Technical Basis and Treatment Status Report for EnBiorganic Pond Bioaugmentation at Sands Point Preserve, Sands Point, NY

Authors: Frank Piccininni, J.D. MSc, Malcolm Burbank, Ph.D.; Rodney Dickerson, PE
Valerie Vivian-Rogers, MSc

Abstract

The Sands Point Preserve Conservancy has recently undertaken an ambitious effort to restore the ecological structure and function of the preserve. As part of this work, the Conservancy has retained Spadefoot Design and Construction and EnBiorganic Technologies to treat the nutrient pollution and sediment overload in a man-made freshwater pond within the preserve through the bioaugmentation of a consortium of probiotic *Bacillus* bacteria. We began treatment on 8/1/2024 and have measured water and sediment quality along with measurements of sediment depth. Sediment depth measurements were analyzed with Inverse Distance Weighting, which is a spatial interpolation technique. Preliminary results of this treatment are promising in that the bioaugmented bacteria appeared to first digest the nutrient-rich sediment at the interface between the water column and the sediment. Subsequent measurements demonstrated that the bacteria then began to restore water quality. Sediment depth measurements also show the remarkable action of the bioaugmented *Bacillus. An* estimated 1390 cubic yards of sediment—the equivalent of 70 large dumpsters full—has left the system in three months of active treatment. The measured data is consistent with visual observations of the pond; there has been a striking improvement in pond water clarity. This report provides the technical basis for the EnBiorganic technology along with an assessment of treatment status.

Introduction

Eutrophication is a nearly ubiquitous phenomenon caused by overloading of nutrients—primarily nitrogen and phosphorus—into the water column and stored within sediments.[i] This degradation of water and sediment quality happens gradually over time, with nutrient loading emanating from wastewater, the input of fertilizer, sedimentation due to runoff, and nitrogen deposition from the atmosphere.[ii] The deleterious impact of eutrophication includes anoxic and hypoxic conditions, the release of toxins from cyanobacteria blooms, habitat loss for fish and wildlife, and the degradation of recreational opportunities.[iii] These impacts are increasingly exacerbated by climate change as extreme weather becomes more frequent and intense.[iv]

Researchers have long pointed to the potential of bioaugmentation for the reduction of eutrophication in waters. In particular, certain species of *Bacillus* bacteria are noted to have a probiotic effect on pond ecology in digesting existing nutrient loads, including toxins released by cyanobacteria[v], leading to water and sediment quality improvements.[vi] Probiotic *Bacillus* bacteria are ubiquitous in the environment, and are known to be present in soil[vii], sediment[viii], surface waters[ix], air[x], and even within the human body.[xi] The family *Bacillaceae* owes its wide distribution mainly due to their remarkable ability to form endospores; these "dormant" cells last for many years and can withstand extreme heat, radiation, irradiation, chemical contamination, desiccation, and resource limitation.[xii] Within a pond ecosystem, *Bacillus* act as nitrogen fixers, but they also simultaneously nitrify and denitrify, facilitating the nitrogen cycle and enhancing the natural digestion of nitrogen.[xiii] Further, *Bacillus* also digest carbon and solubilize phosphorus, making the latter bioavailable to plants and non-harmful algal species.[xiv]

Conventional bioaugmentation methods show theoretical promise but lack practical scalability due to high costs associated with off-site production, manual deployment, and limitations of spore-state microbes. Fortunately, the patent pending EnBiorganic system overcomes these barriers by automating *Bacillus* bacteria's on-site generation and activation with continuous adaptation and dispersion. This innovation ensures a scalable, autonomous application of active-state *Bacillus* for nutrient reduction and the elimination of cyanotoxins.

The EnBiorganic Unit has been employed in many locations across the United States and Canada to reduce nutrient loads in wastewater treatment plants and surface waters. This report details the methods and preliminary results of a pilot project at the Sands Point Museum and Preserve in Sands Point, NY.

Methods:

Site Description and Project Background

The Sands Point Preserve is physically situated on the coast of Long Island's north shore within what is referred to as Manhasset Neck. Manhasset Neck was formed by the retreating glaciers, which left behind unconsolidated sediments and layers of sand and clay confining units.[xv] Currently, the site is comprised of a rich variety of habitats including, meadows, maritime hardwood forests, coastal bluff communities, and the Long Island Sound.

The Sands Point Preserve was undeveloped until it was purchased by the son of the railroad robber baron Jay Gould in 1900. It was then sold to wealthy miner, Daniel Guggenheim in 1917, who summered at the estate until his death in 1930. After being donated to the Institute of Aeronautical Sciences in 1942, it was then sold to the United States Navy in 1946. The Navy used the site as a weapons testing facility. At one point, it housed both the Navy staff and an additional 800 civilian staff members. In 1971, most of the original Gould estate was acquired by the County of Nassau for recreational purposes. In 2008, the Sands Point Preserve Conservancy was granted the authority to manage the affairs of the facility.[xvi]

Given the varied historic and current land uses at the preserve, the remaining patchwork of intact native habitat is fragmented and threatened by the continued proliferation of invasive species. Fortunately, within the last few years, The Sands Point Preserve Conservancy has undertaken an ambitious ecological restoration project, which includes the removal of more than 5 acres of invasive species removal and the restoration of a native meadows and forested ecosystems. As part of this effort, the Sands Point Preserve Conservancy has sought out solutions to restore the ecological health of the constructed freshwater wetland at the preserve.

Like many waterbodies on Long Island and beyond, the pond at the Sands Point Preserve suffers from eutrophication, with sediment depths of the pond exceeding 2-feet deep in places. This organic sediment is rich in polluting nutrients, including nitrogen and phosphorus. The ¾ of an acre man-made pond is lined by concrete and is fed by the aquifer via a pumped well. The pond is also receiving drainage from a 1 ¼ acre parking lot, along with overland flow and sheet flow from the surrounding watershed. See **Appendix 1** for a map of the pond and associated built infrastructure, and **Appendix 2** for a map of the site's topography.

Monitoring and Analysis of EnBiorganic Bioaugmentation

To monitor the efficacy of the bioaugmentation at Sands Point Preserve, we have devised a protocol to measure water and sediment quality parameters. Specifically, we have sent samples for a 3rd party laboratory analysis for the following parameters:

Sediment	**Reference Method**	**Limit of Quantification**
Total Organic Carbon (mg/kg)	L. Kahn	100
Nitrate as N (mg/kg)	EPA 300.0	0.05
Nitrite as N (mg/kg)	EPA 300.0	0.05
Ammonia Nitrogen as N (mg/kg)	SM 4500-NH3 D	0.05
Phosphorus, Total (mg/kg)	SM 4500-P B5/E	0.02
Total Kjeldahl Nitrogen (mg/kg)	SM 4500-N Org D	0.4
Total Nitrogen (mg/kg)	SM 4500-N B	0.05
Total Organic Nitrogen (mg/kg)	SM 4500-N	0.1
Total Solids (%)	SM 2540D-2015	10
WATER:		
Nitrate as N (mg/L)	EPA 300.0	0.05
Nitrite as N (mg/L)	EPA 300.0	0.05
Ammonia Nitrogen as N (mg/L)	SM 4500-NH3 D	0.05
Phosphorous, Total (mg/L)	SM 4500-P B5/E	65.5
Total Kjeldahl Nitrogen (mg/L)	SM 4500-N Org B	131
Total Nitrogen (mg/L)	SM 4500-N B	0.05
Total Organic Carbon (mg/L)	SM 5310B-2014	100
Total Suspended Solids (mg/L)	SM 2540D-2015	10
Total Organic Nitrogen (mg/L)	SM 4500-N	0.1

In addition to "grab samples" for sediment and water quality, we established nine permanent sample locations to measure the depth of the sediment. Sediment depth was measured using a "Sludge Judge", which is a cylindrical shaped tool designed to measure the depth of settleable solids in a water column. See **Appendix 3** for a photograph of our field technician using the sludge judge.

For each of the nine plots established in a grid across the pond, we took four sludge judge samples and averaged them to provide a representative sample. The plots were then georeferenced and subjected to Inverse Distance Weighting (IDW) for analysis. IDW is an exact, deterministic technique that operates under the single assumption that values close to the sampled points are more similar than those far away.[xvii]

Preliminary Results and Discussion

Pond nutrient and carbon dynamics is an exceedingly complex subject that depends on a host of feedback cycles within the pond (i.e., autochthonous factors)[xviii] and variables emanating from outside of the pond (allochthonous factors).[xix] These factors interact across a variety of spatial and temporal scales to produce a system that is difficult to predict and analyze. Nevertheless, despite these challenges, preliminary results of this pilot study suggest a strong impact from our efforts at bioaugmentation of the Sands Point Pond.

When reviewing the data, it is important to note that the microbial generator employed produces approximately 1,500 gallons of active state microbiology each day, and that these bacteria continue to multiply rapidly within the pond ecosystem. As a consequence, we are adding a large amount of organic nitrogen, phosphorus, and carbon to the system in the form of *Bacillus* bacteria itself. Any measured value in water or sediment quality parameters includes the bioaugmented bacterial load. Given this fact, we have observed that water and sediment measures tend to fluctuate when we augment wastewater or surface waters; measured parameters can initially increase until the *Bacillus* begin to dominate the system and have a chance to catch up and digest the nutrient loads. Even though we continue to add nutrients, the data from this pilot project show a trend of microbial digestion of nutrients that is consistent with our expectations for how the

Bacillus act on the system.

The consortium of *Bacillus* bacteria that we utilized exhibit "chemotaxis", in that they detect gradients of resources and move towards more favorable environments.[xx] In the initial post-discharge sample, bioaugmented bacteria appeared to preferentially target the interface between the sediment and the water column, as we measured a 80% decrease in organic nitrogen in the sediment within the first two weeks of the beginning of our bioaugmentation efforts (See **Appendix 4**). The decrease in organic nitrogen was coupled with a measured increase of sediment ammonia. The increase in sediment ammonia is likely attributable to several processes driven by the *Bacillus*, including the fact that when organic nitrogen is broken down, a resulting byproduct is ammonia.[xxi] Further, carbon within the sediment increased during the initial period after we started the bioaugmentation, which may simply reflect the loading of bioaugmented bacteria settling into the nutrient rich sediment layer.

Conversely, with the first data point post-bioaugmentation, we measured an increase in concentration of total organic nitrogen and phosphorus within the water column. Such an observation could reflect the increased load associated with the bioaugmentation and the fact that the bacterial action on the sediment causes a disturbance, promoting the release of nutrients from the sediment into water column. The observed increase in phosphorus and nitrogen could also be, in part, driven by atmospheric deposition as there was a fair amount of rainfall prior to the sample (See **Appendix 4**).

Although *Bacillus* bacteria are facultative anaerobes,[xxii] aerobic respiration is the preferred pathway as it is more efficient. As such, after resources were depleted within the sediment/water interface, instead of moving into the anoxic/deeper sediment, the *Bacillus* appeared to target the nitrogen load within the water column. Between August 16 and October 7, organic nitrogen loads within the water column began to decrease. These measured decreases were contemporaneous with a visible change in water quality, as an apparent algal bloom—newly starved of nutrients—appeared to die back. See **Appendix 5** for photographs of the pond clarity. Like planting a tree, constructing a bioswale, or installing an advanced wastewater treatment system, bioaugmented *Bacillus* inhibits cyanobacteria by reducing the availability of nutrients that fuel harmful algal blooms. The visible changes in water quality were also reflected in the data for total suspended solids, which was non-detectable by the lab in our latest sample (**Appendix 4**).

As the water quality continued to improve, the concentration of organic nitrogen and ammonia in the sediment began to rebound slightly (**Appendix 4**). Such an observation is likely explained, in part, by the die-back of cyanobacteria populations which settle out to the bottom upon their death and release organic nitrogen and, after decomposition, ammonia. Despite the uptick in organic nitrogen and ammonia, sediment carbon levels have continued to plummet, suggesting that the bioaugmented *Bacillus* is continuing to work on the system (**Appendix 4**). Over time, as nutrient loads in the water column reach a healthy level, we expect the *Bacillus* to return to the pond sediment layer, "fluff it up", and begin to digest the load in the deeper/anoxic sediment layer—either aerobically or anaerobically.

It is also important to consider the fate of phosphorus in the system. Unlike nitrogen and carbon, phosphorus does not volatilize (i.e., turn into a gas) and leave the system. Instead, phosphorus is solubilized and made bioavailable to plants, non-harmful algae, and is sequestered within the cells of the *Bacillus* bacteria and other native microorganisms.[xxiii] As such, the fairly steady phosphorus numbers reflect the fact that it does not leave the system but, because it is used by beneficial macro- and microorganisms, is less available to fuel harmful algal blooms (**Appendix 4)**.

Notably, measured water quality improvements are coming at a time when pond ecosystems typically experience a natural uptick in nitrogen concentration.[xxiv] During the fall, due to decreased metabolism within the pond and shoreline ecosystem (e.g., from plants and other microorganisms), nitrogen that was previously bound up within pond microbes and plants are released back into the water column.[xxv] In the pond at Sands Point, however, the *Bacillus* continues to voraciously digest the nitrogen load. It will continue to do so throughout the winter, albeit at a slower rate as the temperature continues to fall.

Further evidence of the EnBiorganic unit working as expected can be found in the data from the "sludge judge". The baseline data demonstrates that the flow of the system—from the source of the groundwater feeding the pond to the overflow/discharge point—caused a buildup of sediment towards the discharge point (**Appendix 6**). Just two weeks into the treatment, however, the models demonstrated that the pond sediment has begun to "fluff up" throughout most of the system. As the Bacillus breaks down the loosely compacted sediment layers, the sediment begins to fluff up, increasing the pore volume and allowing more oxygen to diffuse through it. The one notable exception is the area where pond sediment was originally the deepest where we presume the bacterial load is settling out due to the eddies caused by morphology of the pond and due to chemotaxis; the area that was originally the deepest lost approximately nine inches of sediment. Overall, in the August sample the minimum sediment depth increased markedly from 3.7" to 9.25" whereas the maximum sediment depth decreased from 25.99" to 19.25".

As the bioaugmentation treatment continued, we have measured a remarkable decrease in overall sediment depth. In November, the hot spots of activity/fluffing appear to be towards the on the North end of the pond, where the bioaugmented bacteria are presumably working on the deeper sludge and where, perhaps, some of the "fluffed up" sediment from other parts of the pond are settling due to the pond circulation. In contrast, the sediment depth in the rest of the pond appears to be decreasing. Overall, the average decrease of sediment is 1.17", which amounts to an estimated reduction of 1396 cubic yards of sediment having been digested in just three short months.

This assessment provides valuable insights into the current water and sediment conditions at Sands Point. The results underscore the importance of continued monitoring to measure the efficacy of the treatment and the long-term health and sustainability of this valuable resource. We look forward to updating this report as we continue to collect samples.

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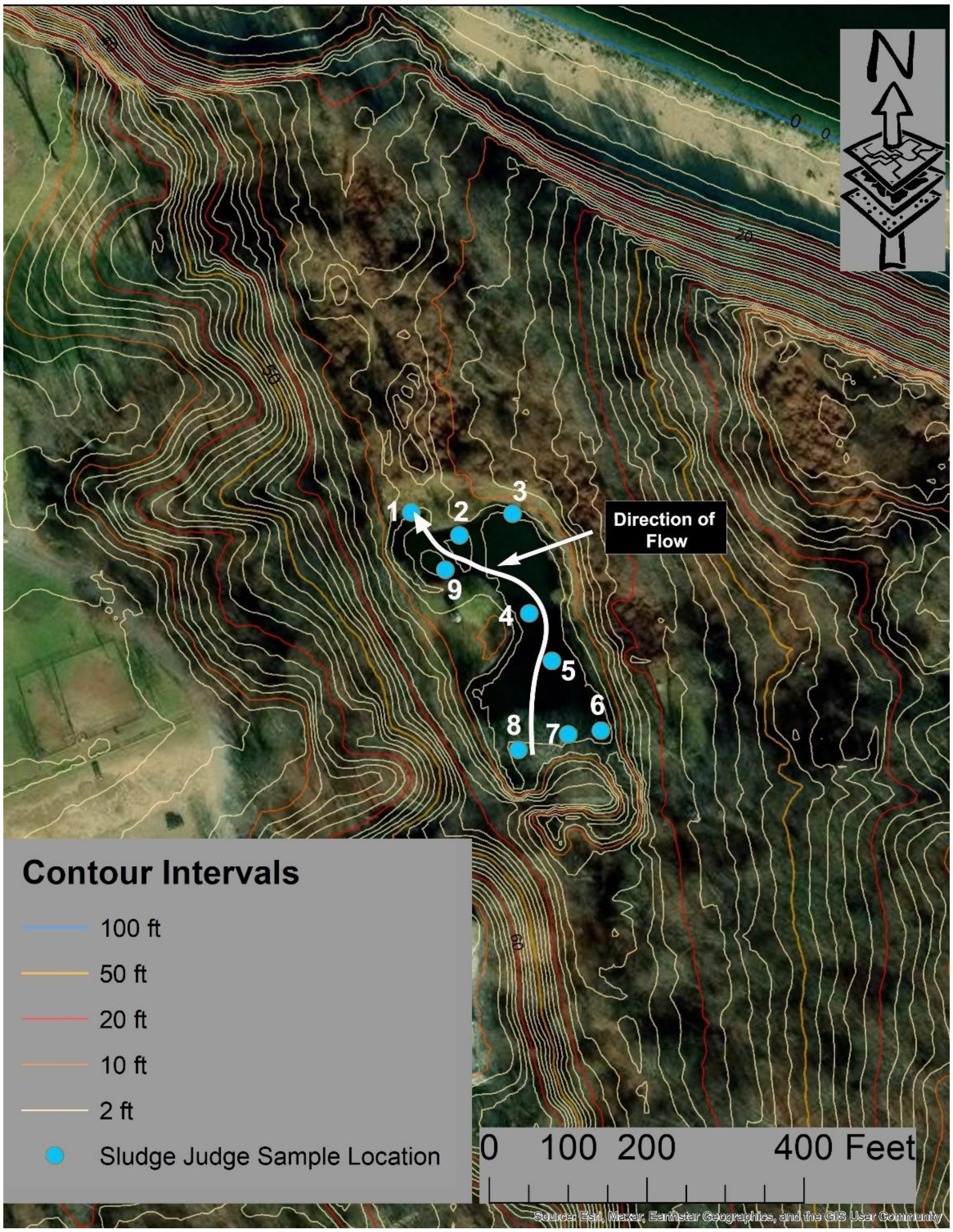
SPADEFOOT DESIGN & CONSTRUCTION

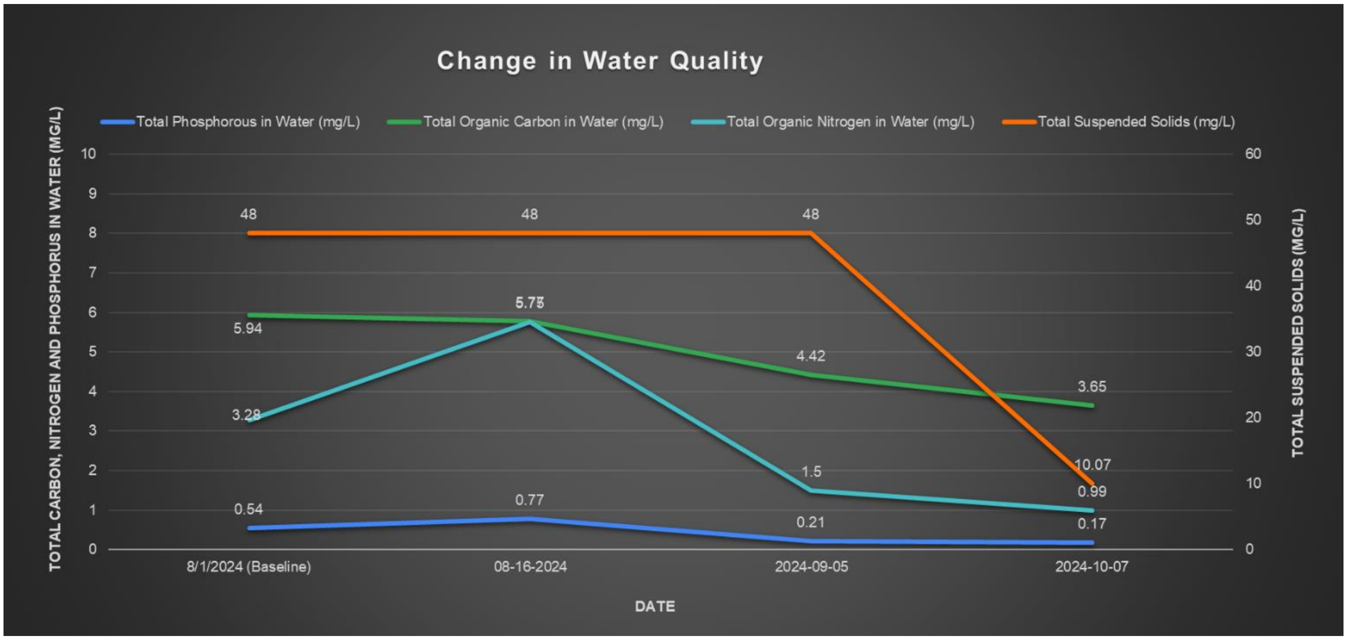
Appendix 1-A Map of the Pond and Associated Infrastructure

Appendix 2- A Topographic Map of the Project Location

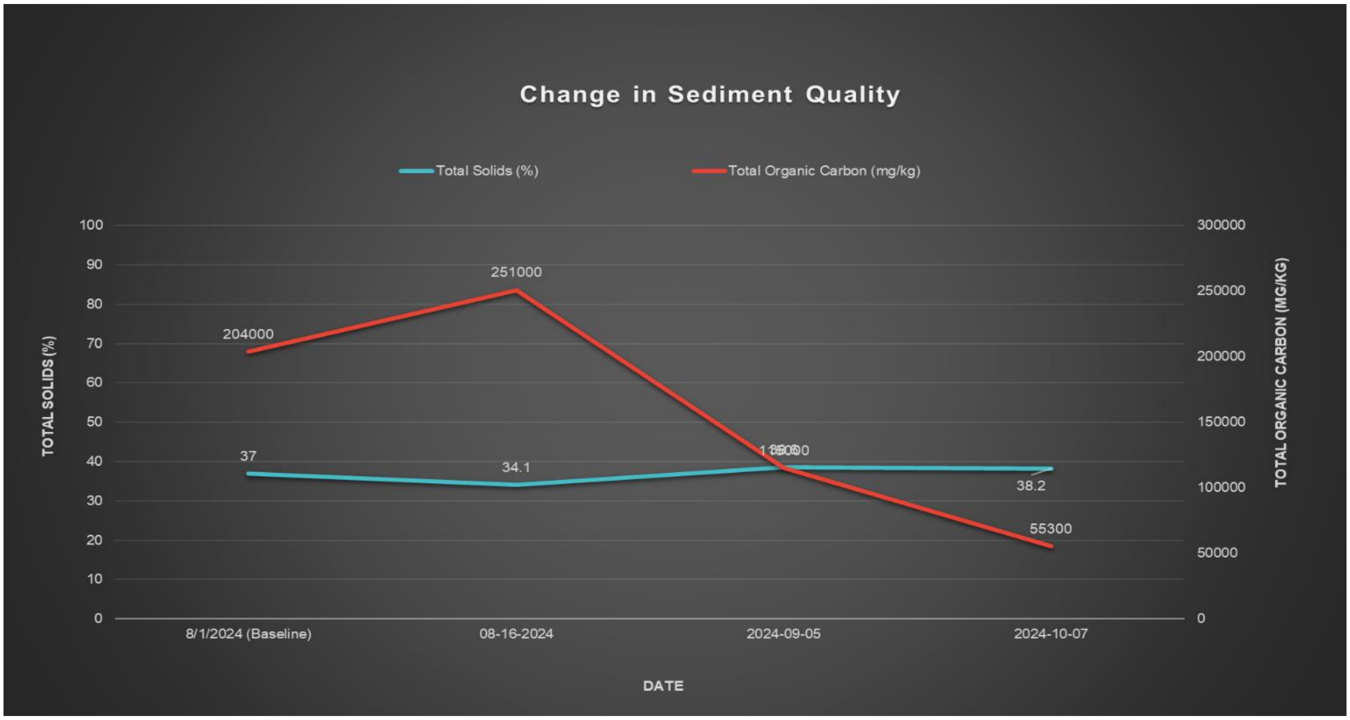


Appendix 3- A Photograph of Our Field Technician Using a Sludge Judge to Record Pond Sediment Depth

Appendix 4- Sediment and Water Quality Data




Change in Sediment Quality
Ammonia Nitrogen as N (mg/kg)
Phosphorus, Tot (mg/kg)
Total Organic Nitrogen (mg/kg)
AMMONIA-N (MG/KG)
TOTAL PHOSPHORUS AND TOTAL ORGANIC NITROGEN (MG/GKG)
20
18
16
14
12
10
8
6
4
2
0
7000
6000
5000
4000
3000
2000
1000
0
6450
10.5
2970
7.49
12.5
3340
1380
240
0
408
309
247
8/1/2024 (Baseline)
08-16-2024
2024-09-05
2024-10-07
DATE


Change in Sediment Quality
Total Solids (%)
Total Organic Carbon (mg/kg)
TOTAL SOLIDS (%)
TOTAL ORGANIC CARBON (MG/KG)
100
90
80
70
60
50
40
30
20
10
0
300000
250000
200000
150000
100000
50000
0
204000
251000
55300
37
34.1
38.2
8/1/2024 (Baseline)
08-16-2024
2024-09-05
2024-10-07
DATE

Appendix 5—Photographs Documenting the Change of Water Clarity. The Top Photograph is from September 19, 2024 and the Bottom Photograph is from September 23, 2024.

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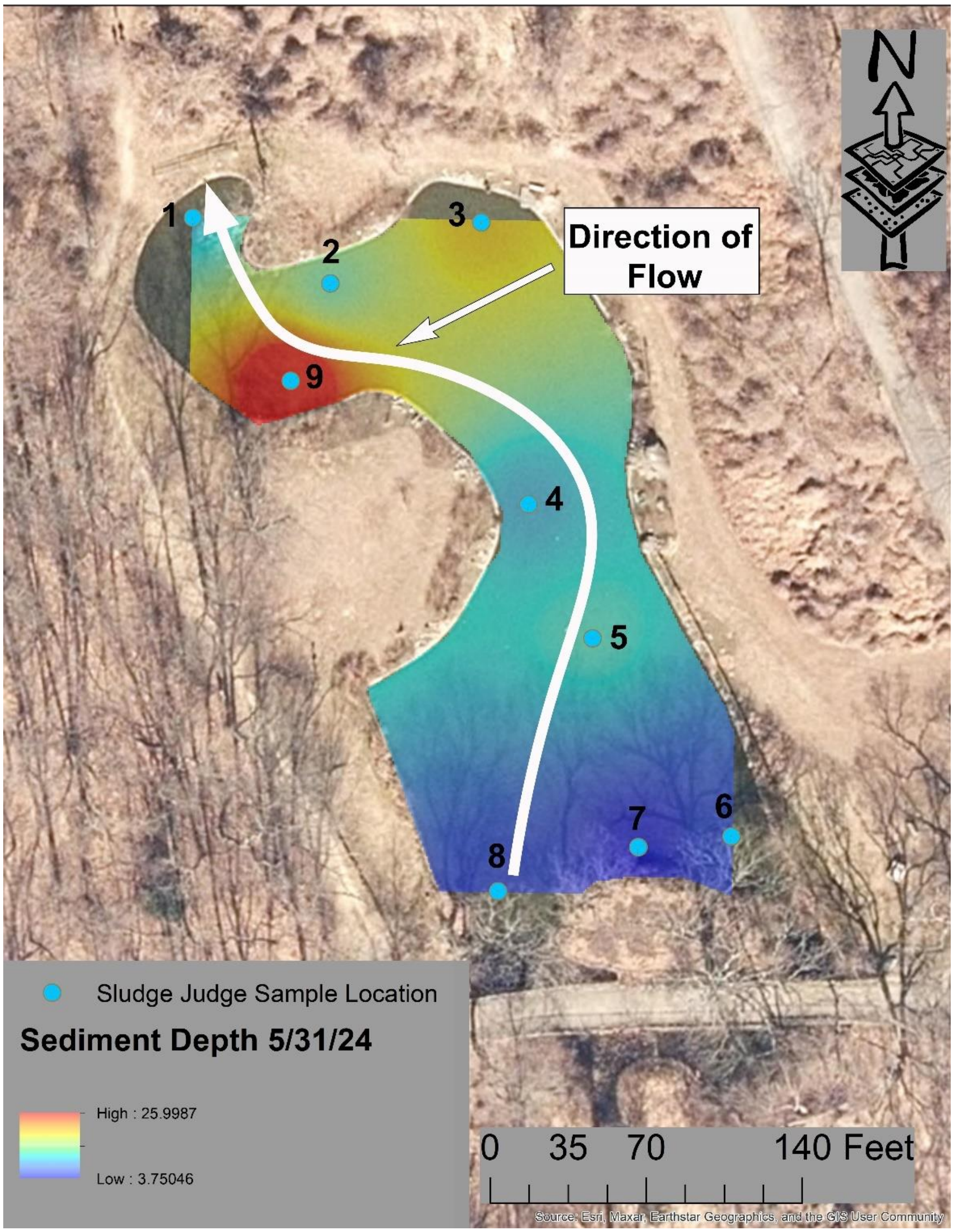
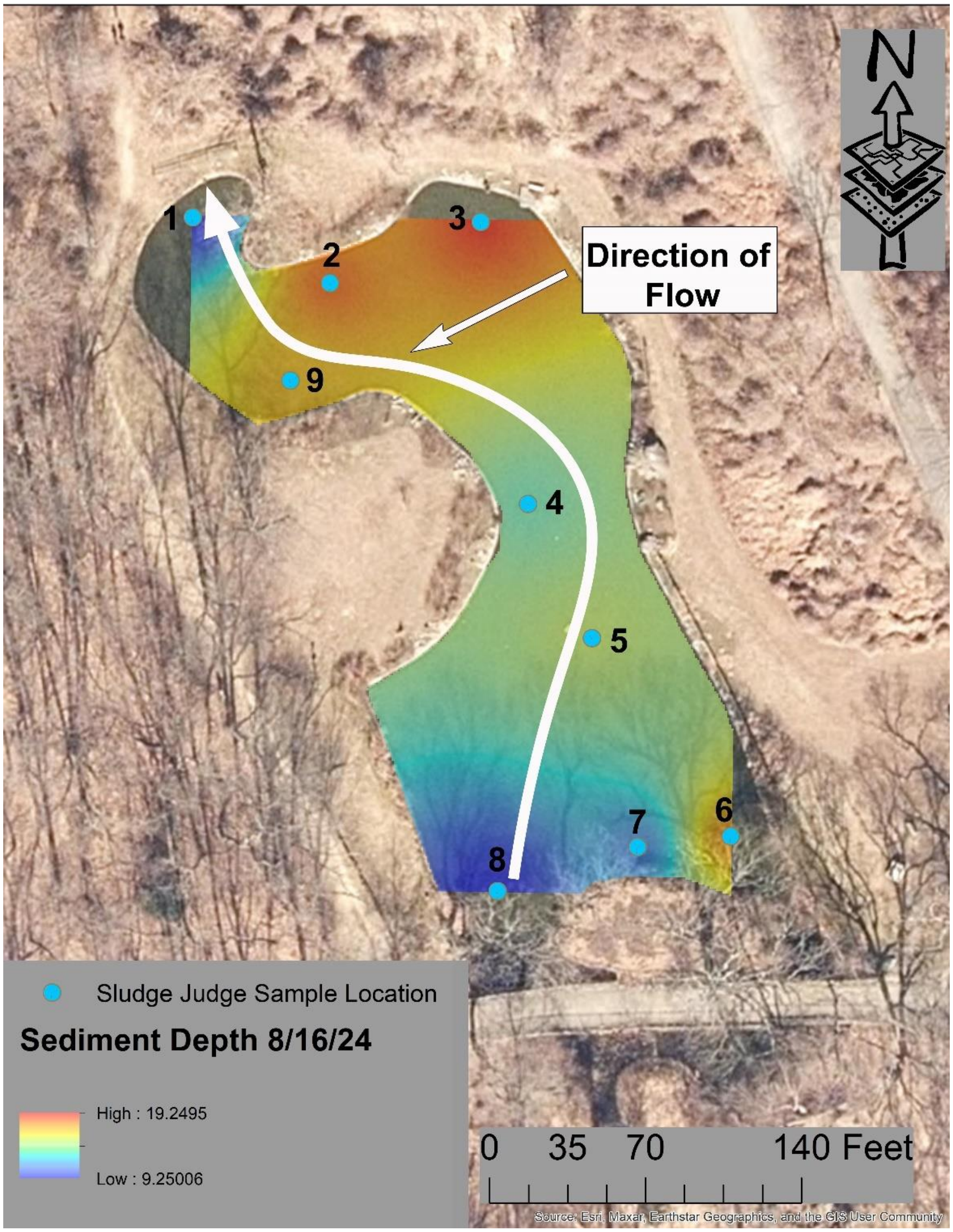
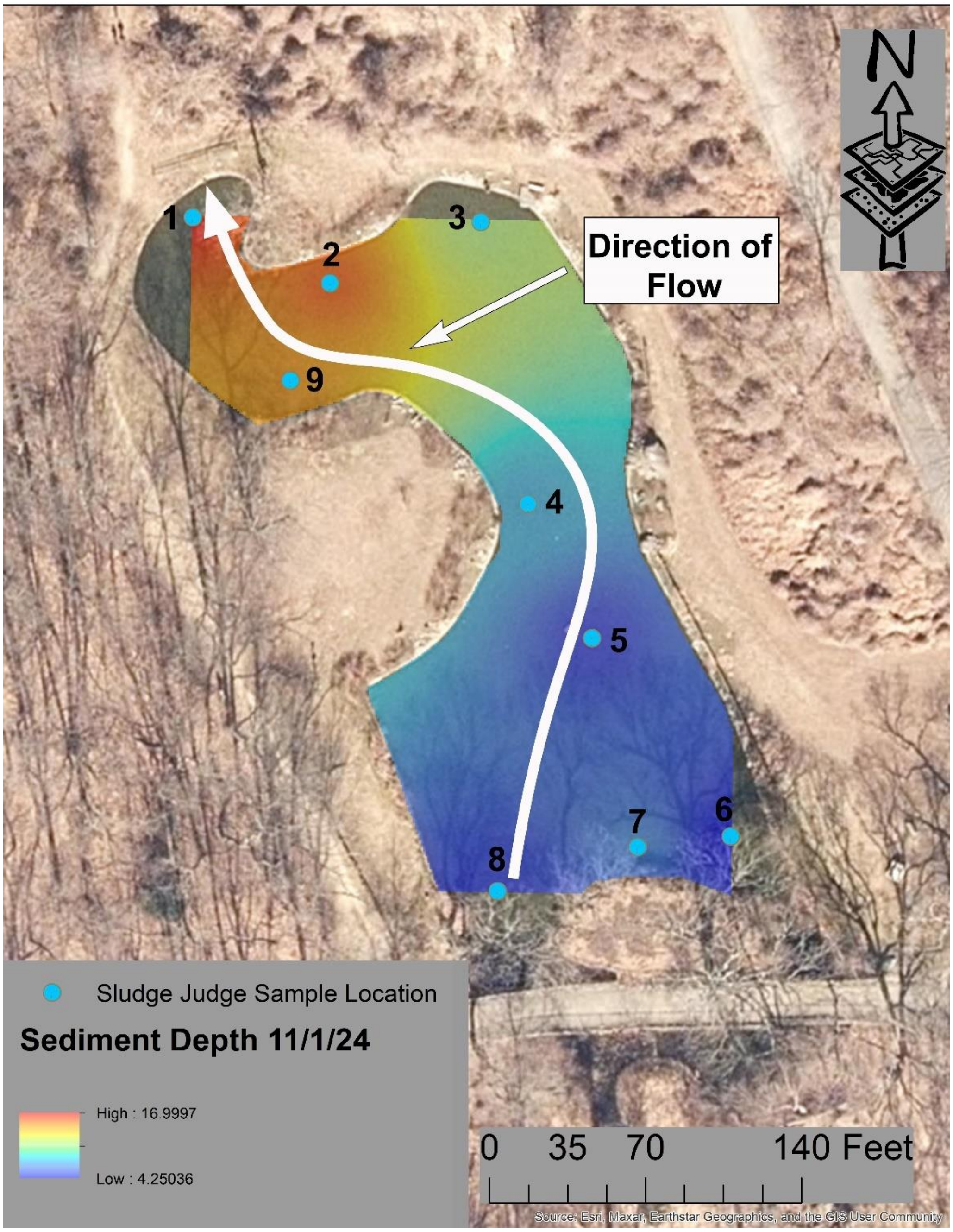
SPADEFOOT DESIGN & CONSTRUCTION

Appendix 6-Spatial Interpolations (Inverse Distance Weighting for Sludge Judge Data)





N
1
2
3
Direction of Flow
9
4
5
6
7
8
Sludge Judge Sample Location
Sediment Depth 8/16/24
High : 19.2495
Low : 9.25006
0 35 70 140 Feet
Source: Esri, Maxar, Earthstar Geographics, and the GIS User Community


N
1
2
3
Direction of Flow
9
4
5
6
7
8
Sludge Judge Sample Location
Sediment Depth 11/1/24
High : 16.9997
Low : 4.25036
0 35 70 140 Feet
Source: Esri, Maxar, Earthstar Geographics, and the GIS User Community

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[i] *See* Khan and Mohammad, "Eutrophication: Challenges and Solutions. In: Ansari, A., Gill, S. (eds) Eutrophication: Causes, Consequences and Control", Springer, Dordrecht (2014). https://doi.org/10.1007/978-94-007-7814-6_1;.

[ii] *See generally* Monti et al., "Nitrogen load estimates from six nonpoint sources on Long Island, New York, from 1900 To 2019", *U.S. Geological Survey, Scientific Investigations Report 2024–5047* (2024) (providing comprehensive estimates of the source of nitrogen load on LI); *see also* Ashton et al., "Invasive species accelerate decomposition and litter nitrogen loss in a mixed deciduous forest" 15 *Ecological Applications* (2005): 1263-1272 (finding that invasive plant species increase nitrogen loss from terrestrial ecosystems).

[iii] *See* Anderson et al., "Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences", *Estuaries* 25 (2002):704-726.

[iv] See Piccininni, "Adaptation to Climate Change and the Everglades Ecosystem", *Environmental Claims Journal* 26 (2014): 63, 80–82 (reviewing the projected impact of climate change on rates of eutrophication); *see also* Piccininni and Perret, "SMPIL Comments on the Long Island Nitrogen Action Plan", *Environmental Claims Journal* 28 (2016): 346-352 (discussing Long Island's nutrient reduction initiative in the context of climate change and land use); Kuhnert et al., "Effects of weather extremes on the nutrient dynamics of a shallow eutrophic lake as observed during a three-year monitoring study", 14 *Water* (2022): 1-16 (measuring the impact of extreme weather on temporal patterns of

water quality impairment).

[v] *See* Kansole and Lin, "Microcystin-LR Biodegradation by Bacillus sp.: Reaction Rates and Possible Genes Involved in the Degradation", 8 *Water* (2016): 1-19;

[vi] *See* Hlordi et al., "The use of Bacillus species in maintenance of water quality in aquaculture: A review", 18 *Aquaculture Reports* (2020): 1-12 (describing the promise of *Bacillus* bacteria in maintaining water quality); *see also* James et al., "Bacillus as an aquaculture friendly microbe", 29 *Aquaculture International* (2021): 323–353 (noting the ways in which bacillus contribute to ecosystem functioning, including by benefiting other co-existing microbes). Incredibly, *Bacillus* bacteria has been shown to degrade other harmful toxins including petroleum contamination, heavy metals, and PFAS/PFAO. *See* Nedoroda et al., "Analysis of Petroleum Biodegradation by a Bacterial Consortium of Bacillus amyloliquefaciens ssp. Plantarum and Bacillus subtilis" 22 *Journal of Ecological Engineering* (2021): 36-42; Dai et al., Potential impact of bacteria on the transport of PFAS in porous media", *Water Resources* (2023): 1-28; Wrobel et al., "Bioremediation of heavy metals by the genus Bacillus", 20 *International Journal of Environmental Research and Public Health* (2023): 1-17.

[vii] *See* Saxena et al., "Bacillus species in soil as a natural resource for plant health and nutrition", 128 *Journal of Applied Microbiology* (2019): 1583-1594 (noting that *Bacillus* species are common in soil ecosystems and provide many beneficial functions including facilitating nutrient cycling and conferring stress tolerance to plants); *see also* Rigamonte et al., "The role of mycorrhization helper bacteria in the establishment and action of ectomycorrhizae associations", *Brazilian Journal of Microbiology* 41 (2010) 832-840 (referencing *Bacillus* as "helper bacteria" that work with fungal mycorrhizae to drive plant growth and healthy ecosystem functioning); Nyugen et al. "Distribution of the bacterial genera *Pseudomonas* and *Bacillus* within ectomycorrhizae of Gray Birch (*Betula populifol*ia Marsh.) in a northern New Jersey forest", 73 Bios (2002): 91-97 (describing mutualistic symbiotic relationships between plants and bacteria).

[viii] *See* Fortu and Antai, "The dynamics of bacterial population during growth and decomposition of phytoplankton in a tropical productive pond water ecosystem", *African Journal of Microbiology Research* 7 (2013): 5625-5631 (discussing the role of *Bacillus* in pond sediments).

[ix] *Id.*

[x] Striluk et al., "The effect of season and terrestrial biome on the abundance of bacteria with plant growth-promoting traits in the lower atmosphere", 32 *Aerobiologia* (2016): 1-13 (finding an abundance of *Bacillus* in the atmosphere to be related to the nature of the underlying terrestrial biome).

[xi] Hong et al. "Bacillus subtilis isolated from the human gastrointestinal tract", 160 *Research in Microbiology* (2009): 134-143.

[xii] *See generally* Ines Mandic-Mulec et al., "Ecology of Bacillaceae", 3 *Microbiology Spectrum* (2015): 1-24 (reviewing the remarkable adaptability of *Bacillus* bacteria, including its ability to facilitate ecosystem processes and promote

plant health). Perhaps even more remarkably, species of *Bacillus* have been shown produce two types of cells, i.e., sporulating cells and non-sporulating cells. Under nutrient limitation, the group of bacteria exhibit a social behavior where sporulating cells produce toxins to kill the non-sporulating cells. The dead nonsporulating cells are then consumed by their sporulating "sister cells" to delay sporulation, which is a resource intensive process. This allows the population to take advantage if nutrients become available. *See* Gonzalez-Pastor, "Cannibalism: a social behavior in sporulating *Bacillus subtilis*", *Microbiology Review*, 35 (2011):415-424; Gonzalez-Pastor et al., "Cannibalism by Sporulating Bacteria", *Science*, 301 (2003): 510-513; Nandy and Venkatesh, "Effect of Carbon and Nitrogen on the Cannibalistic Behavior of *Bacillus subtilis*", *Applied Biochemistry and Biotechnology*, 151 (2008): 424-432.

[xiii] He et al., "Understanding and application of *Bacillus* nitrogen regulation: A synthetic biology perspective" 49 *Journal of Advanced Research*, 49 (2023):1-14 (reviewing the ways in which *Bacillus* drives the nitrogen cycle); Kim et al., "Aerobic nitrification-denitrification by heterotrophic Bacillus strains", 96 *Bioresource Technology* (2005): 1897-1906 (using laboratory experiments to demonstrate simultaneous nitrification and denitrification).

[xiv] *See* Li et al., "*Characteristics of inorganic phosphate-solubilizing bacteria from the sediments of a eutrophic lake"*, 16 *International Journal of Environmental Research and Public Health* (2019): 1-15; Stulke and Hillen, "Regulation of carbon catabolism in *Bacillus* species" 54 *Annual Review of Microbiology* (2000): 849-880.

[xv] *See generally*, Stumm et al., Hydrogeology and Extent of Saltwater Intrusion on Manhasset Neck, Nassau County, New York, Water-Resources Investigations Report 00-4193 (2002).

[xvi] *See* Sands Point Preserve Conservancy, http://sandspointpreserveconservancy.org/about/mission-history/

[xvii] See ArcMap Model Documentation, https://pro.arcgis.com/en/pro-app/latest/tool-reference/3d-analyst/how-idw-works.htm#:~:text=Inverse%20distance%20weighted%20(IDW)%20interpolation,of%20a%20locationally%20dependent%20variable.

[xviii] *See* Wang et al., "Seasonal pattern of nutrient limitation in a eutrophic lake and quantitative analysis of the impacts from internal nutrient cycling", *Environmental Science and Technology* 53 (2019): 13675-13686 (noting that nutrient loads stored in the sediment influence seasonal patterns of nitrogen and phosphorus limitation).

[xix] Monti et al., "Nitrogen load estimates from six nonpoint sources on Long Island, New York, from 1900 To 2019", *U.S. Geological Survey, Scientific Investigations Report 2024–5047* (2024).

[xx] *See* Rao et al., "The three adaptation systems of Bacillus subtilis chemotaxis", 16 *Trends Microbiology* (2008):480-487 (discussing the chemical pathways *Bacillus* use to "see" their environment); *see also* Garrity and Ordal, "Chemotaxis in *Bacillus subtilis*: How bacteria monitor environmental signals", *Pharmacology and Therapeutics* 68 (1995): 87-104 (noting the swimming behavior exhibited by *Bacillus* bacteria in response to chemical gradients).

[xxi] *See* Robinson and Tartar, "The decomposition of protein substances through the action of bacteria", *The Journal of Biological Chemistry* 30 (1917): 135-144.

[xxii] Hartig and Jahn, "Regulation of the Anaerobic Metabolism in *Bacillus subtilis*", 61 *Advances of Microbial Physiology*

(2012): 195-216.

[xxiii] *See* Li et al., "*Characteristics of inorganic phosphate-solubilizing bacteria from the sediments of a eutrophic lake*", 16 *International Journal of Environmental Research and Public Health* (2019): 1-15 (discussing *Bacillus* bacteria as having an ability to release phosphorus from the sediment).

[xxiv] Markou et al., "Water quality of Vistonis Lagoon, Northern Greece: seasonal variation and impact of bottom sediments", 210 *Desalination* (2007): 83-97 (measuring an uptick in nitrogen concentration in the fall);

[xxvxxv] Suratman et al., "Seasonal variability of inorganic and organic nitrogen in the North Sea", 610 *Hydrobiologia* (2008): 83-98; Murray et al., "Nitrogen wet deposition stoichiometry: the role of organic nitrogen, seasonality, and snow", 160 *Biogeochemistry letters* (2022): 301-314.