ERDC/EL TR-24-6

Environmental Laboratory

U.S. ARMY
US Army Corps of Engineers®
ERDC

Dredging Operations Technical Support Program

Evaluating Soil Conditions to Inform Upper Mississippi River Floodplain Restoration Projects

Aaron M. McFarlane, Nia R. Hurst, Carina M. Jung, and Charles H. Theiling

April 2024

DISTRIBUTION STATEMENT A. Approved for public release; distribution is unlimited.

The US Army Engineer Research and Development Center (ERDC) solves the nation's toughest engineering and environmental challenges. ERDC develops innovative solutions in civil and military engineering, geospatial sciences, water resources, and environmental sciences for the Army, the Department of Defense, civilian agencies, and our nation's public good. Find out more at www.erdc.usace.army.mil.

To search for other technical reports published by ERDC, visit the ERDC online library at https://erdclibrary.on.worldcat.org/discovery.

Dredging Operations Technical Support Program

ERDC/EL TR-24-6
April 2024

Evaluating Soil Conditions to Inform Upper Mississippi River Floodplain Restoration Projects

Aaron M. McFarlane

US Army Corps of Engineers
St. Paul District
180 Fifth Street E., Suite 700
St. Paul, MN 55101

Nia R. Hurst, Carina M. Jung, and Charles Theiling

Environmental Laboratory
U.S. Army Engineer Research and Development Center
3909 Halls Ferry Rd
Vicksburg, MS 39180

Final report

DISTRIBUTION STATEMENT A. Approved for public release; distribution is unlimited.

Prepared for USACE St. Paul District
St. Paul, MN
US Army Engineer Research and Development Center (ERDC)
Environmental Laboratory (EL)
Vicksburg, MS 39180

Under Funding account code U4372374
AMSCO code 086000

Abstract

The US Army Corps of Engineers (USACE) has designed and constructed thousands of acres of ecosystem restoration features within the Upper Mississippi River System. Many of these projects incorporate island construction to restore geomorphic diversity and habitat, including floodplain forests. Soils are the foundation of the ecological function and successful establishment of floodplain forests as they are the basis through which plants obtain water and nutrients and provide critical ecosystem services. To improve floodplain forest island restoration outcomes, three natural and four recently (<10 years) constructed restoration sites were studied to compare soil physical, chemical, microbial, and fungal characteristics. Constructed islands had lower soil organic matter and dissolved organic carbon and differed in nutrient concentrations, bacterial assemblages, and fungal communities compared to reference sites. However, soil enzyme activity and some microbial community characteristics were functionally similar between the natural and created sites. Results align with previously established restoration trajectory theories where hydrological and basic microbial ecosystem functions are restored almost immediately, but complex biologically mediated and habitat functions require more time to establish. Data from this and future studies will help increase the long-term success of USACE floodplain forest restoration, improve island design, and help develop region-specific restoration trajectory curves to better anticipate the outcomes of floodplain forest creation projects.

DISCLAIMER: The contents of this report are not to be used for advertising, publication, or promotional purposes. Citation of trade names does not constitute an official endorsement or approval of the use of such commercial products. All product names and trademarks cited are the property of their respective owners. The findings of this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

DESTROY THIS REPORT WHEN NO LONGER NEEDED. DO NOT RETURN IT TO THE ORIGINATOR.

Contents

Abstract ii

Figures and Tables v

Preface vii

1 Introduction 1

1.1 Background 1
1.2 Objective 3
1.3 Approach 3

2 Methods 4

2.1 Study area 4
2.2 Study site selection 5
2.2.1 Land cover 5
2.2.2 Elevation 7
2.2.3 Hydrology 7
2.2.4 Historical land use 8
2.2.5 Constructed island information 8
2.3 Selected study sites 9
2.3.1 Belvedere Island, Pool 5 – Natural Reference 9
2.3.2 McGregor Lake, Pool 10 – Natural Reference 10
2.3.3 Sny McGill Lower, Pool 10 – Natural Reference 11
2.3.4 Pool 8 Islands – Phase III – Islands C4 and C8 – Constructed 12
2.3.5 Capoli Slough Habitat Rehabilitation and Enhancement Project – Islands 'C' and 'K' – Constructed 13
2.4 Sampling 15
2.4.1 Plot selection and layout 16
2.4.2 Flora survey methods 17
2.4.3 Soil survey and collection methods 19
2.5 Processing and lab analyses 21
2.5.1 Soil physical, chemical, and microbial activity analyses 21
2.5.2 Microbiome - Bacterial 16S rRNA sequencing 24
2.5.3 Mycobiome - Internal Transcribed Spacer (ITS) rRNA sequencing 25

3 Results and Discussion 27

3.1 Plot locations and physical characteristics 27
3.2 Flora 27
3.3 Soil physical properties 33
3.4 Soil chemical properties 35
3.5 Soil microbial biomass and enzymatic activity properties 37
3.6 Soil correlations 40
3.7 Soil bacterial microbiome 42
3.8 Soil fungal mycobiome 48

3.9 Conclusions 56
3.9.1 Restoration trajectories and expected outcomes 57
3.9.2 Management implications and future directions 58

References 60

Appendix A: Study Site Maps 65

Appendix B: List of Plant Species Observed at All Study Sites 87

Appendix C: Tree Location Plots 90

Appendix D: Soil Profile Photos 100

Appendix E: Soil Data 118

Appendix F: Microbiome and Mycobiome PCoA Plots 123

Acronyms and Abbreviations 132

Report Documentation Page

Figures and Tables

Figures

Figure 1. Study area showing the seven selected study sites; three naturally occurring and four recently constructed. 4
Figure 3. Satellite image of the Belvedere Island reference site. 10
Figure 4. Satellite image of the McGregor Lake reference site. 11
Figure 5. Satellite image of the Sny McGill Lower reference site 12
Figure 6. Satellite image of the Pool 8 Islands Habitat Rehabilitation and Enhancement Project constructed site. 13
Figure 7. Satellite image of the Capoli Slough Habitat Rehabilitation and Enhancement Project Islands constructed site. 15
Figure 8. Sample plot photo, Belvedere Island site 3. Note orange plot center stake located in foreground, and plot boundary stakes located in background highlighted by the orange arrows. 16
Figure 9. Sample plot layout detail showing location of soil samples collected within each plot 17
Figure 10. Floral survey plot layout detail. Ground cover vegetation for each plot was identified to species and assigned an estimated percent cover for each sextant. 18
Figure 11. Soil collection techniques. On *left*, example of upper horizons (0–10 cm and 10–20 cm) collected with a 5 cm diameter, open-ended tube. On *right*, example of lower horizons (20–45 cm and 45+ cm) collected with 2.1 cm diameter soil probe. 20
Figure 12. Microbial soil sample collection. Centrifuge tube collection of soil samples used a mallet to tap the open-ended, sterile tube into the soil and remove the soil intact for collection. 21
Figure 13. Tree composition compared to basal area in each plot. 30
Figure 14. Percent of ground cover vegetation type per plot. 31
Figure 15. Wetland indicator status of ground cover plants per plot, compared to flooding regime (mean days inundation) by site. 32
Figure 16. Wetland indicator status of tree species per plot, compared to flooding regime (mean days inundation) at site 33
Figure 17. Site soil texture profiles. 34
Figure 18. Soil extractable nutrients (soluble reactive phosphorus (SRP), ammonium (NH_4^+), and nitrate-nitrate (NOx)), dissolved organic C (DOC), and total carbon (TC) and nitrogen (TN) concentrations in natural and constructed sites for each soil depth (0-10 and 10-20 cm). Results are displayed as a mean and error bars represent ± 1 SE. 37
Figure 19. Soil microbial biomass carbon and enzyme activity (β-glucosidase (β-gluc), Cellobiohydrolase (Cello), Xylosidase (Xylo), Phosphatase (Phos), and N-acetyl-glucosaminidase (NAG)) in natural and constructed sites. Results are displayed as a mean and error bars represent ± 1 SE. 39

Figure 20. Normalized bacterial relative abundance (>1.0%) of averaged soil samples (n=3) at each study plot. 43
Figure 21. Bacterial orders with statistically significant differences in relative abundance (>1.0%); Kruskal Wallis (FDR p<0.05). 44
Figure 22. Bacterial alpha diversity per sample plot. 45
Figure 23. Bacterial principal coordinates analysis plots. 46
Figure 24. Ratio of possible KEGG pathways in natural and constructed sites, inferred using MiSeq 16S rRNA data in PICRUSt. *Bars* represent ratios between different sites. *Purple* is the ratio of natural to manmade sites while *green* is the ratio of manmade without trees to manmade with trees sites. 48
Figure 25. Normalized fungal relative abundance (>1.0%) at the class level of averaged soil samples at each study plot (n = 3, except for NMCG01 and WTCAP02 where n = 2). 50
Figure 26. Fungal alpha diversity per sample plot. 51
Figure 27. Fungal principal coordinates analysis plots. 52
Figure 28. Normalized percentage of assigned fungal guild OTUs represented at each site. OTUs taxonomically parsed by ecological guild using the FUNGuild tool (Nguyen et al. 2016b) and limited to “highly probable” or “probable” confidence ranking of guild assignment. Unassigned OTUs excluded. 54
Figure 29. Proportion of ectomycorrhizal OTUs at each strata (fungal family or genus). 55
Figure 30. Conceptual restoration trajectory theorizing the recovery of ecological function based on time post-restoration (Source: Berkowitz et al. 2021). 58

Tables

Table 1. Summary of sample plot locations and related data. 29
Table 2. Site biophysical properties within natural and constructed sites. Data presented as a mean, plus or minus (±) one standard error (SE). 35
Table 3. (*a*) Comparison of bulk density at natural and constructed sites analyzed in this study, and (*b*) how those bulk densities may impact plant root growth based on the different soil textures observed at each type of study site. 35
Table 4. (*a*) Comparison of soil pH values at natural and constructed sites analyzed in this study, and (*b*) how those pH levels may impact microorganism communities at each type of study site. Note the typically lower preferred pH range of fungi. 36
Table 5. Correlation matrix for each measured parameter (r values). Bold values indicate a positive correlation, italicized values indicate a negative correlation. Only statistically significant r values are shown (n = 30, d.f. = 28, alpha = 0.05, critical value = 0.361. ns=no significance. 41

Preface

This study was conducted through Mr. Aaron McFarlane's participation in the 2020 Class of the US Army Engineer Research and Development Center (ERDC) University program. Mr. McFarlane is a Biologist in the St. Paul District Office. Dr. Charles Theiling of the ERDC Environmental Laboratory (EL) was the mentor and advisor for the project.

Project funding and support was provided by the Dredging Operations Technical Support Program (DOTS; Dr. Burton Suedel, program manager); the St. Paul District Regional Planning and Environment Division North (CEMVP-PD) and Recreation and Natural Resources offices (CEMVP-OP-RNR); and the Upper Mississippi River Restoration Program.

Soil physical and chemical analyses were performed by Ms. Nia R. Hurst (Wetlands and Coastal Ecology Branch) and soil microbial analyses were performed by Dr. Carina M. Jung and Mr. Matthew Carr (Environmental Processes Branch) of ERDC-EL. At the time of publication, Ms. Patricia M. Tolley was branch chief of the Wetlands and Coastal Ecology Branch, and Mr. Mark Farr was the Ecosystem Evaluation and Engineering (EE) division chief; Mr. William Jones was the acting branch chief of Environmental Processes and Mr. Warren Lorentz was division chief of Environmental Processes and Engineering (EP). The deputy director of ERDC-EL was Dr. Brandon Lafferty, and the director was Dr. Edmund Russo.

Thanks to Mr. Eric Hanson (CEMVP-PDP) for help with field work, soil profile collection, and plant identification. Mr. Addison Scufsa (CEMVP-PDG), Ms. Mackinzie Schafer-Smiley, and Mr. Marshal Johnson (CEMVP-OP-RNR) assisted with field work and sample collection. Mr. Andy Meier (CEMVP-OP-RNR) provided forestry data and analyses that led to site selection and study direction. The US Army Corps of Engineers (USACE) St. Paul District provided logistical support throughout the project.

COL Christian Patterson was commander of ERDC; and Dr. David W. Pittman was the director.

1 Introduction

1.1 Background

The US Army Corps of Engineers (USACE) has planned and constructed a significant number of environmental restoration projects in the Upper Mississippi River System (UMRS) through a variety of authorities. The largest authority for environmental restoration on the UMRS is the Upper Mississippi River Restoration (UMRR) Program, which to date has completed 56 floodplain habitat restoration projects to improve wildlife and fish habitats on over 42,900 ha (106,000 acres) in Illinois, Iowa, Minnesota, Missouri, and Wisconsin. Many restoration projects on the UMRS include construction of islands to restore geomorphic diversity in lower portions of navigation pools where impoundment has led to island erosion, creating wide expanses of open water. Island design has evolved to achieve larger systemic ecosystem goals by improving surrounding hydrologic connectivity, reducing wind fetch and wind-generated waves, and developing backwater bathymetric diversity while supporting the USACE navigation mission (Theiling et al. 2015). The UMRS contains dozens of successful large-scale ecosystem restoration efforts, including the Pool 8 Islands Project, the Spring Lake Islands Habitat Project, and the Capoli Slough Islands Project in Minnesota, Wisconsin, and Iowa, respectively.

Constructed floodplain islands within the Upper Mississippi River (UMR) have been built using a mix of river sediments and typically use sand dredged from the UMR 9-Foot Navigation Channel Project as the fill material for the island base. Sand is the primary earth material used and can constitute over 90% of the total material for island construction (USACE 2012). After building the sand base, fine sediments (consisting primarily of silt and clay-sized particles) are dredged from backwater areas near the project and placed on the island surface to establish a topsoil layer, which typically ranges from 15–45 cm (6–18 in.) in depth and is sometimes incorporated into the sand by disking. The backwater dredging sites are selected to provide the additional benefit of restoring deep backwater aquatic habitat that has experienced sedimentation.

Island design and vegetation planting is designed to meet specific habitat objectives of the project. In general, most islands are planted with various

native grasses or legume species on the island crests and sandbar willows along the edges. More recently, UMRS resource managers have begun to focus restoration goals on improving floodplain forest habitats, and islands have undergone native tree plantings with desirable hard mast-producing species such as swamp white oak (*Quercus bicolor*), northern red oak (*Quercus rubra*), and bitternut hickory (*Carya cordiformis*). This is in response to studies suggesting the UMRS floodplain forest community is becoming less diverse in the face of disease, introduced pests, invasive species, and altered hydrology (Yin et al. 2009; Romano 2010; DeJager et al. 2012).

Soils are the basis of the ecological function and successful establishment of terrestrial communities. Soil physical and chemical characteristics create the baseline conditions upon which plants physically establish, and they serve as the conduit through which plants obtain water, nutrients, and minerals necessary for growth. Soil systems also support a great diversity of bacteria and fungi that decompose organic matter and interact with the physical and biotic communities by regulating plant nutrient and water uptake, providing protection against stress, and regulating plant pathogens. These services, which occur in well-established ecosystems, may explain why trees grow better in their naturally developed soils relative to built systems, even when environmental conditions change (Remke et al. 2020).

Many studies have documented relationships between soil biota and above-ground flora. Van der Heijden et al. (2008) summarize a wide range of known direct and indirect ecosystem processes facilitated by bacteria and fungi. Direct effects include nitrogen fixation by bacteria, plant nutrient acquisition facilitated by symbiotic mycorrhizal fungi, and plant productivity regulation by microbial pathogens. Indirect effects identified include various processes of nutrient conversion within the soil and nutrient storage. At a community level, many studies have documented specific correlations between the types of above-ground plant communities and the associated soil microorganisms in a broad variety of ecosystems (e.g., Nguyen et al. 2016a; Mathews et al. 2019). Although there are previous floodplain forest studies in the lower Mississippi river, there are no known studies focused specifically on floodplain forest communities or restoration in the UMR (Berkowitz 2013; Hurst et al. 2016; Berkowitz et al. 2018). Therefore, it is important to evaluate the

impact of soil properties in constructed sites on both site and plant development.

The goal of this study was to investigate soils on islands constructed as part of UMRS ecosystem restoration projects. Specifically, this study sought to examine the physical, chemical, and biotic properties of soils at restoration sites and compare them with natural floodplain forest reference sites to identify soil properties related to desired habitat development and growth. Results can inform future floodplain forest creation and management projects and ultimately increase restoration project success.

1.2 Objective

The project objective was to compare physical, chemical, and biological differences between (1) UMR reference site soils (those formed in situ) that presently support desired floodplain forest communities and (2) soils in constructed sites that were created for ecosystem restoration purposes. The intent of this research is to provide insight and recommendations for improving floodplain restoration projects in the UMRS and increase the success of vegetative plantings in created sites that beneficially utilize dredged materials from navigation projects. Project outcomes can also provide valuable data to inform future efforts and serve as a baseline in the development of chrono sequences for the sites studied.

1.3 Approach

The current study was developed and executed as an initial investigation on a small set of natural and constructed sites within the UMRS. Sites were located within the USACE St. Paul District, with plans to expand the research throughout the UMRR area of operation. The study investigated several natural and constructed sites distributed across 200 river kilometers (150 river miles) and analyzed (1) geospatial and physical, (2) vegetation, and (3) soil physical, chemical, and microbiome characteristics.

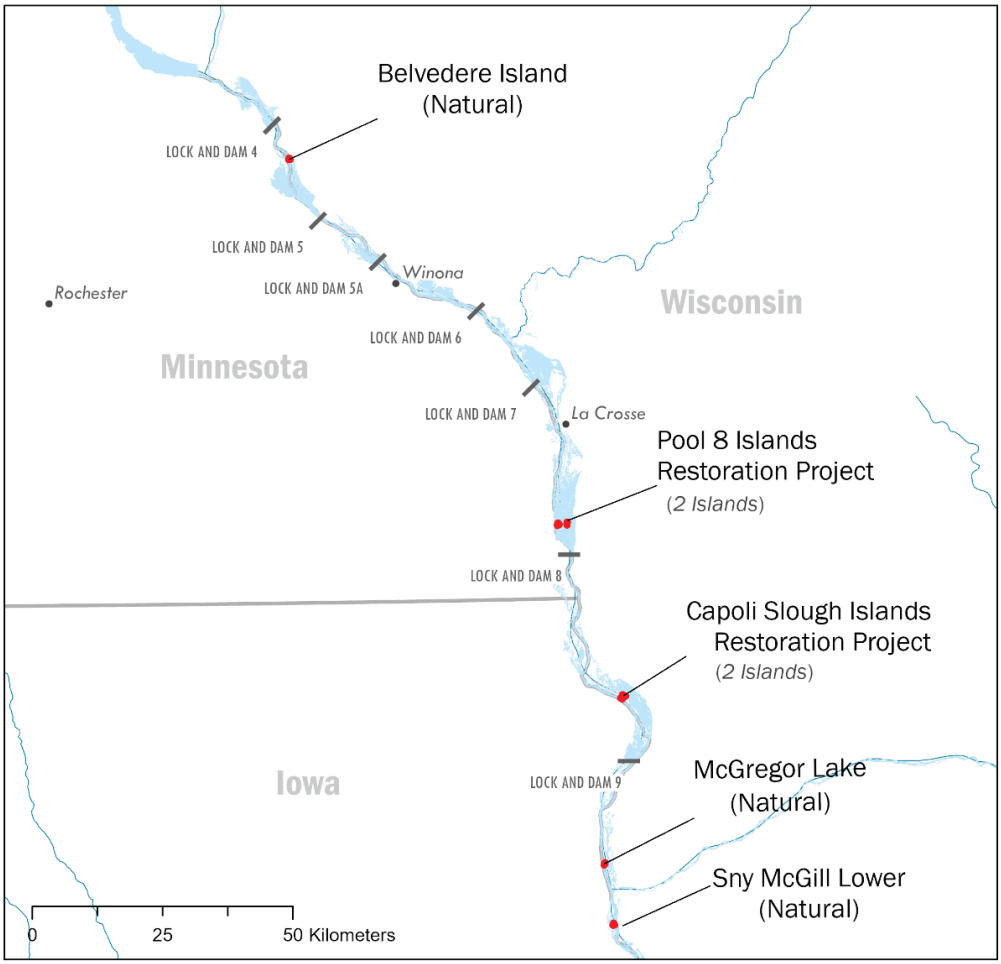
The report begins with an introduction to the study area and selection of study sites. The methods of each study factor are discussed individually. Finally, the results and discussion from each of the study factors are presented with overall study conclusions.

2 Methods

2.1 Study area

Study sites were situated along the UMR in Minnesota, Wisconsin, and Iowa. The study sites span 200 river km (125 river miles) in Navigation Pools 5, 8, 9, and 10 (Figure 1). Within the UMR, the term "pool" is used to describe the area between navigation dams. For example, Pool 5 is the area upstream of Lock and Dam Number 5 extending to Lock and Dam Number 4. The dams on this portion of the Mississippi River were authorized in the River and Harbors Act of 1930 and are designed to maintain a minimum 9 ft (2.7 m) deep navigation system from St. Paul, Minnesota to St. Louis, Missouri (USACE 2000). At high river flows, the dams operate at run-of-the-river, and do not provide flood abatement.

Figure 1. Study area showing the seven selected study sites; three naturally occurring and four recently constructed.



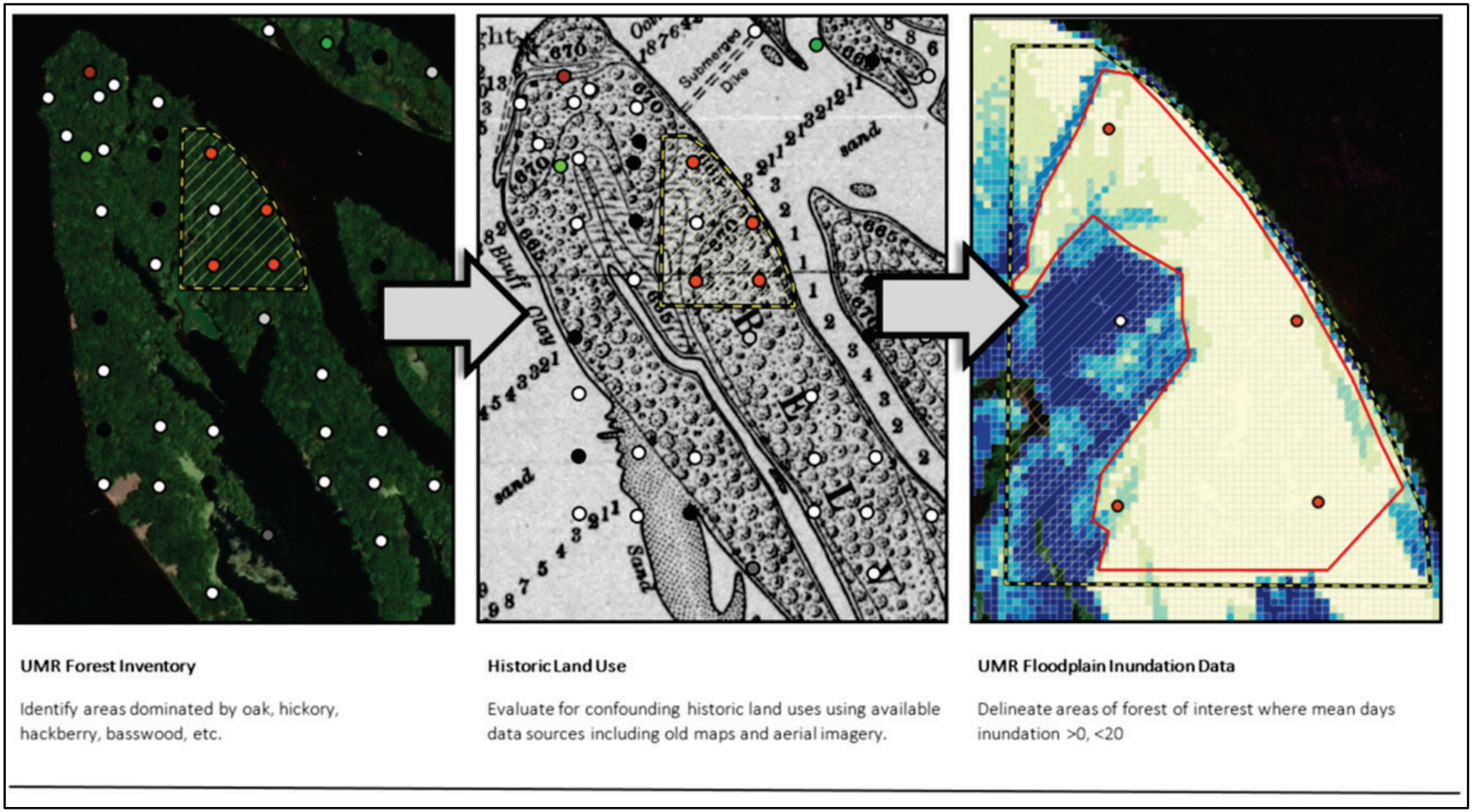
2.2 Study site selection

Site selection was conducted as a two-part process with areas of interest identified first ("study sites"), and specific sampling points within those sites were selected in the field ("sampling plots," described in Section 2.4.1). First, "study sites" with the desired characteristics were identified using desktop analysis to standardize variables such as hydrology and historic land use. Geospatial and physical site characteristics including cover type, elevation, hydrology, historic land use, and construction details for constructed sites were compared using existing geospatial information system (GIS) datasets, construction plans, and as-built documentation. Each variable is described below. Figure 2 shows a visual representation of these variables and how they contributed to delineating a study site.

2.2.1 Land cover

The St. Paul District's Forest Inventory GIS data was used to locate areas of natural floodplain forest that fit the interests of the study. As of 2020, the St. Paul District's Forest Inventory contained data from systematic forest surveys conducted between 2014 and 2018 and is continually updated with new monitoring data. Systemic forest surveys were conducted using the Regional Phase II Forest Inventory Data Protocol (Meier 2016). In general, survey points are distributed in a grid, spaced at 330 ft apart, and include data about species composition, height, age, and forest health. Plot data were evaluated for occurrence and age of target species based on inventory estimates of average basal area and records of average tree diameter. Mixed-species, mast-producing native forest stands (indicative of stakeholder-desired floodplain forest habitat) were targeted to include swamp white oak (*Quercus bicolor*), red oak (*Quercus rubra*), bitternut hickory (*Carya cordiformis*), common hackberry (*Celtis occidentalis*), American basswood (*Tilia americana*), and American elm (*Ulmus americana*). Sites dominated by silver maple (*Acer saccharinum*), which are common, were avoided because they typically represent an undesirable, monotypic forest community type and flood regime that do not represent the diverse species desired on restoration project sites.

Figure 2. Typical study strata identification and delineation process



2.2.2 Elevation

Elevation was determined using digital elevation models (DEMs) generated from high-resolution Light Detection and Ranging (LiDAR) data collected from 2007 to 2011, the latest available, by the USACE and the Iowa Department of Natural Resources. LiDAR data were processed into DEMs by the US Geological Survey's Upper Midwest Environmental Sciences Center (UMESC). The restoration project sites selected for the study (Pool 8 Islands and Capoli Slough Islands) were not yet constructed when the LiDAR data were collected; therefore, construction as-built drawings were referenced to determine elevations where DEMs were not available.

2.2.3 Hydrology

Hydrology is an important factor that influences vegetation communities in the UMR floodplain (e.g., De Jager et al. 2012; Ingvalson et al. 2020). Hydrology also influences soil development and biological soil communities. The average annual flood duration at sites was selected as an appropriate measure of hydrologic character and was standardized across sites to reduce the impact of hydrologic variability. Floodplain inundation attributes are available in raster format for the UMRS from UMESC (Van Appledorn et al. 2018). This data set uses the LiDAR-based DEM referenced above in section 2.2.2 and compares the land elevations to river gage elevations from 1972–2011 to calculate the average number of days any given area of the floodplain was flooded during the growing season.

Average annual flood duration data were not available within the UMESC dataset (Van Appledorn 2018) for many of the Corps' restoration project features. This was the case when projects were constructed after the most recent LiDAR data were collected in those areas. Since the island elevations were not included within the LiDAR data, they were not captured in the UMESC models. Therefore, average annual flood duration for restoration projects was instead determined by simple interpolation. The final constructed island elevations and the river mile were correlated to land at similar elevations at the same river mile to select the appropriate floodplain inundation attributes for comparison to natural sites.

The range of average annual flooding selected for this study was >0 days - <20 days. High elevation sites not commonly affected by flooding were eliminated by the minimum criteria (>0 days inundation). The maximum

level of flooding was subjectively selected based on past studies and observations while searching for sites. De Jager (2012) observed that 70 days or more of growing season inundation was a threshold at which floodplain forest communities lost diversity and became dominated by silver maple (*Acer saccharinum*). Invgalson and colleagues (2020) studied the flood tolerance of specific species and observed that forest communities containing the species of interest identified in 2.2.1 ranged between 4.1 to 66.8 days of flooding. When searching for sites within the UMR forest inventory (section 2.2.1), sites that contained the target species for this study tended to have far fewer days of inundation. Additionally, restoration projects with trees planted were built at higher elevations, receiving far fewer than 70 days of flooding. A target maximum of 20 days of annual flooding was selected to minimize variation, standardize with existing restoration projects, and increase the chances of encountering the target floodplain forest communities.

2.2.4 Historical land use

Historical land use was reviewed to avoid sites manipulated in the past by land-disturbing activities such as farming or development. Aerial imagery and historical maps were examined for indications of past land use for each site. Sites that exhibited signs of manipulation in any current or historical imagery were removed from consideration. Maps and imagery sources included

- 1890 Mississippi River Commission Map, digitized and ortho-referenced by UMESC,
- 1939 Brown Survey Map, digitized and ortho-referenced by UMESC,
- John R. Borchert Map Library's *Minnesota Historical Aerial Photographs Online* webpage, including imagery ranging from the 1920s to the 2000s, and
- historical land cover classification developed by the UMESC based on the symbology from the 1890 Mississippi River Commission Map. The dataset includes 15 classes of land cover, and includes classes such as 'wet forest,' 'upland forest,' 'agriculture,' and 'developed.'

2.2.5 Constructed island information

Construction documentation for all restored islands was used to obtain site information and select appropriate areas for sampling. Variables

considered included final constructed elevation, depth of fine material placed as topsoil, and vegetative planting details.

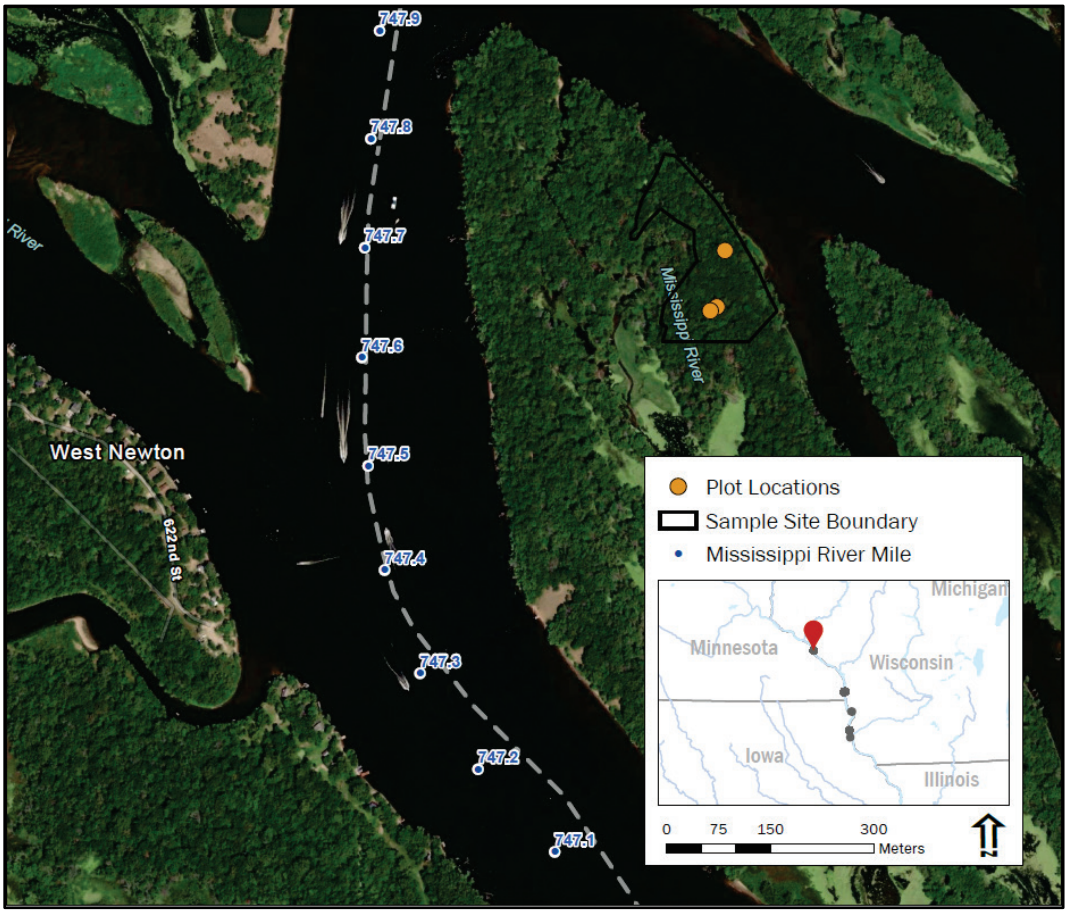
2.3 Selected study sites

Sections 2.3.1–2.3.5 describe the characteristics of each study site selected for sampling. Descriptions are based on the data sources described above used for study site selection. Additional maps of each study site are presented in Appendix A.

2.3.1 Belvedere Island, Pool 5 – Natural Reference

Belvedere island (Figure 3) is adjacent to the main navigation channel in upper Pool 5 in Wisconsin. UMR forest inventory data were used to locate an area of the island dominated by mixed-mast species of interest. The dominant species in the study area based on measures of density and basal area are, in descending order, northern red oak, bitternut hickory, basswood, and hackberry. Live basal area within the selected study area averaged approximately 36.7 m^2/ha (160 ft^2/ac) during a forestry survey in 2017. Live trees within the study area had an average diameter at breast height of 27 cm (10.7 in.). A total of nine tree species were recorded within the selected area. The 1890 Mississippi River Commission map portrays the area as a forested island. Aerial imagery from 1939 and 1949 were also available from the John R. Borchert Map Library's Minnesota Historical Aerial Photographs Online webpage and showed the islands with similar land mass to today and with forest cover. This land is owned by the USACE.

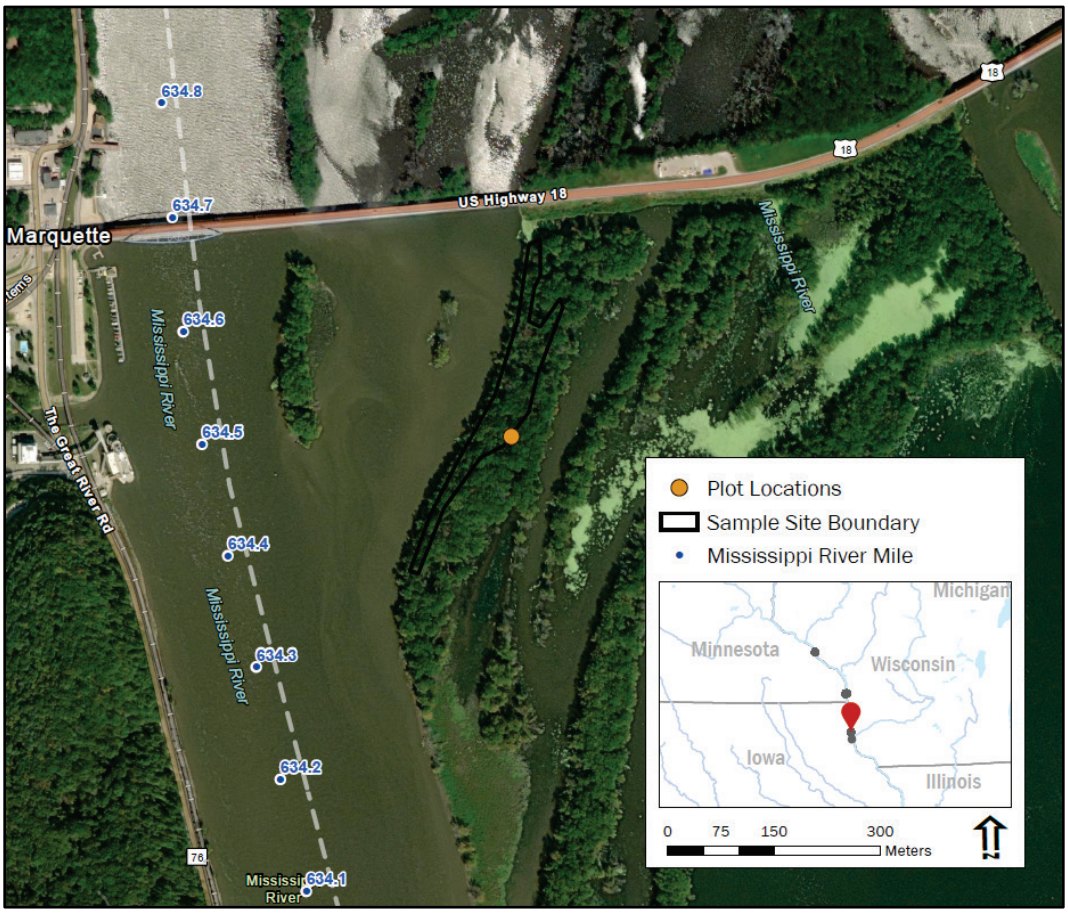
Figure 2. Satellite image of the Belvedere Island reference site.



2.3.2 McGregor Lake, Pool 10 – Natural Reference

McGregor Lake (Figure 4) is an area near Prairie du Chien, Wisconsin that is the setting for an upcoming USACE restoration project. Using the UMR forest inventory data, an area to the west of McGregor Lake was identified with a substantial population of swamp white oak based on measurements of density and basal area. Other species documented on the site include silver maple, green ash, and American elm. Six total tree species were observed in the project area. The UMR forest inventory data show basal area within the selected study area averaged 29.4 m^2/ha (128 ft^2/ac), and diameter at breast height of trees surveyed averaged 40.3 cm (15.9 in.) in 2015. The 1890 Mississippi River Commission map portrays the area as forested. Aerial imagery from 1930 shows the area as having forest cover and landform similar to present day - although many of the surrounding areas have since been lost. This land is owned by the US Fish and Wildlife Service (USFWS).

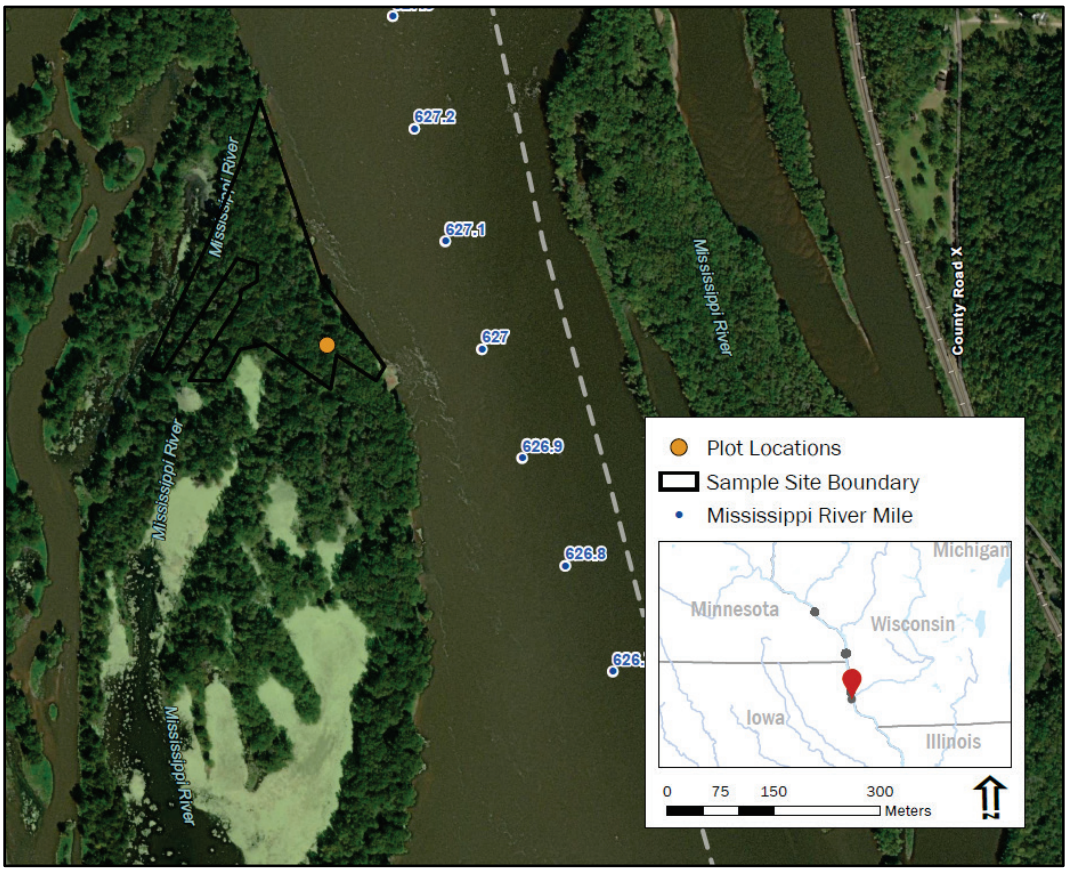
Figure 3. Satellite image of the McGregor Lake reference site.



2.3.3 Sny McGill Lower, Pool 10 – Natural Reference

There is a large area of floodplain forest around the confluence of Sny McGill Creek with the Mississippi River, downstream of McGregor, Iowa (Figure 5). Using the UMR forest inventory data, two areas high in mast-producing tree species were identified. The site is dominated by silver maple at a few points, but also had a substantial presence of green ash, swamp white oak, and American elm. Other species documented on the site include cottonwood, hackberry, bur oak, and red oak. The live basal area within the selected area averaged 24.8 m^2/ha (108 ft^2/ac), and the trees had an average diameter at breast height of 47.0 cm (18.5 in.) based on forestry survey data from 2017. Eight total tree species were observed. The 1890 Mississippi River Commission map portrays the area as forested. Aerial imagery from 1930 shows the area as having forest cover. This land is owned by the USFWS.

Figure 4. Satellite image of the Sny McGill Lower reference site.



2.3.4 Pool 8 Islands – Phase III – Islands C4 and C8 – Constructed

The Pool 8 Islands complex (Figure 6) of habitat restoration features includes three phases constructed over 25 years, and together constitutes one of the largest restoration projects on the UMR. The project includes over 19.2 km (63,000 linear feet, 11.9 mi) of islands. Islands C4 and C8 were constructed recently (2012) and were selected for this study. Island C8 was selected because recent monitoring found oak plantings growing successfully. In contrast, the western portion of Island C4 was not planted with trees and, since there are no known differences in design or construction, was used to evaluate soils in constructed islands without trees. These islands were constructed using a sand base of dredged material from the main navigation channel, and then topped with a layer of finer-grained silt- and clay-dominated dredged materials to help create a topsoil. The islands were constructed with fine material layers ranging between 22.9–45.7 cm (9–18 in.) deep. Two samples on Island C4 were collected where fines were placed 30.5 cm (12 in.) deep, and one sample was collected from an area where fines were placed 45.7 cm (18 in.) deep.

All samples collected on Island C8 were in areas where fines were placed 30.5 cm (12 in.) deep. Fact sheets, project details, and reports can be found at the St. Paul District Habitat Rehabilitation and Enhancement Projects website*.

Figure 5. Satellite image of the Pool 8 Islands Habitat Rehabilitation and Enhancement Project constructed site.

2.3.5 Capoli Slough Habitat Rehabilitation and Enhancement Project – Islands 'C' and 'K' – Constructed

The Capoli Slough project (Figure 7) was constructed between 2012 and 2014, with tree plantings occurring in spring of 2016. Monitoring Island C

* Phase I: https://www.mvr.usace.army.mil/Missions/Environmental-Stewardship/Upper-Mississippi-River-Restoration/Habitat-Restoration/St-Paul-District/Pool-8-Islands-Phase-I/

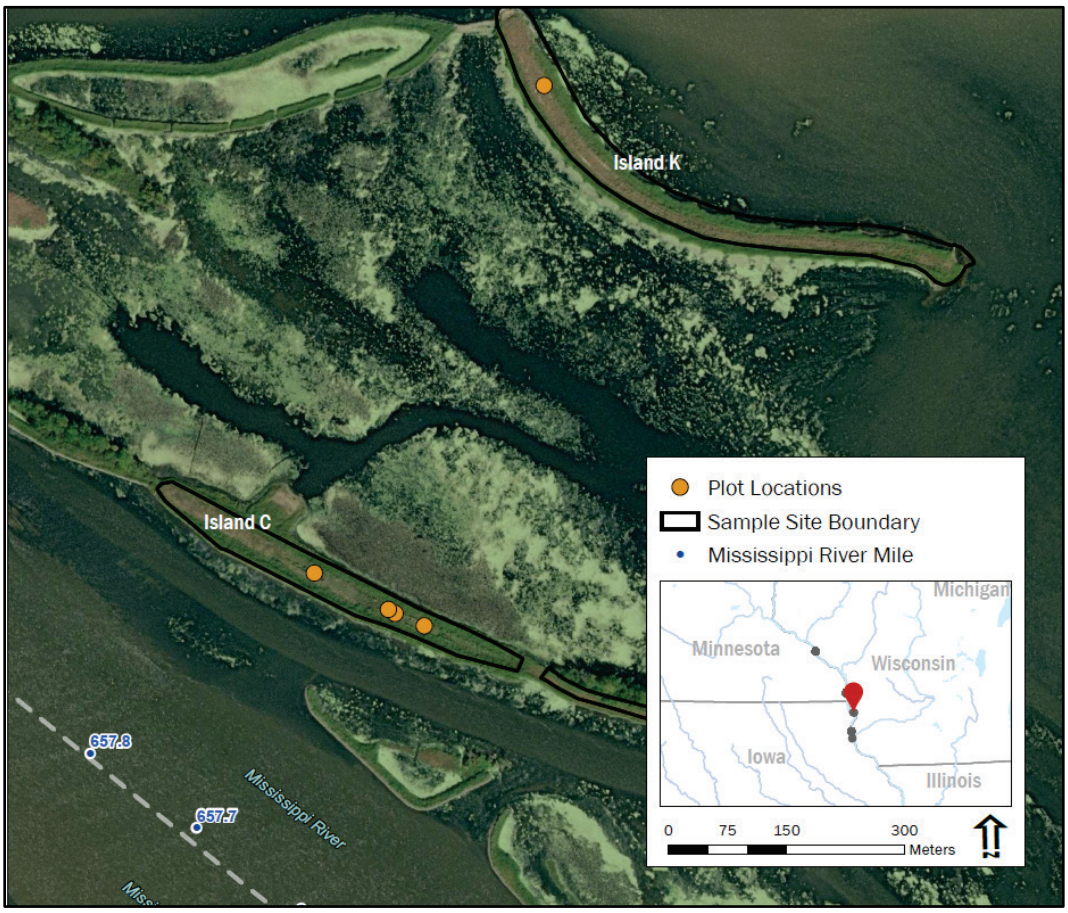
Phase II: https://www.mvr.usace.army.mil/Missions/Environmental-Stewardship/Upper-Mississippi-River-Restoration/Habitat-Restoration/St-Paul-District/Pool-8-Islands-Phase-II/

Phase III: https://www.mvr.usace.army.mil/Missions/Environmental-Stewardship/Upper-Mississippi-River-Restoration/Habitat-Restoration/St-Paul-District/Pool-8-Islands-Phase-III/

(Figure 7) in 2018 indicated trees had at least three successful growing seasons. Island C was tied into a remnant island, and is located near the main channel, along the upstream end of the Capoli Slough side channel. These islands were constructed using a sand base of dredged material from the main navigation channel, and then topped with a layer of finer-grained silt- and clay-dominated dredged materials. The Capoli Slough Islands were constructed with substantial and experimental variation in fine material layers, ranging from 22.9–137.2 cm (9–54 in.) deep. Island 'C' was selected because recent surveys indicated good tree growth. Sites here had 45.7 cm (18 in.) of fine sediment placement. Island 'K' contained either failed or extremely stunted tree plantings and was constructed at a higher elevation and with a shallower 22.9 cm (9 in.) layer of fines. Tree species planted on Island C were pin oak, bur oak, northern red oak, cottonwood, sycamore, hackberry, silver maple, river birch, black cherry, and sandbar willow. Fact sheets, project details, and reports can be found at the St. Paul District Habitat Rehabilitation and Enhancement Projects website*.

* https://www.mvr.usace.army.mil/Missions/Environmental-Stewardship/Upper-Mississippi-River-Restoration/Habitat-Restoration/St-Paul-District/Capoli-Slough/

Figure 6. Satellite image of the Capoli Slough Habitat Rehabilitation and Enhancement Project Islands constructed site.



2.4 Sampling

Sampling was conducted at Belvedere Island and the Pool 8 Islands restoration project sites 20–23 July 2020, and at the McGregor Lake, Sny McGill Lower, and Capoli Slough Island sites 27–29 July 2020. Fifteen circular 0.01 acre (0.004 ha) plots were sampled, including five plots within natural reference sites, five within restoration sites with planted trees, and five within restoration sites without trees.

Sampling methods are described in the following sections. At each plot, data were collected to describe plot vegetation including tree presence and ground cover. Photos were taken of each site from multiple directions for future reference. Soil profiles were described by either digging a small pit or using a soil probe to document soil texture characteristics at the center of the plot. Soil samples were collected to analyze physical and chemical characteristics for laboratory analysis. Separately, additional soil samples

were collected to analyze microbiome communities using high-throughput DNA sequencing.

2.4.1 Plot selection and layout

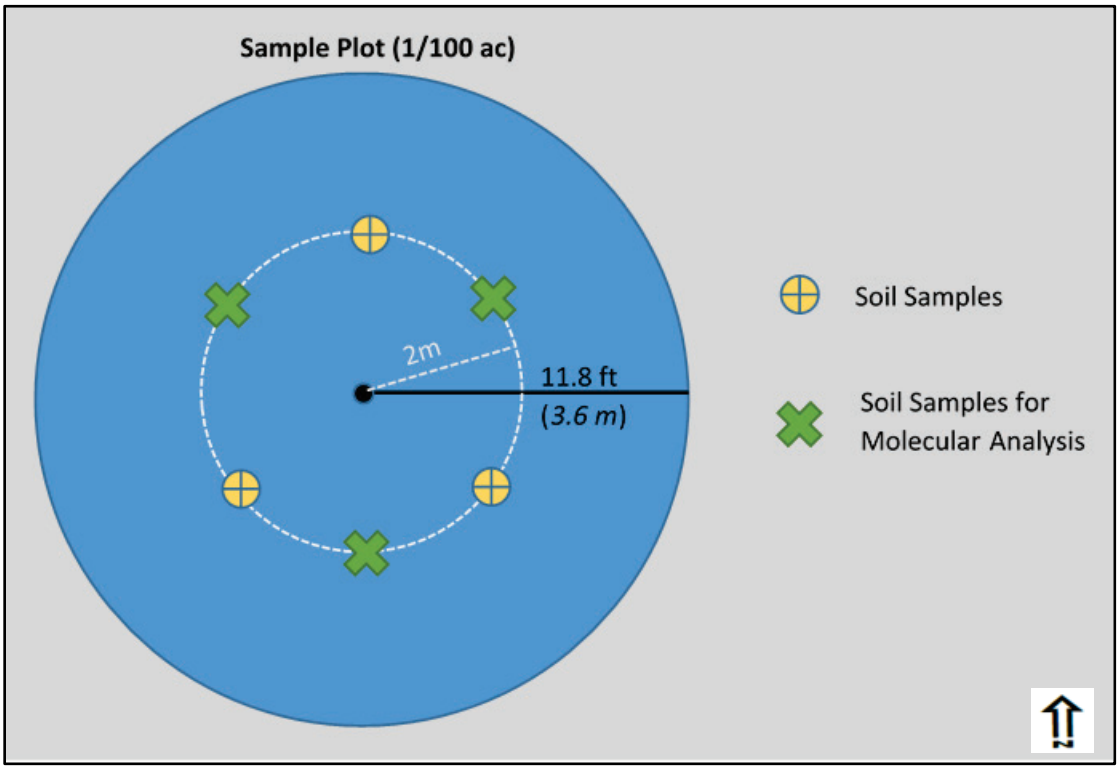
Plot selection was conducted in the field within the larger study sites and were based on observed site conditions. This method ensured the limited number of plots for this study captured the types of habitats defined by each stratum. Randomized plot generation within these larger sites (3-12 acres; 1.2-4.6 ha) would not have guaranteed the collection of data for the target strata. This was especially a concern for ensuring that molecular soil samples were sufficiently close to target tree species to capture the rhizosphere biota.

Once a sampling site was selected, the center stake was inserted (Figure 8). A compass and measuring tape were used to insert stakes delineating the outer plot boundary 11.8 ft (3.6 m) from the center at 0, 60, 120, 180, 240, and 300 degrees. Photos were collected of the plot from each stake pointing toward the center, and from the center toward each stake.

Figure 7. Sample plot photo, Belvedere Island site 3. Note orange plot center stake located in foreground, and plot boundary stakes located in background highlighted by the orange arrows.

Figure 9 shows the layout of each sample plot with the locations of soil and molecular sequencing samples. Note that while plot locations were subjectively selected from the broader site, the locations of soil and microbiome samples within the plot were dictated by this pre-determined plot layout. All soil samples were collected two meters from plot center at bearings of 0, 120, and 240 degrees, while microbiome samples were all collected two meters from plot center at bearings of 60, 180, and 300 degrees.

Figure 8. Sample plot layout detail showing location of soil samples collected within each plot.



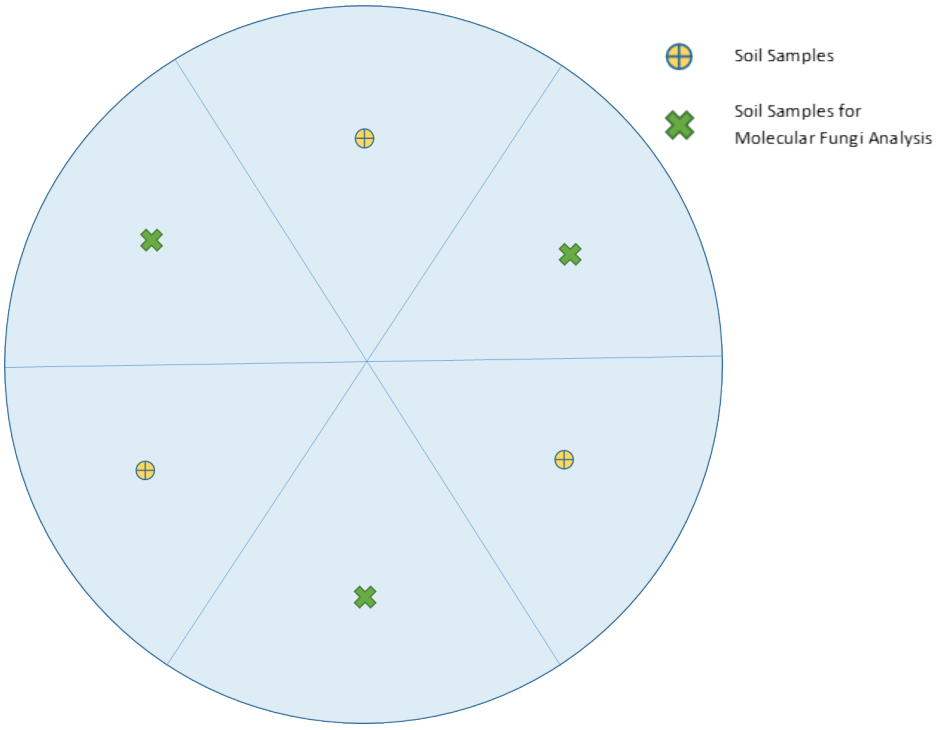
2.4.2 Flora survey methods

Site floral data was collected in two categories – trees and ground cover. All live and dead standing trees within the plots were individually inventoried. Trees immediately adjacent to the edge of the plot borders were also included in the inventory. Once a plot was delineated, the distance and bearing from the center of the plot to each tree was recorded to document each tree's position and proximity to other trees and soil samples. All trees were identified to species. For each tree over 2.5 cm in diameter, the tree's diameter at breast height (DBH) was recorded. For tree saplings at created islands where DBH was less than 2.5 cm (0.5 in.),

DBH was not recorded; instead, approximate tree height was recorded. In basal area calculations, unmeasured saplings were assigned a DBH of 2.5 cm (0.5 in.).

All other vegetation within the plots, including shrubs, forbs, grasses, and vines, were documented to species and the percentage ground cover of each species was estimated. Each plot was split into equal sextants, with the soil collection points centered within the sextants (Figure 10). In this manner, the ground cover vegetation around each soil and microbial soil sample was documented. Plants not identifiable to species were identified to the lowest taxonomic level possible, and at a minimum the growth habitat was recorded (e.g., forb, graminoid, etc.). Photos were taken of unidentified species to reference for further identification efforts. Plant species observed and sample plot layouts are in Appendix B and Appendix C, respectively.

Figure 9. Floral survey plot layout detail. Ground cover vegetation for each plot was identified to species and assigned an estimated percent cover for each sextant.



2.4.3 Soil survey and collection methods

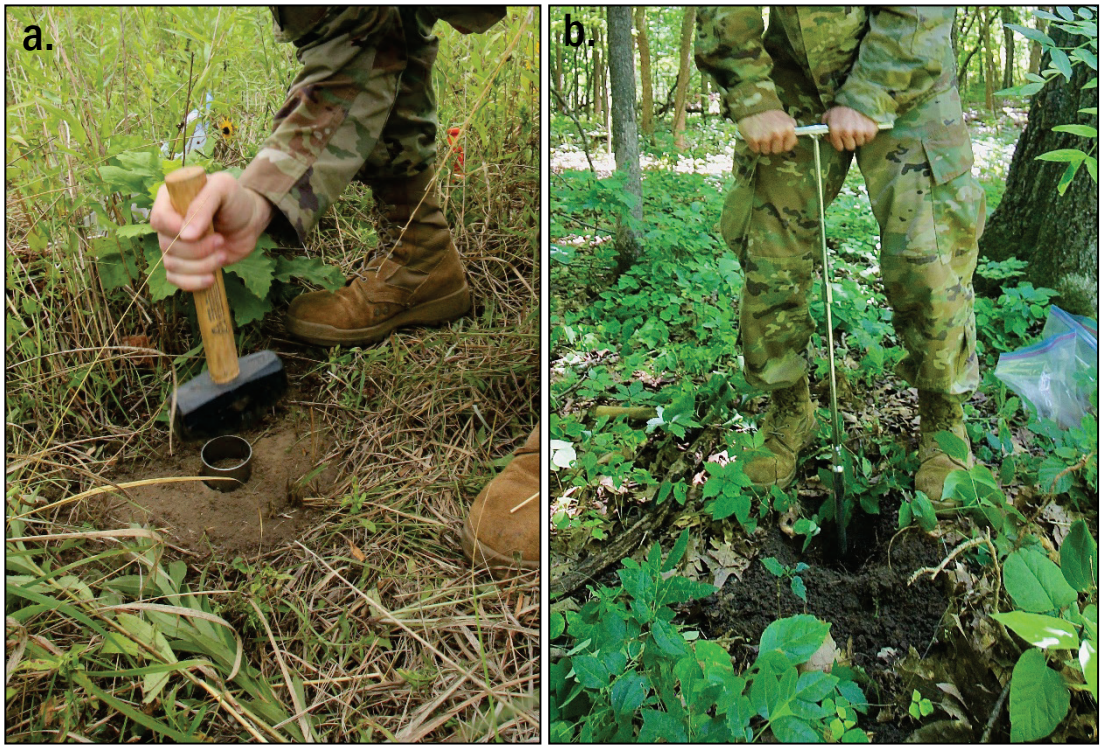
2.4.3.1 Soil profile descriptions

Soil profiles were examined at each site to document soil textures, colors, and redoximorphic features. At the Belvedere and Pool 8 Island sites, small pits were dug and extracted by shovel for study. At the Capoli Islands, McGregor, and Sny McGill sites, soil cores were extracted and examined. Photos of soil profiles were collected and are presented in Appendix D.

2.4.3.2 Soil physical and chemical sample collection

Soil samples for physical and chemical analyses were collected from three, pre-determined locations within each plot (Figures 9–10). Soil was collected from the three sampling locations in each plot in four incremental depths: 0-10 cm, 10-20 cm, 20-45 cm, and 45+ cm. The three soil samples collected at each plot were combined by depth to increase site representation, resulting in one homogenized sample from each plot. The two shallow samples (to 20 cm) were collected with a 5 cm-diameter, 10 cm depth soil core (Figure 11a). The soil core was driven into the soil until flush with the surface or previous sample depth. A soil knife was used to remove soil around the outside of the core and samples were placed into Ziploc bags. For the deeper samples (>20 cm), a 30.5cm (12 in.) Oakfield Model B Tube Sampler Soil Probe was inserted into the existing hole for sample collection (Figure 11b). The first sample collected using the probe (20–45 cm) was removed and placed in a Ziploc bag. The final sample depth was conducted either to the length of the probe (approximately 95 cm), or to as deep as possible before encountering a hard or compacted layer which the probe would not penetrate. Sample bags were sealed and placed into a cooler on ice for storage and transport. Samples were shipped overnight to the ERDC Soil and Wetland Biogeochemistry Lab in Vicksburg, Mississippi, where samples were stored at 4°C until processing.

Figure 10. Soil collection techniques. On *left*, example of upper horizons (0–10 cm and 10–20 cm) collected with a 5 cm diameter, open-ended tube. On *right*, example of lower horizons (20–45 cm and 45+ cm) collected with 2.1 cm diameter soil probe.



2.4.3.3 Microbiome sample collection

Soil samples for microbial analyses were collected from three, pre-determined locations within each plot, as shown in Figures 9–10. The sample location was first identified by measuring two meters from the center of the plot at the bearing for that sample. Vegetation and surface litter were gently pushed away from the sampling location to create a bare soil surface. Soil samples were collected as intact cores using sterile 50 mL, conical-bottom centrifuge tubes, approximately 2.8 cm wide and 9.5 cm long. For each sample, a centrifuge tube was removed from the packaging, and a flame-sterilized utility knife was used to cut the tip off the conical bottom of the tube around the 5 mL mark. The cut side of the tube was immediately placed at the prepared sample location and tapped down to the cap of the centrifuge tube using a mallet (Figure 12). The tube was retrieved from the soil and immediately placed inside a clean, zippered, plastic bag. A rubber band was secured around the open tip of the tube to prevent soil from dislodging from the tube, and the sample was placed inside of a second clean plastic, zippered bag to further protect the

sample. Samples were immediately placed on ice in a cooler, shipped overnight to the ERDC Environmental Microbiology Team lab in Vicksburg, Mississippi, where samples were transferred to a freezer and stored at -20°C until processing.

Figure 11. Microbial soil sample collection. Centrifuge tube collection of soil samples used a mallet to tap the open-ended, sterile tube into the soil and remove the soil intact for collection.

2.5 Processing and lab analyses

2.5.1 Soil physical, chemical, and microbial activity analyses

Soil physical, chemical, and microbial properties were analyzed in natural and constructed sites to assess soil conditions associated with successful floodplain forest establishment and growth. As described previously, triplicate samples (which themselves were each homogenized triplicates) to two separate depths (0–10 and 10–20 cm) were collected in each plot and analyzed for the following soil properties: moisture content (MC), bulk density (BD), percent organic matter (OM), soil pH, extractable ammonium (NH_4^+), nitrate (NO_3^-), and soluble reactive phosphorus (SRP), dissolved organic carbon (DOC), total carbon (TC), total nitrogen (TN), microbial biomass carbon (MBC), and Beta-glucosidase (BG),

Phosphatase (Phos), Xylosidase (Xylo), Cellobiosidase (Cello), and N-acetyl-glucosaminidase (NAG) enzyme activities. Analysis was completed on the deeper soil cores (20+ cm) for bulk density, moisture content, and percent organic matter, which can be found in Appendix E.

2.5.1.1 Soil physical properties

Field moist soil was thoroughly homogenized, and 50 g subsamples were dried at 105°C until constant weight. Soil moisture content was calculated using the wet and dry weight of each subsample. Dry weight soil bulk density was determined by dividing the total dry mass of soil in each sample by the sample volume. Dried soil was ground using a ball mill grinder, homogenized, and combusted at 550°C for 4 hr to measure percent organic matter using the loss on ignition (LOI) method.

2.5.1.2 Soil chemical properties

Extractable NH4+, NO3-, and SRP and DOC were determined by 2M KCl extraction of field moist soil. Samples were placed on a longitudinal shaker for 1 hr and centrifuged at 5,000 × g for 10 min. Extractant was filtered through a 0.45 µm membrane filter, acidified to a pH<2, and stored at 4°C until analysis. Soil extractable nutrients were analyzed calorimetrically using a Seal AQ2 Auto-mated Discrete Analyzer with method detection limits of 0.04 mg N L^{-1}, 0.015 N mg L^{-1}, and 0.04 P mg L^{-1} for $NH4^{+}$, $NO3^{-}$, and SRP, respectively (SEAL Analytical Inc, Mequon, Wisconsin; Methods 350.1, 353.2, and 365.1, respectively; USEPA, 1993a, b, c). Dissolved organic *C* was determined using a Shimadzu TOC-LCSH analyzer (Shimadzu Instruments, Kyoto, Japan). Soil pH was measured by creating a 1:1 soil slurry of field moist soil and DI water following the methods described by Reddy et al. (2013).

2.5.1.3 Microbial biomass and soil enzymatic activity

Microbial biomass *C* was determined using the chloroform-fumigation extraction method after Vance et al. (1987) with modifications by White and Reddy (2000). For the non-fumigated samples, field moist subsamples were extracted with 2M KCl, placed on a longitudinal shaker for 1 hr, and centrifuged at 5,000 × g for 10 min. A duplicate set of samples were fumigated in a vacuum-sealed desiccator with a beaker containing 50 mL of chloroform for 24 hr. Fumigate samples were then extracted with 2M KCl as described above. Extracts were filtered through a 0.45 µm membrane filter, acidified with H_2SO_4 to a pH <2, and

refrigerated at 4°C until analysis. Microbial biomass *C* was determined as the difference between the DOC of fumigated and nonfumigated samples, determined using a Shimadzu TOC-LCSH analyzer (Shimadzu Instruments, Kyoto, Japan).

Enzyme activities were fluorometrically analyzed using 4-methylumbelliferone (MUF, a fluorescent substrate) for standardization and its associated fluorescent labeled MUF substrates. Enzymes catalyze the breakdown of organic plant material, facilitating nutrient release. Five enzymes were assessed in this study: β-glucosidase (β-gluc); Cellobiohydrolase (Cello); Xylosidase (Xylo); Phosphatase (Phos); and N-acetyl-glucosaminidase (NAG). Enzymes are typically released either in proportion to the substrate they are acting upon (constitutive) or in response to the demand of the nutrient they release (inducible). β -gluc is indicative of *C* acquisition from labile sources, degrading cellobiose into glucose (Dick 2015). Cello, a constitutive enzyme, degrades cellulose into cellobiose, with its activity indicative of relatively recalcitrant *C*. Xylo is a constitutive enzyme that degrades hemicellulose and indicates the most recalcitrant from of *C* measured in this study. Phos activity is an inducible enzyme that releases phosphate ions. Finally, NAG, a constitutive enzyme, degrades chitin and acts as an indicator of *N* acquisition.

For microbial enzyme activity assays, 0.5 g of field moist soil from each sample was shaken with 39 mL of DI water for 1 hr, creating a 1:80 soil slurry. The subsequent sediment slurry was pipetted into 96-well plates along with separate MUF-labeled substrates. Plates were read fluorometrically using a BioTek Synergy HTX microplate reader (BioTek Instruments, Inc., Winooski, Vermont, USA) immediately after substrate addition and then again 24 hr later. Linear regression analysis was used to determine rate of enzyme activity. All enzyme activities were measured within 48 hr of field collection.

2.5.1.4 Data analysis

Data was analyzed using analysis of variance (ANOVA) tests in Microsoft Excel (Version 2108). Results from the two constructed strata were not determined to be significantly different; thus, results from all constructed sites were pooled for comparison to natural sites. To better relate physical, chemical, and microbial data to bacterial analysis, only the upper soil layers (0–20 cm) are discussed below. A correlation table was produced and included all soil data previously discussed in addition to tree DBH,

basal area, percent ground cover, and mean days of flooding for comparison.

2.5.2 Microbiome - Bacterial 16S rRNA sequencing

A semi-quantitative survey method of 16S rRNA community analysis via next generation sequencing with the Illumina MiSeq platform (Illumina, Inc., San Diego, California) was performed on all soil samples. Soil was thawed and roughly homogenized by hand and vigorous shaking for 3 min after extruding the soil from the collection tubes into the collection bag. DNA was extracted from 0.2 g of homogenized soil using Qiagen's Power Soil DNA Isolation Kit (Valencia, California). Resultant DNA was amplified with uniquely barcoded primers specifically designed for paired-end sequencing with the Illumina MiSeq from the 515–806 bp region of the 16S rDNA gene (*E. coli* numbering) as described in Caporaso et al. (2011). Amplicons were normalized and combined to 20 pmol and further combined with 11% PhiX control according to Illumina MiSeq instructions. The sample was added to a 300-cycle MiSeq kit for sequencing. Relative abundances of microbial community composition between samples were compared via QIIME I software for downstream multivariate statistical analysis of sequencing data (Caporaso et al. 2011, 2012). Briefly, the sequencing data from the Illumina runs were trimmed, demultiplexed, and quality filtered with Illumina MiSeq Reporter software. The sequences were then joined by paired-end assembly and OTU (operational taxonomic units – sequences) assignment was performed with the open reference OTU clustering package implemented in QIIME I. The package performs an initial closed reference clustering against the Greengenes database with a 97% sequence similarity threshold followed by two rounds of sequential *de novo* clustering with sequences that failed to align in the database using each cluster centroid as a reference sequence against the Greengenes database. Principal coordinates analysis (PCoA) was conducted using pairwise distances between communities (Bray–Curtis and UniFrac distances). Total relative abundances were obtained using a 1% total abundance cutoff and these data were normalized to 100% relative abundance. An analysis of variance (one-way ANOVA) across all experimental samples was conducted using Kruskal Wallis within the QIIME environment to look only at bacteria that were unique in at least one sample. Alpha diversity was determined by raw observed OTUs and Faith's Phylogenetic Diversity (PD Wholetree) which both give an estimation of sample diversity and can be used to infer perturbations or relative health of the sample; larger numbers mean more diversity.

Functional gene analysis was inferred via KEGG pathways through PICRUSt1 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; Langille et al. 2013). PICRUSt was performed at the phylogenetic level of Family.

2.5.3 Mycobiome - Internal Transcribed Spacer (ITS) rRNA sequencing

A semi-quantitative survey method of ITS (internal transcribed spacer) rRNA community analysis via next generation sequencing with the Illumina MiSeq platform (Illumina, Inc., San Diego, California) was performed. Soil was thawed and roughly homogenized by hand and vigorous shaking for 3 min after extruding the soil from the collection tubes into the collection bag. DNA was extracted from 0.2 g of homogenized soil using Qiagen's Power Soil DNA Isolation Kit (Valencia, California). Resultant DNA was amplified with uniquely barcoded primers used for 16S (Caparoso 2011) attached to the ITS1 and ITS2 primers (Thompson 2017). These primers amplify a small portion of the 18S gene, through the entire ITS1 region to the 5.8S gene. Amplicons were normalized and combined to 20 pmol and further combined with 11% PhiX control according to Illumina MiSeq instructions. The sample was added to a 300-cycle MiSeq kit for sequencing. Relative abundances of fungal community composition between samples were compared via QIIME I software for downstream multivariate statistical analysis of sequencing data (Caporaso et al. 2011, 2012). Briefly, the sequencing data from the Illumina runs were trimmed, demultiplexed, and quality filtered with Illumina MiSeq Reporter software. The sequences were then joined by paired-end assembly and OTU (operational taxonomic units – sequences) assignment was performed with the open reference OTU clustering package implemented in QIIME I. The package performs an initial closed reference clustering against the NCBI BLAST database with a 97% sequence similarity threshold followed by two rounds of sequential *de novo* clustering with sequences that failed to align in the database using each cluster centroid as a reference sequence. Principal coordinates analysis (PCoA) was conducted using pairwise distances between communities (Bray–Curtis and UniFrac distances). Total relative abundances were obtained using a 1% total abundance cutoff and these data were normalized to 100% relative abundance. Alpha diversity was determined by raw observed OTUs which gives an estimation of sample diversity and can be used to infer perturbations or relative health of the sample; larger numbers mean more diversity.

Ecosystem functional roles were inferred from sequencing data using FUNGuild (Fungi + fUNctional + Guild) bioinformatics software package to assign ecosystem functional roles to OTUs (Nguyen et al. 2016b). The FUNGuild tool searches fungal OTUs in a community-annotated database to match each with trophic strategies or ecological guilds. The guilds assigned within FUNGuild are animal pathogens, arbuscular mycorrhizal fungi, ectomycorrhizal fungi, ericoid mycorrhizal fungi, foliar endophytes, lichenicolus fungi, lichenized fungi, mycoparasites, plant pathogens, undefined root endophytes, undefined saprotrophs, and wood saprotrophs. FUNGuild includes a confidence level to each guild assignment ("highly probable," "probable," and "possible"). Only guild assignments of "highly probable" and "probable" were used in this analysis.

3 Results and Discussion

3.1 Plot locations and physical characteristics

A total of 15 plots (five in each of three strata) were sampled, resulting in a total area sampled of 0.15 acres (0.06 ha; Table 1). Table 1 displays the plot names, along with coordinates and physical information collected for each. This includes elevation and mean days of inundation, along with constructed topsoil depth for constructed sites.

3.2 Flora

Sixty-six plant varieties were documented across all sites, 59 of which were identified to species level. Sixteen species of trees were documented. Introduced species were present in 6 of 15 plots and were only found within constructed sites. Reed canarygrass (*Phalaris arundinacea*), which is not introduced but considered an ecologically invasive species in the UMR, was present throughout all the Pool 8 Islands plots. Appendix B lists all plant species recorded within sample plots.

Tree composition is summarized in Figure 13, and tree locations are mapped for each sample plot in Appendix C. Tree composition differed significantly between strata and between some sites and plots. Basal area was different between natural and constructed strata and averaged over 30 times higher at natural sites (47.8 m^2/ha; 208.0 ft^2/ac) than at constructed sites (1.5 m^2/ha; 6.7 ft^2/ac). This is not surprising as the natural reference sites surveyed are much older and contain mature trees compared to the constructed sites where trees have had less than 10 years of growth. Tree species composition varied among all sites. Tree species richness was not significantly different between plots and ranged from 1–5 species per plot. However, the created sites had a greater dominance and number of oak species, likely because oaks were generally the most common species planted at the sites and have been a focus of restoration projects. The species that were common to both natural and created sites were northern red oak (*Quercus rubra*) and swamp white oak (*Quercus bicolor*). Species unique to natural site plots were hackberry (*Celtis occidentalis*), American basswood (*Tilia americana*), American elm (*Ulmus americana*), and bitternut hickory (*Carya cordiformis*). Species unique to the created sites were sycamore (*Platanus occidentalis*), northern pin oak (*Quercus palustris*), and bur oak (*Quercus macrocarpa*). Silver maple (*Acer*

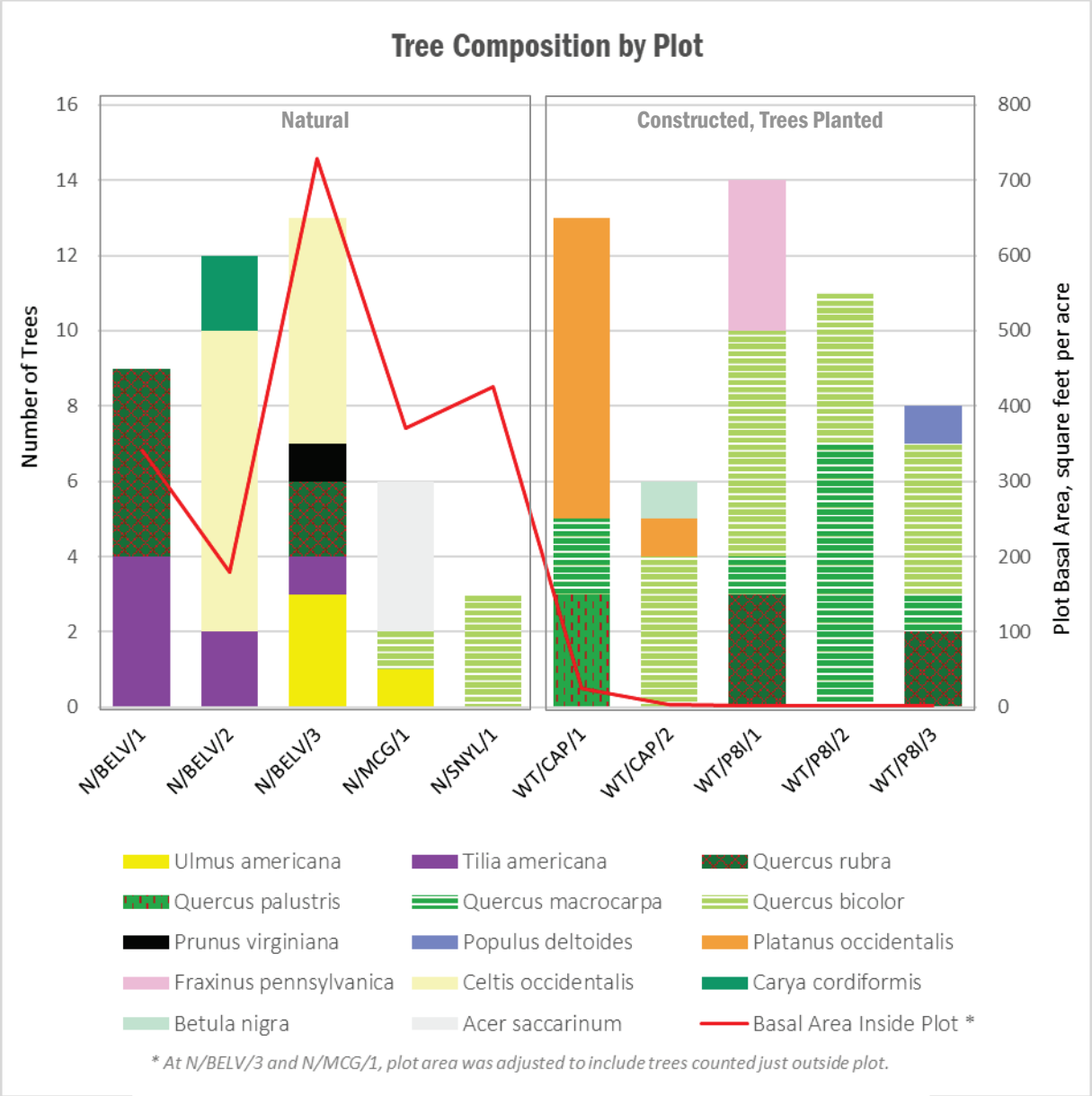
saccharinum) was encountered as mature trees in natural sites and observed as naturally established seedlings at the created sites.

Table 1. Summary of sample plot locations and related data.

Plot Name	Strata	Site / Location	Year Built	Pool[1]	Easting[2]	Northing[2]	Mean Days Inundation[3]	Elevation[4]	Topsoil Depth (in)
N/BELV/1	Natural	Belvedere Island	N/A	5	588594.7	4902169.3	1.8	666.4	N/A
N/BELV/2	Natural	Belvedere Island	N/A	5	588582.9	4902087.8	1.3	666.6	N/A
N/BELV/3	Natural	Belvedere Island	N/A	5	588573.4	4902082.1	1.6	665.6	N/A
N/MCG/1	Natural	McGregor Lake	N/A	10	649059.3	4767017.4	13.7	617.6	N/A
N/SNYL/1	Natural	Sny McGill Lower	N/A	10	650864.6	4755324.2	15.7	615.8	N/A
WT/P8I/1	Constructed With Trees	Pool 8 Islands; Island "C8"	2012	8	641786.2	4832148.0	3	633.5	12
WT/P8I/2	Constructed With Trees	Pool 8 Islands; Island “C8”	2012	8	641789.3	4832167.6	3	633.5	12
WT/P8I/3	Constructed With Trees	Pool 8 Islands; Island "C8"	2012	8	641795.1	4832219.7	3	633.5	12
NT/P8I/1	Constructed No Trees	Pool 8 Islands; Island "C4"	2012	8	639991.3	4832126.7	2	634	12
NT/P8I/2	Constructed No Trees	Pool 8 Islands; Island "C4"	2012	8	639974.3	4832036.2	2	634	18
NT/P8I/3	Constructed No Trees	Pool 8 Islands; Island "C4"	2012	8	640009.8	4832179.7	2	634	12
WT/CAP/1	Constructed With Trees	Capoli Slough Islands; Island “C”	2014	9	652225.0	4798778.0	9.2	624.3	18
WT/CAP/2	Constructed With Trees	Capoli Slough Islands; Island "C"	2014	9	652323.1	4798729.9	12.2	623.8	18
NT/CAP/1	Constructed No Trees	Capoli Slough Islands; Island "C"	2014	9	652364.8	4798711.1	15.6	623.3	18
NT/CAP/2	Constructed No Trees	Capoli Slough Islands; Island "K"	2012	9	652517.2	4799397.3	3	627.7	9

1 – Upper Mississippi River System Navigation Pool Number
2 – Coordinates presented UTM Zone 15N NAD83
3 – e.g., average number of days site is flooded annually, reference Chapter 2.2.3
4 – Elevation in North American Vertical Datum of 1988 (NAVD 88)

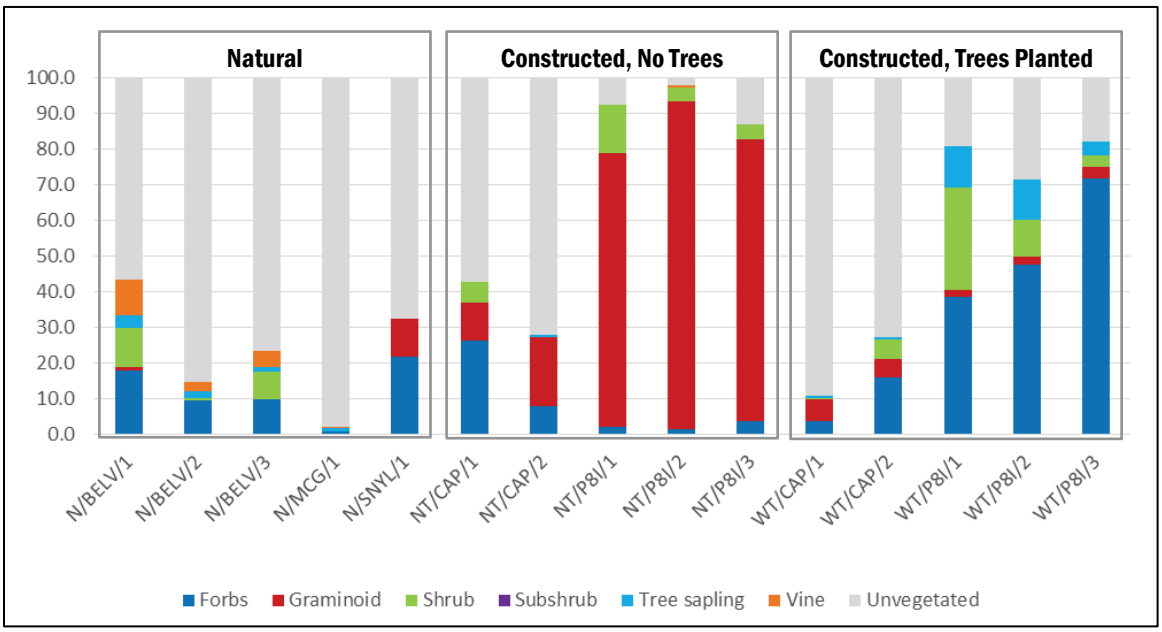
Figure 12. Tree composition compared to basal area in each plot.



Ground cover vegetation varied across plots and showed trends across strata and sites. Figure 14 shows ground cover percentage and types by site. In general, sites with trees had less ground cover and more bare soil than sites without trees. Natural reference sites had an average of 23.3% ground cover (range 2.2–43.3 %), whereas constructed sites with trees averaged 54.5% (10.8–82.0%), and constructed sites without trees averaged 69.6 (28.0–97.8%). The wide range for constructed sites is because the Pool 8 Islands and Capoli Islands had substantially different levels of ground cover compared to one another. The Pool 8 Island sites consistently had the highest levels of ground cover regardless of trees:

85.2% (71.3–97.8%), whereas Capoli Islands averaged closer to the natural sites: 27.3% (10.8–42.8%). Within each site, the trend toward higher percentage cover at sites without trees is still apparent.

Figure 13. Percent of ground cover vegetation type per plot.

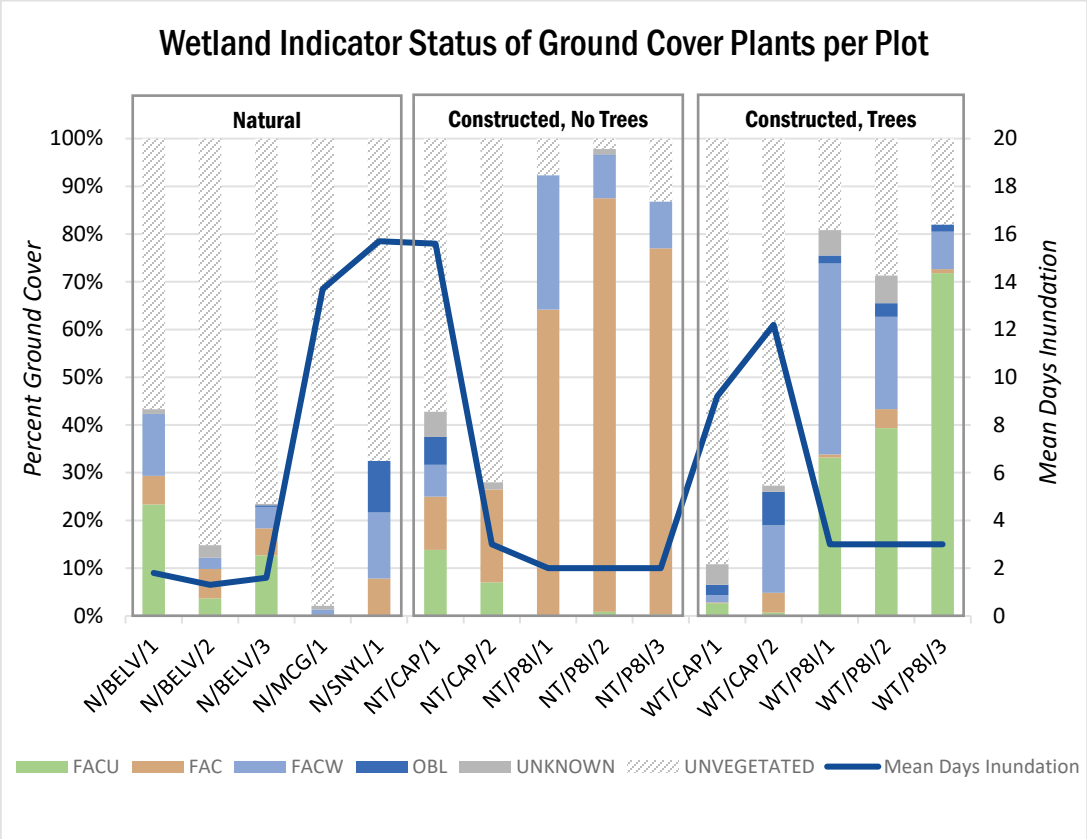


Composition of growth habits also differed substantially between sites. On sites without trees, graminoid species were more common, while forbs generally dominated ground cover in sites with trees.

Indicators of wetland site characteristics were compared across sites to determine if characteristics were correlated with hydrologic parameters. Site flooding characteristics (mean days of annual inundation) were compared to wetland indicator status for plant species found at that site (National Wetland Plant List, USDA 2020). Ground cover for all six sextants of each plot were combined to determine the total percentage cover of each species at the plot level. The percentage of unvegetated area is included to create absolute percentages (Figure 15). In general, wetter sites (9.2–15.7 mean days inundation) contained greater percentages of plant species categorized as “obligate wetland” (OBL) species, and fewer “facultative upland” (FACU) species. Strong differences in wetland plant indicators are apparent between the created Pool 8 Islands sites with and without trees, even though the sites do not have substantially different flooding characteristics. The Pool 8 Islands plots with trees receive an estimated 3 days of inundation annually and had high percentages of FACU species, dominated by tall goldenrod, which is believed to have

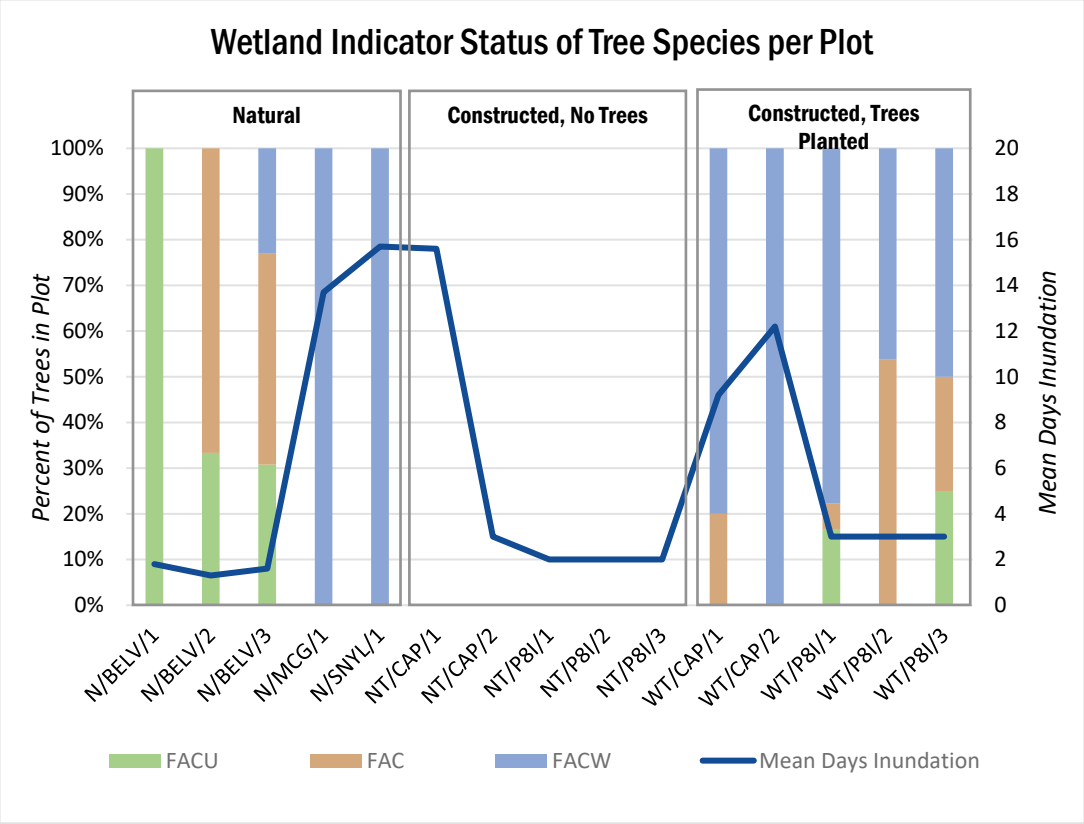
naturally colonized the islands post-construction. The Pool 8 Islands plots without trees receive an estimated 2 days of inundation but were dominated by facultative (FAC) plant species, such as switch grass, which was seeded during construction. These groups of plots were on two different islands, which were planted with the same seed mixes.

Figure 14. Wetland indicator status of ground cover plants per plot, compared to flooding regime (mean days inundation) by site.



Trees were compared on a separate plot for sites where trees were present (Figure 16). Tree species correlated very strongly with hydrologic characteristics. The wettest four sites had 80–100% facultative wetland (FACW) tree species, whereas drier sites exhibited more variation in wetland indicator status of trees (Figure 16).

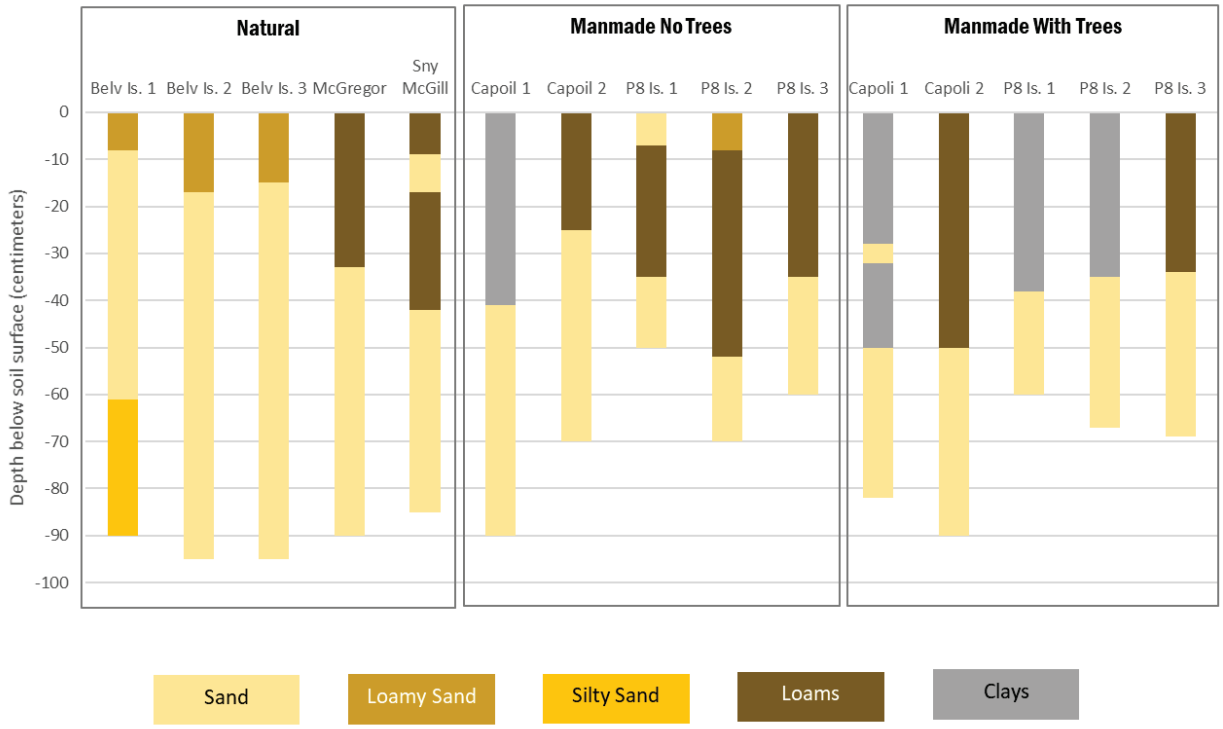
Figure 15. Wetland indicator status of tree species per plot, compared to flooding regime (mean days inundation) at site.



3.3 Soil physical properties

Natural reference sites contained a greater percentage of sandy soils, including sand, silty sand, and loamy sand. Constructed sites contained relatively greater amounts of loams and clays, especially in the surface, compared to the soils in natural sites (Figure 17). Subsurface soils for all sites generally contained high levels of sand and/or silty sand (Figure 17).

Figure 16. Site soil texture profiles.



Soil moisture content was 15–45% higher in constructed sites compared to natural reference sites (Table 2). Moisture content generally decreased with soil depth while bulk density increased with depth. Bulk density was 20–30% higher on average in constructed sites compared to natural sites; however, this numerical difference doesn't fully capture the comparative conditions (Table 3a). The effects of bulk density on plant growth and soil biotic systems are relative to the soil texture. As soil texture becomes finer with increased presence of silts and clays, the bulk density associated with root growth restriction decreases (See Table 3b, USDA Natural Resources Conservation Service 2008). Natural sites in this study were markedly sandier, whereas created sites contained higher proportions of silts and clays. The average bulk density values found in the natural sites studied were within the ideal range for plant growth, regardless of depth, especially considering their sandier textures. In contrast, the created sites had bulk densities that were on the edge of ideal for silty soils while the average lower horizon results (1.52 ± 0.05 g/cm^3) would restrict root growth in clayey soils. Although subjective, collecting the deeper sample cores (20–95 cm) during field sampling was often unachievable to the full 95 cm depth at sites and required repeated striking of the soil probe with a

mallet. In contrast, at natural sites the soil probe was easily pushed down using bodyweight.

Table 2. Site biophysical properties within natural and constructed sites. Data presented as a mean, plus or minus (±) one standard error (SE).

Site	Depth (cm)	Moisture Content (%)	Bulk Density (g/cm³)	Organic Matter (%)	Soil pH
Natural	0-10	13.74 ± 1.9	1.13 ± 0.1	5.34 ± 1.1	7.15 ± 0.3
	10-20	10.55 ± 1.8	1.17 ± 0.1	2.85 ± 0.9	7.17 ± 0.3
Constructed	0-10	15.9 ± 1.2	1.35 ± 0.04	2.83 ± 0.2	7.94 ± 0.1
	10-20	15.31 ± 1.2	1.52 ± 0.05	2.53 ± 0.2	8.04 ± 0.1

Table 3. (*a*) Comparison of bulk density at natural and constructed sites analyzed in this study, and (*b*) how those bulk densities may impact plant root growth based on the different soil textures observed at each type of study site.

a) Bulk density results, this study.

	Depth (cm)	Bulk Density (g/cm³)
Natural	0-10	1.13 ± 0.1
	10-20	1.17 ± 0.1
Manmade	0-10	1.35 ± 0.04
	10-20	1.52 ± 0.05

b) General relationship between soil bulk density to root growth based on soil texture (NRCS June 2008)

Soil Texture	Ideal bulk densities for plant growth (g/cm³)	Bulk densities that restrict root growth (g/cm³)
Sandy	< 1.60	> 1.80
Silty	< 1.40	> 1.65
Clayey	< 1.10	> 1.47

Organic matter content decreased with depth and was up to 88% higher in the surface soil of natural sites relative to those in constructed (Table 2), which could be attributed to organic material from surface activity, such as input from plant biomass and sedimentation. Despite moderately higher bulk density in constructed sites, organic material addition from plant biomass and fine-grained sedimentation in the long-term would be expected to decrease bulk density over time.

3.4 Soil chemical properties

Soil pH was generally higher in constructed sites and increased minimally with depth (Table 4a). Soil pH can influence soil biotic communities as there are different optimum ranges for different types of microorganisms (see Table 4b, USDA Natural Resources Conservation Service 2011; Rousk et al. 2009). Soil pH values observed at constructed sites were higher than

those typically tolerated by soil-dwelling fungi. This may be important as many of the floodplain forest tree families of interest (e.g., Fagaceae, Juglandaceae, and Betulaceae) associate with fungal symbionts (Tedersoo and Brundrett 2017) and are ecologically dependent upon one another under typical natural conditions (Smith and Read 2008). Further research is needed to investigate the impact of soil pH on fungal communities within the constructed sites.

Table 4. (*a*) Comparison of soil pH values at natural and constructed sites analyzed in this study, and (*b*) how those pH levels may impact microorganism communities at each type of study site. Note the typically lower preferred pH range of fungi.

a) Soil pH results, this study.

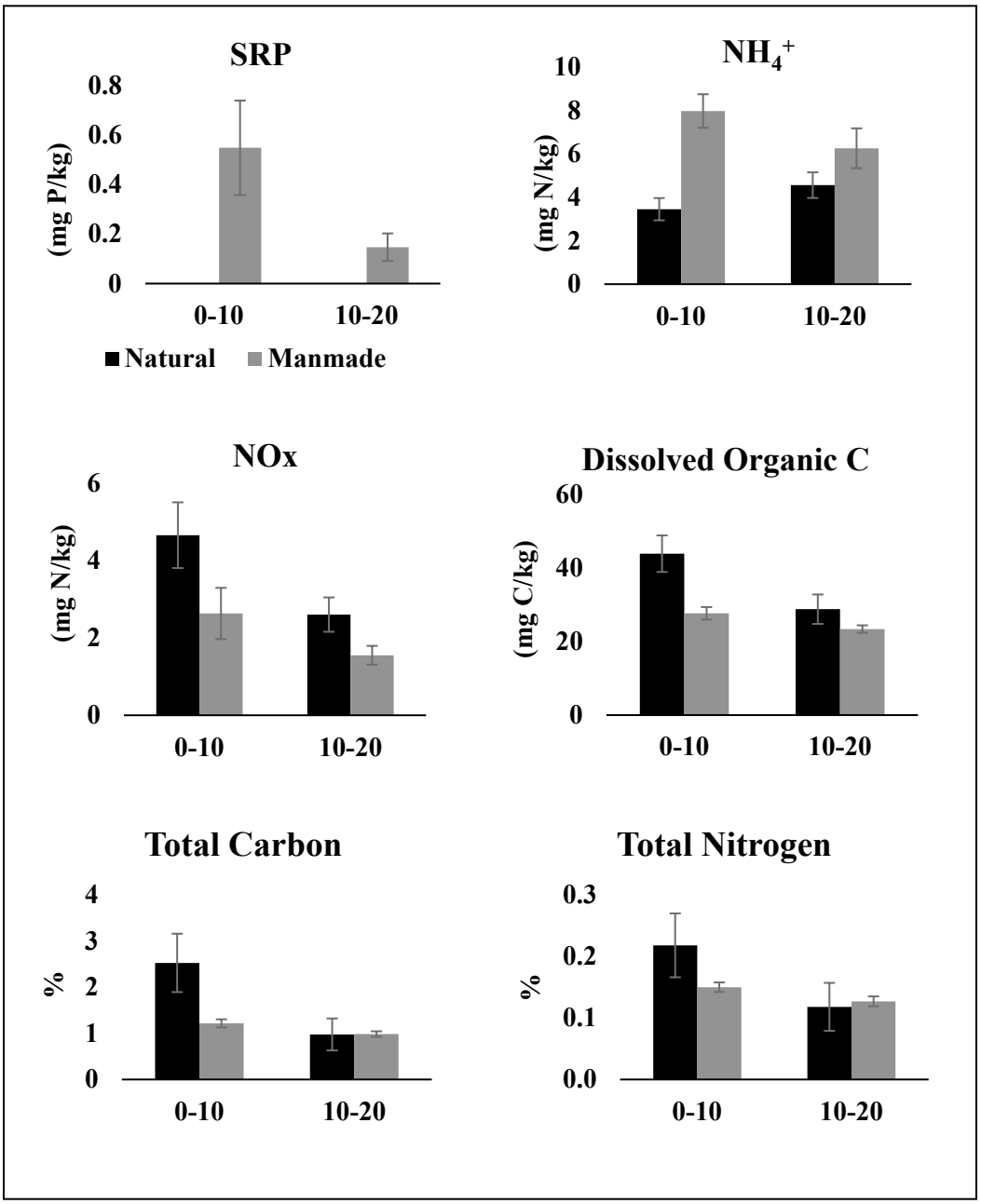
	Depth (cm)	Soil pH
Natural	0-10	7.2 ± 0.3
	10-20	7.2 ± 0.3
Manmade	0-10	7.9 ± 0.1
	10-20	8.0 ± 0.1

b) Maximum, minimum, and optimum pH values for microbial groups (NRCS 2011).

Microorganisms	Range	Optimum
Bacteria	5 - 9	7
Actinomycetes	6.5 - 9.5	8
Fungi	2 - 7	5
Blue green bacteria	6 - 9	> 7
Protozoa	5 - 8	> 7

Extractable SRP concentrations were higher in constructed sites and functionally absent (below detection) in natural sites, suggesting *P* may be a limiting nutrient within these areas and readily taken up by plants, as observed in floodplains elsewhere (Gallardo 2003; Figure 18). Ammonium concentrations were also up to 130% higher in the soils of created sites. Higher nutrient content (SRP and $NH4^{+}$) in constructed sites could be due to lower rates of plant uptake, with nutrients supplied from those inherent to the original dredged sediment, as well as those mineralized by the active microbial community (Figure 18). Conversely, nitrate was up to 40% lower in constructed sites, suggesting the majority of *N* in those sites are in the ammonium form (Figure 18). Dissolved OC, an indicator of labile *C* input, was also 23–59% lower in constructed sites, which is likely related to the lower OM content in these sites relative to natural ones (Figure 18). Like DOC and OM, both TC and TN were lower in the surface soils of constructed sites, but similar between the sites within subsurface soils.

Figure 17. Soil extractable nutrients (soluble reactive phosphorus (SRP), ammonium (NH_4^+), and nitrate-nitrate (NOx)), dissolved organic C (DOC), and total carbon (TC) and nitrogen (TN) concentrations in natural and constructed sites for each soil depth (0-10 and 10-20 cm). Results are displayed as a mean and error bars represent ± 1 SE.

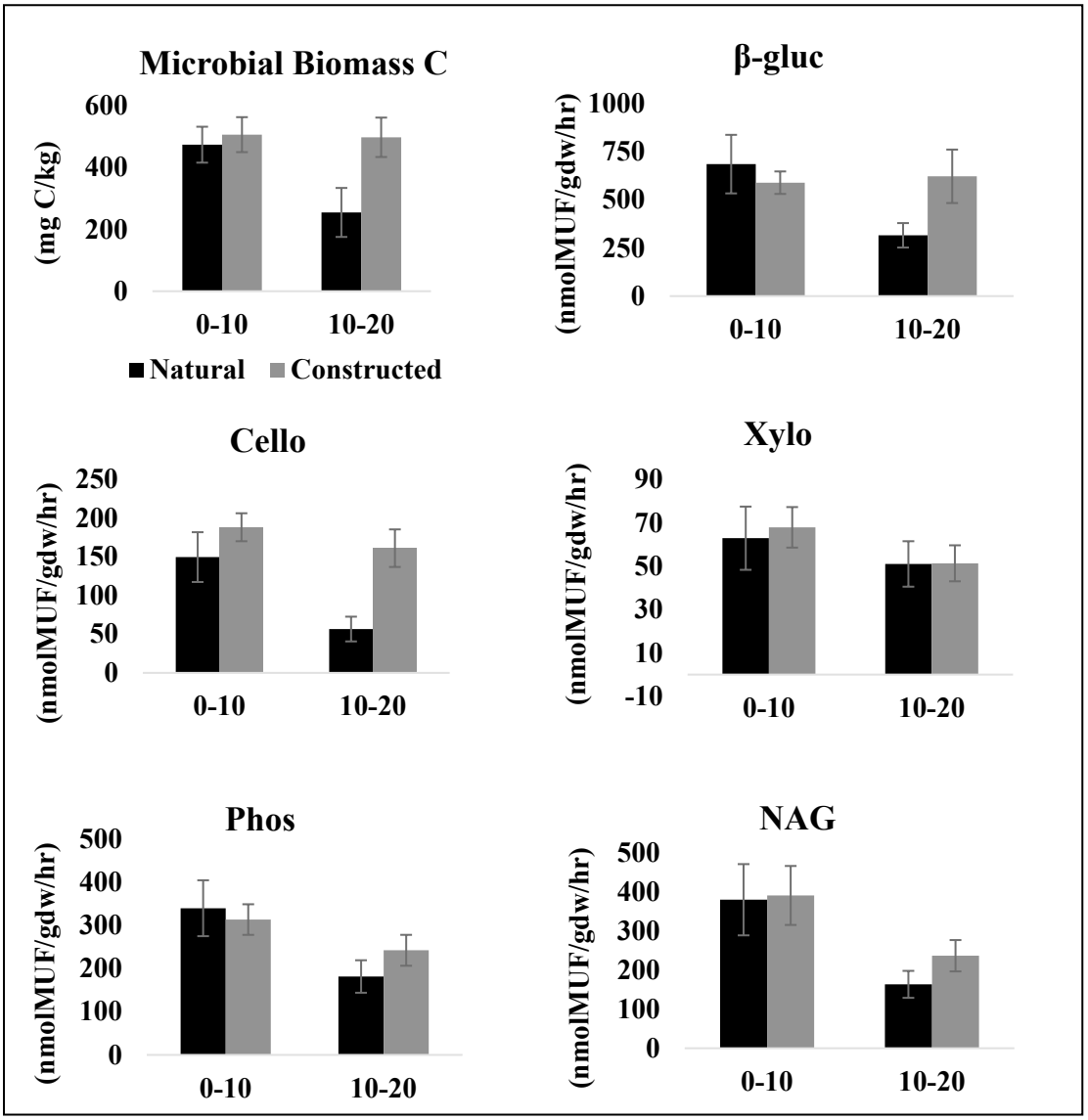


3.5 Soil microbial biomass and enzymatic activity properties

Microbial biomass *C* was similar in the surface soils of the two strata (natural and constructed), but the subsurface soils of constructed sites exhibited greater MBC (~48% higher). Differences in MBC between depths

were more exaggerated in the natural sites while remaining similar across depths in the constructed sites. Similarly, β-gluc activity was elevated in the surface soils of natural sites relative to the subsurface but were similar throughout the soil profile in the constructed sites. β -gluc had the most activity of any enzyme, indicating the soils in both sites contain labile *C*. Cello enzyme activity generally decreased with depth and was greater in constructed sites, particularly in the subsurface depth where it was nearly 300% higher (Figure 19). Increased Cello activity in constructed sites indicate the presence of relatively recalcitrant *C* in their soil that have yet to be degraded. Xylo activities were similar between the sites and did not vary with depth. Phos enzyme activity was also similar among depths for both sites, but generally higher in surface soils (Figure 19). Increased Phos activity in surface soils suggest a greater need for *P* in the root zone, but the demand isn't different between sites despite greater SRP concentrations in the constructed sites. NAG activity also decreased with depth and was higher, on average, in the constructed sites, implying larger amounts of N availability, which was supported by an increased availability of NH_4^+ in those sites (Figure 19).

Figure 18. Soil microbial biomass carbon and enzyme activity (β-glucosidase (β-gluc), Cellobiohydrolase (Cello), Xylosidase (Xylo), Phosphatase (Phos), and N-acetyl-glucosaminidase (NAG)) in natural and constructed sites. Results are displayed as a mean and error bars represent ± 1 SE.



Neither bacterial community (see Section 3.7) or biogeochemical properties differed between constructed sites with planted trees and constructed sites without trees. The lack of difference in soil properties between the sites despite a difference in tree presence suggests the current soil properties in the constructed sites are either (1) indicative of the physical environment and conditions inherent to the soil material utilized or (2) are currently more influenced by herbaceous vegetation rather than tree presence. The distinction between surface and subsurface soils in natural sites (supported by differences in OM, MBC, NOx, DOC, TC, TN,

B-Gluc, Phos, Cello, and NAG measurements) highlights the influence of surface activity, such as inundation and plant biomass. Although a few parameters displayed some differences in soil depth in the constructed sites (e.g., SRP, NOx, NAG), similarities throughout the soil profiles measured in the constructed sites suggests the lack of surface development and immaturity of these recently created sites.

3.6 Soil correlations

Soil correlations were evaluated to identify the relationships between the soil, habitat, and environmental conditions studied, helping identify variables most related to desired project outcomes, such as basal area or ground cover. Percent ground cover, basal area, bulk density, and OM displayed the most correlations out of the metrics evaluated (see Table 5). Percent ground cover was the driver for several correlations and was well related to soil nutrients and enzyme activity. Ground cover was inversely related to tree basal area, DBH, and flooding frequency; sites with trees, especially those with mature trees, typically contained less ground cover vegetation compared to those without trees (or with only saplings) present. Based on correlations and similarity of nutrient cycling and microbial activity, soil and biogeochemical functioning in the constructed sites currently appear to be supported by groundcover vegetation and system hydrology rather than tree presence specifically. Previously established forest restoration trajectory curves implied that ground cover vegetation increases rapidly following restoration, but eventually decreases 15–20 years post-restoration due to canopy closure and subsequent light limitation (Berkowitz 2013). For example, plots in natural sites were occupied by only 23% of ground cover, while plots in created sites experienced 54–70% ground cover vegetation. It is likely that ground cover influences soil properties during the early stages of island development, while trees have a greater influence as the island matures. A study by Kreiling et al. (2015) found inundation frequency patterns and duration can influence nutrient availability and organic matter content more than vegetation communities. Although this current study was designed to minimize the effect of flooding, flooding frequency was positively correlated to MBC, tree DBH, and OM, but negatively related to the percentage of ground cover and BD, reinforcing the strong influence flooding has on floodplain forest development.

Table 5. Correlation matrix for each measured parameter (r values). Bold values indicate a positive correlation, italicized values indicate a negative correlation. Only statistically significant r values are shown (n = 30, d.f. = 28, alpha = 0.05, critical value = 0.361. ns=no significance.

	MC	BD	OM	Soil pH	SRP	NO_X	NH_4^+	MBC	DOC	Phos	Cello	Xylo	B-gluc	NAG	Avg. DBH	Basal Area	% Cover	# of Trees	Flood Days	TC
BD	ns																			
OM	**0.428**	*-0.660*																		
Soil pH	ns	**0.582**	ns																	
SRP	ns	ns	ns	**0.387**																
NOX	ns	ns	ns	ns	ns															
NH4	ns	ns	ns	**0.372**	ns	**0.985**														
MBC	**0.910**	ns	**0.610**	ns	ns	ns	ns													
DOC	ns	*-0.505*	**0.461**	*-0.614*	ns	ns	ns	ns												
Phos	ns	ns	ns	ns	ns	ns	ns	ns	ns											
Cello	ns	ns	ns	**0.594**	**0.404**	ns	ns	ns	ns	**0.740**										
Xylo	ns	ns	ns	ns	**0.713**	ns	ns	ns	ns	ns	ns									
Bgluc	ns	ns	ns	ns	**0.560**	ns	ns	ns	ns	**0.626**	**0.664**	**0.755**								
NAG	ns	ns	ns	ns	**0.564**	ns	ns	ns	**0.419**	ns	ns	**0.536**	**0.474**							
Avg. DBH	ns	*-0.592*	**0.496**	*-0.384*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns						
Basal Area	ns	*-0.617*	**0.437**	*-0.717*	ns	ns	ns	ns	**0.575**	ns	*-0.371*	ns	ns	ns	**0.757**					
% Cover	ns	**0.618**	ns	**0.411**	ns	**0.432**	**0.437**	*-0.425*	ns	ns	ns	ns	**0.458**	ns	*-0.576*	*-0.531*				
# of Trees	ns	ns	ns	*-0.572*	*-0.446*	ns	ns	ns	ns	ns	*-0.435*	ns	*-0.413*	ns	ns	ns	ns			
Flood Days	**0.604**	*-0.395*	**0.403**	ns	ns	ns	ns	**0.669**	ns	ns	*	ns	ns	ns	**0.361**	ns	*-0.650*	ns		
TC	ns	*-0.537*	**0.873**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**0.382**	**0.454**	ns	ns	ns	
TN	**0.428**	*-0.509*	**0.858**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**0.947**

3.7 Soil bacterial microbiome

Bacterial microbiomes associated with samples collected at all 15 study plots were compared; three samples were collected at each plot. One sample at plot WTCAP02 was excluded from analysis due to low-quality reads (143 sequences). The 44 remaining total samples yielded 2.8 million sequences with a mean read count per sample of 63,000 ± 17,000.

Figure 20 presents averaged (n = 3), normalized bacterial order relative abundance for each study plot. The bacterial class Acidobacteria-6 and order Rhizobiales were strongly represented across all sites, accounting for a combined total of 15.4–25.5% of relative abundance at each averaged (n = 3) study plot (Figure 20). Between 23 and 32 bacterial orders were represented above 1% relative abundance within each plot (Figure 20). A total of 43 different orders were observed across all plots at higher than 1% relative abundance. Figure 21 shows the 20 bacterial orders that differed significantly between strata (Kruskal Wallis (FDR $p<0.05$)). Alpha Diversity (OTUs) was similar between all samples and strata, indicating no loss of diversity from natural, constructed with planted trees, or constructed islands without trees planted (Figure 22).

Figure 19. Normalized bacterial relative abundance (>1.0%) of averaged soil samples (n=3) at each study plot.

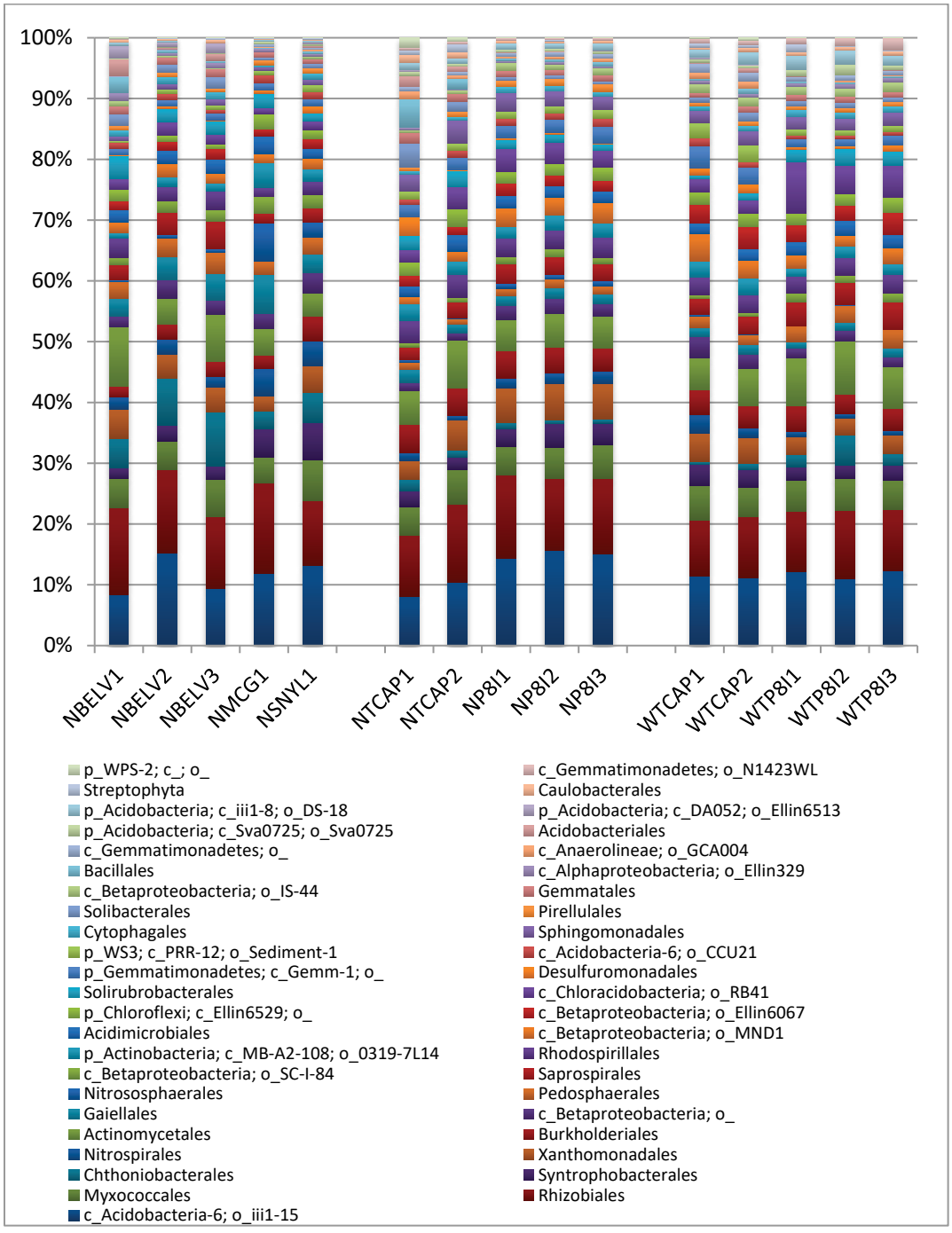


Figure 20. Bacterial orders with statistically significant differences in relative abundance (>1.0%); Kruskal Wallis (FDR p<0.05).

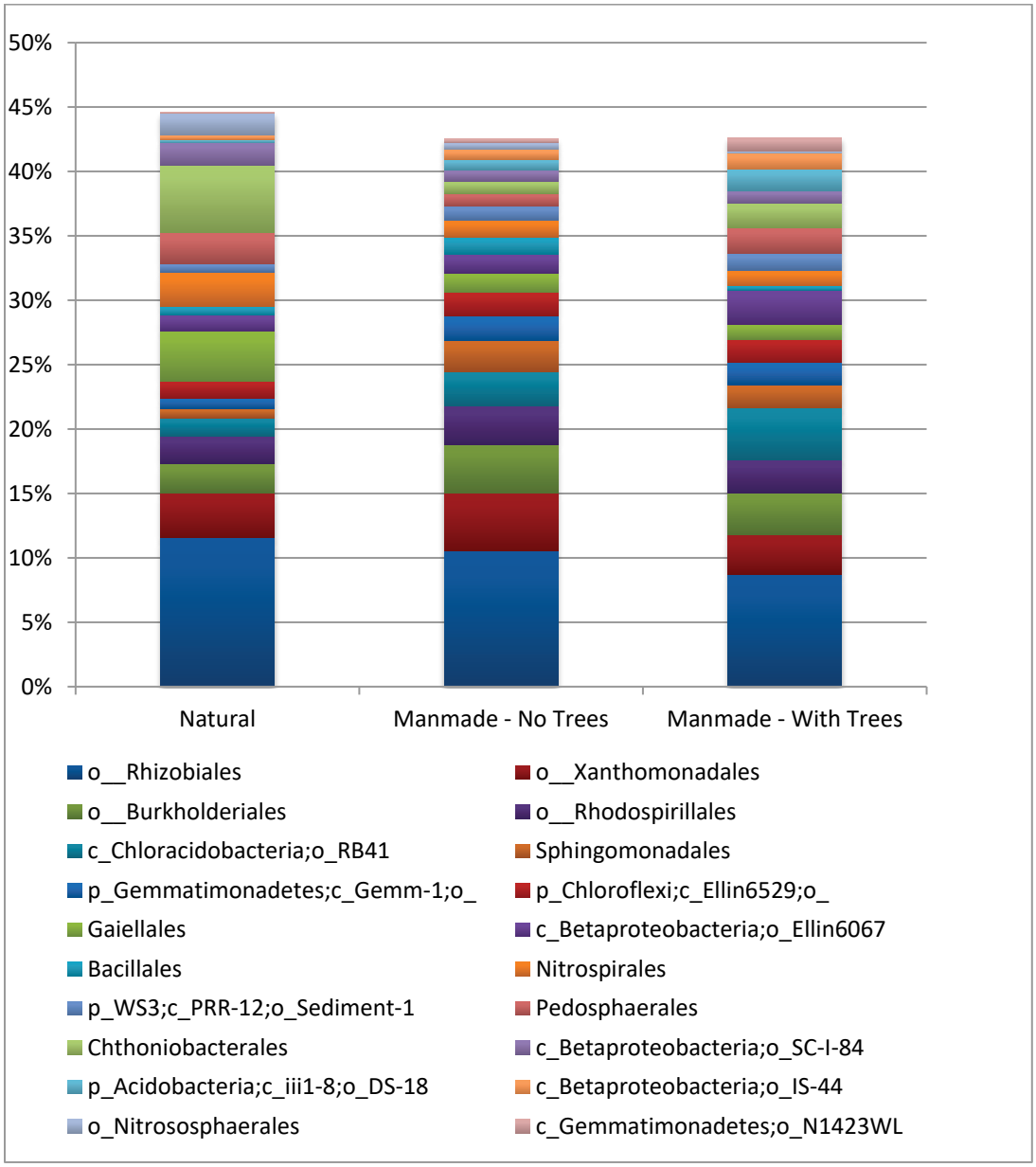
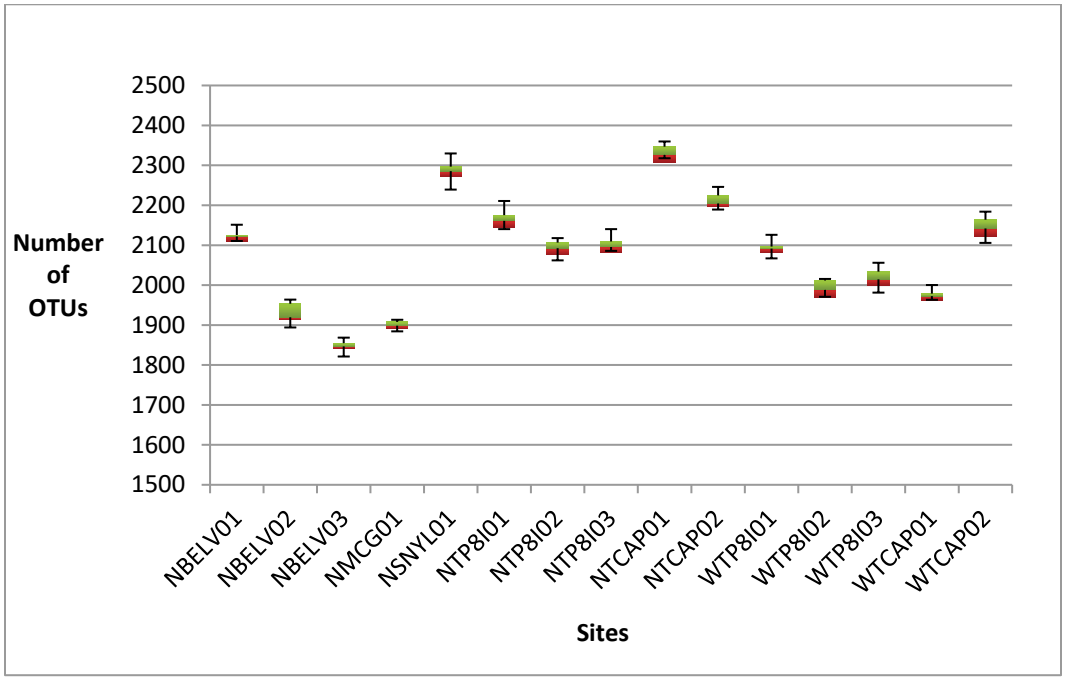
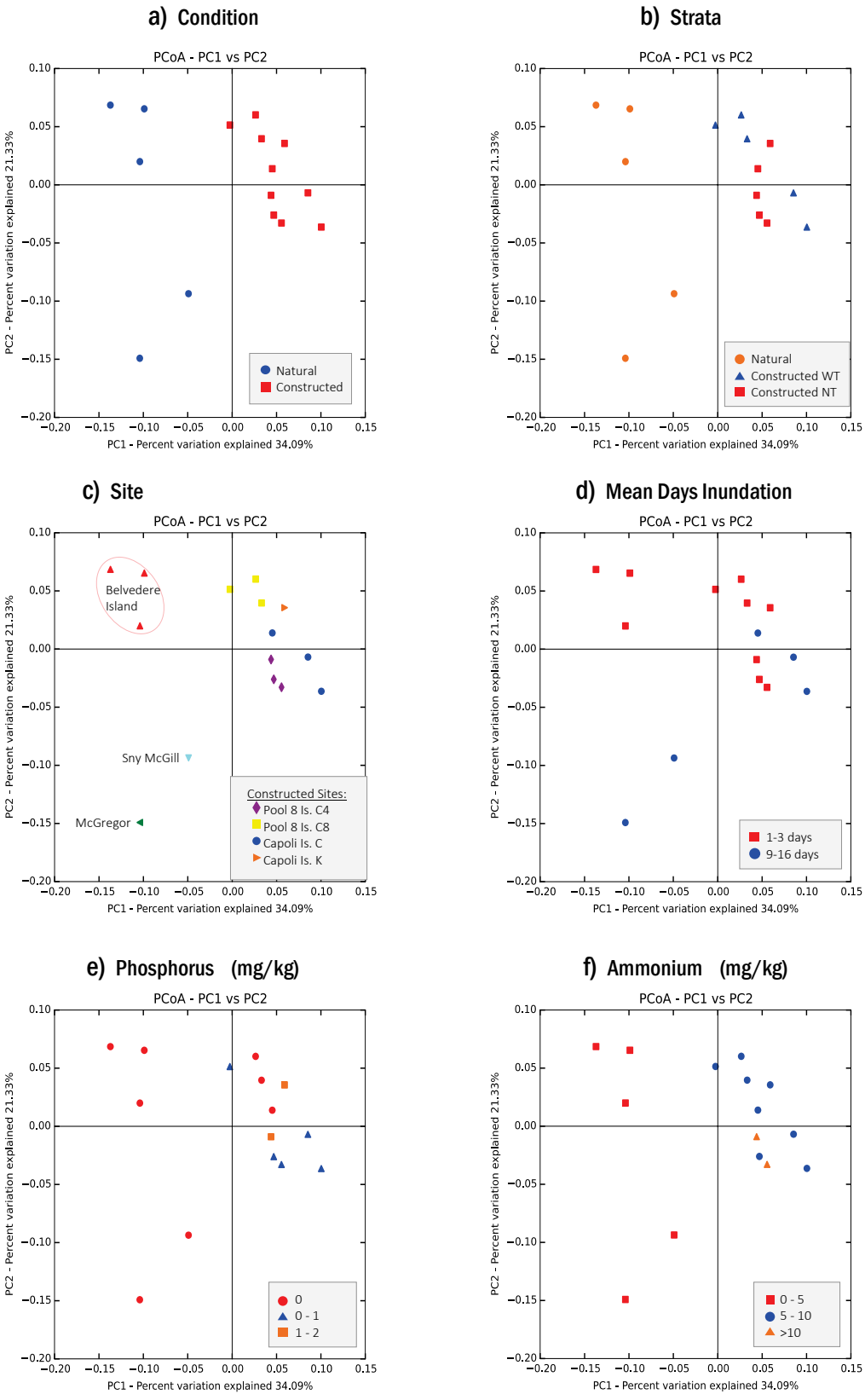


Figure 21. Bacterial alpha diversity per sample plot.



Principal coordinates analysis (PCoA) using pairwise distances between communities (Bray–Curtis and UniFrac distances) showed a clear separation between natural reference and constructed sites (condition, Figure 23a), but no separation between constructed sites with trees and constructed sites without trees (Figure 23b). There was also an apparent, but less significant separation between sites (Figure 23c). In all, PCoA plots were analyzed to study differences between the bacterial microbiome and 21 site variables, including many of the soil test results (nutrients, pH, enzyme activity, etc.) and site characteristics (mean days inundation, vegetation types present, etc.). All PCoA plots are provided in Appendix F. Many site characteristics did not show any apparent separation between sites (e.g., mean days inundation, Figure 23d). Other variables, such as phosphorus and ammonium which showed significant differences between sites, also showed a similar separation on the PCoA plots (Figure 23e and f). Based on our study data, it seems reasonable to conclude that the bacterial communities are most correlated with site condition (natural versus constructed), and the sites themselves, but not as closely to any specific site condition measured.

Figure 22. Bacterial principal coordinates analysis plots.



The 16S rRNA sequencing data was analyzed using PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) bioinformatics software package to infer potential differences in functional roles of the bacterial microbiomes at study sites (Langille et al. 2013). The resulting predicted metagenome functions were collapsed into Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways to compare sites. Gene counts of each KEGG pathway were averaged across sites and normalized to compare sites by condition and strata. Figure 24 displays the ratio of inferred KEGG pathways at natural sites compared to constructed sites, and between constructed sites with and without trees. The natural sites had higher gene counts for every pathway with the single exception of cell communication. Although the differences were consistent, they were relatively minimal and were therefore not further investigated or statistically analyzed. However, the differences are consistently greater between constructed and natural sites than between constructed sites with or without trees, again suggesting that the constructed sites in this study are more different from natural sites than they are from one another.

Variation is present between bacterial microbiomes - including some statistically significant differences - but no strong patterns or trends clearly differentiate the sites or strata. Further, no differences were identified between site condition or strata pointed to any known functional differences in the bacterial microbiomes or correlate strongly with measured site metrics. Taken together, this may suggest the restored sites sampled in this study have developed ecologically functioning bacterial microbiomes in the years following construction. Regardless, principal coordinates analysis did show a clear separation between natural sites and constructed sites. The data should be revisited as our knowledge of bacterial functions in soil increases and as data is expanded to include additional sites.

Figure 23. Ratio of possible KEGG pathways in natural and constructed sites, inferred using MiSeq 16S rRNA data in PICRUSt. *Bars* represent ratios between different sites. *Purple* is the ratio of natural to manmade sites while *green* is the ratio of manmade without trees to manmade with trees sites.

3.8 Soil fungal mycobiome

Fungal mycobiomes associated with samples collected at 14 of the 15 study plots were compared; three samples were collected at each plot. All three samples from the plot NSNYL01 and one sample each from plots NMCG01 and WTCAP02 failed during sequencing for unknown reasons. The 40

remaining total samples yielded 1.3 million sequences with a mean read count per sample of 32,000 ± 24,000.

Figure 25 presents averaged (n = 3, except for NMCG01 and WTCAP02 where n = 2), normalized fungal class relative abundance for each study plot. Between 12 and 17 fungal classes were represented above 1% relative abundance within each plot. A total of 19 different classes were observed across all plots at higher than 1% relative abundance. The fungal classes Agaricomycetes and Mortierellomycotina (*Incertae Sedis*) were strongly represented across all sites, accounting for a combined total of 12–72% of relative abundance at each averaged study plot. Pezizomycetes made up a large percentage of some sites – between 5–27% at natural sites and between 3–46% at constructed sites with trees. Alpha Diversity (OTUs) was variable among samples and strata, with no clear differences in diversity between natural, constructed with planted trees, or constructed islands without trees planted (Figure 26).

Figure 24. Normalized fungal relative abundance (>1.0%) at the class level of averaged soil samples at each study plot (n = 3, except for NMCG01 and WTCAP02 where n = 2).

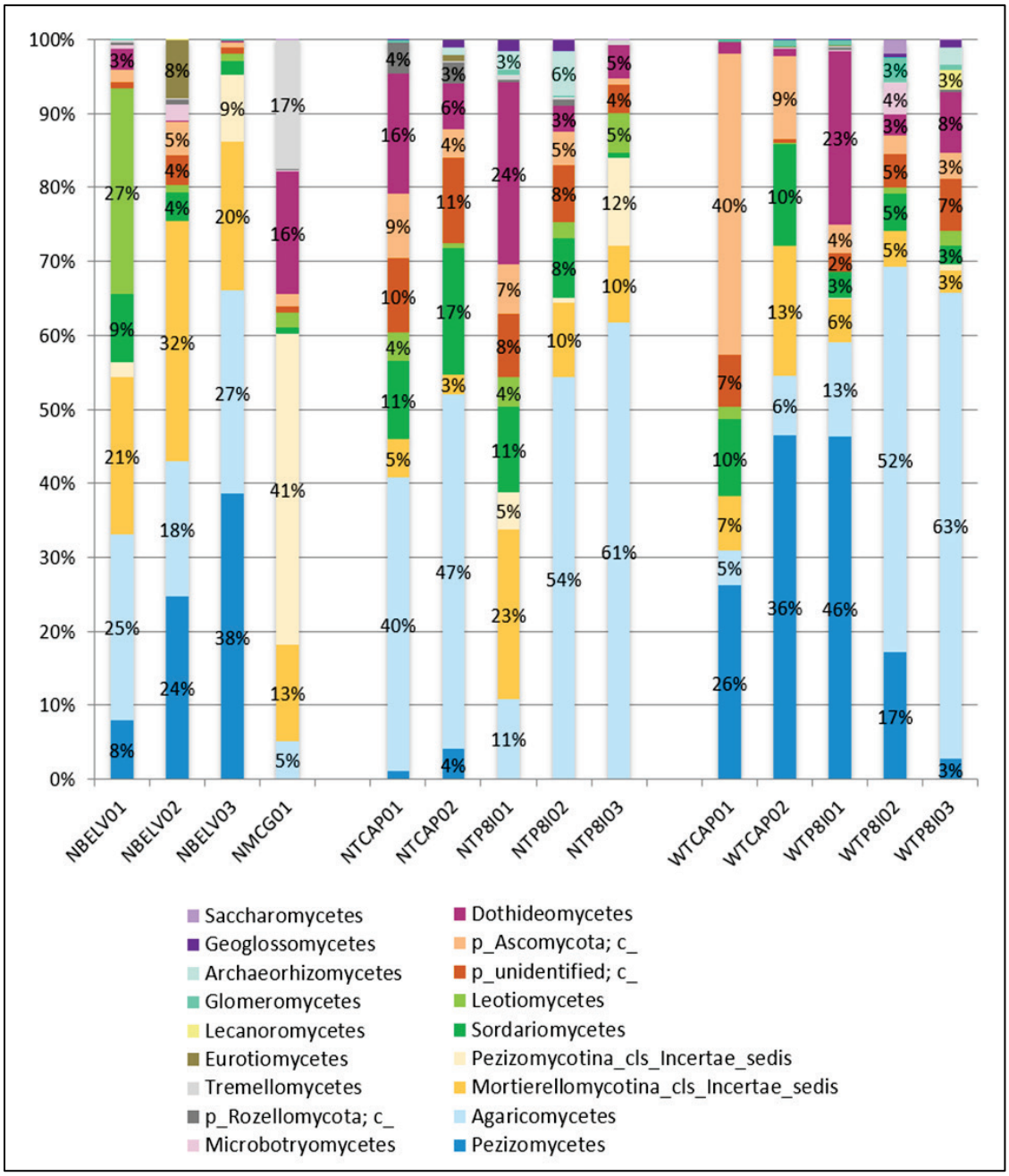
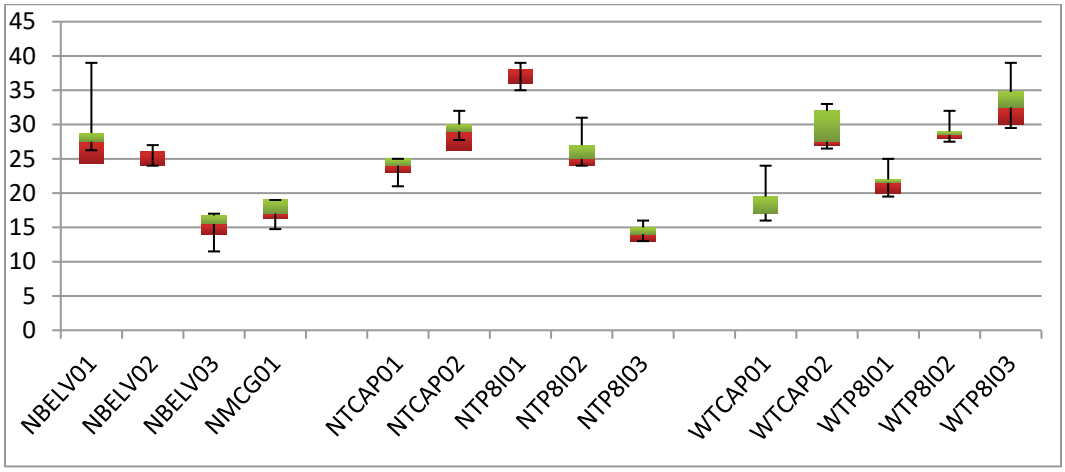


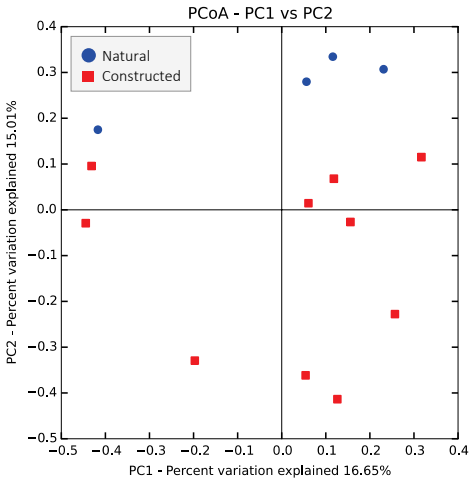
Figure 25. Fungal alpha diversity per sample plot.



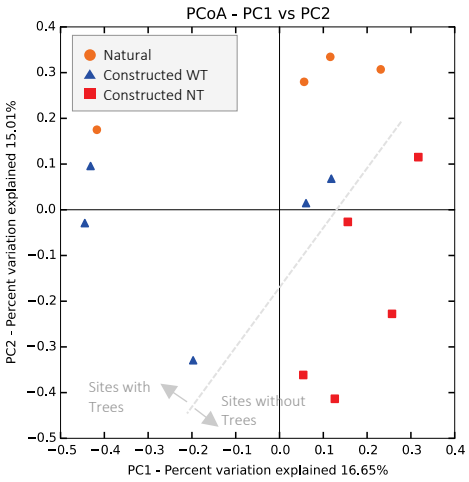
PCoA using pairwise distances between communities (Bray–Curtis and UniFrac distances) showed that samples were separated between natural and constructed conditions (Figure 27a). Within the constructed sites, sites with and without trees each clustered together, and those sites with trees were closer to the natural sites than constructed sites without trees (Strata, Figure 27b). Some sample sites tended to cluster together, such as Belvedere Island and Pool 8 Islands C4 (Figure 27c). In all, PCoA plots were analyzed to study differences between the fungal microbiome and 21 site variables, including many of the soil test results (nutrients, pH, enzyme activity, etc.) and site characteristics (mean days inundation, vegetation types present, etc.). All PCoA plots are provided in Appendix F. Many site characteristics did not reveal any distinct clustering or separation (e.g., mean days inundation and phosphorus; Figure 27d and e). Ammonium, which was significantly different between sites, also appeared separated on the PCoA plot (Figure 27f).

Figure 26. Fungal principal coordinates analysis plots.

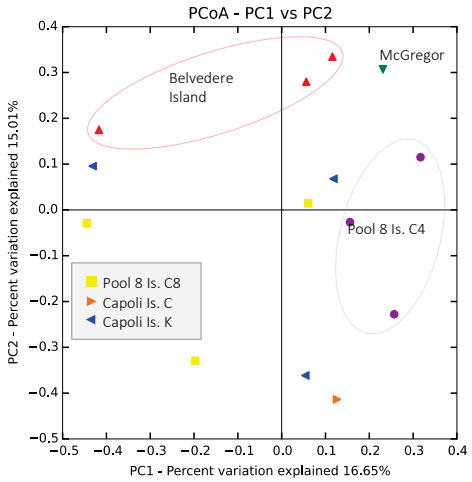
a) Condition



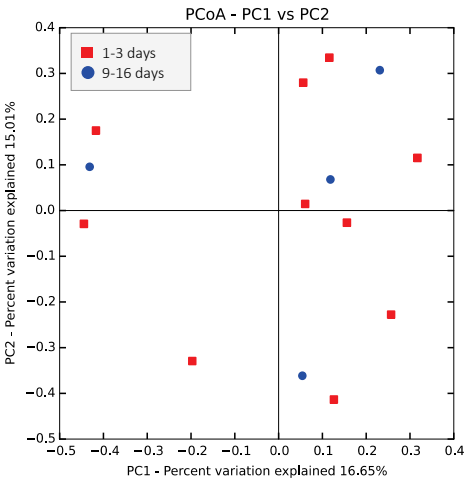
b) Strata



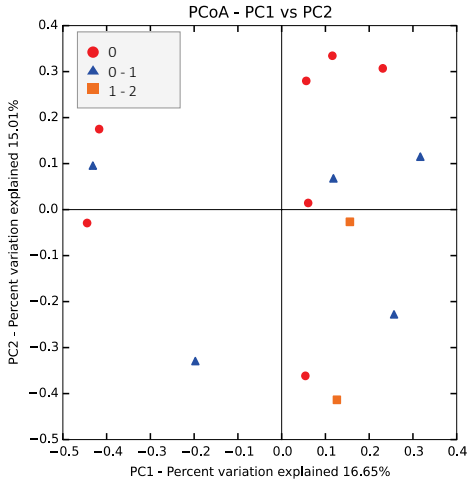
c) Site



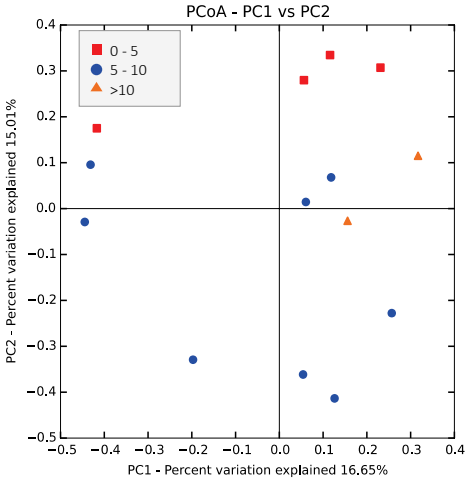
d) Mean Days Inundation



e) Phosphorus (mg/kg)



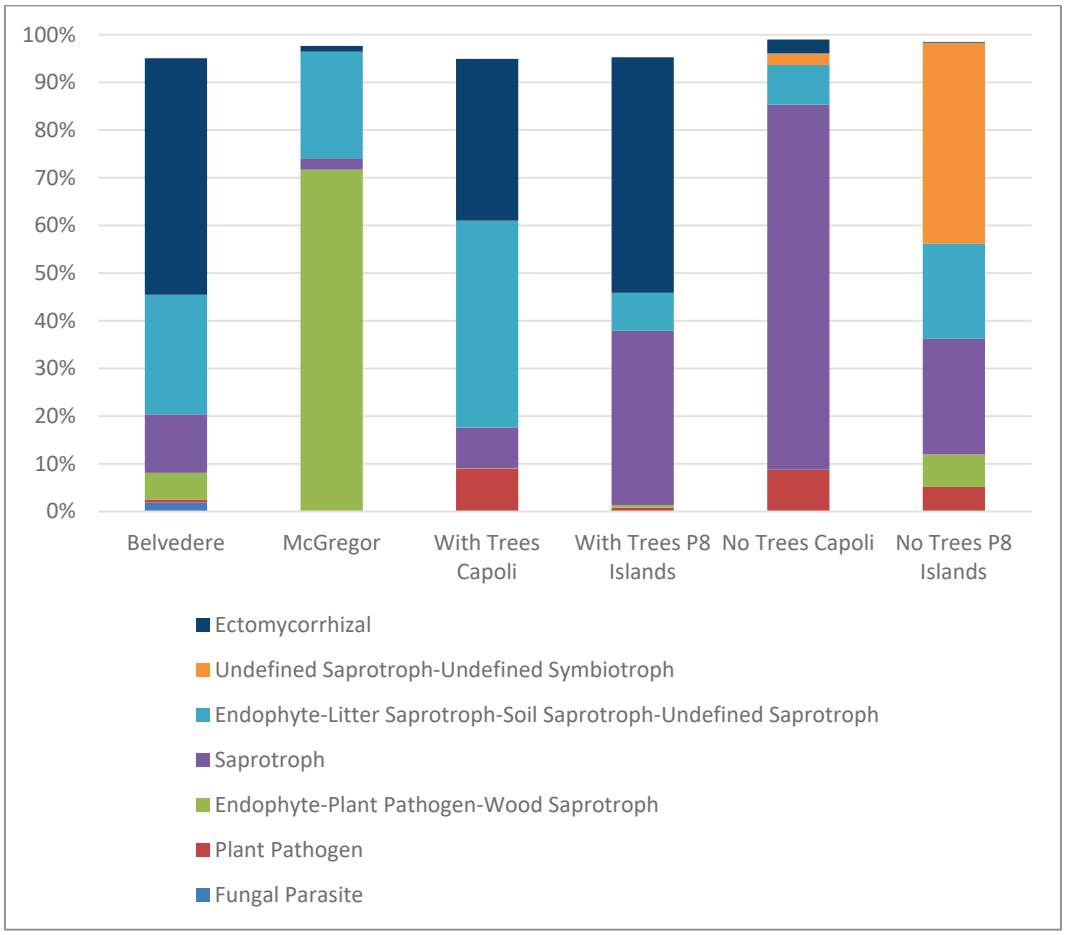
f) Ammonium (mg/kg)



The FUNGuild bioinformatics software identified ecological guilds for 56% of all OTUs at either "highly probable" or "probable" confidence levels. Natural sites had a higher rate of guild assignment at 77% of OTUs, whereas constructed sites averaged 47% of OTUs assigned to a fungal guild. Figure 28 displays the fungal guilds represented within each site strata. Seven guilds accounted for over 95% of all identified OTUs. Guilds were not evenly represented across sites. Of particular interest for this study, ectomycorrhizal fungi were more prevalent at the Belvedere Island natural site and the constructed sites with trees. Ectomycorrhizal fungi form symbiotic relationships with plants – primarily trees – wherein the fungi provide nutrients, minerals, and water collected from the soil in exchange for carbohydrates photosynthesized by the plant. Ectomycorrhizal fungi are estimated to be involved in up to 80% of plant *N* acquisition, up to 70% of plant phosphorus uptake, and provide many other important ecosystem services including enhancing seedling survival (van der Heijden 2015) and facilitating nutrient exchange and communication among trees (Simard 2018). This suggests that it is important to have a microbiome that includes ectomycorrhizal fungi at restoration sites where floodplain forests are the desired end state.

The results of the FUNGuild analysis indicate that ectomycorrhizal fungi are present at the restoration sites in areas where trees have successfully established. While this finding is promising, it is important to note these sites were selected to focus on areas with the best planted tree performance and are therefore biased towards the most likely locations for ectomycorrhizal fungi to be found. It is unclear how widespread they are throughout the island, the degree of correlation with tree planting success, or whether these fungi were introduced to the site via the roots of the planted trees or from natural colonization. Further research is required to validate these preliminary findings.

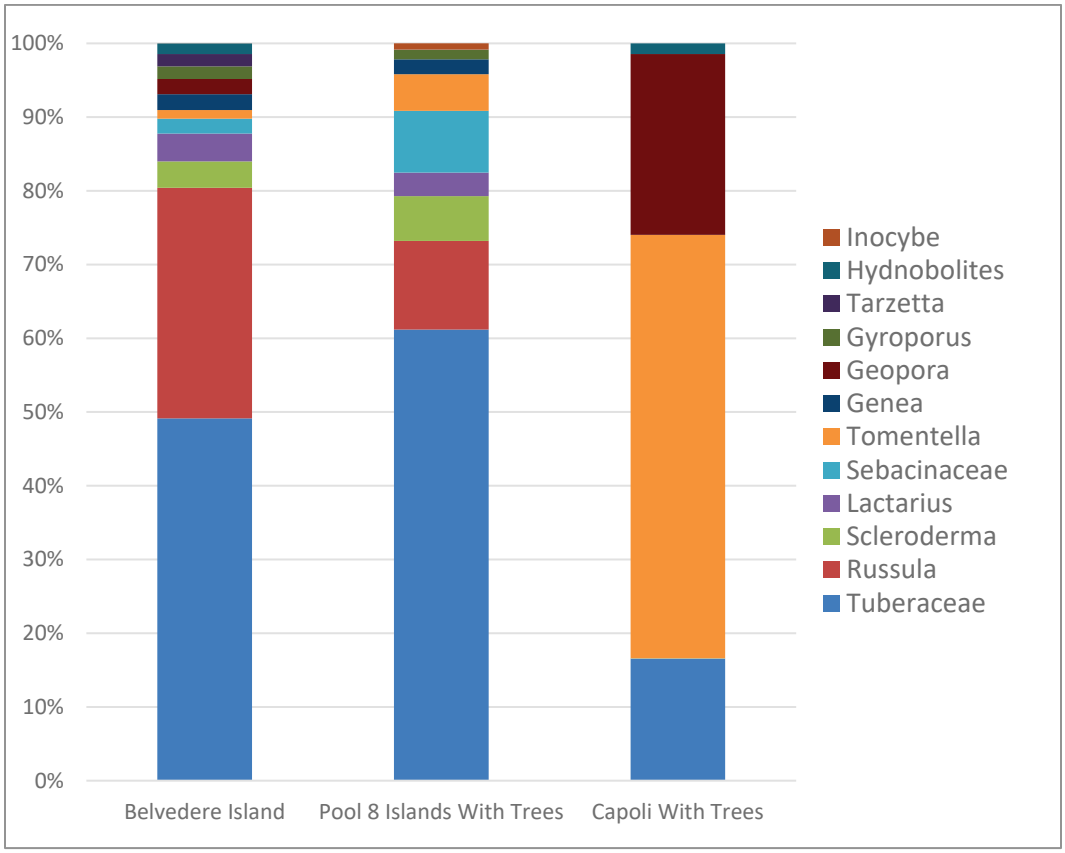
Figure 27. Normalized percentage of assigned fungal guild OTUs represented at each site. OTUs taxonomically parsed by ecological guild using the FUNGuild tool (Nguyen et al. 2016b) and limited to "highly probable" or "probable" confidence ranking of guild assignment. Unassigned OTUs excluded.



The genera or family of OTUs assigned to the ectomycorrhizal guild at each site (Figure 29) were also compared. Different species of ectomycorrhizal fungi fill many different functional niches within the ecosystem, and research suggests that increased complexity and co-occurrence of certain species can cause synergistic effects and improved ecosystem function beyond those ascribed to generalized guilds discussed here (Waag 2019). Diversity was highest at the natural Belvedere Island site which was dominated by species from the family *Tuberaceae* (~50%) and from the genera *Russula* (~30%) (Figure 29). The remaining 20% was evenly split among nine additional groups (Figure 29). The Pool 8 Islands site had a similar composition with slightly reduced diversity. The Capoli Islands site had a substantially reduced diversity and different composition, comprised of fungi of the genus *Tomentella* (57%), *Geopora*

(25%), and the family *Tuberaceae* (17%) (Figure 29). The two constructed restoration soils sampled are similar-aged sites (5–10 years old), and although ectomycorrhizal fungi had a similar relative abundance at Capoli, the sites clearly differ in diversity and composition. There is evidence that ectomycorrhizal fungi communities progress through successional trajectories along with forest stand development (e.g., Twieg et al. 2007). The differences between restoration sites could also be related to microbial communities brought to the site on the roots of the planted trees or could be a function of mycobiomes in the environments surrounding the sites. Additional sampling and analysis of natural reference sites and restoration sites of varying ages in the UMR could establish if and where mycobiome development fits into restoration trajectories and identify actions that may improve outcomes. Experimental plantings of tree species groupings or inoculation of sites with native microbiota could help determine if restoration success could be increased through manipulation of micro- or mycobiomes.

Figure 28. Proportion of ectomycorrhizal OTUs at each strata (fungal family or genus).



Ectomycorrhizal species were not prevalent at the McGregor natural site. This may be because the site was affected by flooding and beaver damage in recent years that had killed many of the trees at the site. The fungal guild predominant at the McGregor site was instead "Endophyte-Plant Pathogen-Wood Saproptroph," The most abundant OTU was a soft rot fungus in the genus *Chalara* (*Insertae sedis*). The Sny McGill natural site experienced similar recent flooding and would have been interesting for comparison, but the sequencing failed for all samples from that site for unknown reasons. Several studies suggest that the increasing frequency and magnitude of high-water events in the UMR could impact both the floodplain forest tree communities and their associated underground fungal microbiomes. Högberg et al. (2001) detected a loss of ectomycorrhizal fungi only two months after girdling trees in experimental plots but observed no decline in saprotrophic fungi. Sumorok et al. (2008) studied periodically inundated plant communities on the Pilica River floodplain in Poland and found that the diversity and abundance of ectomycorrhizal fungi increased as inundation frequency decreased. Piotrowski et al. (2008) studied a chrono sequence of riparian forests in Montana, USA, and found that the abundance of ectomycorrhizal fungi increased with abundance of host plants and that regular flooding could shift the dominance of fungal communities away from ectomycorrhizal fungi towards arbuscular mycorrhizal fungi.

3.9 Conclusions

The current study investigated the physical, chemical, and microbial characteristics of soils in floodplain islands constructed with dredged material in the UMR to improve floodplain island restoration outcomes. Soil conditions in natural, reference floodplain soils display some differences from created sites. These differences were primarily related to OM content, DOC, bulk density, and nutrients, which can likely be attributed to forest age, relative inputs of organic material, and differences in vegetation. Bacterial and fungal communities between natural and constructed sites were also different as observed through genomic sequencing but displayed some functional similarities within soil nutrient cycling as measured by enzyme activity. This may indicate that components of the microbial community are proceeding along a restoration trajectory that may eventually support habitat function.

3.9.1 Restoration trajectories and expected outcomes

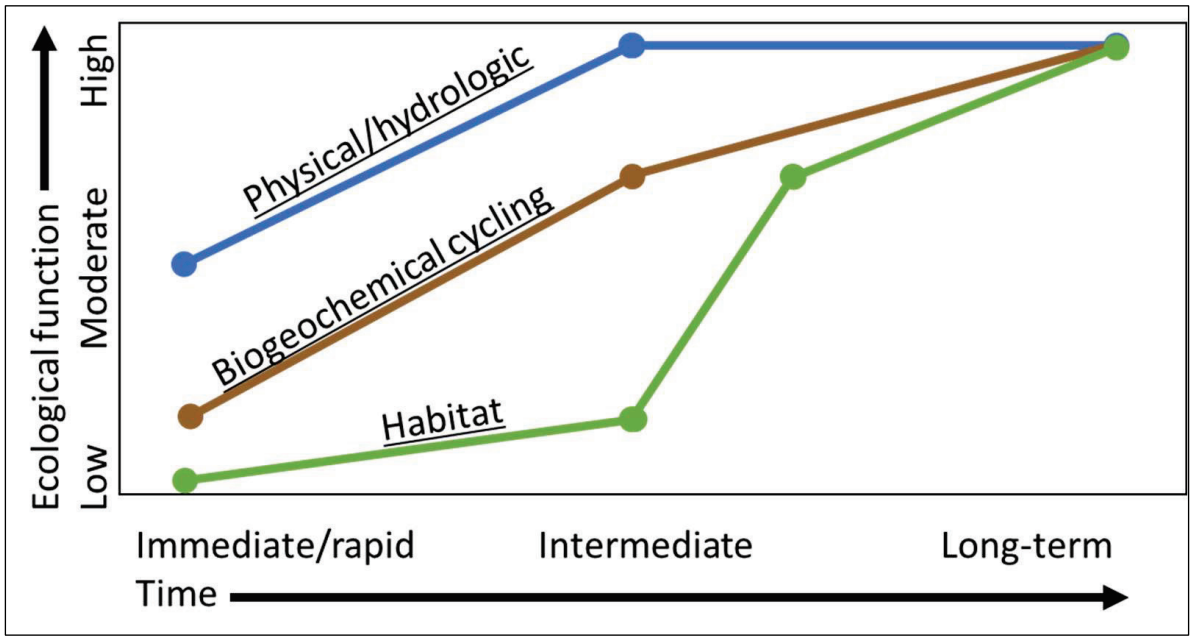
Results observed in this study align with previously established restoration trajectory theories where physical and hydrological functions are restored almost immediately, while more complex biologically mediated biogeochemical and habitat functions take additional time to achieve. For example, carbon storage is an important function of floodplain forests and natural sites had relatively higher *C*-related functions (as indicated by organic matter, dissolved organic *C*, and total *C*), likely due to site age, larger basal areas, and greater tree densities, which provide increased *C* via litter fall and root growth.

Other studies found that microbial communities were rapidly restored after restoration and are often an early indicator of restoration success (Hurst et al. 2016). Longer-term ecosystem processes, such as those related to soil biogeochemistry, require more time to develop as they are mediated by biological drivers. As would be expected by restoration trajectories, nutrient, OC, and OM concentrations differed the most between the created and natural sites in this study, indicating these soil properties require more time to reach the concentrations observed in natural sites. Since tree presence had the greatest correlation to bulk density and *C* cycling (OM content and DOC) amongst all sites, these soil properties in created sites may improve as the vegetative communities in those sites continue to develop (Shoch et al. 2009).

Studies in floodplain forests of the lower Mississippi River observed that significant time (15+ years) is required to achieve increases in tree basal area that provide subsequent increases in some soil properties (Berkowitz 2013; Berkowitz et al. 2018). In the lower Mississippi alluvial valley (LMAV), ground vegetation cover, shrub/sapling density, and O and A horizon thickness were most significantly related to floodplain forest restoration age (Berkowitz 2013). Horizon thickness changes took at least 11–12 years to develop, while tree density and basal area are likely to take 20+ years to display an increase in response to restoration (Smith and Klimas 2002; Berkowitz 2013). Berkowitz et al. (2021) developed a conceptual restoration trajectory diagram comparing ecological functions to their expected recovery time (Figure 30). In the diagram, physical and hydrologic properties are recovered almost immediately, followed by biogeochemical cycling, and eventually habitat properties (Figure 30). The proposed restoration trajectory of the UMRS sites studied here is similar, where (1) physical floodplain properties return rapidly, followed closely by

(2) basic microbial functional properties, which influences the restoration of (3) biogeochemical cycling, and then finally the increase in (4) habitat function recovery (Figure 30).

Figure 29. Conceptual restoration trajectory theorizing the recovery of ecological function based on time post-restoration (Source: Berkowitz et al. 2021).



3.9.2 Management implications and future directions

Future research should incorporate a variety of increasingly older constructed sites within the UMRS to document restoration responses over time and anticipate restoration trajectories via a chrono sequence. Soil properties in floodplain forests generally have a large spatial variability (especially those controlled by biological processes like the ones studied here) that often requires large scale and numerous sample sites to identify trends (Gallardo 2003). Using information provided from this study, along with similar studies along the Mississippi River, a few key variables could be tested to provide a rapid assessment on where constructed sites are located along a regionally developed restoration trajectory curve. Based on the current study, these variables could include ground cover vegetation, basal area, bulk density, organic matter, dissolved OC, and microbial community analysis – particularly fungal community analysis if the desired end state is floodplain forest habitat.

Detecting ectomycorrhizal fungi colocated with tree plantings at constructed sites is promising for the long-term outcome of UMR floodplain forest restoration. Ectomycorrhizal fungi were detected in locations where trees were successfully growing, and not in areas where tree plantings have failed. This study was not designed to determine if this is a cause or an effect of planting success, but these findings warrant further research. Studies of fungal communities at more UMR restoration projects of varying ages and stages of stand development would help USACE analyze and compare restoration site progression and may help identify specific conditions for successful, self-sustaining floodplain forest restoration. Future studies should attempt to determine whether planted tree success can be related to the ectomycorrhizal species and diversity present, and if or how this could be manipulated to increase the chance of success. There is increasing interest in the applications of ectomycorrhizal fungi in restoration (e.g., Policelli et al. 2020) and the results of this study suggest it may be worth considering fungal inoculation via native soil augmentation during construction or tree planting at sites.

Floodplain hydrology and hydroperiod within the UMRS is expected to undergo alteration with future climate change (Jha et al. 2004; Wu et al. 2012). Precipitation, soil temperatures, and nutrient loading in the UMRS are also expected to be influenced by a changing climate (Jha et al. 2015). Evaluating floodplain forest conditions under the current hydrologic regime will allow better understanding, tracking, and documenting future floodplain forest changes within the UMRS as hydrologic patterns change. Additionally, detailed and early (within the first ~ 10 years of restoration) assessment information can be used to document baseline ecological conditions.

Additional research will be necessary to (1) confirm whether these results are representative of the overall soil conditions in UMR natural and constructed sites, and (2) understand whether the soil differences or similarities will be more important in determining the future conditions of the constructed sites. Data gathered from this study can help document ecological responses on restoration sites and develop UMR specific restoration trajectory curves to better anticipate the outcomes of floodplain forest projects that beneficially utilize dredged material.

References

Berkowitz, Jacob F. 2013. "Development of Restoration Trajectory Metrics in Reforested Bottomland Hardwood Forests Applying a Rapid Assessment Approach." *Ecological Indicators* 34: 600–606. https://doi.org/10.1016/j.ecolind.2013.06.025.

Berkowitz, Jacob F., Darrell E. Evans, Kevin D. Philley, Jason P Pietroski, Casey Ehorn, and Nathan R. Beane. 2018. *Restoring Bottomland Hardwood Forests on U.S. Army Corps of Engineers Lands: 2016 Monitoring Report*. ERDC/EL TR-18-4. Vicksburg, MS: US Army Engineer Research Center. https://hdl.handle.net/11681/26652

Berkowitz, Jacob, Nathan Beane, Kevin Philley, Jake Jung, and Nia Hurst. 2021. *An Assessment of Long-Term, Multipurpose Ecosystem Functions and Engineering Benefits Derived from Historical Dredged Sediment Beneficial Use Projects*. ERDC/EL TR-21-4. Vicksburg, MS: US Army Engineer Research Center. https://hdl.handle.net/11681/41382

Caporaso, J. Gregory, Christian L. Lauber, William A. Walters, Donna Berg-Lyons, Catherine A. Lozupone, Peter J. Turnbaugh, Noah Fierer, and Rob Knight. 2011. "Global Patterns of 16S rRNA Diversity at a Depth of Millions of Sequences per Sample." *Proceedings of the National Academy of Sciences of the United States of America* 108, no. supplement_1: 4516–22. https://doi.org/10.1073/pnas.1000080107.

Caporaso, J. Gregory, Christian L. Lauber, William A. Walters, Donna Berg-Lyons, James Huntley, Noah Fierer, Sarah M. Owens, Jason Betley, Louise Fraser, Markus Bauer, Niall Gormley, Gilbert Jack, Geoff Smith, and Rob Knight. 2012. "Ultra-High-Throughput Microbial Community Analysis on the Illumina HiSeq and MiSeq Platforms." *The ISME Journal* 6, no. 8 (August 2012): 1621–24. https://doi.org/10.1038/ismej.2012.8.

De Jager, Nathan R., Meredith Thomsen, and Yao Yin. 2012. "Threshold Effects of Flood Duration on the Vegetation and Soils of the Upper Mississippi River Floodplain, USA." *Forest Ecology and Management* 270: 135–46. https://doi.org/10.1016/j.foreco.2012.01.023.

Dick, Warren A. 2015. "Kinetics of Soil Enzyme Reactions." In *SSSA Book Series*, 57–69. Madison, WI, USA: American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America. https://doi.org/10.2136/sssabookser9.c3.

Gallardo, Antonio. 2003. Spatial Variability of Soil Properties in a Floodplain Forest in Northwest Spain. *Ecosystems* 6(6): 564–76. http://www.jstor.org/stable/3658998.

Hurst, Nia, John R. White, and Joseph Baustian. 2016. "Nitrate Reduction in a Hydrologically Restored Bottomland Hardwood Forest in the Mississippi River Watershed, Northern Louisiana." *Soil Science Society of America Journal. Soil Science Society of America* 80, no. 6: 1698–1705. https://doi.org/10.2136/sssaj2016.08.0250.

Högberg, P., A. Nordgren, N. Buchmann, A. F. Taylor, A. Ekblad, M. N. Högberg, G. Nyberg, M. Ottosson-Löfvenius, and D. J. Read. 2001. "Large-Scale Forest Girdling Shows That Current Photosynthesis Drives Soil Respiration." *Nature* 411, no. 6839 (14 June 2001): 789–92. https://doi.org/10.1038/35081058.

Ingvalson, Derek S., Marcella A. Windmuller-Campione, and Andrew R. Meier. 2020. "Identification of Annual Flood Durations Associated with Tree Species in the Upper Mississippi River System Floodplain, with Applications to Forest Restoration Staff Paper Series No. 258 Department of Forest Resources." University of Minnesota Twin Cities. https://doi.org/10.13140/RG.2.2.24792.32007.

Jha, Manoj K., Philip W. Gassman, and Yiannis Panagopoulos. 2015. "Regional Changes in Nitrate Loadings in the Upper Mississippi River Basin under Predicted Mid-Century Climate." *Regional Environmental Change* 15(3): 449–60. https://doi.org/10.1007/s10113-013-0539-y.

Jha, Manoj, Zaitao Pan, Eugene S. Takle, and Roy Gu. 2004. "Impacts of climate change on streamflow in the Upper Mississippi River Basin: A regional climate model perspective." *J. Geophys. Res.* 109, D09105, https://doi.org/:10.1029/2003JD003686.

Kreiling, Rebecca M., Nathan R. De Jager, Whitney Swanson, Eric A. Strauss, and Meredith Thomsen. 2015. "Effects of Flooding on Ion Exchange Rates in an Upper Mississippi River Floodplain Forest Impacted by Herbivory, Invasion, and Restoration." *Wetlands (Wilmington, N.C.)* 35(5): 1005–12. https://doi.org/10.1007/s13157-015-0675-x.

Langille, Morgan G. I., Jesse Zaneveld, J. Gregory Caporaso, Daniel McDonald, Dan Knights, Joshua A. Reyes, Jose C. Clemente, Deron Burkepile, Rebecca Vega Thurber, Rob Knight, Robert Beiko, and Curtis Huttenhower. 2013. "Predictive Functional Profiling of Microbial Communities Using 16S rRNA Marker Gene Sequences." *Nature Biotechnology* 31(9): 814–21. https://doi.org/10.1038/nbt.2676.

Matthews, A., S. Pierce, H. Hipperson, and B. Raymond. 2019. "Rhizobacterial Community Assembly Patterns Vary between Crop Speices." *Frontiers in Microbiology* 10: 1–13.

Meier, Andrew. 2016. MVP-ES Forest Inventory Phase II Protocol, Version 1.2. US Army Corps of Engineers, St. Paul District. https://ecos.fws.gov/ServCat/DownloadFile/157228?Reference=105804

Nguyen, Nhu H., Laura J. Williams, John B. Vincent, Artur Stefanski, Jeannine Cavender-Bares, Christian Messier, Alain Paquette, Dominique Gravel, Peter B. Reich, and Peter G. Kennedy. 2016a. "Ectomycorrhizal Fungal Diversity and Saprotrophic Fungal Diversity Are Linked to Different Tree Community Attributes in a Field-Based Tree Experiment." *Molecular Ecology* 25(16): 4032–46. https://doi.org/10.1111/mec.13719.

Nhu, Nguyen, Zewei Song, Scott T. Bates, Sara Branco, Leho Tedersoo, Jon Menke, Jonathan S. Schilling, and Peter Kennedy. 2016b. "FUNGuild: An Open Annotation Tool for Parsing Fungal Community Datasets by Ecological Guild." *Fungal Ecology* 20: 241–48.

Piotrowski, Jeff S., Ylva Lekberg, Mary J. Harner, Philip W. Ramsey, and Matthias C. Rillig. 2008. "Dynamics of Mycorrhizae during Development of Riparian Forests along an Unregulated River." *Ecography* 31(2): 245–53. https://doi.org/10.1111/j.0906-7590.2008.5262.x.

Policelli, Nahuel, Thomas R. Horton, Aimée T. Hudon, Taylor R. Patterson, and Jennifer M. Bhatnagar. 2020. "Back to Roots: The Role of Ectomycorrhizal Fungi in Boreal and Temperate Forest Restoration." *Frontiers in Forests and Global Change* 3. https://doi.org/10.3389/ffgc.2020.00097.

Reddy, K. R., M. W. Clark, R. D. DeLaune, and M. Kongchum. 2013. "Physicochemical Characterization of Wetland Soils." In *Methods in Biogeochemistry of Wetlands*, 41–54. Madison, WI, USA: American Society of Agronomy and Soil Science Society of America. https://doi.org/10.2136/sssabookser10.c3.

Remke, Michael J., Tonny Hoang, Thomas Kolb, Catherine Gehring, Nancy C. Johnson, and Matthew A. Bowker. 2020. "Familiar Soil Conditions Help *Pinus Ponderosa* Seedlings Cope with Warming and Drying Climate." *Restoration Ecology* 28(S4). https://doi.org/10.1111/rec.13144.

Romano, Susan P. 2010. "Our Current Understanding of the Upper Mississippi River System Floodplain Forest." *Hydrobiologia* 640(1): 115–24. https://doi.org/10.1007/s10750-009-0063-8.

Rousk, Johannes, Philip C. Brookes, and Erland Bååth. 2009. "Contrasting Soil pH Effects on Fungal and Bacterial Growth Suggest Functional Redundancy in Carbon Mineralization." *Applied and Environmental Microbiology* 75(6): 1589–96. https://doi.org/10.1128/aem.02775-08.

Simard, Suzanne W. 2018. "Mycorrhizal Networks Facilitate Tree Communication, Learning, and Memory." In *Memory and Learning in Plants*, 191–213. Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-75596-0_10.

Smith, Ronald, and Charles Klimas. 2002. A regional guidebook for applying the hydrogeomorphic approach to assessing wetland functions of selected regional wetland subclasses, Yazoo Basin, Lower Mississippi River Alluvial Valley. ERDC/EL TR-02-4. Vicksburg, MS: US Army Engineer Research and Development Center.

Smith, Sally, and David Read. 2008. *Mycorhizal Symbiosis*. Mycorrhizal Symbiosis. https://doi.org/10.1016/B978-012652840-4/50001-2.

Shoch, David T., Gary Kaster, Aaron Hohl, and Ray Souter. 2009. "Carbon Storage of Bottomland Hardwood Afforestation in the Lower Mississippi Valley, USA." *Wetlands (Wilmington, N.C.)* 29(2): 535–42. https://doi.org/10.1672/08-110.1.

Sumorok, Beata, Krzysztof Kosiński, Dorota Michalska-Hejduk, and Edyta Kiedrzyńska. 2008. "Distribution of Ectomycorrhizal Fungi in Periodically Inundated Plant Communities on the Pilica River Floodplain." *International Journal of Ecohydrology & Hydrobiology* 8(2–4): 401–10. https://doi.org/10.2478/v10104-009-0032-x.

Tedersoo, Leho, and Mark C. Brundrett. 2017. "Evolution of Ectomycorrhizal Symbiosis in Plants." In *Biogeography of Mycorrhizal Symbiosis*, 407–67. Ecological Studies: Analysis and Synthesis. Berlin, Heidelberg, New York NY. Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-56363-3_19.

Theiling, Charles H., Jeffrey A. Janvrin, and Jon Hendrickson. 2015. "Upper Mississippi River Restoration: Implementation, Monitoring, and Learning since 1986." *Restoration Ecology* 23(2): 157–66. https://doi.org/10.1111/rec.12170.

Thompson, Luke R., Jon G. Sanders, Daniel McDonald, Amnon Amir, Joshua Ladau, Kenneth J. Locey, Robert J. Prill, Anupriya Tripathi, Sean Gibbons, Gail Ackermann, Jose Navas-Molina, Stefan Janssen, Eyguenia Kopylova, Yoshiki Vázquez-Baeza, Antonio González, James Morton, Sjavash Mirarab, Zhenjiang Xu, Lingjing Jiang, and Hongxia Zhao. 2017. "A Communal Catalogue Reveals Earth's Multiscale Microbial Diversity." *Nature* 551(7681): 457–63. https://doi.org/10.1038/nature24621.

Twieg, Brendan D., Daniel M. Durall, and Suzanne W. Simard. 2007. "Ectomycorrhizal Fungal Succession in Mixed Temperate Forests." *The New Phytologist* 176(2): 437–47. https://doi.org/10.1111/j.1469-8137.2007.02173.x.

US Army Corps of Engineers. 2000. *Upper Mississippi River and Illinois Waterway Cumulative Effects Study, Volume 1: Geomorphic Assessment*. Prepared by WEST Consultants, Inc. for U.S. Army Engineer District, Rock Island, Illinois.

US Army Corps of Engineers. 2012. *Upper Mississippi River Restoration Environmental Management Program Environmental Design Handbook*.

USEPA. 1993a. "Determination Of Ammonia Nitrogen By Semi-Automated Colorimetry." Revison 2.0. *Usepa Environ. Monitor. Syst. Lab.*, Cincinnati, OH.

USEPA. 1993b. "Determination Of Nitrate-Nitrite Nitrogen By Automated Colorimetry." Revison 2.0. *Usepa Environ. Monitor. Syst. Lab.*, Cincinnati, OH.

USEPA. 1993c. "Determination Of Phosphorus By Semi-Automated Colorimetry." Revison 2.0. *Usepa Environ. Monitor. Syst. Lab.*, Cincinnati, OH.

USDA Natural Resources Conservation Service. 2008. *Soil Quality Indicator Sheets: Bulk Density*. https://www.nrcs.usda.gov/wps/PA_NRCSConsumption/download?cid=nrcs142p2_051591&ext=pdf

USDA Natural Resources Conservation Service. 2011. *Soil Quality Indicator Sheets: Soil pH*. https://www.nrcs.usda.gov/wps/PA_NRCSConsumption/download?cid=nrcs142p2_052474&ext=pdf

USDA, NRCS. 2020. The PLANTS Database (http://plants.usda.gov). National Plant Data Team, Greensboro, NC USA.

Vance, E. D., P. C. Brookes, and D. S. Jenkinson. 1987. "An Extraction Method for Measuring Soil Microbial Biomass C." *Soil Biology & Biochemistry* 19(6): 703–7. https://doi.org/10.1016/0038-0717(87)90052-6.

Van Appledorn, Molly, Nathan R. DeJager, and Jason Rohweder. 2018. "UMRS Floodplain Inundation Attributes." US Geological Survey. https://doi.org/10.5066/F7VD6XRT.

Van der Heijden, Marcel, R.D. Bardgett, and Nico M. van Straalen. 2008. "The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems." *Ecology Letters* 11(3): (296-310). https://doi.org/10.1111/j.1461-0248.2008.01199.x.

Heijden, Marcel G. A., Francis M. Martin, Marc-André Selosse, and Ian R. Sanders. 2015. "Mycorrhizal Ecology and Evolution: The Past, the Present, and the Future." *The New Phytologist* 205(4): 1406–23. https://doi.org/10.1111/nph.13288.

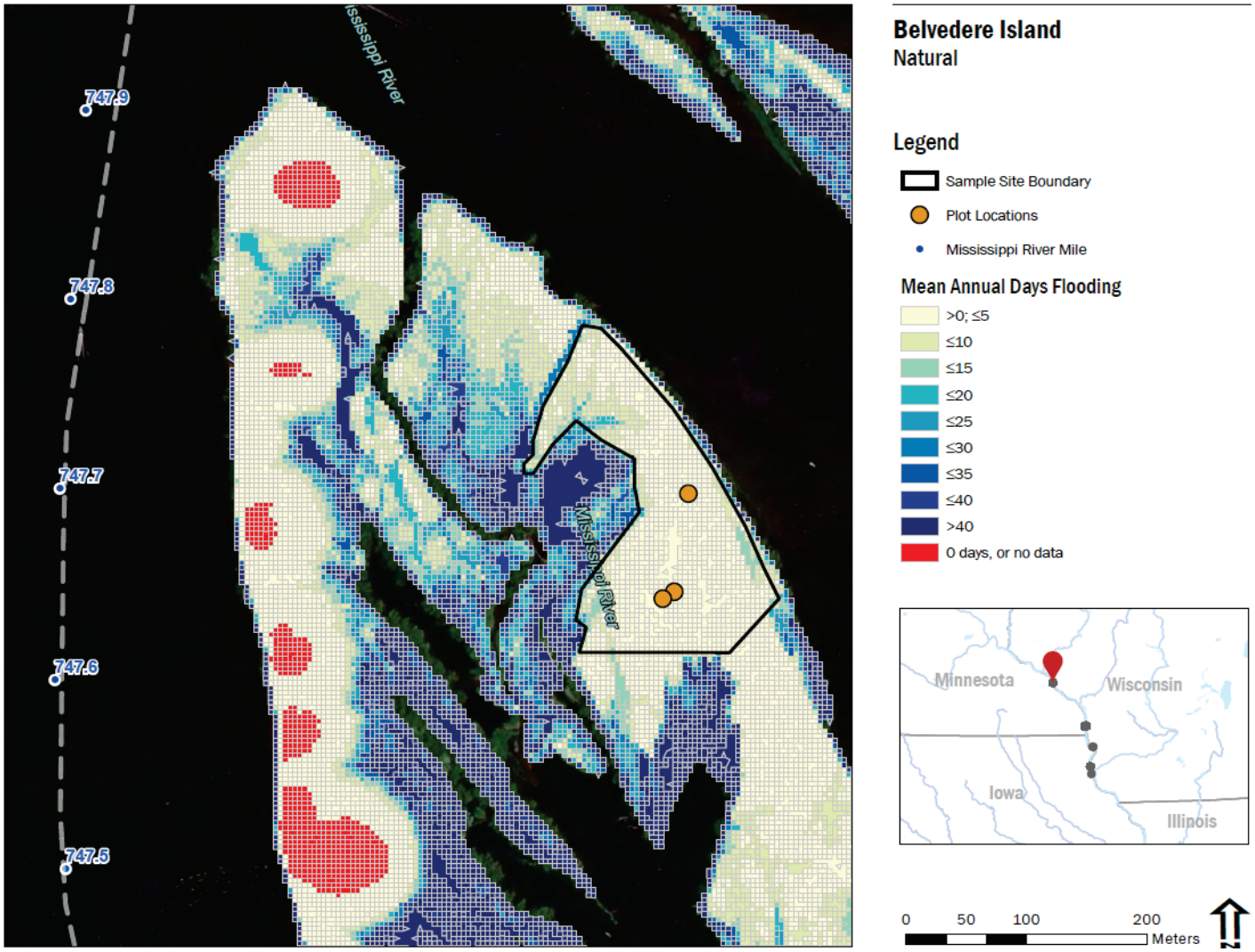
Wagg, Cameron, Klaus Schlaeppi, Samiran Banerjee, Eiko E. Kuramae, and Marcel G. A. van der Heijden. 2019. "Fungal-Bacterial Diversity and Microbiome Complexity Predict Ecosystem Functioning." *Nature Communications* 10(1). https://doi.org/10.1038/s41467-019-12798-y.

White, J. R., and K. R. Reddy. 2000. "Influence of Phosphorus Loading on Organic Nitrogen Mineralization of Everglades Soils." *Soil Science Society of America Journal. Soil Science Society of America* 64(4): 1525–34. https://doi.org/10.2136/sssaj2000.6441525x.

Wu, Yiping, Shuguang Liu, and Omar I. Abdul-Aziz. 2012. "Hydrological Effects of the Increased CO2 and Climate Change in the Upper Mississippi River Basin Using a Modified SWAT." *Climatic Change* 110(3–4): 977–1003. https://doi.org/10.1007/s10584-011-0087-8.

Yin, Yao, Yegang Wu, Steve M. Bartell, and Robert Cosgriff. 2009. "Patterns of Forest Succession and Impacts of Flood in the Upper Mississippi River Floodplain Ecosystem." *Ecological Complexity* 6(4): 463–72. https://doi.org/10.1016/j.ecocom.2009.08.004.

Appendix A: Study Site Maps


747.9
747.8
747.7
747.6
747.5
Mississippi River
Mississippi River
Belvedere Island
Natural
Legend
Sample Site Boundary
Plot Locations
Mississippi River Mile
Mean Annual Days Flooding
>0; ≤5
≤10
≤15
≤20
≤25
≤30
≤35
≤40
>40
0 days, or no data
Minnesota
Wisconsin
Iowa
Illinois
0
50
100
200
Meters

Natural – Belvedere Island

N/BELV/01:

Standing at 240° plot edge post; Facing 60°

Standing at 180° plot edge post; Facing 0°

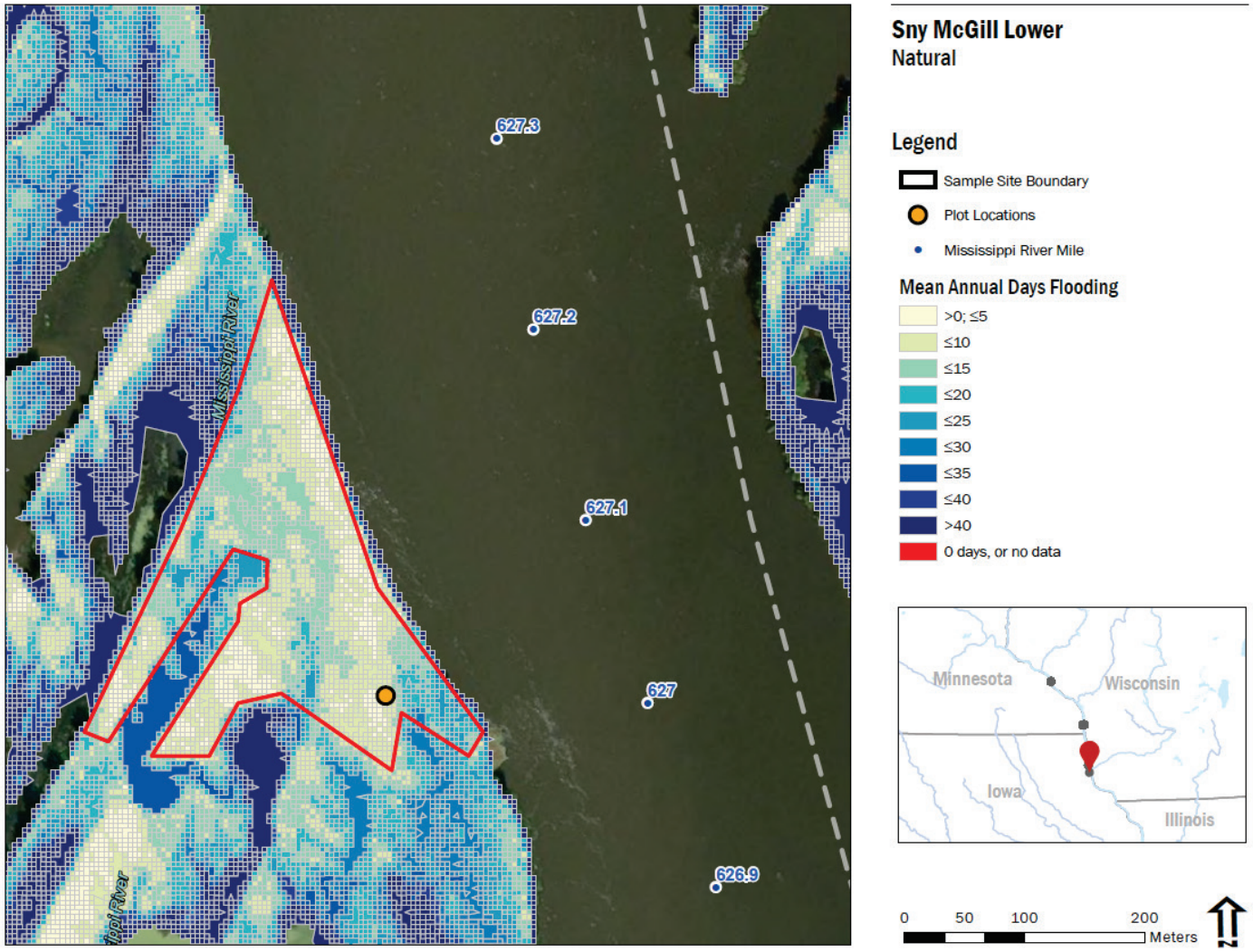
Standing at 120° plot edge post; Facing 300°

Natural – Belvedere Island

N/BELV/02: Standing at 240° plot edge post; Facing 60°

Natural – Belvedere Island

N/BELV/03: Standing at 180° plot edge post; Facing 0°



Sny McGill Lower
Natural

Legend

- Sample Site Boundary
- Plot Locations
- Mississippi River Mile

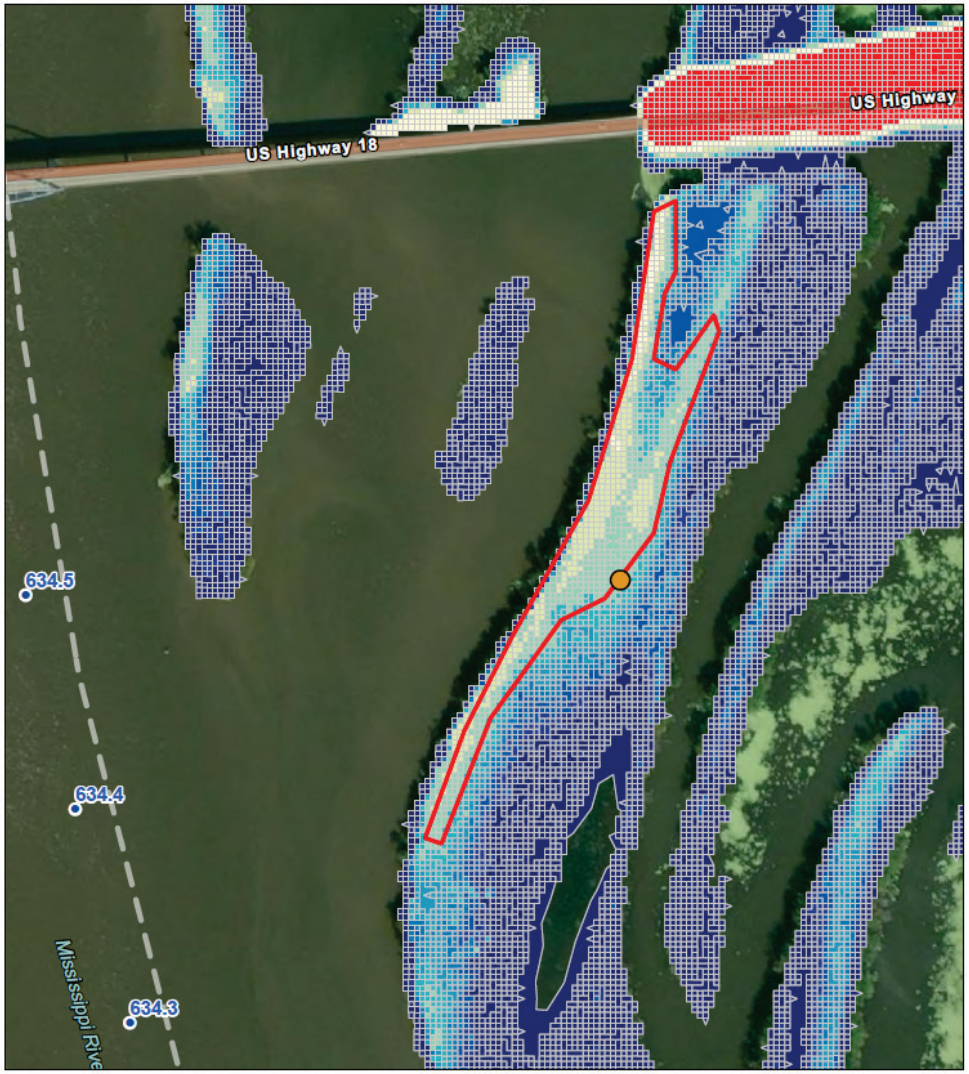
Mean Annual Days Flooding

- >0; ≤5
- ≤10
- ≤15
- ≤20
- ≤25
- ≤30
- ≤35
- ≤40
- >40
- 0 days, or no data

0 50 100 200 Meters

Natural – Sny McGill Lower

N/SNYL/01: Standing at plot center; Facing 120°



McGregor
Natural

Legend

- Sample Site Boundary
- Plot Locations
- Mississippi River Mile

Mean Annual Days Flooding

- >0; ≤5
- ≤10
- ≤15
- ≤20
- ≤25
- ≤30
- ≤35
- ≤40
- >40
- 0 days, or no data

0 50 100 200 Meters

Natural – McGregor

N/MCG/01: Standing at 0° plot edge post; Facing 180°

Pool 8 Islands - No Trees
Manmade

Legend

Sample Site Boundary

Topsoil depth // elevation

Plot Locations

Mississippi River Mile

Island C4 was constructed during Phase III of the Pool 8 Islands Project in 2012. Topsoil Depth and elevation are show from construction documentation.

No Trees – Pool 8 Islands (Island "C4")

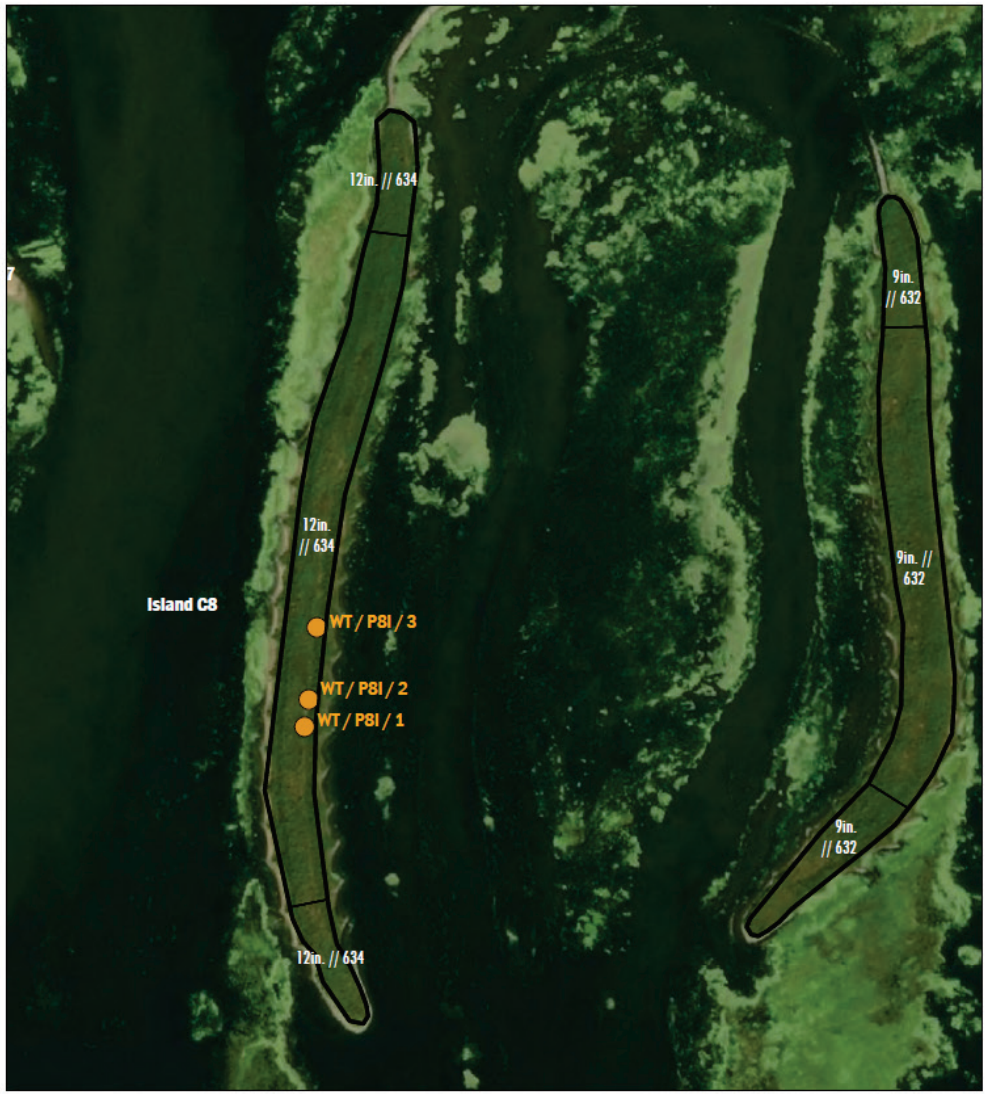
NT/P8I/01: Standing at 180° plot edge post; Facing 0°

No Trees – Pool 8 Islands (Island "C4")

NT/P8I/02: Standing at 180° plot edge post; Facing 0°

No Trees – Pool 8 Islands (Island "C4")

NT/P8I/03: Standing at 180° plot edge post; Facing 0°



Pool 8 Islands - With Trees
Manmade

Legend

- Sample Site Boundary
  - Topsoil depth // elevation
- Plot Locations
- Mississippi River Mile

Island C8 was constructed during Phase III of the Pool 8 Islands Project in 2012. Topsoil Depth and elevation are show from construction documentation.

With Trees – Pool 8 Islands (Island "C8")

WT/P8I/01: Standing at 180° plot edge post; Facing 0°

With Trees – Pool 8 Islands (Island "C8")

WT/P8I/02: Standing at 180° plot edge post; Facing 0°

With Trees – Pool 8 Islands (Island "C8")

WT/P8I/03: Standing at 180° plot edge post; Facing 0°

Capoli Islands
Manmade

Legend

Sample Site Boundary
Topsoil depth // elevation

Plot Locations

Island C was constructed during Phase II of the project, and was planted in 2016.

Island K was constructed during Phase I of the project, which was completed in 2013.

Topsoil depths and elevations were taken from construction documents.

0 50 100 200
Meters

With Trees – Capoli Islands (Island "C")

WT/CAP/01: Standing at Plot Center; Facing 180°

With Trees – Capoli Islands (Island "C")

WT/CAP/02: Standing at 180° plot edge post; Facing 0°

No Trees – Capoli Islands (Island "C")

NT/CAP/01: Standing at 0° plot edge post; Facing 180°

No Trees – Capoli Islands (Island "K")

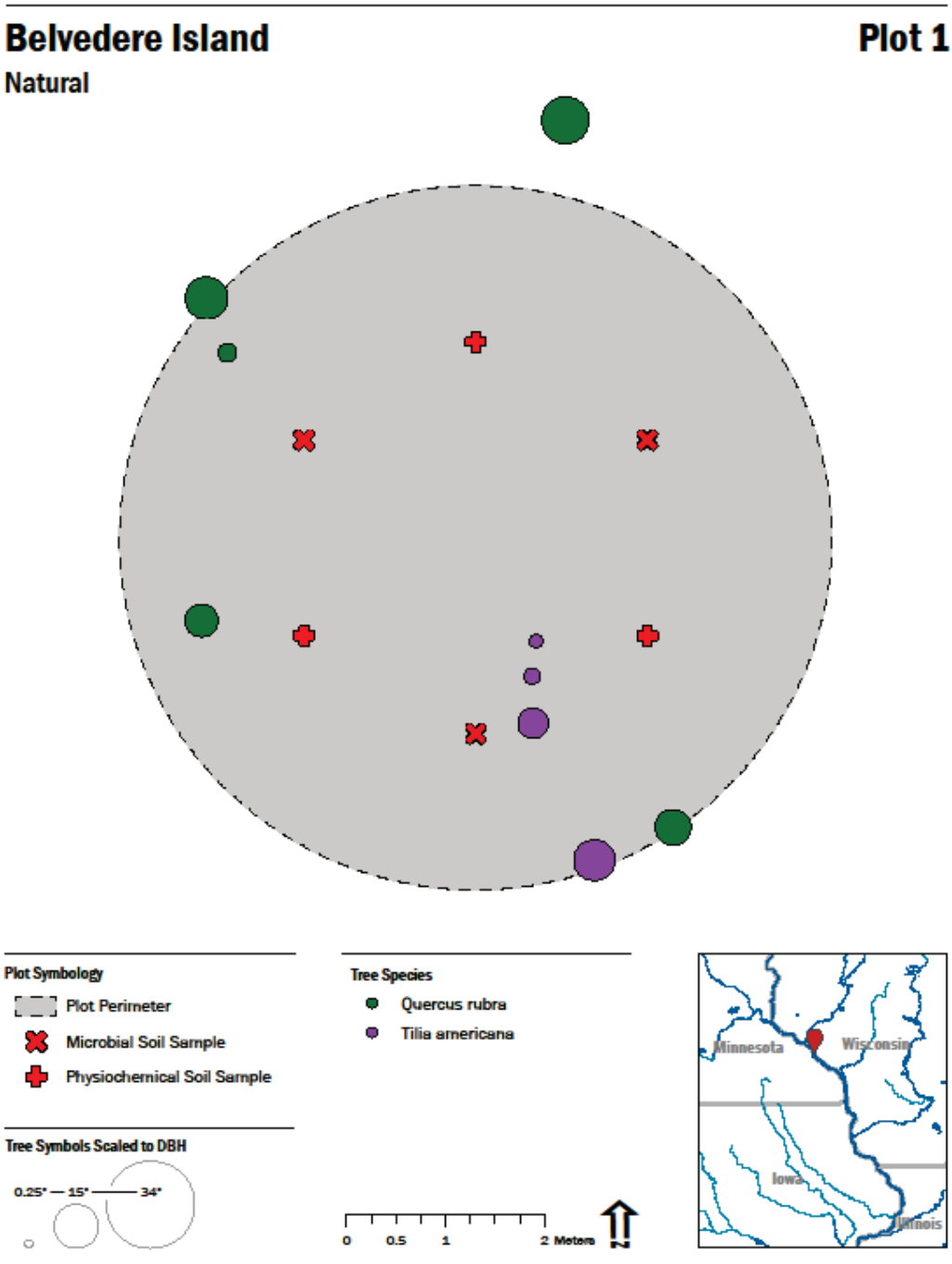
NT/CAP/02: Standing at 300° plot edge post; Facing 120°

Appendix B: List of Plant Species Observed at All Study Sites

							Presence at Study Sites							
Common Name	Scientific Name	Symbol	Growth Habit	Duration	Wetland Status	Native Status	Belvedere	McGregor	Sny McGill	P8 Island C4	P8 Island C8	Capoli Is. C	Capoli Is. K	
Alpine enchanter's nightshade	*Circaea alpina*	CIAL	Forb	Perennial	FACW	Native	x							
American elm	*Ulmus americana*	ULAM	Tree	Perennial	FACW	Native	x	x			x			
American sycamore	*Platanus occidentalis*	PLOC	Tree	Perennial	FACW	Native						x		
Aster spp.			Forb									x		
Awl aster	*Symphyotrichum pilosum*	SYPIP3	Forb	Perennial	None	Native					x			
Basswood	*Tilia americana*	TIAM	Tree	Perennial	FACU	Native	x							
Big Bluestem	*Andropogon gerardii*	ANGE	Graminoid	Perennial	FAC	Native					x			
Bitternut hickory	*Carya cordiformis*	CACO15	Tree	Perennial	FACU	Native	x							
Black eyed susan	*Rudbeckia hirta*	RUHI2	Forb	Annual/Biennial/Perennial	FACU	Native				x	x			
Blue verbena	*Verbena hastata*	VEHA2	Forb	Biennial/Perennial	FACW	Native				x		x		
Box elder	*Acer negundo*	ACNE2	Tree	Perennial	FAC	Native	x							
Bristly greenbrier	*Smilax tamnoides*	SMTA2	Vine	Perennial	FAC	Native	x							
Bull thistle	*Cirsium vulgare*	CIVU	Forb	Biennial	FACU	Introduced						x		
Bur oak	*Quercus macrocarpa*	QUMA2	Tree	Perennial	FAC	Native					x	x		
Canada clearweed	*Pilea spp.*	PILEA	Forb	Annual	FACW	Native		x						
Chokecherry	*Prunus virginiana*	PRVI	Tree	Perennial	FACU	Native	x							
Common hackberry	*Celtis occidentalis*	CEOC	Tree	Perennial	FAC	Native	x							
Cottonwood	*Populus deltoides*	PODE	Tree	Perennial	FAC	Native					x	x	x	
Daisy spp.			Forb										x	
Fall witch grass	*Digitaria cognate*	DICO6	Graminoid	Perennial	None	Native					x			
False indigo bush	*Amorpha fruticosa*	AMFR	Shrub	Perennial	FACW	Native				x	x			
False nettle	*Boehmerica sylindrica*	BOCY	Forb	Perennial	OBL	Native				x				
False solomons seal	*Maianthemum racemosum*	MARA7	Forb	Perennial	FACU	Native								
Giant goldenrod	*Solidago gigantea*	SOGI	Forb	Perennial	FACW	Native				x		x		
Gooseberry			Shrub	Perennial			x							
Grass spp.			Graminoid				x					x		
Green ash	*Fraxinus pennsylvanica*	FRPE	Tree	Perennial	FACW	Native		x			x			
Hairy sweet cicely	*Osmorhiza claytonii*	OSCL	Forb	Perennial	FACU	Native	x							
Hog peanut	*Amphicarpaea bracteata*	AMBR2	Forb	Annual/Perennial	FAC	Native	x							
Horse tail	*Equisetum hyemale*	EQHY	Forb	Perennial	FACW	Native	x							
Jewelweed	*Impatiens capensis*	IMCA	Forb	Annual	FACW	Native			x					
Lady fern	*Athyrium filix-femina*	ATFI	Forb	Perennial	None	Native	x							
Marsh skullcap	*Scutellaria galericulata*	SCGA	Forb	Perennial	OBL	Native						x		
Moonseed	*Menispermum canadense*	MECA3	Vine	Perennial	FAC	Native		x						
Northern pin oak	*Quercus palustris*	QUPA2	Tree	Perennial	FACW	Native						x		
Northern red oak	*Quercus rubra*	QURU	Tree	Perennial	FACU	Native	x				x			
Plantain	*Plantago major*	PLMA2	Forb	Perennial	FAC	Introduced						x		
Poison ivy	*Toxodendron radicans*	TORA2	Forb	Perennial	FAC	Native	x		x					
Prairie fleabane	*Erigeron strigosus*	ERST3	Forb	Annual/Biennial/Perennial	FACU	Native					x			
Prickly ash	*Zanthoxylum americanum*	ZAAM	Shrub	Perennial	FACU	Native	x							
Purple loosestrife	*Lythrum salicaria*	LYSA2	Forb	Perennial	OBL	Introduced					x	x		
Quercus		QUERC	Tree	Perennial		Native		x				x	x	
Raspberry			Subshrub				x							
Reed canarygrass	*Phalaris arundinacea*	PHAR3	Graminoid	Perennial	FACW	Native				x	x			
Rice cutgrass	*Leersia oryzoides*	LEOR	Graminoid	Perennial	OBL	Native			x			x		
River birch	*Betula nigra*	BENI	Tree	Perennial	FACW	Native					x	x		
Riverbank grape	*Vitis riparia*	VIRI	Vine	Perennial	FACW	Native		x		x				
Sandbar willow	*Salix interior*	SAIN3	Shrub	Perennial	FACW	Native				x	x	x		
Silver maple	*Acer saccharinum*	ASCA2	Tree	Perennial	FACW	Native		x				x		
Smartweed			Forb	Annual		Native		x						
Sneezeweed	*Helenium*	HELEN	Forb	Perennial	FACW	Native				x				

							Presence at Study Sites						
Common Name	Scientific Name	Symbol	Growth Habit	Duration	Wetland Status	Native Status	Belvedere	McGregor	Sny McGill	P8 Island C4	P8 Island C8	Capoli Is. C	Capoli Is. K
Stinging nettle	*Urtica dioica*	URDI	Forb	Perennial	FACW		x						
Sugar maple	*Acer saccharum*	ACSA3	Tree	Perennial	FACU	Native	x						
Sumac	*Rhus typhina*	RUHTY	Shrub	Perennial		Native				x			
Swamp white oak	*Quercus bicolor*	QUBI	Tree	Perennial	FACW	Native	x	x	x		x		
Sweet white clover	*Melilotus officinalis*	MEOF	Forb	Annual/Biennial/Perennial	FACU	Introduced					x		x
Switchgrass	*Panicum virgatum*	PAVI2	Graminoid	Perennial	FAC	Native				x		x	x
Tall goldenrod	*Solidago altissima*	SOAL6	Forb	Perennial	FACU	Native					x	x	x
Three-way sedge	*Dulichium arundinaceum*	DUAR3	Graminoid	Perennial	OBL	Native	x						
Virginia creeper	*Parthenocissus quinqufolia*	PAQU2	Vine	Perennial	FACU	Native	x						
Virginia knotweed	*Persicaria virginiana*	POVI2	Forb	Annual/Perennial	FAC	Native			x				
Virginia wild rye	*Elymus virginicus*	ELVI3	Graminoid	Perennial	FACW	Native						x	
Wild cucumber	*Echinocystis lobata*	ECLO	Vine	Annual	FACW	Native	x		x				
Wild rye	*Elymus canadensis*	ELCA4	Graminoid	Perennial	FACU	Native						x	
Wood nettle	*Laportea canadensis*	LACA3	Forb	Perennial	FACW	Native	x		x				
				Total Species Observed at Study Site:			25	9	7	11	18	21	6

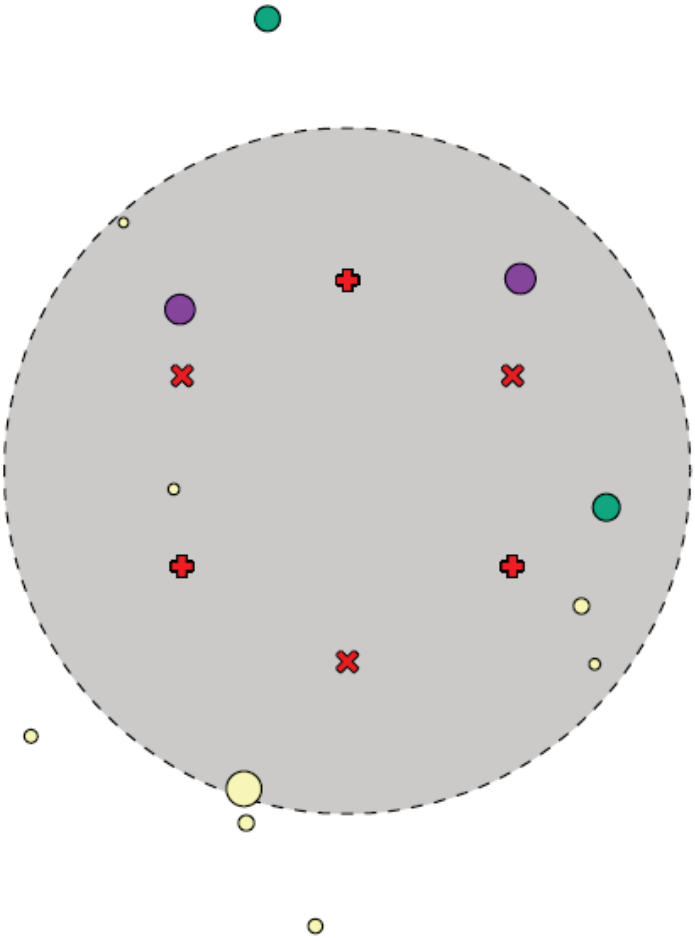
Appendix C: Tree Location Plots



Belvedere Island

Natural

Plot 2



Plot Symbology

- Plot Perimeter
- Microbial Soil Sample
- Physiochemical Soil Sample

Tree Symbols Scaled to DBH

0.25" — 15" —— 34"

Tree Species

- Carya cordiformis
- Celtis occidentalis
- Tilia americana

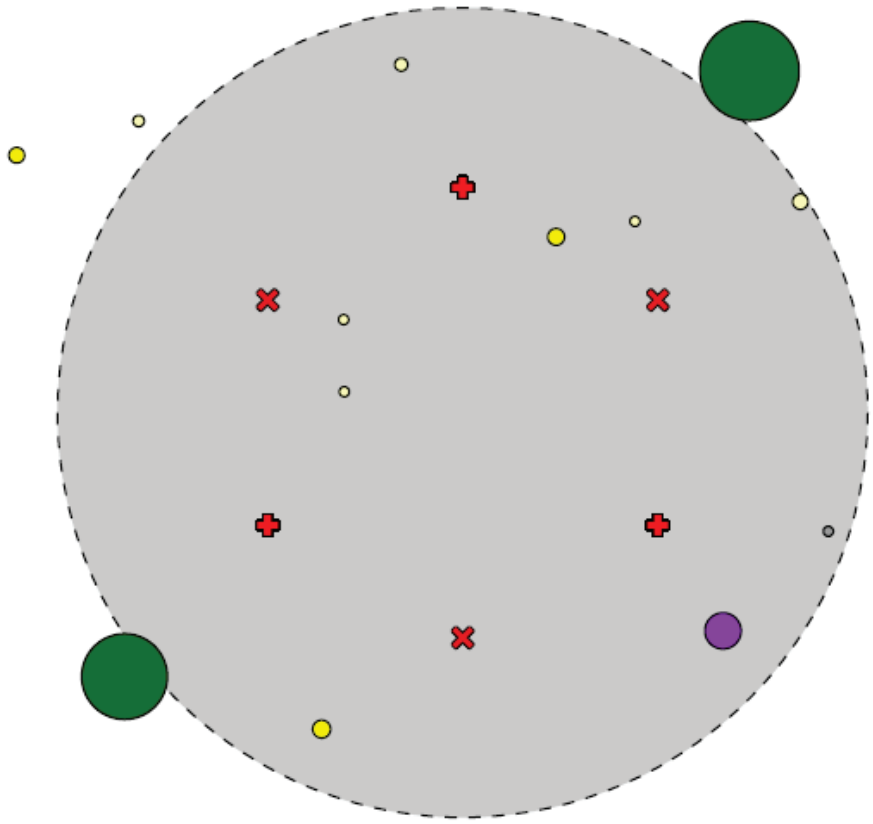
0 0.47 0.95 1.9 Meters

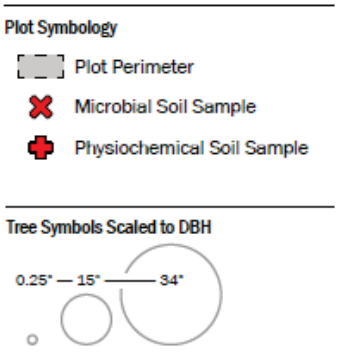
N

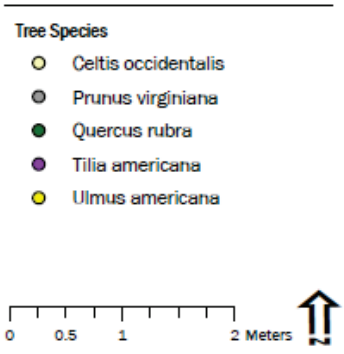
Belvedere Island

Natural

Plot 3



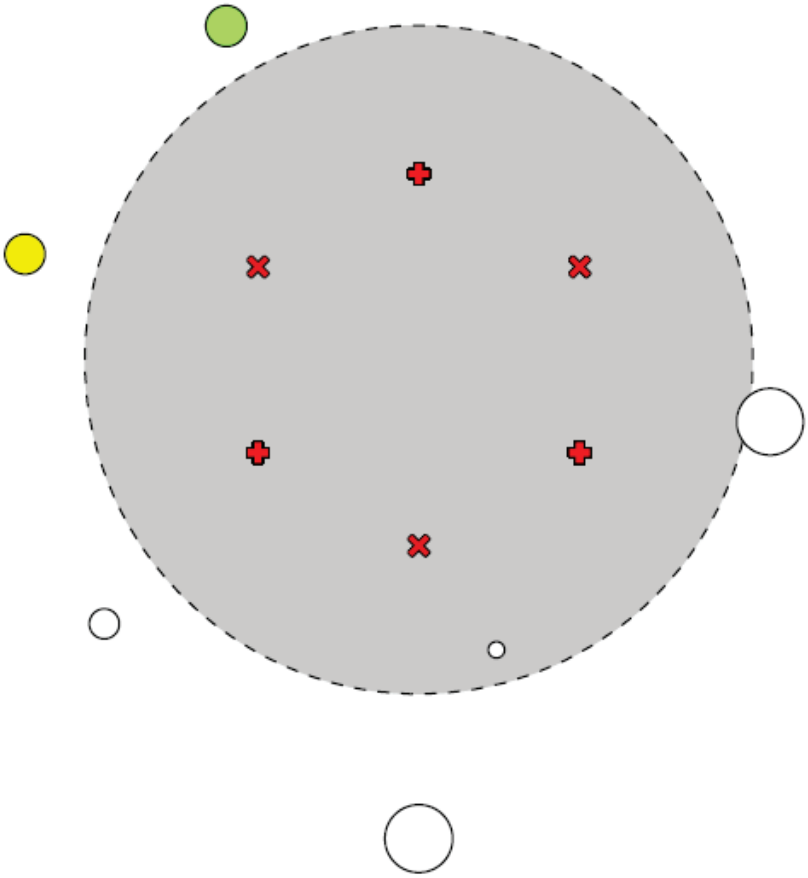




McGregor Lake

Natural

Plot 1



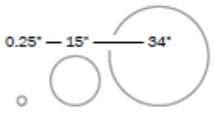
Plot Symbology

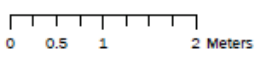
- Plot Perimeter
- Microbial Soil Sample
- Physiochemical Soil Sample

Tree Species

- Acer saccarinum
- Quercus bicolor
- Ulmus americana

Tree Symbols Scaled to DBH

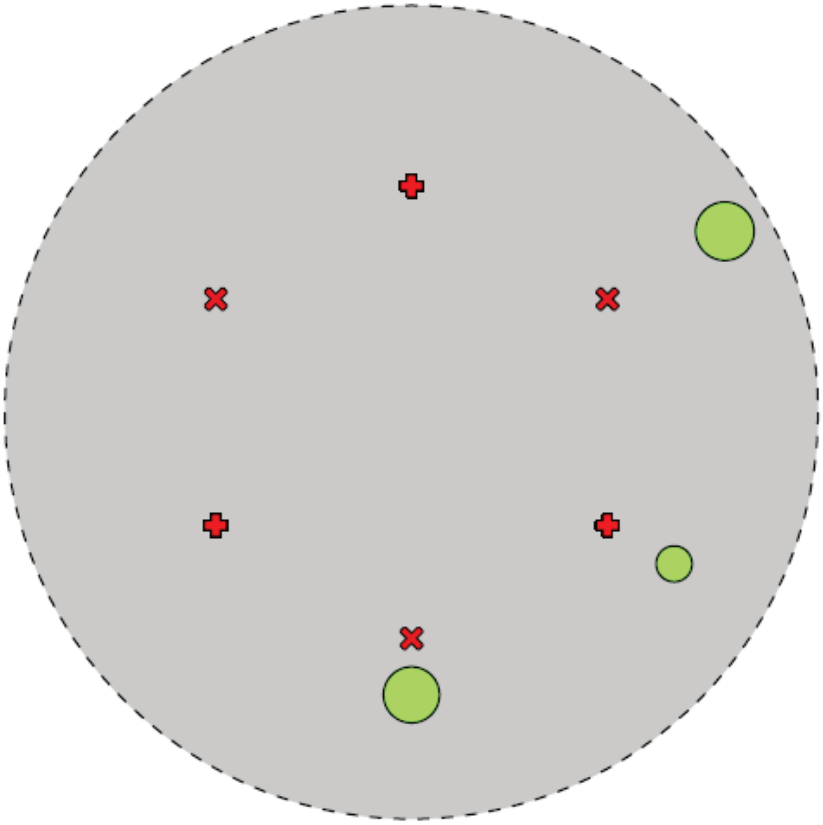




Sny McGill Lower

Natural

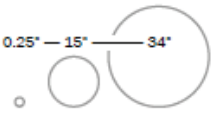
Plot 1



Plot Symbology

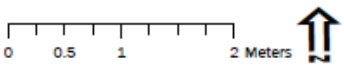
- Plot Perimeter
- Microbial Soil Sample
- Physiochemical Soil Sample

Tree Symbols Scaled to DBH



Tree Species

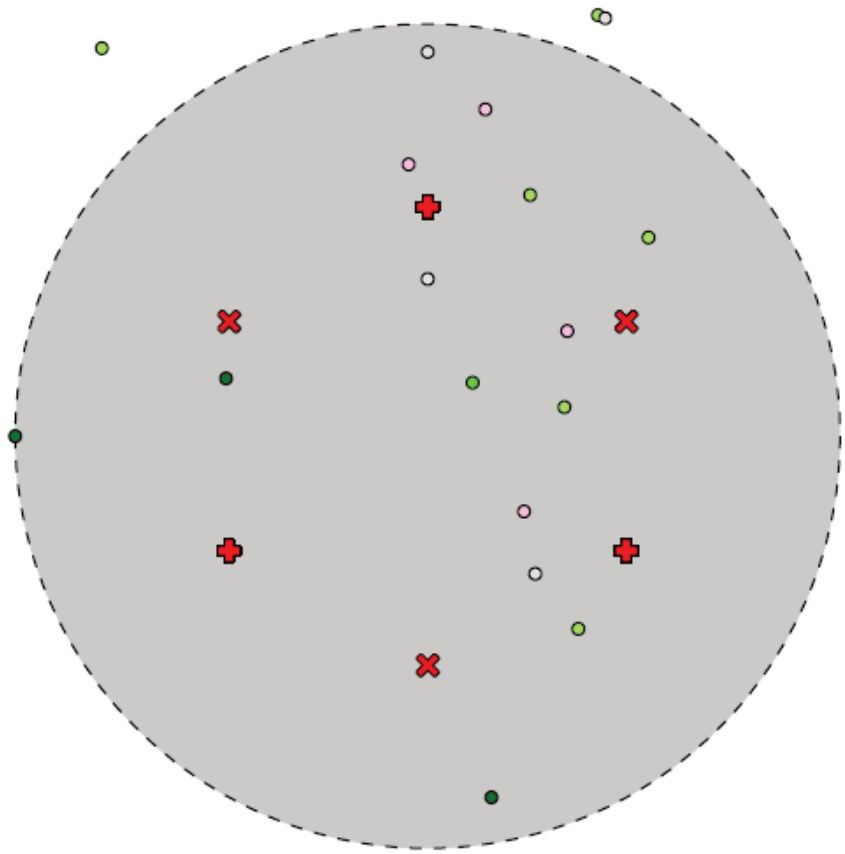
- Quercus bicolor



Pool 8 Islands Habitat Restoration Project

Plot 1

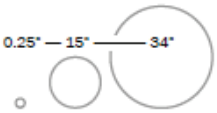
Artificial / With Trees



Plot Symbology

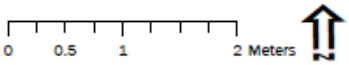
- Plot Perimeter
- Microbial Soil Sample
- Physiochemical Soil Sample

Tree Symbols Scaled to DBH



Tree Species

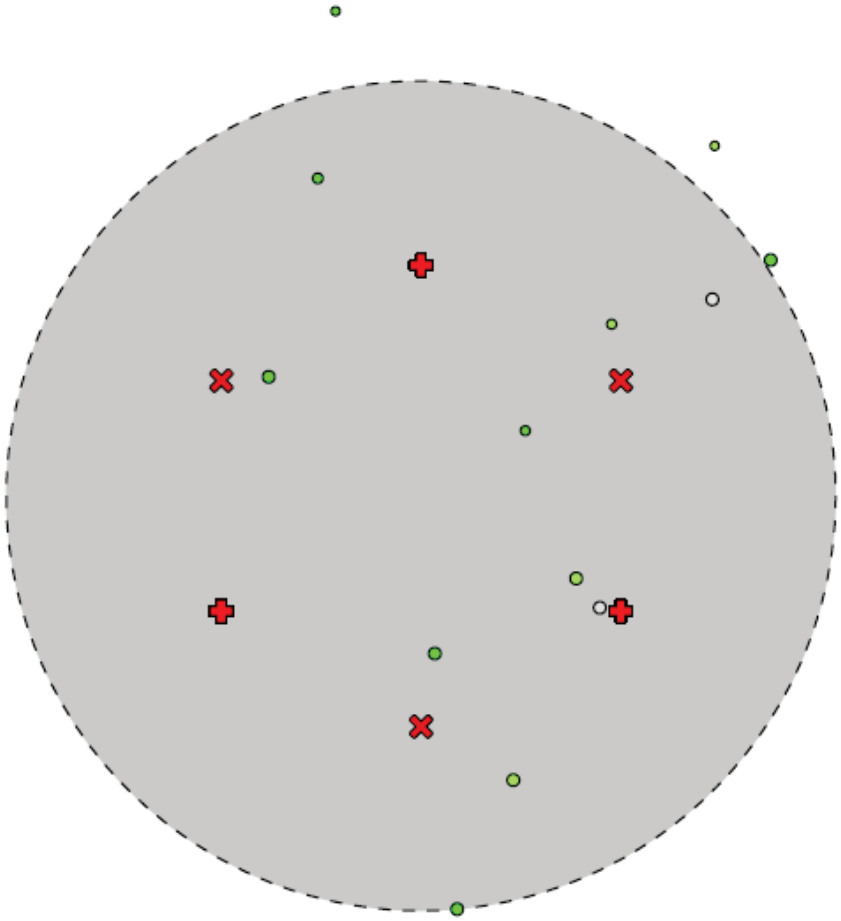
- Amorpha fruticosa
- Fraxinus pennsylvanica
- Quercus bicolor
- Quercus macrocarpa
- Quercus rubra



Pool 8 Islands Habitat Restoration Project

Plot 2

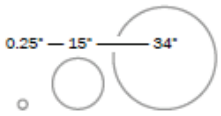
Artificial / With Trees



Plot Symbology

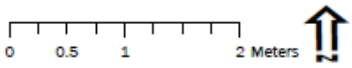
- Plot Perimeter
- Microbial Soil Sample
- Physiochemical Soil Sample

Tree Symbols Scaled to DBH



Tree Species

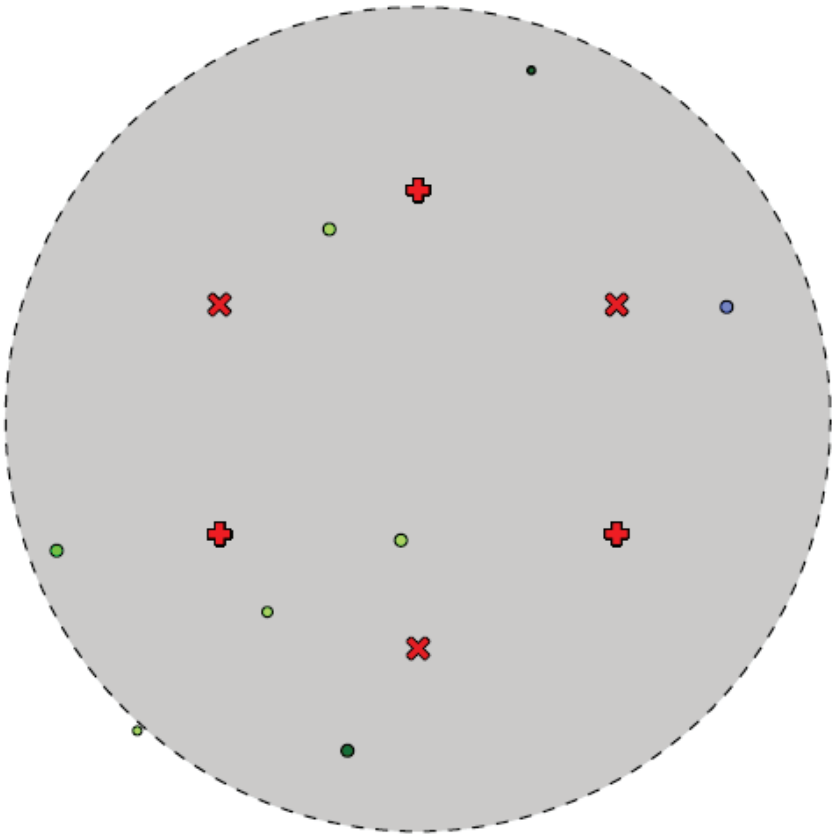
- Amorpha fruticosa
- Quercus bicolor
- Quercus macrocarpa



Pool 8 Islands Habitat Restoration Project

Plot 3

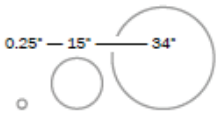
Artificial / With Trees



Plot Symbology

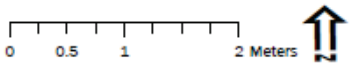
- Plot Perimeter
- Microbial Soil Sample
- Physiochemical Soil Sample

Tree Symbols Scaled to DBH



Tree Species

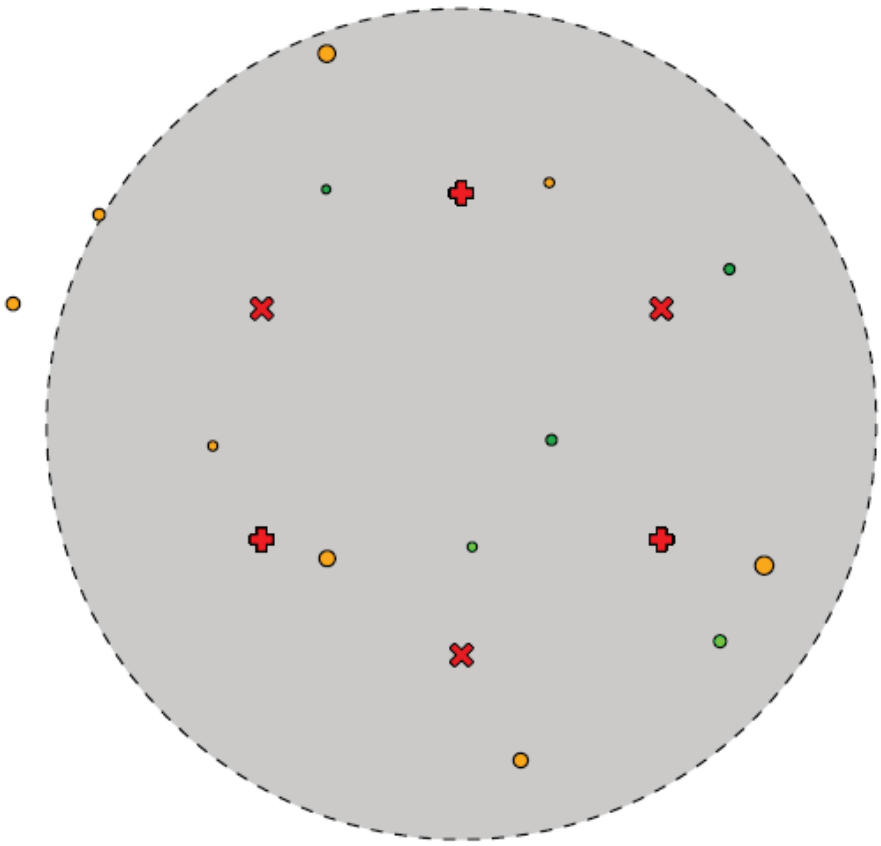
- Populus deltoides
- Quercus bicolor
- Quercus macrocarpa
- Quercus rubra



Capoli Slough Island Habitat Restoration

Plot 1

Artificial / With Trees



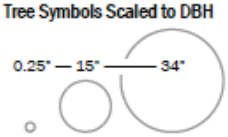
Plot Symbology

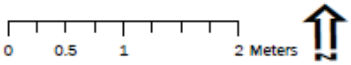
- Plot Perimeter
- Microbial Soil Sample
- Physiochemical Soil Sample

Tree Species

- Platanus occidentalis
- Quercus ellipsoidalis
- Quercus macrocarpa

Tree Symbols Scaled to DBH

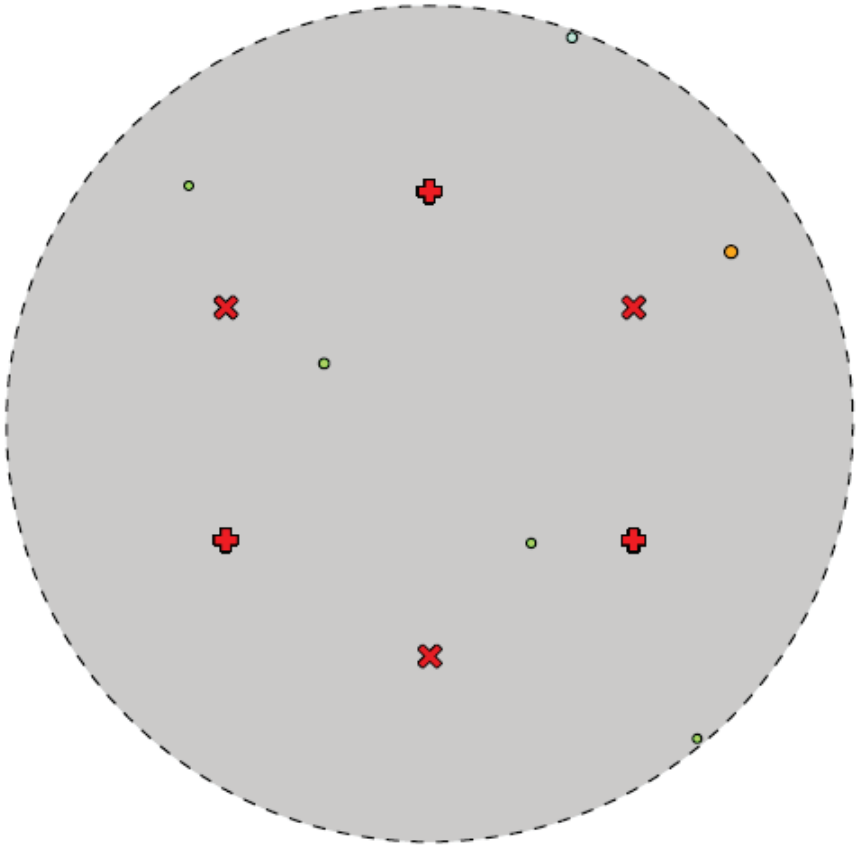




Capoli Slough Island Habitat Restoration

Plot 2

Artificial / With Trees



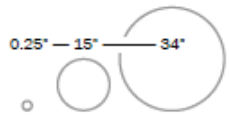
Plot Symbology

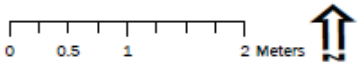
- Plot Perimeter
- Microbial Soil Sample
- Physiochemical Soil Sample

Tree Species

- Betula nigra
- Platanus occidentalis
- Quercus bicolor

Tree Symbols Scaled to DBH





Appendix D: Soil Profile Photos

Natural – Belvedere Island

Figure 1 - N/BELV/01: 0-70 cm

Natural – Belvedere Island

Figure 2 - N/BELV/02: 0-100 cm

Natural – Belvedere Island

Figure 3 - N/BELV/03: 0-90 cm

Natural – Belvedere Island

Figure 4 - N/BELV/01: Redox Features

Figure 5 - N/BELV/03: Pit face

Natural – McGregor

Figure 6 - N/MCG/01: 0-25 cm

Figure 7 - N/MCG/01: 25-50 cm

Figure 8 - N/MCG/01: 45-68 cm

Natural – Sny McGill Lower

Figure 9 – N/SNYL/01: 0-25 cm

Figure 10 – N/SNYL/01: 25-50 cm

No Trees – Pool 8 Islands

Figure 11 – NT/P8I/01: 0-55cm

No Trees – Pool 8 Islands

Figure 12 – NT/P8I/02: 0-75cm

No Trees – Pool 8 Islands

Figure 13 – NT/P8I/03: 0-55cm

With Trees – Pool 8 Islands

Figure 14 – WT/P8I/01: 0-50cm

With Trees – Pool 8 Islands

Figure 15 – WT/P8I/02: 0-55cm

With Trees – Pool 8 Islands

Figure 16 – WT/P8I/03: 0-60cm

No Trees – Capoli Islands

Figure 17 – NT/CAP/01: 0-25cm

Figure 18 – NT/CAP/01: 0-25cm

Figure 19 – NT/CAP/01: 45-70cm

No Trees – Capoli Islands

Figure 20 – NT/CAP/02: 0-25cm

Figure 21 – NT/CAP/02: 21-43cm

No Trees – Capoli Islands

Figure 22 – NT/CAP/02: Example redox features

With Trees – Capoli Islands

Figure 23 – WT/CAP/01: 0-25cm

Figure 24 – WT/CAP/01: 25-50cm

Figure 25 – WT/CAP/01: 50-75cm

With Trees – Capoli Islands

Figure 26 – WT/CAP/02: 0-25cm

Figure 27 – WT/CAP/02: 25-50cm

Appendix E: Soil Data

Sample	Rep	Depth	MC	Bulk Density	OM	Soil pH
		(cm)	(%)	(g/cm3)	(%)	
N-BELV	1	0-10	7.85	1.21	2.96	7.14
N-BELV	1	10-20	3.91	1.42	0.50	7.16
N-BELV	2	0-10	13.76	1.12	4.46	6.63
N-BELV	2	10-20	7.67	1.14	1.97	6.73
N-BELV	3	0-10	13.19	1.03	5.94	6.39
N-BELV	3	10-20	7.75	1.13	1.97	6.41
N-MCG	1	0-10	20.09	0.91	9.41	7.46
N-MCG	1	10-20	19.49	1.12	5.88	7.59
N-SNYL	1	0-10	13.83	1.38	3.94	8.13
N-SNYL	1	10-20	13.93	1.03	3.94	7.98
NT-CAP	1	0-10	19.14	1.29	2.46	7.76
NT-CAP	1	10-20	21.74	1.41	2.96	7.62
NT-CAP	2	0-10	18.13	1.15	2.96	8.02
NT-CAP	2	10-20	17.61	1.46	3.45	8.15
NT-P8I	1	0-10	16.08	1.50	2.40	8.17
NT-P8I	1	10-20	12.17	1.59	2.44	8.26
NT-P8I	2	0-10	11.72	1.42	1.97	8.35
NT-P8I	2	10-20	11.88	1.48	2.44	8.24
NT-P8I	3	0-10	12.37	1.41	2.97	8.35
NT-P8I	3	10-20	15.02	1.84	1.47	8.25
WT-CAP	1	0-10	19.55	1.36	2.45	7.85
WT-CAP	1	10-20	17.00	1.47	3.00	7.97
WT-CAP	2	0-10	20.32	1.20	3.47	7.95
WT-CAP	2	10-20	20.95	1.36	3.37	7.90
WT-P8I	1	0-10	18.38	1.53	2.48	7.61
WT-P8I	1	10-20	12.19	1.53	1.49	7.97
WT-P8I	2	0-10	10.02	1.41	2.79	7.48
WT-P8I	2	10-20	12.77	1.65	2.28	8.04
WT-P8I	3	0-10	13.30	1.25	4.39	7.83
WT-P8I	3	10-20	11.73	1.37	2.42	7.96

Sample	Rep	Depth (cm)	SRP	NOx	NH4+	DOC	TC	TN
			(mg/kg)				%	
N-BELV	1	0-10	0.00	2.86	2.64	38.46	0.720	0.070
N-BELV	1	10-20	0.00	1.29	3.40	17.26	0.090	0.00
N-BELV	2	0-10	0.00	4.25	3.19	52.29	2.34	0.220
N-BELV	2	10-20	0.00	2.92	4.01	34.35	0.640	0.120
N-BELV	3	0-10	0.00	4.45	2.15	58.84	4.02	0.310
N-BELV	3	10-20	0.00	2.97	4.17	40.45	0.620	0.070
N-MCG	1	0-10	0.00	7.88	4.64	37.90	3.86	0.350
N-MCG	1	10-20	0.00	6.41	4.40	27.65	2.02	0.220
N-SNYL	1	0-10	0.00	3.85	4.65	32.21	1.69	0.140
N-SNYL	1	10-20	0.00	3.24	6.83	24.44	1.51	0.180
NT-CAP	1	0-10	0.00	1.79	5.06	21.11	0.770	0.120
NT-CAP	1	10-20	0.00	1.31	4.79	17.07	0.700	0.070
NT-CAP	2	0-10	1.51	0.88	8.67	24.60	1.15	0.150
NT-CAP	2	10-20	0.46	0.95	6.38	22.33	0.810	0.120
NT-P8I	1	0-10	1.50	11.56	12.37	36.41	0.930	0.120
NT-P8I	1	10-20	0.40	11.50	12.68	28.35	0.930	0.110
NT-P8I	2	0-10	0.34	5.78	7.36	32.86	1.02	0.130
NT-P8I	2	10-20	0.00	2.83	5.36	25.01	0.920	0.120
NT-P8I	3	0-10	0.64	63.59	61.13	28.61	1.24	0.130
NT-P8I	3	10-20	0.29	37.08	39.03	25.77	1.32	0.150
WT-CAP	1	0-10	0.23	1.87	9.57	21.78	1.19	0.160
WT-CAP	1	10-20	0.01	1.91	6.48	20.34	0.880	0.130
WT-CAP	2	0-10	0.15	1.30	7.79	25.84	1.54	0.180
WT-CAP	2	10-20	0.00	1.14	5.93	23.53	1.19	0.160
WT-P8I	1	0-10	0.00	1.64	6.36	26.14	1.25	0.160
WT-P8I	1	10-20	0.04	1.16	4.77	24.05	0.900	0.130
WT-P8I	2	0-10	0.11	2.12	5.16	24.58	1.36	0.160
WT-P8I	2	10-20	0.12	1.42	3.72	21.67	1.02	0.130
WT-P8I	3	0-10	0.00	5.33	9.09	35.07	1.71	0.190
WT-P8I	3	10-20	0.15	64.44	59.01	26.04	1.17	0.150

Sample	Rep	Depth (cm)	MBC (mg/kg)	Phos	Cello	Xylo	Bgluc	NAG
				(nmol/gdw/hr)				
N-BELV	1	0-10	301.73	252.59	86.61	99.38	404.63	369.06
N-BELV	1	10-20	82.50	62.26	29.06	23.26	39.46	58.25
N-BELV	2	0-10	538.80	408.90	118.89	62.23	993.60	484.45
N-BELV	2	10-20	223.82	264.17	61.07	67.10	213.34	161.84
N-BELV	3	0-10	548.39	559.70	88.19	89.84	667.13	536.50
N-BELV	3	10-20	246.77	254.03	36.38	33.58	209.05	273.98
N-MCG	1	0-10	918.15	279.96	224.27	42.34	1061.56	127.41
N-MCG	1	10-20	736.94	136.86	99.96	50.78	372.66	176.01
N-SNYL	1	0-10	506.42	199.46	229.83	20.78	301.57	980.00
N-SNYL	1	10-20	465.85	191.51	219.44	80.45	468.25	145.96
NT-CAP	1	0-10	663.94	155.30	124.36	37.42	365.84	168.49
NT-CAP	1	10-20	662.68	179.26	85.24	35.86	270.57	123.97
NT-CAP	2	0-10	690.71	296.87	197.23	116.06	555.03	711.74
NT-CAP	2	10-20	811.04	381.25	215.98	54.05	449.81	260.24
NT-P8I	1	0-10	434.51	461.17	272.39	310.16	2169.11	856.54
NT-P8I	1	10-20	290.75	1147.87	487.87	98.53	1301.41	334.86
NT-P8I	2	0-10	325.94	419.23	156.57	45.03	797.33	413.55
NT-P8I	2	10-20	301.43	347.83	197.05	86.28	958.93	428.15
NT-P8I	3	0-10	354.07	357.19	192.56	77.18	815.08	480.38
NT-P8I	3	10-20	423.69	779.61	303.56	67.59	1280.24	329.98
WT-CAP	1	0-10	734.70	391.17	279.70	62.71	704.47	287.91
WT-CAP	1	10-20	646.70	227.50	114.60	31.08	401.79	139.71
WT-CAP	2	0-10	688.32	227.35	231.13	62.11	595.30	201.06
WT-CAP	2	10-20	741.37	289.13	145.22	20.84	489.35	118.53
WT-P8I	1	0-10	550.59	419.30	160.57	62.76	466.98	379.20
WT-P8I	1	10-20	291.15	124.56	101.76	55.81	399.90	126.72
WT-P8I	2	0-10	265.82	255.62	140.78	39.66	323.95	115.58
WT-P8I	2	10-20	479.37	133.66	81.90	24.30	278.78	109.53
WT-P8I	3	0-10	354.32	155.06	125.94	107.35	635.28	288.27
WT-P8I	3	10-20	328.71	136.77	165.07	39.02	655.06	391.86

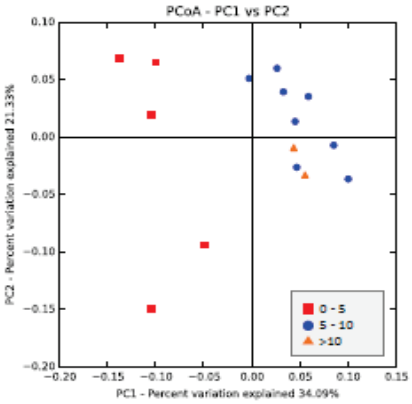
Sample	Rep	Depth (cm)	Bulk Density (g/cm3)	MC	OM
				%	
N-BELV	1	0-10	1.21	7.85	2.96
N-BELV	1	10-20	1.42	3.91	0.50
N-BELV	1	20-45	1.30	4.48	0.49
N-BELV	1	45-90	1.40	5.35	0.50
N-BELV	2	0-10	1.12	13.76	4.46
N-BELV	2	10-20	1.14	7.67	1.97
N-BELV	2	20-45	0.99	4.14	0.99
N-BELV	2	45-95	1.03	3.49	0.49
N-BELV	3	0-10	1.03	13.19	5.94
N-BELV	3	10-20	1.13	7.75	1.97
N-BELV	3	20-45	1.19	4.44	1.47
N-BELV	3	45-95	1.18	5.47	1.48
N-MCG	1	0-10	0.91	20.09	9.41
N-MCG	1	10-20	1.12	19.49	5.88
N-MCG	1	20-45	1.29	19.49	3.40
N-MCG	1	45-90	1.39	20.54	1.97
N-SNYL	1	0-10	1.38	13.83	3.94
N-SNYL	1	10-20	1.03	13.93	3.94
N-SNYL	1	20-45	1.36	17.58	2.45
N-SNYL	1	45-85	1.16	17.71	1.97
NT-CAP	1	0-10	1.29	19.14	2.46
NT-CAP	1	10-20	1.41	21.74	2.96
NT-CAP	1	20-45	1.45	19.09	1.49
NT-CAP	1	45-90	1.00	6.55	0.49
NT-CAP	2	0-10	1.15	18.13	2.96
NT-CAP	2	10-20	1.46	17.61	3.45
NT-CAP	2	20-45	1.45	9.83	0.99
NT-CAP	2	45-70	1.74	6.71	0.49
NT-P8I	1	0-10	1.50	16.08	2.40
NT-P8I	1	10-20	1.59	12.17	2.44
NT-P8I	1	20-45	1.94	19.72	2.94
NT-P8I	1	45-50	1.91	10.13	1.93
NT-P8I	2	0-10	1.42	11.72	1.97
NT-P8I	2	10-20	1.48	11.88	2.44
NT-P8I	2	20-45	2.06	13.06	2.45
NT-P8I	2	45-70	1.58	8.61	0.99

Sample	Replicate	Depth (cm)	Bulk Density	MC	OM
			(g/cm3)	%	
NT-P8I	3	0-10	1.41	12.37	2.97
NT-P8I	3	10-20	1.84	15.02	1.47
NT-P8I	3	20-37	2.05	11.18	1.99
NT-P8I	3	37-60	0.71	8.38	0.98
WT-CAP	1	0-10	1.36	19.55	2.45
WT-CAP	1	10-20	1.47	17.00	3.00
WT-CAP	1	20-45	1.57	20.77	3.47
WT-CAP	1	45-82	1.20	12.48	2.49
WT-CAP	2	0-10	1.20	20.32	3.47
WT-CAP	2	10-20	1.36	20.95	3.37
WT-CAP	2	20-45	1.44	22.24	4.21
WT-CAP	2	45-90	1.15	7.31	1.79
WT-P8I	1	0-10	1.53	18.38	2.48
WT-P8I	1	10-20	1.53	12.19	1.49
WT-P8I	1	20-45	1.57	7.52	1.49
WT-P8I	1	45-60	1.17	4.39	0.91
WT-P8I	2	0-10	1.41	10.02	2.79
WT-P8I	2	10-20	1.65	12.77	2.28
WT-P8I	2	20-45	1.51	8.91	2.36
WT-P8I	2	45-67	1.35	5.01	0.98
WT-P8I	3	0-10	1.25	13.30	4.39
WT-P8I	3	10-20	1.37	11.73	2.42
WT-P8I	3	20-45	1.63	9.37	1.47
WT-P8I	3	45-70	1.21	4.44	1.49

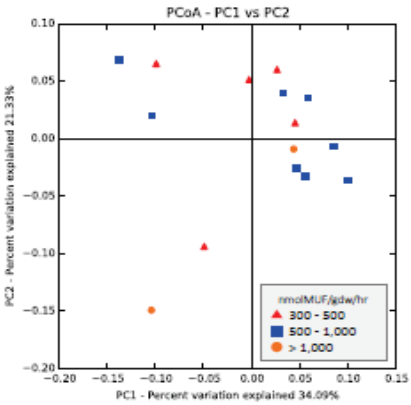
Appendix F: Microbiome and Mycobiome PCoA Plots

Microbiome - Bacteria - 16S PCoA Plots

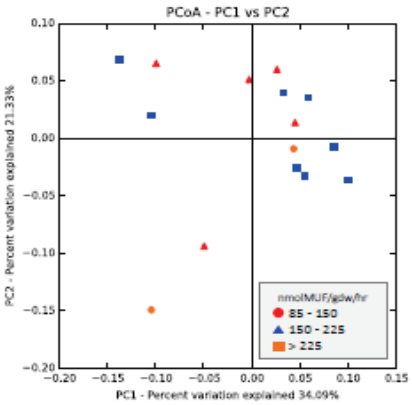
a) Ammonium



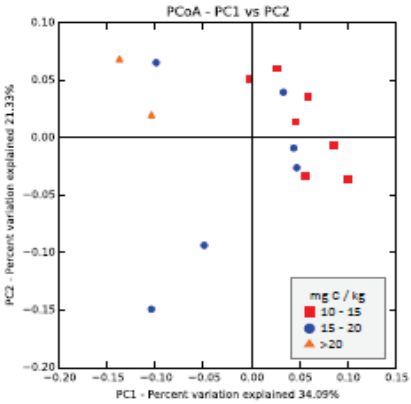
b) β-Glucosidase



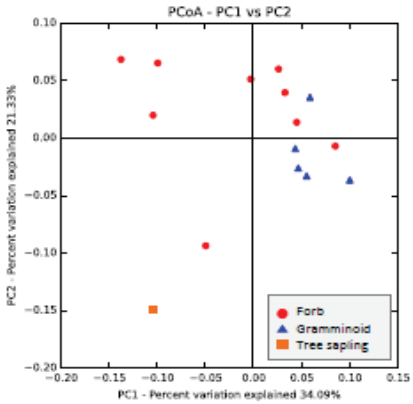
c) Cellobiosidase



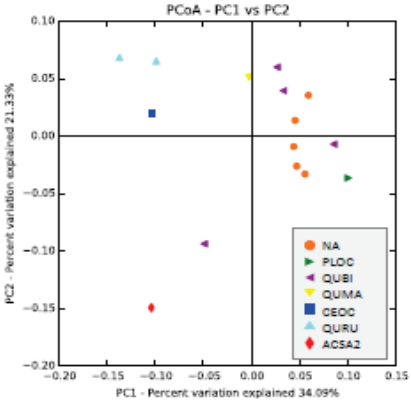
d) Disolved Organic Carbon



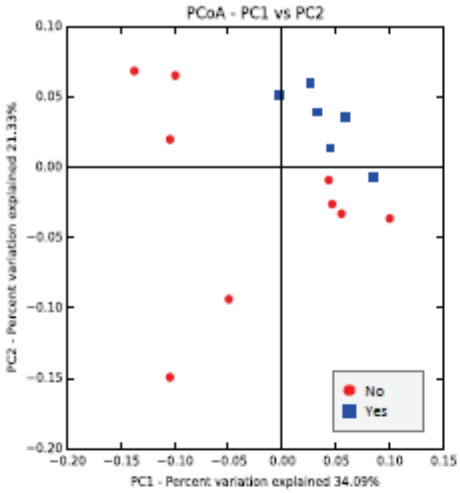
e) Dominant Ground Cover Growth Habit (plot)



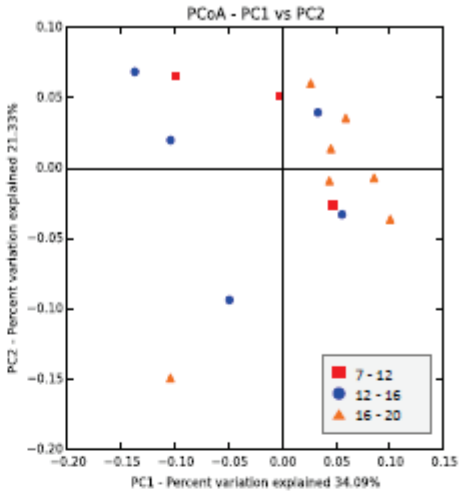
f) Dominant Tree Species (plot)



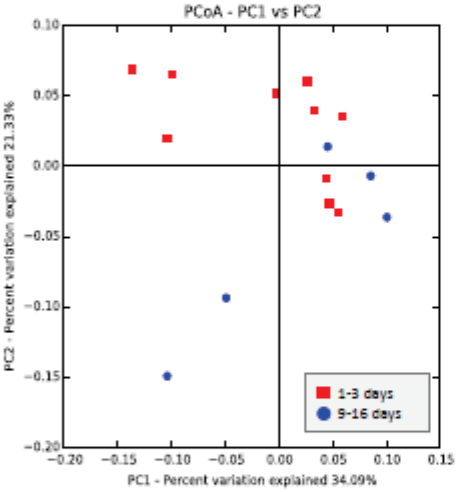
g) Introduced Species Presence



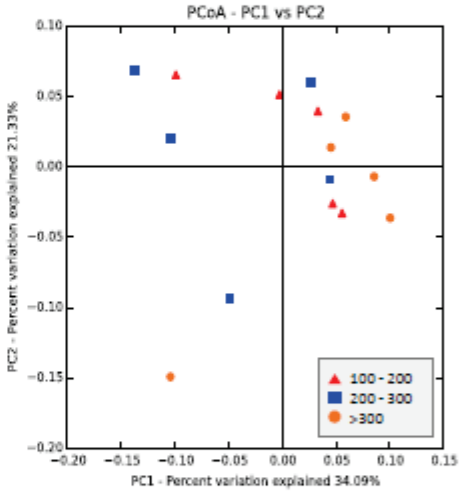
h) Moisture Content (%)



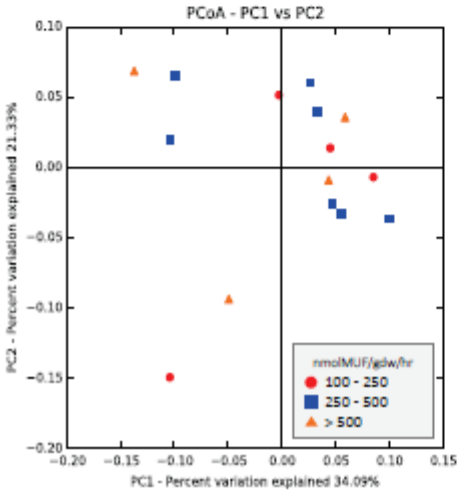
i) Mean Days Inundation



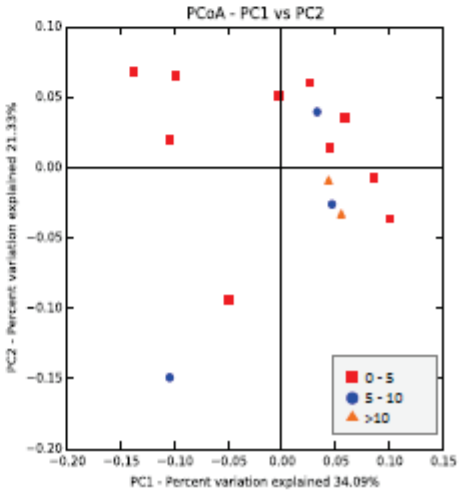
j) Microbial Biomass Carbon (mg C/kg)



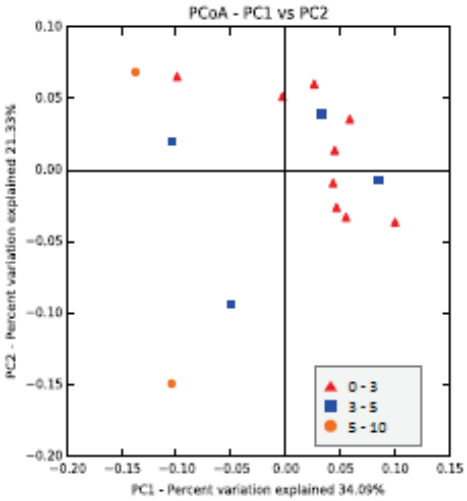
k) N-acetyl-glucosaminidase



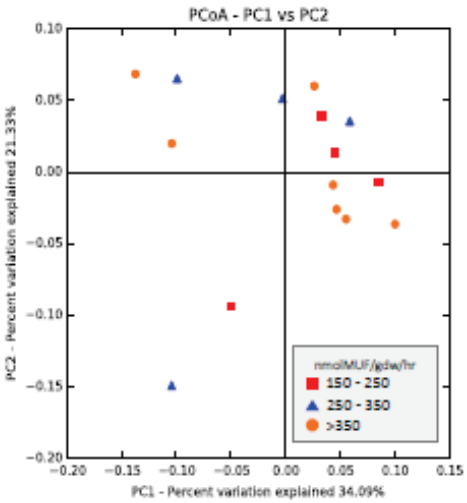
l) Nitrate (mg/kg)



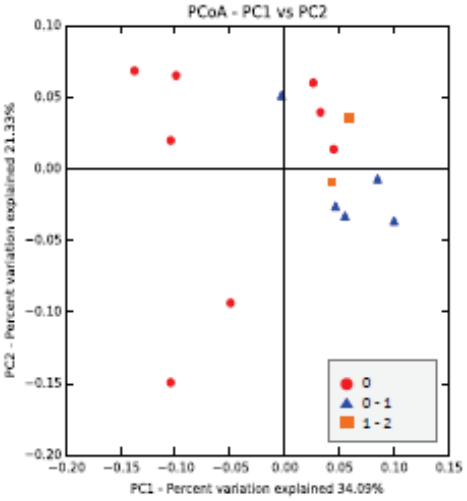
m) Organic Matter (%)



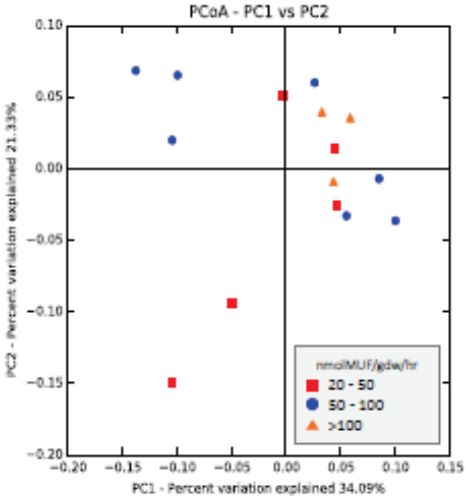
n) Phosphatase



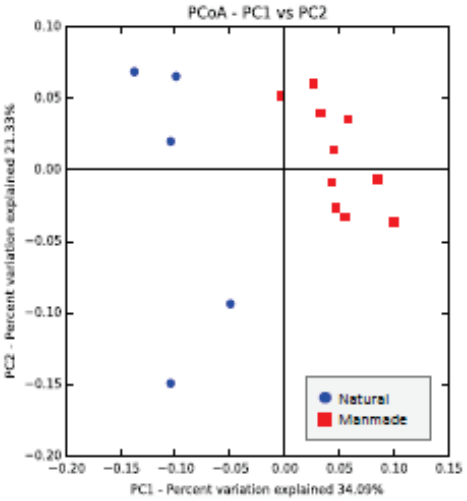
o) Phosphorus (mg/kg)



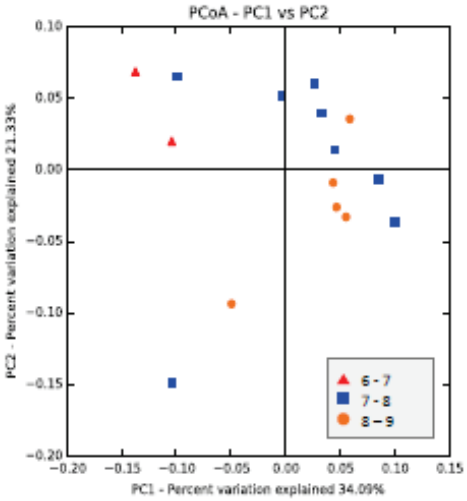
p) Xylosidase



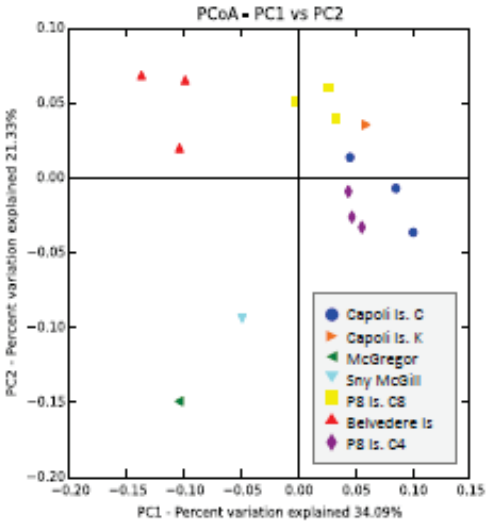
q) Condition



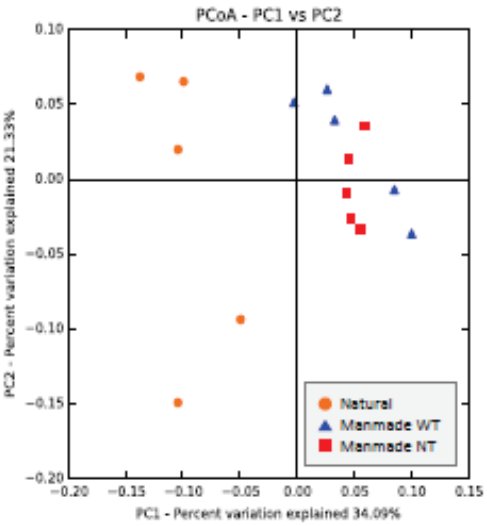
r) pH



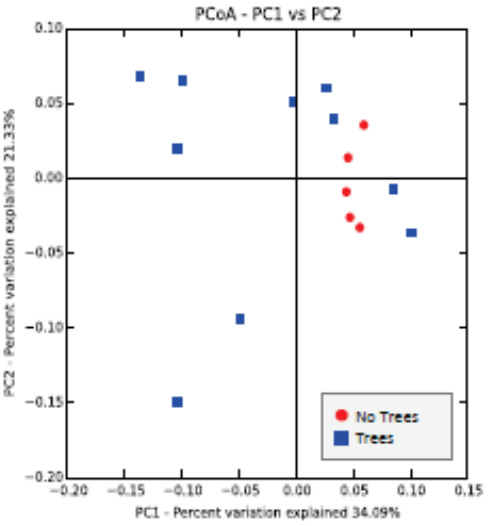
s) Site Location



t) Strata

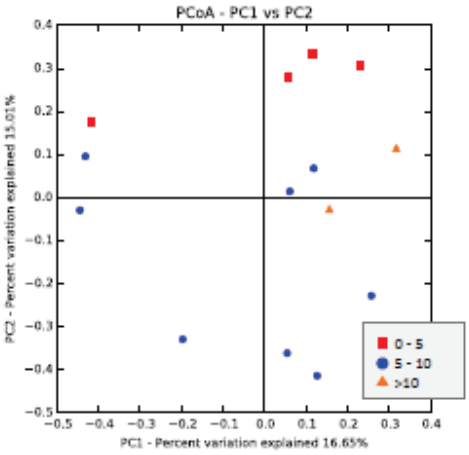


u) Trees

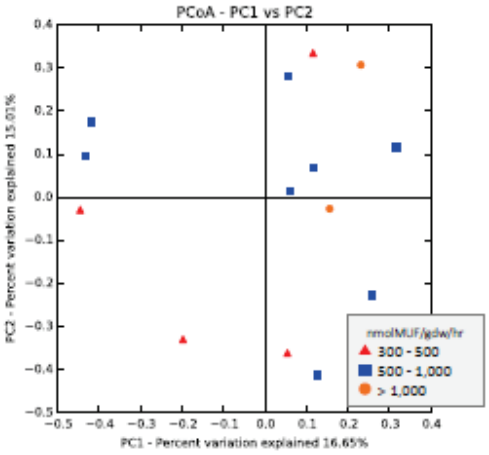


Mycobiome - Fungi - ITS PCoA Plots

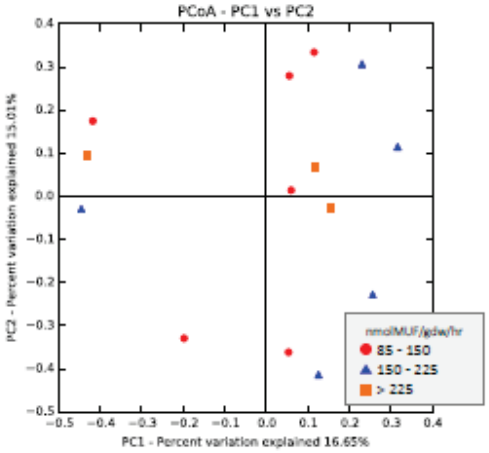
a) Ammonium



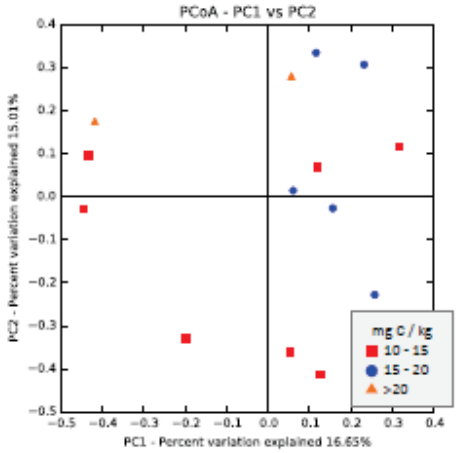
b) β-Glucosidase



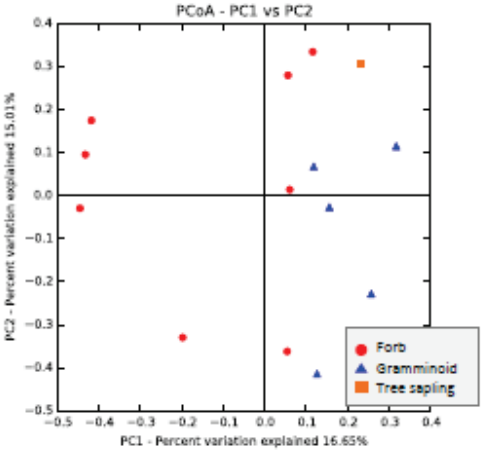
c) Cellobiosidase



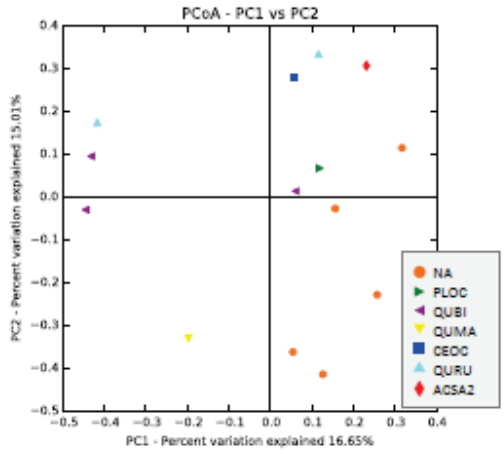
d) Disolved Organic Carbon



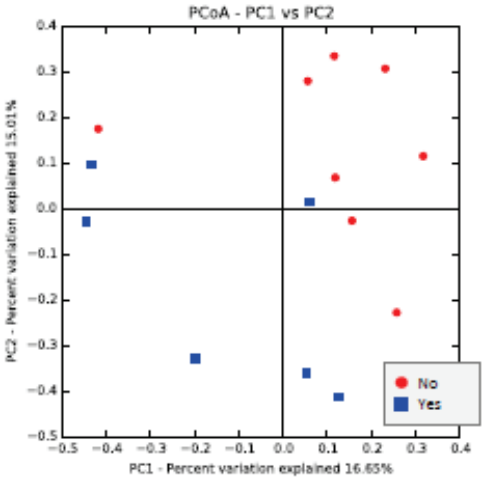
e) Dominant Ground Cover Growth Habit (plot)



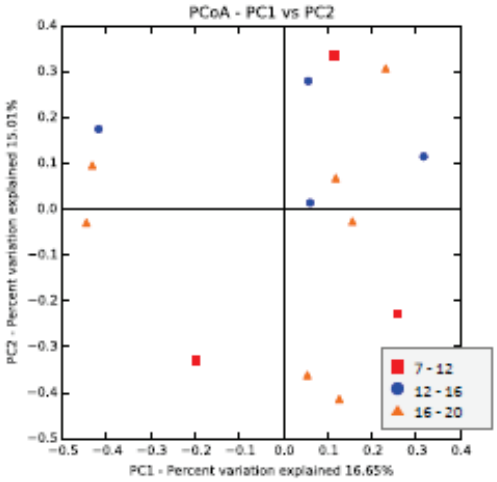
f) Dominant Tree Species (plot)



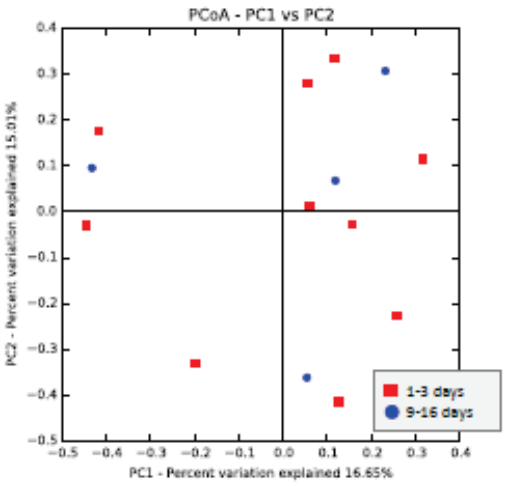
g) Introduced Species Presence



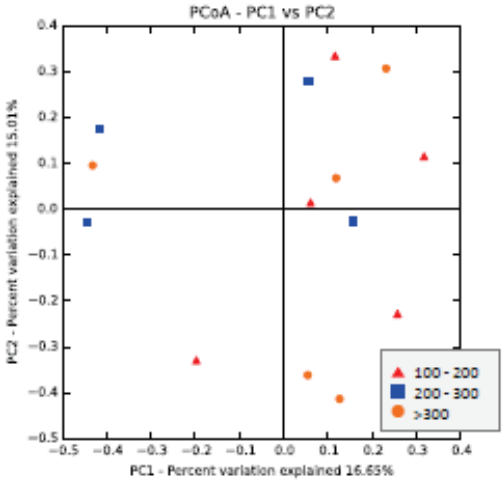
h) Moisture Content (%)



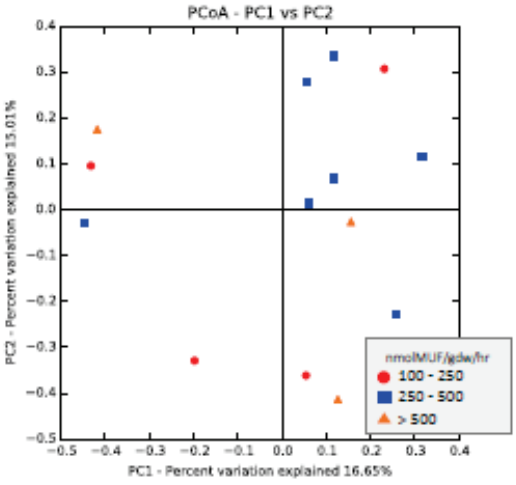
i) Mean Days Inundation



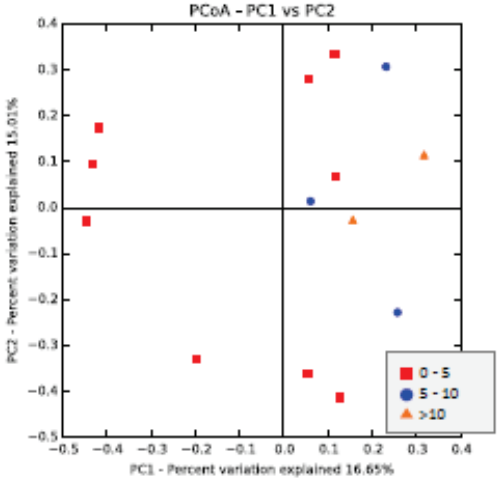
j) Microbial Biomass Carbon (mg C/kg)



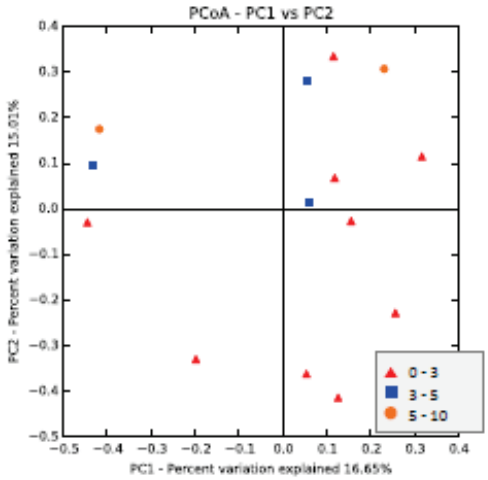
k) N-acetyl-glucosaminidase



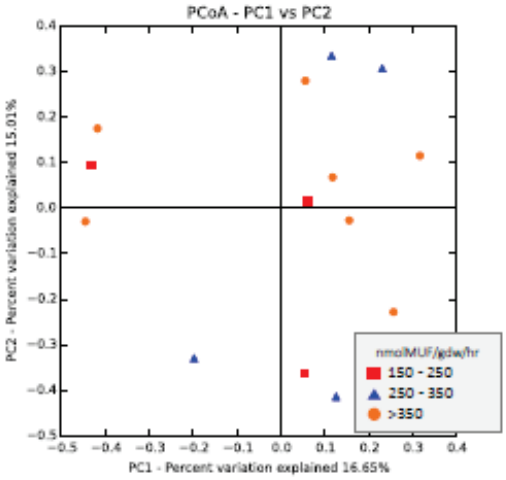
l) Nitrate (mg/kg)



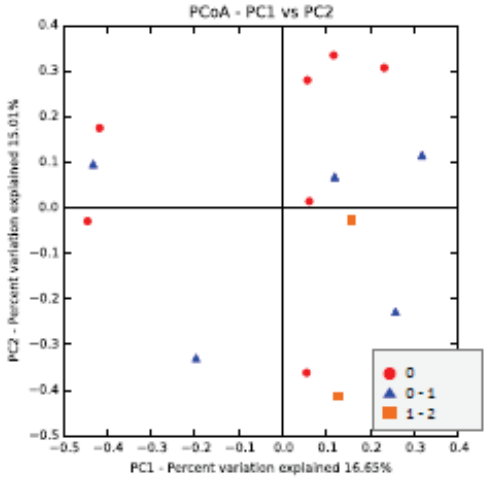
m) Organic Matter (%)



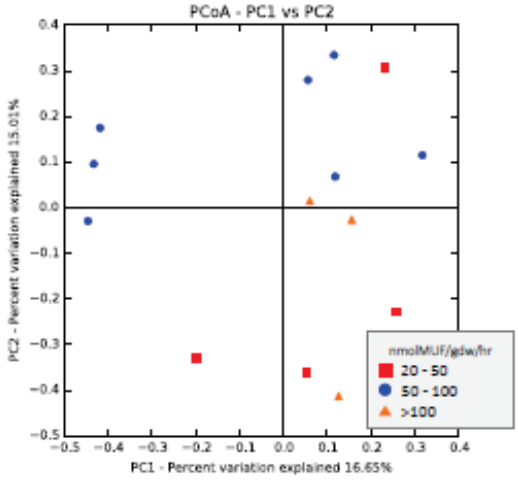
n) Phosphatase



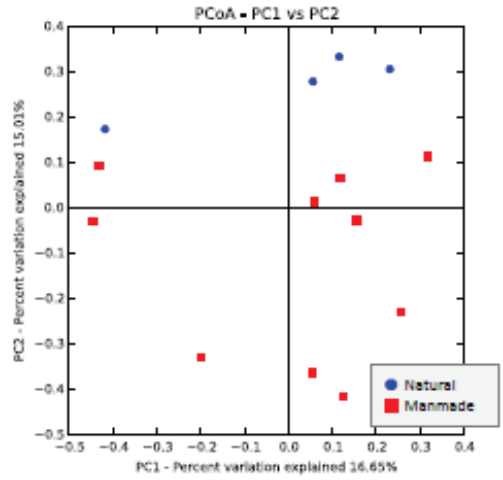
o) Phosphorus (mg/kg)



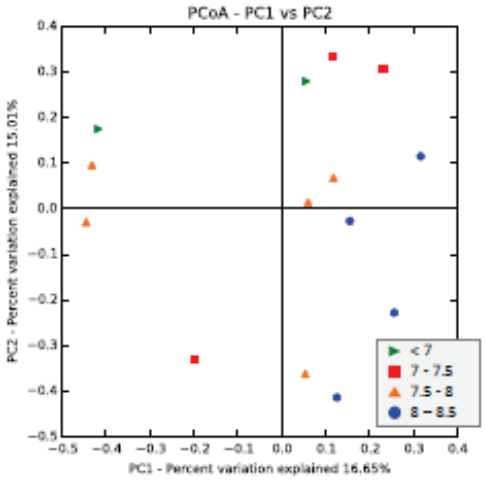
p) Xylosidase



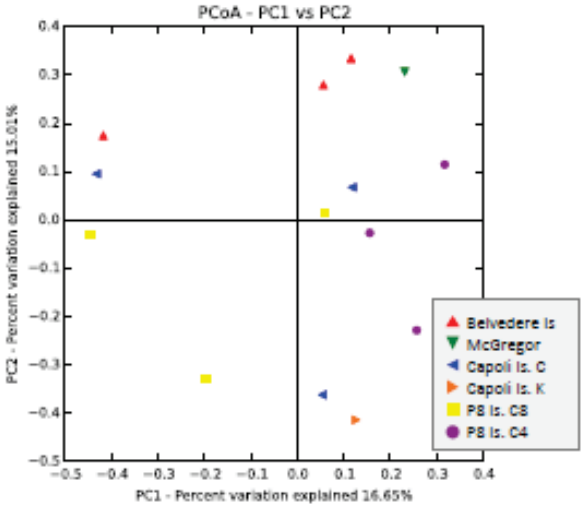
q) Condition



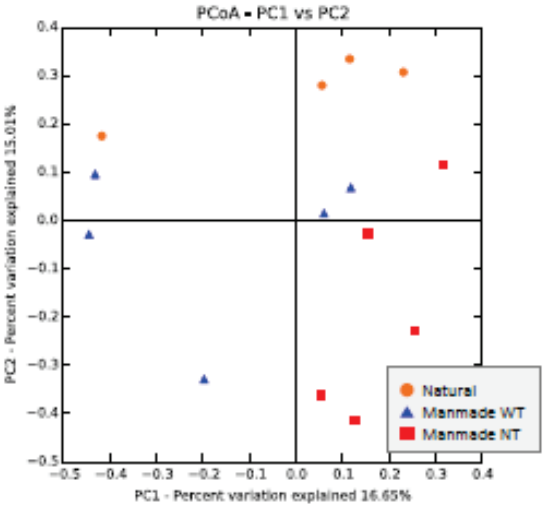
r) pH



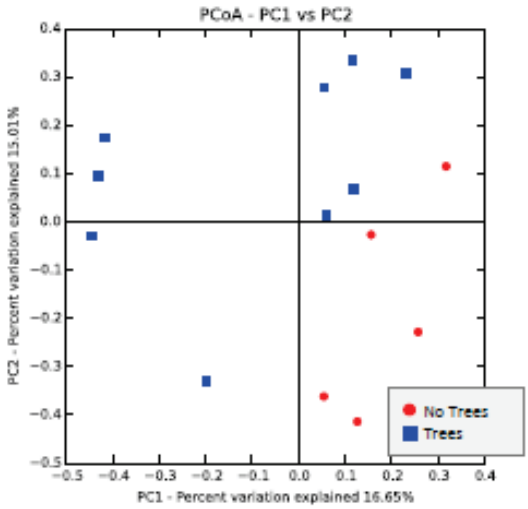
s) Site Location



t) Strata



u) Trees



Site	Type	Basal Area (BA) (m2/ha)	Diameter at Breast Height (DBH) (cm)	# of Tree Species	Tree Species Observed	Constructions Year(s)	Sediment Placement Depth (cm)
Belvedere Island	Natural	36.7 m2/ha (160 ft2/ac)	27 cm (10.7 in)	9	northern red oak, bitternut hickory, basswood, hackberry		N/A
McGregor Lake	Natural	29.4 m2/ha (128 ft2/ac)	40.3 cm (15.9 in)	6	silver maple, green ash, American elm, swamp white oak		N/A
Sny McGill Lower	Natural	24.8 m2/ha (108 ft2/ac)	47.0 cm (18.5 in)	8	silver maple, green ash, swamp white oak, American elm, cottonwood, hackberry, bur oak, and red oak		N/A
Capoli Slough Island K	Constructed	N/A				2012-2014	22.9 – 137.2 cm (9-54 in)
Capoli Slough Island C	Constructed	N/A			pin oak, bur oak, northern red oak, cottonwood, sycamore, hackberry, silver maple, river birch, black cherry, and sandbar willow.	2012-2014	22.9 – 137.2 cm (9-54 in)
Pool 8 **Islands – Phase III – Islands C4**	Constructed	N/A		0	N/A	2012	22.9 – 45.7 cm (9-18 in) 30.5 cm (12 in) deep (1st) 45.7 cm (18 in) deep (2nd)
Pool 8 Islands - Phase III- Island C8	Constructed	N/A		0	maple, green ash, and American elm, Oak	2012	22.9 – 45.7 cm (9-18 in) 30.5 cm (12 in) deep

Acronyms and Abbreviations

ANOVA	Analysis of Variance
DBH	Diameter at Breast Height
DEM	Digital Elevation Model
DNA	Deoxyribonucleic acid
EL	Environmental Laboratory
ERDC	U.S. Army Engineer Research and Development Center
FAC	Facultative
FACU	Facultative Upland
FACW	Facultative Wetland
LiDAR	Light Detection and Ranging
NAVD88	North American Vertical Datum of 1988
OBL	Obligate Wetland
OTU	Operational Taxonomic Unit
PCoA	Principal Coordinates Analysis
rRNA	Ribosomal ribonucleic acid
QIIME	Quantitative Insights into Microbial Ecology
UMESC	Upper Mississippi Environmental Sciences Center
UMR	Upper Mississippi River
UMRS	Upper Mississippi River System
UMRR	Upper Mississippi River Restoration [Program]
UPL	Obligate Upland
USACE	United States Army Corps of Engineers
USDA	U.S. Department of Agriculture
USFWS	U.S. Fish and Wildlife Service
USGS	U.S. Geological Survey
UTM	Universal Transverse Mercator coordinate system
WRDA	Water Resources Development Act

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
April 2024	Final	

4. TITLE AND SUBTITLE

Evaluating Soil Conditions to Inform Upper Mississippi River Floodplain Restoration Projects

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Aaron M. McFarlane, Nia R. Hurst, Carina M. Jung, and Charles H. Theiling

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

US Army Corps of Engineers
St. Paul District
180 Fifth Street E., Suite 700, St. Paul, MN 55101

Environmental Laboratory
U.S. Army Engineer Research and Development Center
3909 Halls Ferry Rd, Vicksburg, MS 39180

8. PERFORMING ORGANIZATION REPORT NUMBER

ERDC/EL TR-24-6

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

USACE St. Paul District
St. Paul, MN
US Army Engineer Research and Development Center (ERDC)
Environmental Laboratory (EL), Vicksburg, MS 39180

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

DISTRIBUTION STATEMENT A. Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

Funded under Funding account code U4372374 and AMSCO code 086000

14. ABSTRACT

The US Army Corps of Engineers (USACE) has designed and constructed thousands of acres of ecosystem restoration features within the Upper Mississippi River System. Many of these projects incorporate island construction to restore geomorphic diversity and habitat, including floodplain forests. Soils are the foundation of the ecological function and successful establishment of floodplain forests as they are the basis through which plants obtain water and nutrients and provide critical ecosystem services. To improve floodplain forest island restoration outcomes, three natural and four recently (<10 years) constructed restoration sites were studied to compare soil physical, chemical, microbial, and fungal characteristics. Constructed islands had lower soil organic matter and dissolved organic carbon and differed in nutrient concentrations, bacterial assemblages, and fungal communities compared to reference sites. However, soil enzyme activity and some microbial community characteristics were functionally similar between the natural and created sites. Results align with previously established restoration trajectory theories where hydrological and basic microbial ecosystem functions are restored almost immediately, but complex biologically mediated and habitat functions require more time to establish. Data from this and future studies will help increase the long-term success of USACE floodplain forest restoration, improve island design, and help develop region-specific restoration trajectory curves to better anticipate the outcomes of floodplain forest creation projects.

15. SUBJECT TERMS

Restoration ecology
Mississippi River Valley
Floodplains
Soils—Testing
Soils--Evaluation
Environmental management
Environmental protection

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified	SAR	142	