Restoration Research Award Program



Final Report Narrative Questions

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Complete the final report narrative questions below. After completing your narrative questions, save this document on your computer and then submit the document via your Chesapeake Bay Trust Online System account. You can access your account using this link https://www.GrantRequest.com/SID 1520.

1. Awardee Information

Organization Name: University of Delaware

Project Leader: Shreeram Inamdar

Award Number: 22076 Date: Nov 27, 2024

2. Project Summary

a. Describe the project results achieved. Provide a before and after photo of the project, if applicable.

While healthy soils are critical for stream and floodplain restoration, little guidance is available to restoration practitioners on which soil metrics to measure and when to expect the signs of recovery post-restoration. Here, we address this important knowledge gap through a study of 37 soil metrics for 11 restored floodplains and two reference sites across a chronosequence of 0-22 years. Soil metrics differed in their sensitivity and response to restoration and followed different rates of change including fast (0-2 years), moderate (2-10 years), and slow (> 10 years). Physicochemical metrics dominated the first two trajectories, whereas biological metrics, while sensitive, fell into the last trajectory. Soil recovery rates for restored sites differed considerably for reference sites highlighting the need to better define reference conditions. Availability of consistent and sensitive soil health metrics will allow restoration practitioners to better assess restoration recovery and receive regulatory credits for meeting restoration targets.

3. Restoration Research Award Program Narrative Questions

What was/were your key restoration research question(s)?

Soil health is a vital component of ecosystems which includes physical, chemical, and biological. soil attributes that contribute to valuable ecosystem functions and services (Lehmann et al., 2020). For floodplains and riparian zones, such ecosystem functions/services include: water infiltration and retention, erosion control, nutrient cycling, and plant growth. Healthy soils that are porous, well-aggregated, rich in organic matter, and populated with diverse microbial communities enhance these functions and ecosystem services. However, stream and floodplain restoration projects that include streambank grading and sediment removal for streamfloodplain reconnection can severely disturb and compact floodplain and riparian soils. This disturbance and compaction can have a significant detrimental effect on the physical, chemical, and biological soil health attributes and constitutes an unintentional and undesirable tradeoff of restorations (Figure 1). *Our interest in this study was to quantify the soil health tradeoffs due to stream and floodplain restorations and investigate the broader implications of these tradeoffs for floodplain functions and ecosystem services*.

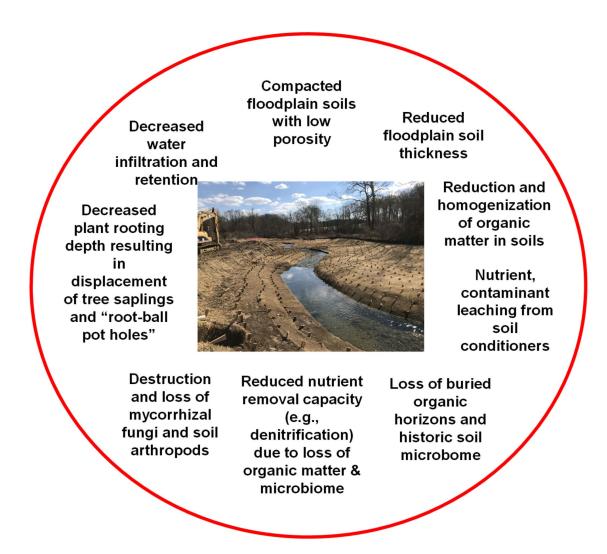


Figure 1: Some of the soils related challenges and trade-offs associated with floodplain restorations.

Typically, soil health is given very little consideration in the restoration projects (Farrell et al., 2020; Inamdar et al., 2023). The reasons for this disregard include: very little information and recognition of how restoration practices impact soil health over the short and long term; absence of specific soil health metrics to evaluate the change in soil conditions pre and post restoration; lack of information on potential "desirable" soil health endpoints or reference conditions; unavailability of design and construction protocols or "best practices" for soil health; and importantly, lack of regulatory incentives or credits for practitioners for enhancing soil health. Here, we assess the key challenges for soil health by addressing the following specific questions:

- How do soil health parameters change following restoration, and which metrics are sensitive and show consistent change?
- How do the restored soil health metrics compare against those for "reference" floodplains and what does this indicate about the choice of reference conditions?

We investigated these questions for a chronosequence of 11 mid-Atlantic restoration sites spanning a post-restoration period of 0 to 22 years. Two relatively undisturbed floodplain wetland sites were also included to characterize reference benchmark or "desirable" soil health conditions. Sampling was performed for a suite of

physical, chemical, and biological soil attributes that are linked to valuable floodplain ecosystem services. We predicted that most soil health parameters will initially be negatively affected by floodplain restoration (shaded region in Figure 1) but will improve and follow different trajectories towards reference conditions including fast (0-2 years), moderate (2-10 years), and slow (> 10 years) (Figure 2). We expected that certain soil chemical attributes would recover early followed by physical and biological parameters. Identifying these metrics and temporal trajectories will contribute to effective soil health in restorations, quantify soil recovery, and allow practitioners to seek regulatory incentives or credits towards enhancing soil health.

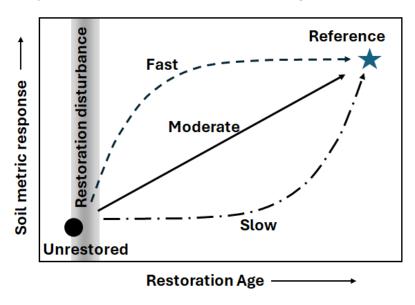


Figure 2. Conceptual model of how soil metrics are expected to evolve with time since restoration. We hypothesized that these trajectories included fast (0-2 years), moderate (2-10 years), and slow (> 10 years) post restoration and initial disturbance. Shaded region indicates post restoration disturbance and initial uncertainty and variability in soil metrics.

a. What are the results for your research question(s)?

Soil samples were collected from a total of 13 sites (11 restored and two reference) across the Mid-Atlantic Piedmont Region of the United States (US) (Galella et al 2024). Reference sites included Gramies Run (GR) forested/shrub wetland in Maryland (MD), and Great Marsh (GM) freshwater emergent wetland in Pennsylvania (PA), (Figure 3 and Table 1).

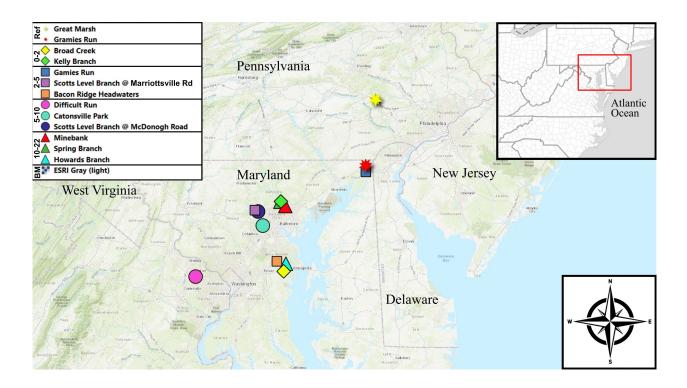


Figure 3: Locations of the 13 sampling sites (11 floodplain restorations + two reference sites) selected for this study in Maryland, Virginia, and Pennsylvania (United States). All sites are within the Mid-Atlantic region (inset). Great Marsh and Gramies Run are reference wetlands, and the remainder of the sites are restored floodplains. The shapes of the symbols represent reference sites and different restoration time frames for restoration sites (indicated in the legend at the top left).

Restored sites were grouped into a chronosequence of four categories: 0-2, 2-5, 5-10, and 10-22 years post restoration. The first category (0-2 years) includes two sites whereas all other categories have three replicate sites (Table 1). Floodplain restorations primarily followed the Natural Channel Design approach coupled with elements of floodplain reconnection and regenerative stormwater conveyance (Wood et al 2020). The Gramies Run forested/shrub wetland reference site was a relatively undisturbed wetland site within immediate proximity of the Gramies Run restoration (Mattern et al 2020). In contrast, the Great Marsh (freshwater emergent marsh) location is one of the few minimally disturbed Holocene wetlands relatively unaffected by anthropogenic landuse activities with organic rich soils 1 – 1.6 m thick and over 10,000 years old (Merritts and Rahnis 2022, Peck et al 2024). We selected the Great Marsh site as an upper or "ideal" endmember and expected that restored floodplains could take significant time to achieve these soil conditions.

Table 1: The 11 floodplain restoration sites used for this study and 2 reference undisturbed wetlands. Restorations are divided into four separate age groups 0-2 years, 2-5 years, 5-10 years, and 10-22 years. The exact coordinates of the sampling site are provided along with the state the site resides in.

Site	Age Range (Years)	Date Restored	State	Latitude / Longitude	
Broad Creek	0-2	2021	MD	38.969415°, -76.568360°	
Kelly Branch	0-2	2021	MD	39.442490°, -76.592562°	
0	0.5	0000	MD	00 0005440	
Gramies Run	2-5	2020	MD	39.686514°, -75.850506°	
Scotts Level Branch at Morriottsville Rd	2-5	2019	MD	39.383717°, -76.819942°	
Bacon Ridge	2-5	2020	MD	39.037240°, -76.627585°	
Difficult Run	5-10	2014	VA	38.933449°, -77.338004°	
Catonsville Park	5-10	2017	MD	39.279201°, -76.750729°	
Scotts Level Branch at McDonogh Rd	5-10	2014	MD	39.374473°, -76.792333°	
Minebank Run	10-22	2005	MD	39.416075°, -76.547572°	
Spring Branch	10-22	2008	MD	39.440247°, -76.597526°	
Howards Branch	10-22	2001	MD	39.021048°,	
Great Marsh	Reference	N/A	PA	40.126773°,	
				-75.765718°	
Gramies Run BT Wetland	Reference	N/A	MD	39.685317°, -75.851134°	

Field sampling

Soil samples were collected using a clean trowel from within the top 20 cm of mineral soil in 1L zip-loc bags. At each floodplain restoration site, ten soil samples were collected which included six (1, 2, and 3 m from the stream from each bank) from the restored reach and four (1 and 3 m from the stream and each bank) from the upstream unrestored section. At reference wetland sites, four to six samples were collected at random for each site. Samples for soil bulk density were collected using a metal cylinder with known dimensions (2 cm inner diameter and 3.2 cm length) and care was taken to obtain exact, uncompressed soil volume by hammering the cylinder vertically through the surficial mineral profile.

Sample Analysis

Soil bulk density samples were dried in a lab oven at 65 °C for 48 hours. Soil bulk density was calculated as the oven-dried mass divided by the known soil cylinder volume. Soil porosity was calculated using standard equation (Porosity = 1-[Bulk density/Particle density]) with a particle density of 2.65 g/cm³ (Weil and Brady, 2017). Gravimetric Water Content (GWC) was the ratio of water mass (wet soil minus dry soil) by the dry soil mass of each sample. Volumetric water content (VWC) was computed as the product of GWC and soil bulk density divided by the water density (assumed 1 g ml⁻¹).

Particle size analysis was performed using standard hydrometer methods (Gee and Bauder 1986). Sieves of > 0.25 mm and < 0.25 mm and > 0.053 mm were used to collect soil macroaggregates and microaggregates, respectively. Soil organic matter (SOM) was determined via loss on ignition in a 400°C furnace. Soil organic carbon (SOC) and total nitrogen (TN) were determined via combustion using an Elementar TC/TN analyzer. A Lachat instrument was used to determine 2M KCl extracted nitrate-N (NO₃-) and ammonium-N (NH₄+) concentrations. Soil organic nitrogen (SON) was determined by subtraction of NO₃- and NH₄+ from soil TN. Soil elemental concentrations were determined through Melich-3 extraction and analyzed via Inductively Coupled Plasma Optical Emission Spectrometer.

Microbial metrics were determined via phospholipid fatty acid (PLFA) analysis measured following Frostegard *et al.* (2011). The full list of all physical, chemical, and biological soil metrics is provided in Table 2.

Statistical Methods

Principal Component Analysis (PCA) was performed to determine differences between unrestored, restored (various age categories), and reference sites, and the soil parameters that explained variation along the first two principal components. To investigate the change in soil parameter values between unrestored and restored age categories, we computed percent change as:

Percent Change = $((Restored - Unrestored) * 100 \div (Unrestored))$

To assess if soil parameter changes shifted towards reference site conditions post-restoration, we computed the Achieved Restoration (AR) index (Marchand *et al* 2021) for each age category as:

Achieved Restoration = $100 * ((Restored - Unrestored \div (Reference - Unrestored)))$

Following Marchand *et al.* (2021), AR values between 0 – 100 indicate successful ecological restoration, while values < 0 indicate failed restoration, and values > 100 indicate that restored sites have exceeded expected reference values. In some data, soil metrics were higher in unrestored and restored soils than in reference soils, yielding erroneous AR results; these data are marked with a yellow star. AR values were computed separately for Gramies Run and Great Marsh reference sites due to substantial differences in reference soil metrics.

Changes between restored and unrestored reaches and across age categories were assessed using box plots and the percent change metric. The AR index was used to assess temporal evolution and recovery of soil metrics towards reference benchmark values. Time (in years) to reach reference benchmark values was estimated by linear extrapolation between restored floodplain values and reference values.

Table 2. Physical, chemical, and biological soil health parameters measured in this study.

Physical	Chemical	Biological
Bulk Density (g/cm³)	Ammonium-N (ppm), Nitrate-N (ppm)	Actinomycetes (ng/g)
Gravimetric Water Content (%)	Total Nitrogen (%), Phosphorus (ppm)	Arbuscular Mycorrhizal Fungi (ng/g)
Volumetric Water Content (%)	Calcium (ppm), Magnesium (ppm)	Functional Group Diversity Index
Macroaggregates (% >0.25mm)	Potassium (ppm), Sodium (ppm)	Gram Negative Bacteria (ng/g)
Microaggregates (% <0.25mm)	Base Saturation (%), CEC (meq/100g)	Gram Positive Bacteria (ng/g)
Sand (%)	Organic Carbon (%), Organic Matter (%)	Saprophytic Fungi (ng/g)
Silt (%)	Soil pH, Boron (ppm)	Total Bacteria (ng/g)
Clay (%)	Cobalt (ppm), Copper (ppm)	Total Fungi (ng/g)
	Iron (ppm), Manganese (ppm)	Total Living Microbial Biomass (ng/g)
	Zinc (ppm)	Undifferentiated (ng/g)

Results:

Distinct clustering of unrestored, restored, and reference sites and key soil parameters that explain the variation

PCA revealed distinct separation among the unrestored and restored age categories, and reference sites (Figure 4a). Principal component (PC) 1 explained 58.9% of total variation, while PC2 explained 15.4% of total variation. Organic matter, organic carbon, volumetric water content, sand, and bulk density loaded the strongest along PC1, while undifferentiated PLFA and soil pH loaded heavily on PC2 (Figure 4b). Unrestored sites across all age categories grouped together (red oval in Figure 4a). In contrast, restored sites differed by age categories with an elongation along PC1 (2-5 and 5-10 age categories) and PC2 (0-2 age category). The oldest age category (10-22 years) displayed the greatest elongation along PC1 (Figure 4a).

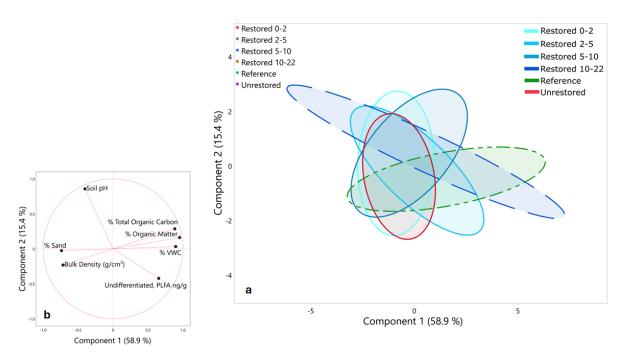


Figure 4. (a) PCA Principal components 1 and 2 (with 95% confidence intervals) grouped by reference sites, unrestored sites, and restored sites by age category; and (b) PCA loading plot.

Changes in soil parameters between restored and unrestored floodplains and recovery with age category Box plots (Figure 5 and 6) and percent change metric (Figure 7) revealed key changes in soil metrics between unrestored and restored reaches with a recovery over time. Variability in soil parameters was found to be higher in restored soils compared to unrestored soils. Interquartile ranges increased with time since restoration for GWC, NH₄⁺, organic matter, sodium and total nitrogen at restored sites (Figure 5). In comparison to physicochemical metrics, PLFA metrics (Figure 6) were more negatively affected by restoration in the initial age categories, with a slower recovery in later age categories.

Percent change (Figure 7) indicated that all metrics other than bulk density, GWC, and NH₄⁺ decreased immediately (red bars and negative values) after restoration (particularly 0-2 year age category), signifying substantial post-restoration disturbance. On average, during the 0-2 year age category, soil parameters decreased by 21% whereas PLFA metrics decreasing by 38.5% compared to the unrestored reaches. Uplift (positive percent change) during the 2-5, year age category was more pronounced with all metrics other than bulk density and NO₃⁻ increasing. Percent change continued to trend positively during the 5–10-year age category with some PLFA metrics decreasing in value (total living microbial biomass, total bacteria, gram positive and actinomycetes). Similar to the 2–5-year age category, all soil metrics increased during the 10–22-year age category except bulk density and NO₃⁻. Soil metrics including GWC, volumetric water content (VWC), SOM, SOC, NH₄⁺ and total nitrogen had consistent positive % change from 2 years post-restoration onwards. Conversely, bulk density and NO₃⁻ exhibited decreasing % change after 2 years post-restoration.

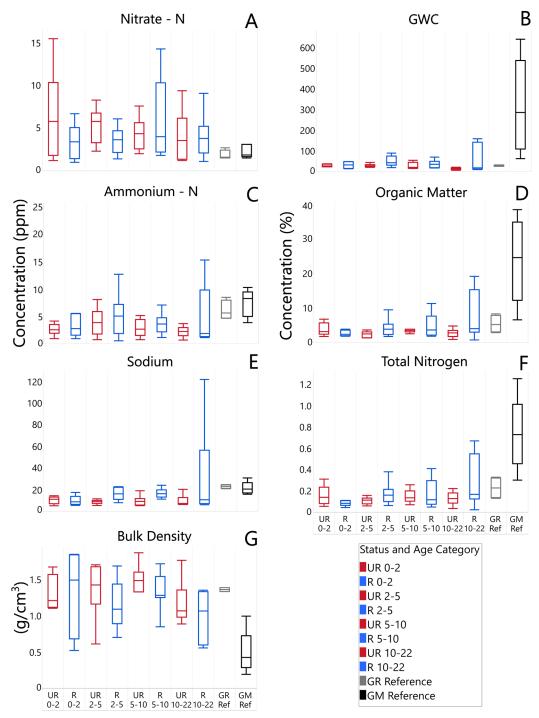


Figure 5. Box and whisker plots of selected soil health parameters that show consistent recovery towards reference conditions over time since restoration-based disturbance. Unrestored sites (UR, red), restored sites (R, blue), Gramies Run (GR) wetland (grey) and Great Marsh (GM, black) show upper and lower quartile ranges with median values shown at the center.

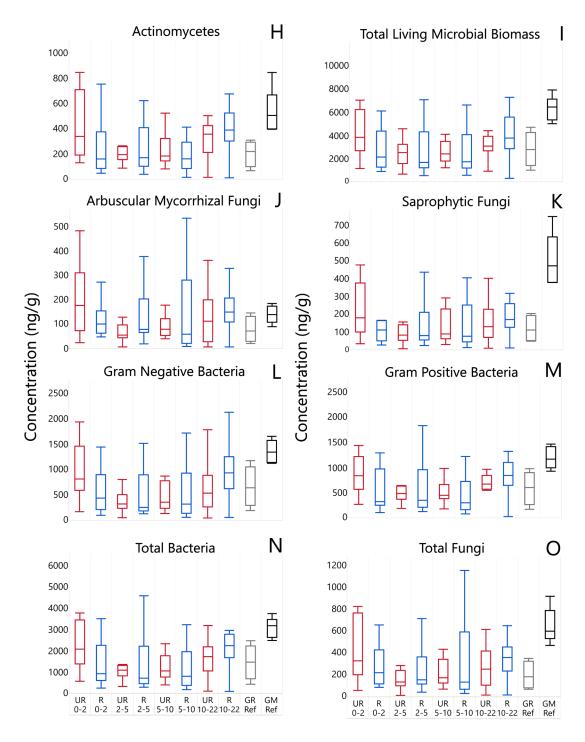


Figure 6. Box and whisker plots of selected soil health parameters that show consistent recovery towards reference conditions over time since restoration-based disturbance. Unrestored sites (UR, red), restored sites (R, blue) Gramies Run (GR) wetland (grey) and Great Marsh (GM, black) show upper and lower quartile ranges with median values shown at the center.

Soil Health Metric	% Chang	ge 0-2	% C	hange 2-5	% Cl	nange 5-10	% Change 10-22	% Change Overall
Bulk Density (g/cm³)		3.06		-11.70		-8.70	-16.23	-9.86
GWC (%)		10.15		64.62		31.93	360.68	87.49
VWC (%)		-4.18		32.75		20.22	170.78	39.75
Organic Matter (%)		-16.62		81.73		34.16	182.58	69.65
NO ₃ - N (ppm)		-47.28		-26.23		41.32	-0.28	-9.96
Na (ppm)		-2.79		115.11		88.24	227.05	117.28
Organic Carbon (%)		-25.63		92.03		70.80	121.94	74.30
NH ₄ ⁺ - N (ppm)		66.89		28.92		29.00	156.87	62.13
Total Nitrogen (%)		-35.88		64.50		18.66	120.89	44.51
Total Living Microbial Biomass PLFA (ng/g)		-36.17		19.89		-14.91	35.25	2.67
Total Bacteria PLFA (ng/g)		-41.86		34.83		-12.10	33.33	4.03
Gram Pos Others PLFA (ng/g)		-38.81		18.09		-24.68	30.53	-1.50
Actinomycetes PLFA (ng/g)		-41.16		21.20		-24.37	22.34	-3.87
Gram Neg Others PLFA (ng/g)		-44.81		68.65		7.36	41.42	13.77
Total Fungi PLFA (ng/g)		-34.13		86.48		10.56	13.18	10.80
Arbuscular Mycorrhizal Fungi PLFA (ng/g)		-35.02		99.55		35.66	0.03	12.96
Saprophytic Fungi PLFA (ng/g)		-33.36		76.98		-6.54	26.38	9.00

Figure 7: Percent change of soil metrics for the four age categories and averaged over the entire study period. Blue bars indicate an increase (improvement, other than bulk density and NO₃-) and red bars indicate a decrease (degradation, other than bulk density and NO₃-) in the soil metric. Measured decreases in bulk density and NO₃- are considered as environmental uplift or improvement.

Soil metrics in restored soils that did not show consistent change compared to unrestored reaches include particle size (sand, silt, and clay) as well as aggregates (micro and macro). Sand, silt and clay did not change noticeably after restoration and did not show consistent change as sites aged. Microaggregates and macroaggregates also did not change consistently over time with microaggregates increasing initially, but then diminishing in concentration as sites aged (5–10-year age category). Mehlich-3 extractable elements generally did not vary with age, but sodium (Na) increased over time (Figure 5). Zinc concentrations initially decreased immediately after restoration (0-2 years) but then markedly increased in all age categories. The sharpest increase in zinc occurred during the 5–10-year age category where concentrations increased by over 400%.

Comparison of restored soil metrics against reference values and time needed to reach reference conditions

Gramies Run and Great Marsh reference sites were significantly different from one another for all values other than NH₄⁺, NO₃⁻, sodium, and arbuscular mycorrhizal fungi (p < 0.05). Except these three parameters, physicochemical and PLFA metrics for Great Marsh exceeded and were more variable than those measured for Gramies Run (Figures 5 and 6) to the extent that maximum concentrations for actinomycetes, total living microbial biomass, saprophytic fungi, and total fungi at Gramies Run were lower than the minimum values recorded at Great Marsh.

AR results (Figure 8) indicate that post-restoration, NH₄⁺, NO₃⁻, and GWC were the only metrics to shift towards reference conditions immediately after restoration during the 0–2-year age category, with all other soil health parameters decreasing or displaying an undesirable increase (bulk density). Based on this AR response and the temporal patterns (Figures 5 and 6), NH₄⁺, NO₃⁻, and GWC were classified into the fast category (Table 2). VWC, bulk density, total nitrogen and organic matter appeared to be more affected by restoration-based disturbance and did not begin to trend towards reference conditions until the 2–5-year age category. Thus, these metrics were grouped under the moderate (2-10) change category in Table 2. PLFA values did not indicate consistent uplift towards reference conditions until 10-20 years after restoration, and fungal communities took the longest to reach recovery (Figure 6). Thus, PLFA metrics were assigned to the slow (> 10) recovery category (Table 2).

Soil Health Metric	AR GR 0	-2	AR GR 2-5	AR GR 5-10	AR GR 10-22	AR G	GR Ref
Bulk Density (g/cm ³)	☆	63.36	☆ -564.05	149.36	-110.48	☆	-298.35
GWC (%)		16294.19	☆ -2506.08	7734.55	302.48	3	527.16
VWC (%)		-55.10	542 <mark>.5</mark> 6	498.14	106.02	2	152.07
Organic Matter (%)		-40.14	58.28	67.94	184.45	5	88.87
NO ₃ - N (ppm)		66.04	39.87	-73.29	0.53	3	16.01
Na (ppm)		-2.22	77.97	59.77	7 163.72	2	83.26
Organic Carbon (%)		-86.44	76.81	163.27	7 989.21	L	171.19
NH ₄ ⁺ - N (ppm)		49.93	50.59	24.24	4 87.78	3	55.82
Total Nitrogen (%)		-82.00	54.93	33.87	7 147.53	3	60.89
Total Living Microbial Biomass PLFA (ng/g)	\bigstar	107.25		☆ 267.45	-376.03	\Rightarrow	- 21 .56
Total Bacteria PLFA (ng/g)	\Rightarrow	115.54	185.68	-134.94	-390.82	☆	<mark>-67</mark> .73
Gram Pos Others PLFA (ng/g)	\bigstar	121.44	☆ -1524.46	-982.78	-396.14	\Rightarrow	15.77
Actinomycetes PLFA (ng/g)	\bigstar	79.70			7 ☆ -65.41	\Rightarrow	12.93
Gram Neg Others PLFA (ng/g)	\bigstar	134.13	99 <mark>.</mark> 10	27.42	1377.70		146.17
Total Fungi PLFA (ng/g)	\bigstar	61.43	307.12	☆ -31.88	-37.15	~ ~	-34.66
Arbuscular Mycorrhizal Fungi PLFA (ng/g)	\bigstar	55.43	552 <mark>.00</mark>	23			-31.55
Saprophytic Fungi PLFA (ng/g)	\Rightarrow	68.24	216.76	☆ 21.00	.126. 7 2	$\stackrel{\circ}{\otimes}$	-39.27
Soil Health Metric	AR GM ()-2	AR GM 2-5	AR GM 5-10	AR GM 10-22	AR C	GM Ref
Bulk Density (g/cm ³)		-4.96	18.67	13.26	5 27.94	1	15.84
GWC (%)		0.99	6.51	3.11	15.29	9	7.24
VWC (%)		-2.01	16.13	10.24	26.43	3	15.26
Organic Matter (%)		-3.12			22.46	n I	9.97
NO ₃ - N (ppm)		3.12	8.38	5.97	7 23.10	, <u> </u>	
		77.71	8.38 49.64				20.79
Na (ppm)					0.79	9	20.79 92.03
		77.71	49.64	-103.61 65.94	0.79 1 181.10)	
Na (ppm)		77.71 -2.47	49.64 86.02	-103.61 65.94 14.29	1 0.79 4 181.10 9 33.28	9	92.03
Na (ppm) Organic Carbon (%)		77.71 -2.47 -5.85	49.64 86.02 11.32	-103.62 65.94 14.29	1 0.79 4 181.10 9 33.28 4 63.92	3	92.03 15.00
Na (ppm) Organic Carbon (%) NH ₄ ⁺ - N (ppm)		77.71 -2.47 -5.85 35.21	49.64 86.02 11.32 30.52	-103.65 65.94 14.29 16.84 4.53	1 0.79 4 181.10 9 33.28 4 63.92 3 24.16	9	92.03 15.00 38.39
Na (ppm) Organic Carbon (%) NH ₄ ⁺ - N (ppm) Total Nitrogen (%)		77.71 -2.47 -5.85 35.21 -9.58	49.64 86.02 11.32 30.52 10.45	-103.65 65.94 14.29 16.84 4.53	1 0.79 4 181.10 9 33.28 4 63.92 3 24.16 0 32.99	2	92.03 15.00 38.39 9.45
Na (ppm) Organic Carbon (%) NH ₄ ⁺ - N (ppm) Total Nitrogen (%) Total Living Microbial Biomass PLFA (ng/g)		77.71 -2.47 -5.85 35.21 -9.58 -70.54	49.64 86.02 11.32 30.52 10.45 15.98	-103.65 65.94 14.29 16.84 4.53 -12.90	1 0.79 4 181.10 9 33.28 4 63.92 3 24.16 0 32.99 2 34.07	9 3 3 5 7	92.03 15.00 38.39 9.45 2.67
Na (ppm) Organic Carbon (%) NH ₄ ⁺ - N (ppm) Total Nitrogen (%) Total Living Microbial Biomass PLFA (ng/g) Total Bacteria PLFA (ng/g)		77.71 -2.47 -5.85 35.21 -9.58 -70.54 110.35	49.64 86.02 11.32 30.52 10.45 15.98 22.20	-103.65 65.94 14.29 16.84 4.53 -12.90 -8.92	1 0.79 4 181.10 9 33.28 4 63.92 3 24.16 0 32.96 2 34.07	33 22 2 77	92.03 15.00 38.39 9.45 2.67 3.89
Na (ppm) Organic Carbon (%) NH ₄ ⁺ - N (ppm) Total Nitrogen (%) Total Living Microbial Biomass PLFA (ng/g) Total Bacteria PLFA (ng/g) Gram Pos Others PLFA (ng/g)		77.71 -2.47 -5.85 3\$.21 -9.58 -70.54 110.35 -98.11	49.64 86.02 11.32 30.52 10.45 15.98 22.20	-103.65 65.94 14.29 16.84 4.53 -12.90 -8.92 -22.39	1 0.79 4 181.10 9 33.28 4 63.92 3 24.16 0 32.99 2 34.07 9 34.19	3 3 5 6 9	92.03 15.00 38.39 9.45 2.67 3.89 -1.75
Na (ppm) Organic Carbon (%) NH ₄ ⁺ - N (ppm) Total Nitrogen (%) Total Living Microbial Biomass PLFA (ng/g) Total Bacteria PLFA (ng/g) Gram Pos Others PLFA (ng/g) Actinomycetes PLFA (ng/g)		77.71 -2.47 -5.85 35.21 -9.58 -70.54 110.35 -98.11 131.94	49.64 86.02 11.32 30.52 10.45 15.98 22.20 17.62 15.99	-103.61 65.94 14.29 16.84 4.53 -12.90 -8.92 -22.39 -18.49 4.45	1 0.79 4 181.10 9 33.28 4 63.92 3 24.16 0 32.96 2 34.07 9 34.19 9 28.40	77	92.03 15.00 38.39 9.45 2.67 3.89 -1.75
Na (ppm) Organic Carbon (%) NH4+- N (ppm) Total Nitrogen (%) Total Living Microbial Biomass PLFA (ng/g) Total Bacteria PLFA (ng/g) Gram Pos Others PLFA (ng/g) Actinomycetes PLFA (ng/g) Gram Neg Others PLFA (ng/g)		77.71 -2.47 -5.85 3\$.21 -9.58 -70.54 110.35 -98.11 131.94 113.79	49.64 86.02 11.32 30.52 10.45 15.98 22.20 17.62 15.99 27.00	-103.61 65.94 14.29 16.84 4.53 -12.90 -8.92 -22.39 -18.49 4.45	1 0.79 4 181.10 9 33.28 4 63.92 3 24.16 0 32.99 2 34.07 9 34.19 9 28.40 5 35.83 5 10.77	77	92.03 15.00 38.39 9.45 2.67 3.89 -1.75 -4.30

Figure 8: Achieved Restoration (AR) of soil health metrics for age categories and averaged over the entire study period. Gramies Run (GR; top) and Great Marsh (GM; bottom) are included separately to illustrate how choosing different reference conditions affects AR values. Blue bars indicate an increase and red bars indicate a decrease in AR values. In some data, soil health metrics were higher in unrestored and restored soils than in reference soils, yielding erroneous AR results; these data are indicated by a yellow star.

Table 2: Classification of soil metrics into rate of recovery categories. Fast recovery began immediately after construction was completed in the 0-2 year age category, moderate recovery began during the 2-10 year age category and slow recovery took >10 years to maintain recovery above unrestored values.

Fast Recovery (0-2 years)	Moderate Recovery (2-10)	Slow Recovery (>10)
Gravimetric Water Content	Volumetric Water Content	Total Living Microbial Biomass
NO ₃ N	Bulk Density	Total Bacteria
NH ₄ ⁺ - N	Total Nitrogen	Gram Positive Bacteria
	Organic Matter	Actinomycetes
		Gram Negative Bacteria
		Total Fungi
		Arbuscular Mycorrhizal Fungi
		Saprophytic Fungi

Time (in years) required for restored soil metrics to reach reference benchmark conditions (separately for Gramies Run and Great Marsh) is presented in Figure 9. Soil conditions at Gramies Run reference wetland were the most achievable, with bulk density, GWC, VWC, actinomycetes, saprophytic fungi and total fungi abundance, meeting, or exceeding reference conditions immediately after restoration (Figure 9). The remainder of the soil health metrics met reference conditions at Gramies Run in less than 25 years, except for arbuscular mycorrhizal fungi abundance and NO₃⁻ (54 and 90 years, respectively, Figure 9). When compared with Great Marsh, most of the soil metrics took 50 years or longer to achieve reference conditions, with GWC, VWC, saprophytic fungi and total fungi taking over a century (top right) to make a full recovery (Figure 9).

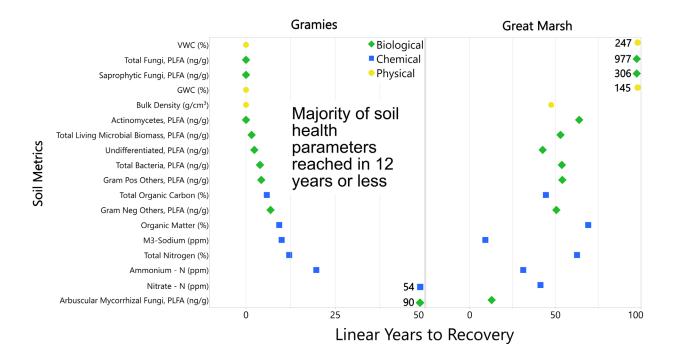


Figure 9: Linear years to recovery for selected soil health metrics when compared against Gramies Run and Great Marsh Reference Wetlands. Biological, chemical, and physical characteristics are shown by green, blue, and yellow points, respectively. Parameter values that exceed the x axis scale are listed with the specific years to recovery (bottom of Gramies panel and top right for Great Marsh panel).

Addressing the hypotheses using results

A full discussion of the results is provided in our published manuscript Galella et al. (2025), included here with this report. A brief response to the hypotheses is provided here.

H1: Floodplain soil health (physical, chemical, and biological characteristics) declines sharply following restoration due to disturbance and compaction.

As indicated in Figure 7, all metrics except GWC and bulk density showed a sharp negative change in the 0–2-year category indicating declining soil health. The slight increase in bulk density is a detrimental soil health response.

H2: Soil health will slowly improve with time following restoration, but individual soil health metrics will differ and recover at different rates.

Figures 8 and 9 and Table 2 clearly show that soil metric recovery follows different trajectories and rates. These results reveal that soil health parameters differ in their level of sensitivity and resilience. These differences however can be leveraged for selection of soil metrics for restoration assessment. The metrics that change fast can be used to assess early gains in floodplain restoration while others could be applied to investigate long-term improvements.

H3: Desired/reference soil health conditions will be achieved after a minimum of 5 years of restoration (rephrased from original proposal following reviewer comments).

The box plots, AR, and the PCA analysis (Figures 4-9) indicate that the "desired"/reference soil health conditions will not be achieved after a minimum of 5 years of restoration. These metrics improve with time and are much better at 22 years post-restoration, but some of them are still unattainable after 22 years of restoration. This suggests that soil health metrics change slowly and will take time. An alternate assessment/interpretation is that we need to reconsider how we define "reference" or "desirable" conditions. Maybe comparing floodplain restorations to undisturbed or "pristine" conditions may not be the best strategy. Future research is needed on how we define "reference" or "desirable" conditions for floodplain restorations. This issue has been raised in CBT pooled monitoring forums and should be addressed by future grant funding announcements.

b. List and describe the regulatory presentations and trainings provided.

The following presentations were made on the results of this study –

- 2025 Annual CBT Symposium in June 2025 (include with the final report)
- 2025 Maryland State Restoration Association (MSRA) presentation in February 2025
- 2024 Stormwater Webinar in April 2024
- c. How can the findings be used for the regulatory community, for practitioners, for researchers, and others?

This study identifies the soil health parameters that reveal a consistent change following restoration. The study determines the parameters that follow a quick, gradual and slow trajectory. These parameters can be then used by restoration agencies to determine the effectiveness of restorations.

d. What future research is needed?

Soil health comparisons such as those performed in this study should be extended to additional sites to strengthen the deductions from this research. We also need to better identify and define "reference" conditions that current restorations can be assessed against.

e. How and when did you provide the data for this project to the Chesapeake Bay Trust?

The data for this project is included with this final report.

f. Provide the citation for the scientific paper in the peer-reviewed literature that was submitted.

Manuscript published -

Galella, J.G., Md. Moklesur Rahman, Eric Moore, Marc Peipoch, Jinjun Kan, Alexis M Yaculak, Matthew Sena, Bisesh Joshi, Sujay S Kaushal, and Shreeram Inamdar. 2025. Soil Health Metrics for Assessment of Floodplain Restorations. Environmental Research Letters, 20 084010.

4. Project Evaluation and Lessons Learned

a. Discuss the project's goal(s) and evaluation(s). Include how the project measured success to meet the goal(s) and to report the outcome(s).

Soil physical, chemical and biological parameters revealed differing changes and trajectories post restoration and across the age categories. This suggests that there are important differences across the soil health metrics and these differences need to be considered and recognized if we are to use the soil health metrics for assessing floodplain restorations.

The other surprise was the large differences in the soil parameters for the reference sites. This indicates that we need to be extremely careful in selecting references. More research needs to be performed to address selection and identification of reference sites for floodplain restoration.

b. Discuss the greatest successes.

The greatest success would likely be the systematic change we observed in some soil health metrics across the restoration age categories. These metrics should be used for evaluating the effectiveness of restorations.

c. Discuss the greatest challenges, including the lessons learned, and potential roadblocks to future progress.

Some of the biggest challenges included in getting permissions and access for sampling of the restored floodplains. Identifying reference sites and getting access to them was also a challenge.

Also, floodplain restorations are performed using varying approaches – NCD, LSR, RSC, etc. Comparing soil health parameters across these diverse approaches is challenging. We limited ourselves to the NCD approach to reduce the variability in restorations, but this also limited the number of sites that were available for us to sample.

d. Based on the results of the project, how would you refine and improve your project or approach in the future?

Based on understanding to date, we would likely want to increase the number of study sites in each restoration age category and particularly in the 10-25 age range (or beyond) where the largest changes are expected to occur. We would also want to increase the number of references sites to sample and study.

If additional funding was available, we would have included additional microbial metrics to assess soil health. Our PLFA data showed that recovery of microbial/biological metrics takes time, and additional metrics would have helped refine this assessment.

e. What advice would you give someone considering a similar project?

Plan ahead in terms of selecting the sites to sample and getting access and permissions lined up. If floodplains with restoration ages exceeding 25 years are available they should be included in the study.

5. Final Project Deliverables

a. Provide all final products (e.g., final report, data, scientific paper, fact sheets, presentation, etc.) and any additional deliverables required per the approved award.

All final products are included – final report, refereed journal publication, fact sheet, and final presentation.

<u>Notes to awardees</u>: 1) products may be externally reviewed and this may lead to revisions that should be factored into the budget and timeline and 2) for questions about your final report, contact the Trust program manager at 410-974-2941.

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Additional guidance and resources are available online at https://cbtrust.org/forms-policies/.

Directions to submit your final report online:

- 1. Sign into your account using this link https://www.GrantRequest.com/SID 1520 and the same username and password as when you applied.
- 2. Once signed in click on the Requirements tab.
- 3. If you do not see your requirement, use the dropdown on the right and in the middle of the page to shift between "Show: New" and "Show: In Progress."
- 4. You should see below the yellow bar your Final Report link.
- 5. Click on the Final Report link and follow the instructions.
- 6. Once complete, click Submit & Review and make sure you have uploaded and entered all of the necessary information.
- 7. If so, click Submit.

To confirm your requirement was successfully submitted use the dropdown to shift between "Show: In Progress" and "Show: Submitted Requirements."

<u>Photos</u>: Upload any photos, digital images, newsletter articles, or press clippings to supplement your written description. These supplemental files can be uploaded into your final report submission under "additional attachments," located on your Chesapeake Bay Trust Online System account.

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