass and survivability by treatment type. Edaphic treatment types in clay settling area (CSA) treatments. The inoculation types are arbuscular mycorrhizae (AM) and fertilizer (F).

Treatment	Average Total Biomass (g)	Average Below Ground Biomass (g)	Average Above Ground Biomass (g)	Root- Shoot Ratio	Survival (%)
CSA	11.68A	6.65 _A	5.04 _A	1.32	90_A
$CSA+AM$	19.13A	10.93 _A	8.21 _A	1.32	100 _A
$CSA+F^*$	14.05 _A	9.0 _A	5.4 _A	1.67	0 _B
$CSA+AM+$ F	18.35 _A	11.04 _A	7.3 _A	1.51	30 _B

*CSA+F biomass was collected from the last 3 seedlings that died two months before the end of the experiment.

CSA Soil Experiment # 2

The overall survival of cedar seedlings was 94% with the addition of organic matter (Table 6). Seedling survival by treatment type did not show any significance in the organic addition treatments $(P=0.74)$. There was a significant difference in biomass between treatment types (Table 6). The greatest total biomass for these seedlings occurred in the peat-only treatment (22.6 g) while the second largest biomass was in the CSA+OM+AM treatment (17.5 g). The lowest total biomass was in the CSA+OM (control) treatment (12.8 g). The average root-shoot ratio for seedlings in CSA soil with the addition of organic matter was 1.03. Small differences in the root-shoot ratio can be seen between the organic soil treatment types, however, the peat-only (control) treatment had a ratio of 1.72 (Table 6.). There was a strong interaction between fertilizer and AM amendments as adding AM only increased biomass when no fertilizer was added (Figure 2).

Table 6. Average organic experiment biomass and survivability by treatment type. Organic treatment types in clay settling area tilled with sphagnum moss (CSAOM). The inoculation types are arbuscular mycorrhizae (AM) and fertilizer (F).

Treatment	Average Total Biomass (g)	Average Below Ground Biomass (g)	Average Above Ground Biomass (g)	Root- Shoot Ratio	Survival $($ %)
Peat Control	22.6 _A	14.3 _A	8.30 _A	1.72	100 _A
$CSA + OM$	12.8 _B	6.3 _B	6.53 _A	0.96	100_A
$CSA+OM+$ AM	17.5 _{AB}	9.3 _{AB}	8.2 A	1.13	90_A
$CSA+OM + F$	18.7 _{AB}	9.8AB	8.9 _A	1.10	90_A
$CSASP+AM+F$	14.1 _B	6.8 _B	7.3 _A	0.93	90 _A

Figure 2. Interaction plots for effects on fertilization (a-d) with mycorrhizal inoculation on NWC survival and total biomass. ($M0 =$ no inoculation, $M1 =$ inoculation, $F0 =$ no fertilizer, $F1$ = fertilizer)

3.1.2 CSA Water Table Experiment

Seedling survival varied along the water table gradient, with the greatest survival

occurring in the driest conditions and decreasing as conditions became wetter (Figure 2).

At the wettest or the highest water-table level, there was no seedling survival, whereas

the driest treatment had just over 70% survival.

Figure 3. Seedling survival across the water table gradient.

3.2 Field Experiment 3.2.1 Environmental Conditions

Hydrology

The average groundwater pH across all years and plots was 7.6 (Table 6). Precipitation totals for growing seasons (May 5 , $-$ October 31) in 2018 were 40.3 cm, 2019 was 74.0 cm, 2020 was 57.4 cm, and 2021 was 36.6 cm. The average growing season precipitation amount between 1991 – to 2020 was 51.1 cm. Water table levels tended to be highest during the spring and early summer and dry out in the late summer and fall (Figure 4). Average water table levels across all sites and years varied between 3 cm and 63 cm below the ground surface (Table 7). Maximum water tables were just above the soil surface at all sites, which was often in the spring (Figure 4). But there were large differences in the minimum water table levels between sites. There also seemed to be a general drying trend from 2018 to 2021.

Site	WT 2021	pH	AVG WT	Max WT	Min WT
	(cm)		$(2018 -$	$2018 -$	$2018 - 2021$
			2021)	2021	(cm)
			(cm)	(cm)	
NF1	-31.7	$7.8\,$	-16.6	$2.5\,$	-31.7
NF2	-57	7.8	-3.3	2.4	-100.5
NF3	-7	$7.8\,$	-6.7	$\mathbf{1}$	-43
NF4	-10	7.3	-20.4	2.7	-99.6
NF ₅	-30	7.8	-22.8	$2.7\,$	-112.7
NF ₆	-50	7.8	-62.5	$\mathbf{1}$	-141
NF7	-65	8.2	-13.5	$\boldsymbol{0}$	-79
NF ₈	-98	8.1	-23.9	$\mathbf{1}$	-98
$1\mathrm{F}$	-54	$7.2\,$	-29.0	1.8	-84
2F	-82	7.2	-22.7	$\mathbf{1}$	-87
NF23	-40	6.8	-11.5	1.7	-55
Average	-47.7	7.6	-21.2	1.6	-85

Table 7. Current water table (WT) levels and the average (AVG), maximum (Max), and minimum (Min) from each of our sites. Table created using our ground water monitoring from July 2018 to July 2021. Negative numbers represent the water table below ground.

Figure 4. A.) Ground water levels during 2019 and **B**.) 2020. Percentages listed next to well ID indicate NWC seedling survival by the plot.

3.2.2 Seedling Survival and Growth

In total, 244 seedlings out of 440 seedlings survived from 2019 -2021, for an average of 55% overall survival (Table 7). There were large differences in survival between plots, ranging from 10-85% (Table 7). There was no overall significance between treatment types (p=0.99) and survival.

The ANCOVA test showed seedling survival was significantly impacted by site, treatment, and MinWT. The mixed model test also showed little significance for treatment type but showed significance for water table levels, pH, and plot location affecting seedling survival (Tables 8 and 9.). Cedar survived better during the drier summer conditions at the plots with a greater minimum average water table (Figure 5). Additionally, there is an interaction between the addition of the OM and AM/fertilizer additions as inoculant was more important when no OM was added (Figure 6).

The average final seedling height across all experimental plots was ~43 cm (Table 10). This was an average change in height of ~1.8 cm. Surviving seedlings that received inoculation and fertilizer treatment showed the largest average increase in height. This same trend was also observed in the bare-root treatment. Indicating that when NWC seedlings survived the biggest observable change (above ground growth) was seen.

Site	BRC	BRI	JPC	JPI Alive	Average
		Alive $(\%)$ Alive $(\%)$ Alive $(\%)$		(%)	$(\%)$
NF1	90	$\overline{0}$	90	$\overline{0}$	45.0
NF ₂	$\boldsymbol{0}$	100	10	80	47.5
NF3	20	$\boldsymbol{0}$	30	20	17.5
NF4	100	30	90	100	80.0
NF ₅	40	100	90	100	82.5
NF ₆	60	90	90	100	85.0
NF7	80	100	30	100	77.5
NF ₈	80	100	60	100	85.0
1F	40	50	$\boldsymbol{0}$	$\boldsymbol{0}$	22.5
2F	$\boldsymbol{0}$	20	20	$\overline{0}$	10.0
NF23	100	30	100	$\overline{0}$	57.5
Average	55.5	56.4	55.5	54.5	55.5

Table 8. Seedling survival across plots by treatment type.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Min WT	1	8.9	8.89974	70.95	< 0.001
Treatment	3	9.574	3.19122	25.44	< 0.001
pH	5	19.817	3.96339	31.6	< 0.001
Min WT [*] Treatment	3	9.325	3.10831	24.78	< 0.001
Treatment*pH	15	14.746	0.98306	7.84	< 0.001
Error	411	51.554	0.12544		
Lack-of-Fit	16	14.265	0.89158	9.44	< 0.001
Pure Error	395	37.289	0.0944		
Total	438	108.383			

Table 9. General Linear Model (GLM) of survival against each variable individually and grouped.

Figure 5. A plot of average NWC survival by plot (%) by minimum average water table (cm).

Figure 6. Interaction plot for the addition of the organic matter against the fertilizer and AM amendments to the NWC planted in the quarry.

Site	AVG Plot Height (cm)	BRC AVG Height (cm)	BRI AVG Height (cm)	JPC AVG Height (cm)	JPI AVG Height (cm)
NF1	38.6	40.8	$\overline{0}$	36.4	$\overline{0}$
NF ₂	43.5	$\boldsymbol{0}$	43.7	40	43.8
NF3	39.2	39	$\boldsymbol{0}$	39.6	39
NF4	46.0	42.7	45.6	49.7	46.1
NF ₅	43.0	37.7	39	47.2	45.5
NF ₆	44.0	42	41.2	44.7	47
NF7	46.0	50	43.5	45	45.2
NF ₈	45.4	47.7	43.7	49.6	42.7
1F	37.7	37.5	38	$\boldsymbol{0}$	$\boldsymbol{0}$
2F	49.5	$\overline{0}$	57	42	$\boldsymbol{0}$
NF23	40.3	39.5	42.3	40.6	$\boldsymbol{0}$

Table 10. Average seedling height by plot and treatment type (2021).

3.2.3 Chemical Analysis

Soils

Analysis of the PRS elemental data showed that the CSA soils did not vary across the site but are very different from natural reference wetlands (Table 4). Particularly, the CSA soils have much lower amounts of total nitrogen (N) and nitrate $(NO₃)$, but much greater amounts of calcium (Ca) and magnesium (Mg). Additionally, we see greater amounts of aluminum (Al), but lower phosphorous (P), potassium (K), and iron (Fe) in the CSA soils.

Foliar nutrient content

We found significant differences between elemental compositions of foliar samples from our planted seedlings compared to native cedar foliage $(P=0.04)$ (Figure 7). This cluster analysis had a high total sum of squares (Total SS 90%). The Cluster Analysis Test by K-Means showed similarities between the natural cedar types but varied by soil pH. The Shapiro test $(P=0.05)$ also indicated that each grouping was significant. With natural cedar foliage samples collected from soils with lower $pH \approx 6.5$) are clustered near the bottom of the natural cedar cluster. Samples collected from soils with a higher pH are seen clustered near the top of the figure (Figure 7). Similarly, the native cedar and greenhouse seedlings with soil amendments clustered together above the non-inoculated

seedlings. The greenhouse samples that received organic amendments clustered the closest to natural cedar.

Figure 7. Cluster Analysis of the elemental composition of foliar samples. $GH =$ greenhouse samples, $CSA =$ field collections, UAU and PSS are native collected cedar. (P, K, S, Mo, Mn, Fe, Ni, Cu, Zn, B, Na, Sr, Ba, Ti, Al, Si, V, Cr, Co, Cd, Pb, As, Se, Mg, Ca.)

		CSA Soil-With	
	CSA Soil-No organics	Organics	Peat
${\bf P}$	311.4	668.9	512.3
$\mathbf K$	2235.5	3859.9	3696.1
$\mathbf S$	198.5	377.7	312.7
Mo	0.65	1.2	1.4
Mn	41.6	48.3	49.7
Fe	58.7	118.4	59.7
Ni	1.5	2.3	2.7
Cu	75.8	43.1	98.1
Zn	8.6	13.7	17.4
B	21.5	29.0	34.1
Na	370.9	464.0	300.1
Sr	53.5	65.8	74.6
Ba	23.0	23.2	52.7
Ti	1.7	3.5	1.7
Al	57.7	98.4	58.3
Si	203.4	274.8	307.3
\mathbf{V}	0.05	0.17	$\boldsymbol{0}$
Cr	0.99	1.9	2.7
Co	0.44	0.67	0.63
C _d	0.03	0.02	0.02
Pb	0.05	0.07	0.07
As	0.16	0.15	0.29
Se	0.39	0.61	0.43
Mg	1747.1	3188.8	2688.8
Ca	25728.3	32823.1	37548.3

Table 11. The average elemental composition from the foliar analysis was taken from seedlings in the greenhouse experiment. These averages represent the pooled foliar samples from CSA soils without organics and from the CSA soil when mixed with organics. The third column is the control peat average.

4 Discussion

The primary objective of the research was to test if it is feasible to create cedar forested wetlands on limestone clay settling areas for wetland mitigation purposes. The results indicate that it is possible to initiate the creation of forested wetlands, but several factors strongly control the survival and growth.

Water table

NWC physiological limitations don't allow for survival in prolonged submerged soils (Chimner et al., 1996, Haynes et al., 2004, Buda et al., 2011, Atkinson., 2019). Our studies saw similar trends that tree survival, root structure, and growth are affected by the inundation period (Chimner et al., 1996, Rogers et al., 2003, Dahl et al., 2004, Stephan et al., 2020). These studies also indicate that forested wetland regeneration heavily relies on hydrologic fluctuation (Haynes et al., 2004, Buda et al., 2011, Atkinson., 2019). Our greenhouse study showed a quick mortality response to excess soil moisture. However, the seedlings in the field (likely not limited by pot size), showed visual rooting changes to cope with soil moisture.

Additionally, clay soils often have a high capillary fringe (Dallaire et al., 2019). We found the capillary fringe in CSA soil is large, upwards of 40 cm. This can alter the inundation periods of our plots and greatly reduce the survival of seedlings. This is why NWC usually grows on hummocks, which are $\sim 20 - 60$ cm above the water table (Chimner et al., 1996, Hofmeyer et al., 2009, Man et al., 2012).

I began a preliminary experiment to test NWC survival in artificial hummocks, which I created on site. I used burlap bags filled with soil and stacked to create hummocks slightly taller than naturally occurring hummocks, $60 - 80$ cm above the water table. These hummocks were created taller to cope with the extra high capillary fringe of the CSA. Despite this increased height, we observed seedlings rooting down through and into the CSA soil.

Soil moisture is often thought of as one of the most important environmental factors to tree survival. It is also shown that within these reclaimed clay quarries that soil moisture along with pH can impact the flow of bioavailable nutrients (Chodak et al., 2010, Naja et al., 2011, Wiedermann et al., 2017, Ortega et al., 2020, Gentili et al., 2020).

Soil Chemistry

Many studies have found that NWC regeneration occurs best in substrates with a pH ranging from 6 - 7.5 (Chimner et al., 1996, Trettin et al., 1997, Pietrzykowski et al., 2015, Kangas et al., 2016). However, highly alkaline soils have been shown to negatively affect cedar seed germination and seedling density (Hofmeyer et al., 2009). The average pH at our field site is 7.9, which is near the high end of the pH range for cedar. High soil pH often creates limitations to bioavailable nutrients that can limit growth rates. Often macronutrients like phosphorous, potassium, and nitrogen are limited (Kooijman et al., 2009). Other chemical changes occur as well, with excess sulfur, and calcium OH ion concentrations (Trettin et al., 2007, Borkenhagen et al., 2018, Wolf et al., 2019, Purre et al., 2020). Our foliar and soil chemistry analysis indicated that nitrogen, potassium, phosphorus, and iron are the likely limiting nutrients on-site. In addition to nutrient

limitations, some elements (e.g., aluminum, lead, and sulfur) appear to be in excess and potentially toxic in the long term. Cedar is a normally slow-growing species reaching \sim 6.0 meters after 50 years (Hannah et al., 2004), but with the unfavorable nutrient levels can be expected to grow even slower.

It was expected that adding fertilizer would improve survival and increase growth rates as the CSA soil was nutrient-limited. We do still believe that fertilizer is needed but as an organic application. As a preliminary soil test, we did apply compost topographically to the CSA soil. The following season these plots showed healthier naturally establishing flora. The decomposition of organic material or the slow release of nutrients should provide the system with all required nutrients. Figure 8 shows the foliar differences for field seedlings with the amended and non-amended field soils.

In the quarry, we also planted cedar in 2 plots that had reclaimed old topsoil. Early on we saw high seedling survival in these fill zones but during our last measurement collections, many were missing. We attribute potential herbivory as the cause because we did observe tracks near these seedlings.

Figure 8. Field images show the difference between two seedling foliage, one from amended soils (A) and the second from bare-root seedlings (B).

Elemental Analysis

The elemental composition of natural cedar was variable across the UP. However, the cluster analysis indicates that parent soil type is affecting elemental composition. We saw that these seedlings seem to be affected by the soil composition, which has been seen before (Ortega et al., 2020). When soils are nutrient deficient, it shows in the foliar analysis as a lower ppm value. Elementally the NWC seedlings we planted at Carmeuse have greater aluminum (Al) and sulfur (S). Both elements are known to be toxic in large amounts (Stanturf et al., 2003, Chodak et al., 2010, Naja et al., 2011, Man et al., 2013, Jagodziński et al., 2015). In addition to elements in greater proportion, we also see two elements lacking, iron (Fe) and zinc (Zn). We think there is possible toxicity and

decreased elemental amounts known to be important for flora growth could increase mortality. Currently, surviving seedlings planted in the amended CSA soils are taller, healthier-looking (Figure 8), and have thicker foliage. Seedlings planted with organics currently look the healthiest, which matches previous results (Anwar et al. 2019). Further investigation into the elemental toxicity and growth needs of NWC will give a better understanding of our findings.

Recommendations

The results of this study indicate that it is possible to create forested wetlands on reclaimed CSA soils, but success comes from controlling nutrient and water stress. Water table levels were found to be critical in cedar survival. Cedar had low survival in both greenhouse and field studies when the average water table was above ~30 cm below the soil surface. However, we cannot make the site too dry as it would not be classified as a wetland and not count towards mitigation credit. We, therefore, recommended that water tables in the spring be allowed to be wet at all sites, and then drop to 30-60 cm below the surface in the summer. This can be done by either planting cedar only in areas with this hydroperiod, controlling water inputs or outputs to maintain desired water table levels, and/or adding microtopographic features such as hummocks, especially in the wetter areas, to create the desired water table levels. We did a small pilot test of planting cedar on small hummocks and they survived (80% survival) and grew much better than the cedar planted on the flat CSA, which matches earlier cedar creation projects (e.g., Kangus et al. 2016).

To improve the growth of cedar, we recommend adding arbuscular mycorrhizae and soil organic matter to the soils; both these improved survival and growth of cedar in the greenhouse and field studies. Organic matter can be added to the surface, such as done at our fill sites, or can be mixed in with the soils. We used peat in the greenhouse, but we also tested compost and other organic matter which seemed to work. It is clear that some organic additions should be added to provide long-term fertilizer to the cedar. After planting cedar, we also recommended planting herbaceous plants native to high pH wetlands. We also recommended protection from deer herbivory if planting on a large scale.

Figure A1. Each sample and individual elements are shown in Cluster Analysis Test.

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Figure A2. Each sample and individual elements are shown in Cluster Analysis Test.

Figure A3. Each sample and individual elements are shown in Cluster Analysis Test.

Table A1. (Next page) Table of nutrient amounts from all samples.

Figure A4. Greenhouse seedlings within 2L pots and attached drip lines.

Figure A5. Example of water table experiment

Figure A6. Field location in 2018 before the planting process began.

Figure A7. Image of the data collection process done in the Fall of 2019.

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