Design of Stormwater BMPs for Surface and Groundwater Protection Based on Site-Scale Soil Properties: Phase I

BDV24-977-43

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The CTS Bold and Gold® Filtration Media tested herein is a proprietary product patented by UCF.

APPROXIMATE CONVERSIONS TO SI UNITS

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
LENGTH				
in	inches	25.4	millimeters	mm
ft	feet	0.305	meters	m
yd	yards	0.914	meters	m
mi	miles	1.61	kilometers	km

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
AREA				
in^2	square inches	645.2	square millimeters	mm^2
ft^2	square feet	0.093	square meters	m^2
yd^2	square yard	0.836	square meters	m^2
ac	acres	0.405	hectares	ha
mi^2	square miles	2.59	square kilometers	km^2

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
VOLUME				
fl oz	fluid ounces	29.57	milliliters	mL
gal	gallons	3.785	liters	L
ft^3	cubic feet	0.028	cubic meters	m^3
yd^3	cubic yards	0.765	cubic meters	m^3
NOTE: volumes greater than 1000 L shall be shown in m^3				

APPROXIMATE CONVERSIONS TO SI UNITS

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
MASS				
oz	ounces	28.35	grams	g
lb	pounds	0.454	kilograms	kg
T	short tons (2000 lb)	0.907	megagrams (or "metric ton")	Mg (or "t")

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
TEMPERATURE (exact degrees)				
°F	Fahrenheit	5 (F-32)/9 or (F-32)/1.8	Celsius	°C

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
ILLUMINATION				
fc	foot-candles	10.76	lux	lx
fl	foot-Lamberts	3.426	candela/m^2	cd/m^2

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
FORCE and PRESSURE or STRESS				
lbf	pounds force	4.45	Newtons	N
lbf/in^2	pounds force per square inch	6.89	kilopascals	kPa

APPROXIMATE CONVERSIONS TO SI UNITS

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
LENGTH				
mm	millimeters	0.039	inches	in
m	meters	3.28	feet	ft
m	meters	1.09	yards	yd
km	kilometers	0.621	miles	mi

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
AREA				
mm^2	square millimeters	0.0016	square inches	in^2
m^2	square meters	10.764	square feet	ft^2
m^2	square meters	1.195	square yards	yd^2
ha	hectares	2.47	acres	ac
km^2	square kilometers	0.386	square miles	mi^2

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
VOLUME				
mL	milliliters	0.034	fluid ounces	fl oz
L	liters	0.264	gallons	gal
m^3	cubic meters	35.314	cubic feet	ft^3
m^3	cubic meters	1.307	cubic yards	yd^3

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
MASS				
g	grams	0.035	ounces	oz
kg	kilograms	2.202	pounds mass	lb
Mg (or "t")	megagrams (or "metric ton")	1.103	short tons (2000 lb)	T

APPROXIMATE CONVERSIONS TO SI UNITS

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
TEMPERATURE (exact degrees)				
°C	Celsius	1.8C+32	Fahrenheit	°F

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
ILLUMINATION				
lx	lux	0.0929	foot-candles	fc
cd/m^2	candela/m^2	0.2919	foot-Lamberts	fl

SYMBOL	WHEN YOU KNOW	MULTIPLY BY	TO FIND	SYMBOL
FORCE and PRESSURE or STRESS				
N	Newtons	0.225	pounds force	lbf
kPa	kilopascals	0.145	pounds force per square inch	lbf/in^2

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16. Abstract

Phase I of this research developed experimental methodologies to quantify and compare the rates and means of nitrogen and phosphorus transformations through diverse soil profiles. Six sites were selected for study from areas of distinct geologic history in Florida. Soils from the six sites were chosen to represent a gradient of clay and organic matter content, two constituents that were hypothesized to influence nutrient remediation potential in soils. A commercially-available engineered infiltration media, Biosorption Activated Media (BAM), was also tested for comparison. Physical, chemical and biological attributes of the six soils and BAM were fully characterized. Each soil was subjected to extensive laboratory and field testing to parametrize hydraulics and nutrient transformation rates within the soil profile, including when exposed to simulated stormwater hydrology and nutrient loads. All soils effectively removed inorganic nitrogen and retained phosphorus; however, there were notable performance differences related to soil characteristics. The data largely support project hypotheses, that clay content and organic matter content positively influence nitrogen and phosphorus remediation. However, soils containing either the highest or lowest organic matter and clay contents did not perform as well as soils that contained moderate amounts of both. BAM also decreased concentrations of nitrogen and phosphorus, though with slightly less efficiency than soils. Though the research is preliminary, results underscore that nutrient cycling potential of project site soils should be understood before soils are amended for the purpose of remediating stormwater nutrients. This Phase I study suggests that nutrient remediation potential may be predictable based on soil properties.

17. Key Word	18. Distribution Statement
Soils, nutrients, stormwater BMPs, roadway runoff, Biosorption Activated Media, nitrogen, nitrate, phosphorus, microbiome	No restrictions

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EXECUTIVE SUMMARY

Much of Earth's nutrient cycling takes place in soils. Characteristics of soils control physical, chemical, and biological processes that determine rates of nutrient fluxes, storage, or transformation. As remediation of excess nutrients in stormwater runoff is one function of stormwater Best Management Practices (BMPs), the soil profile constitutes one of the most important factors of BMP design. Variation observed in BMP effectiveness (e.g., why one BMP design works effectively in one place and not another) can often be explained by variations in the soil profile, either through direct means or by a soil's influence on hydraulics of stormwater flow through the vadose (unsaturated) zone. The objective of this research is to identify soil characteristics most strongly related to nutrient (nitrogen, N, and phosphorus, P) remediation within the soil profile and to apply this understanding to improve both efficiency and cost-effectiveness of stormwater BMP design.

Phase I of this project developed experimental methodologies to quantify and compare the rates and means of nitrogen and phosphorus transformations through diverse soil profiles. Six sites were selected for study from areas of distinct geologic history in Florida. Soils from the six sites were chosen to represent a gradient of clay and organic matter content, two constituents that were hypothesized to influence nutrient remediation potential. A commercially-available engineered infiltration media, Biosorption Activated Media (BAM), was also tested for comparison. Physical, chemical and biological attributes of the six soils and BAM were fully characterized across sites and with profile depth. Each soil was subjected to extensive laboratory and field testing to parameterize hydraulics and nutrient transformation rates within the soil profile, including when exposed to simulated stormwater hydrology and nutrient loads.

Soils consistently decreased concentrations of inorganic nitrogen and phosphorus from simulated runoff. Mean efficiency of nitrate (NO_3^-) removal from soils ranged from 75%–90%, mean decrease of ammonium (NH_4^+) concentration ranged from 31%–90% and mean decrease of total P concentration ranged from 65%–98%. BAM also generally decreased concentrations of N and P, though with slightly less efficiency than soils; mean reductions of NO_3^- and total P by BAM were 60% and 21%, respectively. BAM was the only media tested that was a source of inorganic N. BAM released small amounts of NO_3^- following prolonged exposure to runoff, but increased NH_4^+ concentrations by a mean 43%. Removal of total N varied between soils due to an experimental effect of organic N released by decomposing root matter in soil cores taken from highly vegetated areas. Nitrogen cycling (N removal and N release) were largely balanced in soils immediately after extraction from field sites. However, after being exposed to conditions similar to a stormwater infiltration basin (repeated infiltration of stormwater with a consistent external load of N), N release and N removal were no longer balanced for some soils. The directionality of the imbalance varied within the tested soils; N removal potential increased in some soils but decreased in the soils with the highest clay and highest organic matter.

Though all soils effectively removed inorganic N and retained P, there were notable performance differences related to soil characteristics. The data largely support project hypotheses, that clay content and organic matter content positively influence N and P remediation. However, soils containing either the highest or lowest organic matter and clay contents did not perform as well as soils that contained both. The best nutrient remediation performance overall was observed in two soils that both contained moderate and comparable amounts of clay and organic matter. Results of this preliminary study suggest that soils with clay

content ranging from 5%–8% and organic matter content in the range of 400 g/kg–500 g/kg in the surface 10 cm and 60 g/kg–300 g/kg in 10–30 cm layers were associated with the greatest nutrient remediation potential. Furthermore, soils with pH over 7 and metal content in the range of 10^2 mg/kg–10^3 mg/kg were observed to retain phosphorus at high levels. Importantly, though clay content was similar, the two soils had different overall grain size distributions, which suggests that the organic matter may be as important as the mineral size class when predicting the nutrient remediation potential of a soil. Engineered media, including the BAM tested in this study, do not typically contain organic matter.

Overall, results underscore that properties of project site soils should be understood before soils are amended for the purpose of nutrient remediation. While this preliminary work offers a promising direction for identifying soils that require amendment, thus justifying the material and environmental costs of soil replacement, longer term study under more natural environmental conditions is needed to predict the nutrient remediation potential of heterogeneous soils.

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CHAPTER 1. **Introduction**

As the Floridian population continues to grow and urbanize, there is a vital need for improved transportation networks, and urban stormwater management is an increasingly critical concern. While water quality regulation has traditionally focused on point discharges, distributed loads of non-point source nutrients (nitrogen and phosphorus) from urban and roadway runoff to surface and groundwater resources can cause water quality degradation. Best management practices (BMPs) for managing urban runoff, such as stormwater infiltration basins and vegetative filter strips along highways, are implemented to intercept and infiltrate runoff at the source and reduce the concentration of non-point source pollutants before stormwater reaches receiving surface or groundwaters. However, there is wide variability in nutrient remediation effectiveness of BMPs.

Excess nitrogen (N) in waterways can lead to enhanced productivity, algal growth, and eutrophication. Some inorganic forms of nitrogen (nitrate, NO_3^- and nitrite, NO_2^-) are associated with human health impacts and are therefore strictly regulated in drinking water. Nitrogen removal from stormwater can occur through two basic pathways: (1) biotic processing or (2) temporary storage. In the first pathway, microbial communities or vegetation utilize the N-containing compounds for energy-gain or growth and subsequently transform N into other forms that may be less available (e.g., organic-bound N) or exit the system (harmless N_2 gas). In the second pathway, N-containing compounds are temporarily retained in the soil or soil porewater. Over longer time periods, they may subsequently be biotically processed or released into groundwater. As phosphorus (P) tends to be limiting in many natural aquatic ecosystems, its contribution from stormwater can have outsized impact to eutrophication and algal production in waterbodies. Unlike nitrogen, which eventually can be converted through degradation into a benign gas (N_2) and eventually leave the system, phosphorus remains in either soluble or particulate form, becoming metabolized by organisms and plants, mobilized by groundwater or stormwater, or buried in sediment and soils. The form of N and P (chemical speciation) plays a major role in their bioavailability.

Because many of the processes that change the concentration and/or form of stormwater N and P occur within the shallow soil profile, soils constitute one of the most important factors controlling rates of nutrient transformation within BMPs. Observed variation in BMP effectiveness (e.g., why a BMP design works effectively in one place and not another) can often be explained by variations in the soil profile, either through direct means or by a soil's influence on hydraulics of stormwater flow through the vadose (unsaturated) zone. By influencing the mechanics of infiltration and controlling the movement and residence time of water and dissolved solutes within the soil profile, soils influence the composition and growth of the soil microbiome, as well as the contact time of infiltrated stormwater with the soil and microbes. Soil characteristics vary by geographic location as well as depth. To standardize BMP effectiveness, native soils can be enhanced or replaced by engineered filtration media. However, this will only increase nutrient remediation performance of the BMP if the engineered media are more effective at remediating nutrient pollution as compared to the native site soils. This research is intended to facilitate BMP design by isolating the soil parameters most strongly related to nitrogen and phosphorus removal or sequestration. If a soil's potential for nutrient remediation can be predicted, the decision of whether to amend the soil with an engineered media can be supported and justified.

Phase I of the project developed experimental methodologies to compare rates and means of nitrogen and phosphorus transformations through diverse soil types to isolate the media properties associated with desired nutrient remediation. A commercially-available engineered infiltration media, Biosorption Activated Media (BAM), was also tested for comparison. Experimental methods were developed to preliminarily address the following research questions:

1. What is the nutrient removal/retention potential of unaltered Florida soils of heterogeneous properties?
2. What are the material properties of Florida soils that are most strongly related to phosphorus sequestration and nitrogen removal?
3. How does nutrient remediation in BAM compare to soils of variable properties?

This research tests the hypothesis that soil properties can be an indicator of a soil's potential for phosphorus and/or nitrogen remediation. Initial project hypotheses posited that organic matter content (OMC) and clay content are soil constituents of particular consequence to nutrient remediation. Florida soils of heterogeneous properties along a gradient of OMC and clay content were selected and tested for potential nutrient remediation.

Projects goals were facilitated by the following research tasks:

Task 1: Soils selection, collection and experimental set up

Task 2: Soils testing

Task 3: Draft Final Report

Task 4: Final Report.

Interim reporting on project tasks and all research data are permanently hosted on the UCF STARS data repository (https://stars.library.ucf.edu/fdot/).

CHAPTER 2. **Field Sampling**

Field sites were selected from areas of diverse geologic history in Florida, including clay-containing soils from within the Hawthorn Formation in Alachua and Marion Counties and native sand and organic matter (OM)-dominated soils from the main campus of University of Central Florida (UCF) in Orange County where there is not a confining layer between the surface and Floridan aquifer. Preliminary soil core samples were collected and analyzed to ensure that study soils represented a gradient of characteristics wherein each tested soil was sufficiently unique. BAM CTS media (5% clay, 10% tire crumb, and 85% sand by volume) was procured from a licensed commercial vendor (Environmental Conservation Solutions, LLC).

2.1 Site Selection and Description

Preliminary soil samples were characterized across the soil profile for OMC, clay content, and texture with depth (0-30 cm). This preliminary work resulted in the selection of six diverse soils to be studied: three soils collected from the UCF main campus in Orange County and three soils from the Hawthorn Formation in Alachua and Marion Counties (Table 2.1 and Figure 2.1). When sites were confirmed, permits (UCF R-2021-10, FDEP 02062210) were obtained to proceed with field sampling.

Table 2.1. Study site locations and soil taxonomy based on soil survey; descriptions of soil texture, organic matter and clay content based on field investigation.

Soil	County	Location	Map Unit; Taxonomy	Soil description
1	Orange	Bayhead Swamp UCF Main Campus Orlando, FL	42—Sanibel muck; Sandy, siliceous, hyperthermic Histic Humaquepts	High OMC soil; little mineral content, low clay
2	Orange	Degraded Cypress Dome UCF Main Campus Orlando, FL	99—Water; N/A	Sandy soil; moderate OMC, low to moderate clay
3	Orange	Pine Flatwoods (Unit 11) UCF Main Campus Orlando, FL	44—Smyrna-Smyrna; Sandy, siliceous, hyperthermic Aeric Alaquods	Sandy soil; low OMC, low clay
4	Marion	Riparian Cypress Swamp Ray Wayside Park Silver Springs, FL	19—Bluff sandy clay; Fine-loamy, siliceous, superactive, hyperthermic Typic Endoaquolls	Silty soil; moderate OMC, moderate clay
5	Marion	Cypress Swamp Edge Ray Wayside Park Silver Springs, FL	19—Bluff sandy clay; Fine-loamy, siliceous, superactive, hyperthermic Typic Endoaquolls	Silty soil; low to moderate OMC, moderate clay
6	Alachua	Seasonal Wetland O'Leno State Park Gainesville, FL	21—Newnan sand; Sandy, siliceous, hyperthermic Oxyaquic Alorthods	Silty soil; low to moderate OMC, moderate to high clay

Soil 1 was located on the edge of a bayhead swamp wetland that is consistently flooded with relatively low impact from anthropogenic sources. Site vegetation was predominantly woody, such as sweetgum (*Liquidambar styraciflua*), sweetbay magnolia (*Magnolia virginiana*), and sweet gallberry holly (*Ilex coriacea*), but did include herbaceous vegetation mostly arrow arum (*Peltandra virginica*). Soil 2 was located within a cypress dome swamp that is seasonally inundated; samples were taken on the infrequently flooded dome edge. The dominant site woody vegetation consisted of bald cypress (*Taxodium distichum*), pond cypress (*Taxodium ascendens*), and buttonbush (*Cephalanthus occidentalis*). The dominant herbaceous vegetation included dogfennel (*Upatorium capillifolium*) and rattail fescue (*Festuca myuros*). Soil 3 was located within as upland pine flatwoods ecosystem that receives semi-regular prescribed burns. The dominant vegetation at this site included a mixture of woody and herbaceous vegetation, including longleaf pine (*Pinus palustris*), saw palmetto (*Serenoa repens*), and muhly grass (*muhlenbergia capillaris*).

Figure 2.1. Study site locations. Soils 1-3 were collected from the main campus of UCF in Orange County; Soils 4-5 were collected from Ray Wayside Park in Marion County; Soil 6 was collected from from O'Leno State Park in Alachua County.

Within the Hawthorn Formation, Soils 4 and 5 were located inside the boundaries of Ray Wayside Park in Marion County within a frequently inundated riparian wetland dominated by woody vegetation such as bald cypress (*Taxodium distichum*) and cabbage palm (S*abal palmetto*) but did include a monoculture of herbaceous panic veldtgrass (*Ehrharta erecta*). Soil 6 was located within the bounds of O'Leno State Park in Alachua County. The site was a seasonally inundated depression wetland with dominant herbaceous vegetation of ricefield flatsedge (*Cyperus iria*), and sparse woody vegetation such as buttonbush (*Cephalanthus occidentalis*), river birch (*betula nigra*), and longleaf pine (*Pinus palustris*).

2.2 Collection of Soil Samples

Field soil cores were collected for soil characterization utilizing the push-core technique to manually capture an intact soil profile for subsequent sample collection by depth using a 7 cm diameter, 50 cm long polycarbonate core tube. At each site, five replicate cores were sampled within a 2 m radius of the initial core. Live vegetation was cleared at the surface of each core extraction site but roots were left intact. Once an intact soil core was obtained, it was extruded from the core tube in the field, and the 30-cm soil core was divided with a knife into 10-cm increments (depth of 0-10 cm, 10-20 cm, 20-30 cm) using a premeasured tube collar. The knife used to separate segments within a core was sterilized between cuts using ethanol to limit contamination between core samples and depths. During separation of soil segments, sub-samples of approximately 10 g were removed using a sterile syringe. All samples were placed in air-tight plastic bags and stored on ice for transport to the laboratory. After return from the field, samples were immediately stored at 4°C, while sub-samples for DNA analysis were stored at -20°C.

Soil cores were collected for flow-through study in a similar manner using polycarbonate tubes with a 10-cm diameter and 90-cm length. The push-core technique was employed to collect an intact soil profile that extended from the surface to a depth of approximately 30-40 cm. Five replicate cores were sampled within a 2 m radius of the initial core. After extraction, both ends of the cores were temporarily capped with mechanical stoppers for transport to the lab. Within 24 hours of collection, cores were uncapped at the bottom and fitted with a double layered mesh to prevent soil loss during the flow-through study. During collection of the flow-through cores, the large core tubes were unable to penetrate Soil 3 to the required depth due to compressive forces. Soil 3 was therefore extracted in 10-cm increments to a depth of 30 cm using plastic shovels, keeping the soil at each depth within separate containers. The soil was homogenized for each of the three 10 cm layers, before being packed manually into the core tubes, using water and pressure to compact the soil to the same bulk density observed in the field (~1.18 g/cm^3). The remainder of soils were successfully extracted as intact cores, preserving the structure of the soils. As an engineered media, BAM was not extracted from the field and was manually packed into the core tubes to a uniform dry density of approximately 1.64 g/cm^3. This was completed with additions of BAM in 5 cm increments, measured by mass and compacted with pressure from an extrusion tube, until each core had a soil height of 35 cm.

CHAPTER 3. **Initial Soil Characterization**

Soils provide a natural filter for nutrients during stormwater infiltration. Soil surfaces serve as adsorption sites for P retention and microbial processing of N and P within the soils can convert nutrient forms (e.g., to organic matter) or in the case of denitrification, remove N. Metals such as calcium, magnesium, and aluminum form precipitates with P that become incorporated within the soil matrix. Transition metals such as iron and manganese provide adsorption sites for P and other metals and play important roles during reduction-oxidation reactions. All of these processes contribute to the remediation of nutrient concentrations in incoming stormwater and all are dependent on the physical, chemical and biological characteristics of the soil. Soil samples extracted from the field were analyzed to determine physical properties, metals, and phosphorus speciation, and to characterize the soil microbiome and biogeochemical properties that influence nitrogen cycling. Each analysis was completed for each of the five replicate samples of each soil type, each depth (0-10 cm, 10-20 cm and 20-30 cm). Five replicate samples of BAM were analyzed similarly for comparison.

3.1 Soil Physical Properties

Soil physical properties influence the mechanics of infiltration and control the movement and residence time of water and dissolved solutes within the soil profile. These mechanisms influence the composition and growth of the soil microbiome, as well as the contact time of infiltrated stormwater with the soil and microbes. This study tests the hypothesis that soil physical properties can be an indicator of a soil's potential for phosphorus sequestration and/or nitrogen removal. Physical properties of field moisture content (θ_f), bulk density (ρ_b), porosity (η), OMC, grain-size distribution, and percentage of silt and clay were analyzed (Tables 3.1 and 3.2). Field water content was determined by comparing field wet vs. dry mass of soils, immediately after being taken from the field and after drying the samples overnight in a mechanical convection oven at 100°C, respectively (Eq. 3.1). Soil dry mass was used to calculate bulk density (ρ_b), and porosity (η) by Eqs. 3.2 and 3.3:

$$\theta_f = \frac{m_{wet} - m_{dry}}{m_{wet}} \times 100 \quad \text{(Eq. 3.1)}$$

$$\rho_b = \frac{m_{dry}}{V} \quad \text{(Eq. 3.2)}$$

$$\eta = 1 - \frac{\rho_b}{\rho_p} \quad \text{(Eq. 3.3)}$$

where m_{wet} is the wet mass of the soil from the field, m_{dry} is the oven-dried mass of the soil, V is the volume of the soil sample (84.8 cm^3), and ρ_p is the particle density (2.65 g/cm^3). OMC was determined by loss on ignition. Crucibles were ignited in a furnace and placed in a desiccator to cool before being massed. Five-gram soil samples were placed in each crucible and fired in a ThermoFischer Scientific Thermolyne Benchtop muffle furnace for 16 hours at 550 °C. After ignition, the crucibles were massed, and OMC was calculated as Eq. 3.4:

$$\text{OMC (g/kg)} = \frac{m_{dry} - m_{fired}}{m_{dry}} \times 10^3 \quad \text{(Eq. 3.4)}$$

where m_{fired} is the mass of the sample after ignition in the muffle furnace.

Grain size distributions and proportions of sand, silt and clay were determined through sieve analysis, hydrometer testing, and laser particle size analysis. The Wentworth geological scale (Wentworth, 1922) was adopted to define particle size classes. Organic fractions were removed from all samples, such that grain size distributions are specific to the mineral fractions of soil. The five replicate samples of each soil depth (and five replicate BAM samples) were combined to form composite samples per soil and depth for sieve and hydrometer testing. Dry sieve analysis (ASTM D6913) utilized a set of stacked Gilson Company sieves of decreasing mesh sizes that separated the larger particles from the smaller diameter particles through use of a RO-TAP shaker (RX-29 model). The mass of soil collected on each sieve was recorded. Subsamples were taken from each sieve layer to determine OMC in each size class and mass calculations were adjusted accordingly to reflect mineral fractions only. Hydrometer analysis (ASTM D7928) was used to determine the proportion of particle sizes finer than sand. Soils were sieved to < 2 mm and ignited in the muffle furnace to remove organic matter. A 50 g sample was soaked for 16 hours in 125 mL of a 4% hexametaphosphate solution. The sample was transferred into a baffled dispersion cup and mechanically dispersed in a soil dispersion mixer (Gilson Company model SA-14) for 60 seconds. The sample was then transferred to a 1000 mL hydrometer cylinder, and the water level was filled with distilled water to a marked line. A rubber stopper was placed over the top and the cylinder was agitated for 60 s before being placed on the counter. An ASTM 152H hydrometer was used to take readings at designated time periods of 2, 5, 15, 60, 120, 240, 480, and 1440 minutes. The temperature was noted at each reading.

Each replicate soil sample and depth were tested individually using a CILAS 1090 laser particle analyzer. Soil samples were sieved to below 2 mm and ignited in the muffle furnace to remove organic matter before testing. One (1) gram samples of soil were added to 50 mL beakers, along with 40 mL of 5% hexametaphosphate solution. These samples were allowed to disperse at least 16 hours before being mechanically suspended with a magnetic stir plate until all sediments were fully suspended. 10 mL of the suspended sediment slurry was removed from the beaker using a pipette and the sample was analyzed using the CILAS laser particle size analyzer. To ensure reliability, the test was repeated until error between at least two replicate analyses was within 2%.

Soil 1, the high OMC soil, did not contain enough mineral material to create reliable grain size distributions based on the mineral fraction. Hydrometer and laser testing were not possible for Soil 1. The grain size curve obtained by dry sieving the full sample, including OMC, is shown for comparison in Figure 3.1; however, the curve is truncated because hydrometer testing was not possible for Soil 1. There are not high-fidelity methods for estimating particle size distributions of such high organic-content soils, therefore the curve shown should be considered approximate.

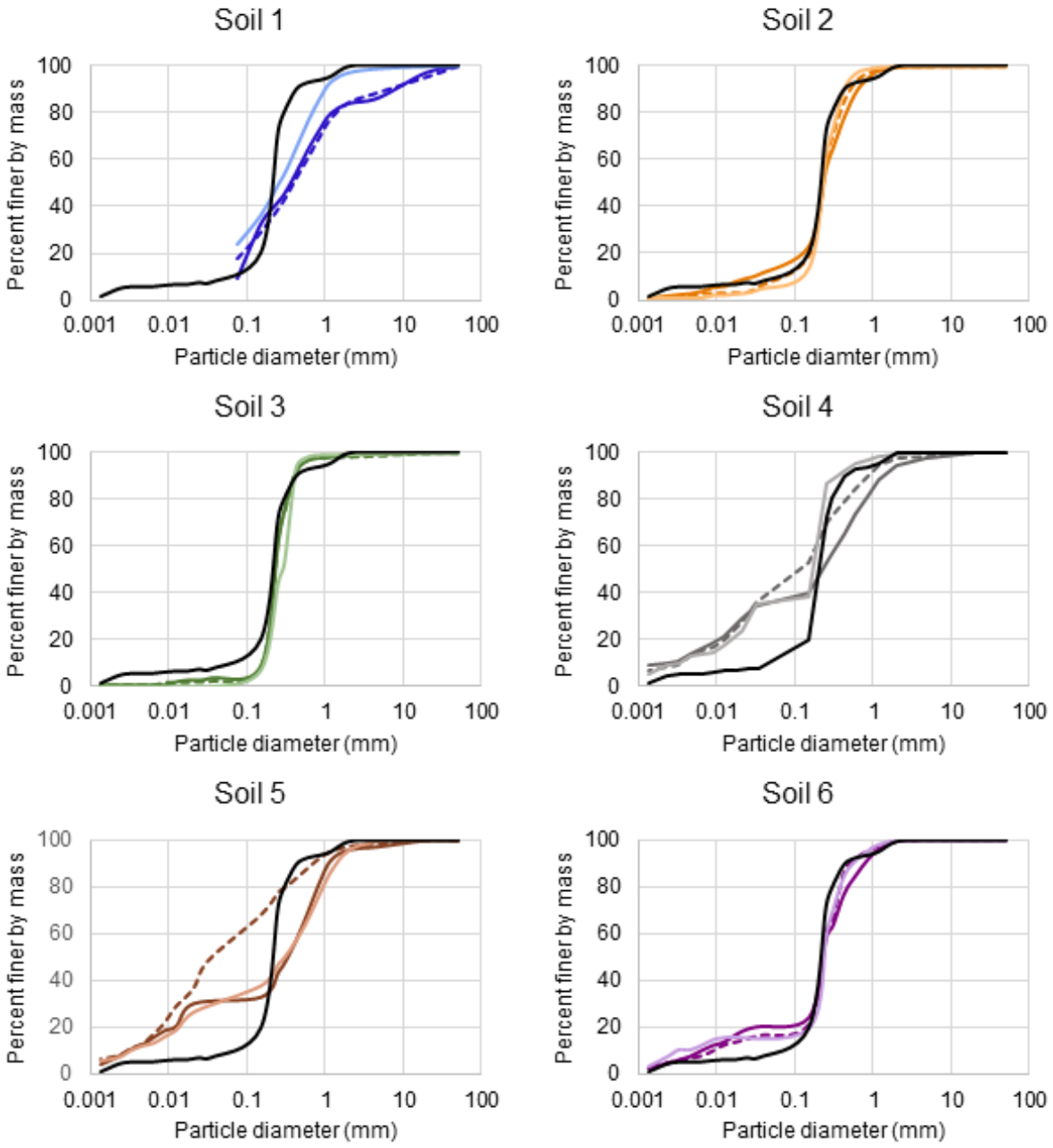

Figure 3.1. Particle size distributions of the mineral fraction of each soil and BAM, by soil depth. For each soil, the darker line represents the surface soil layer (0-10 cm), the dashed line represents the middle layer (10-20 cm), and the lighter shade represents the deepest soil layer (20-30 cm). BAM has the same distribution at all depths and is represented on all plots as the solid black line. Due to high OMC, the grain size distribution curve of Soil 1 is based only on dry sieve analysis, including organic matter.

Table 3.1. Bulk density, porosity and field moisture content of soils and BAM, as mean value ± standard error across the five replicates at each depth.

Depth	0-10 cm			10-20 cm			20-30 cm		
Soil	Bulk density (g/cm^3)	Porosity (cm^3/cm^3)	Field Moisture content (%)	Bulk density (g/cm^3)	Porosity (cm^3/cm^3)	Field Moisture content (%)	Bulk density (g/cm^3)	Porosity (cm^3/cm^3)	Field Moisture content (%)
1	0.171 ± 0.009	0.935 ± 0.003	84.4 ± 0.9	0.146 ± 0.009	0.945 ± 0.004	86.2 ± 0.9	0.187 ± 0.014	0.929 ± 0.004	82.3 ± 0.7
2	0.424 ± 0.047	0.840 ± 0.018	65.6 ± 2.7	0.549 ±0.065	0.793 ± 0.018	57.7 ± 3.8	0.971 ± 0.072	0.634 ± 0.027	38.4 ± 3.0
3	1.177 ± 0.031	0.556 ± 0.012	13.8 ± 0.4	1.303 ± 0.014	0.508 ± 0.005	13.0 ± 0.6	1.459 ± 0.025	0.449 ± 0.009	15.7 ± 0.4
4	0.316 ± 0.019	0.881 ± 0.007	74.1 ± 1.0	0.608 ± 0.065	0.771 ± 0.025	56.9 ± 4.0	0.949 ± 0.022	0.642 ± 0.008	39.8 ± 1.1
5	0.255 ± 0.028	0.904 ± 0.011	75.9 ± 1.6	0.687 ± 0.048	0.741 ± 0.018	48.0 ± 2.4	0.437 ± 0.047	0.835 ± 0.018	62.8 ± 3.2
6	0.972 ± 0.013	0.609 ± 0.023	30.5 ± 0.4	1.246 ± 0.022	0.510 ± 0.024	22.7 ± 0.7	1.543 ± 0.028	0.417 ± 0.012	17.1 ± 0.6
BAM[1]	1.643 ± 0.012	0.380 ± 0.004	NA	--	--	--	--	--	--

[1] BAM is an engineered media. Unlike a soil, properties of BAM do not vary with depth and there is no field moisture content. Bulk density and porosity vary depending on compaction. The mass of dry BAM added to intact cores and the final volume after the flow-through test were applied to determine the bulk density and porosity applicable to this study.

Table 3.2. Clay and silt percentages as determined by laser analysis and OMC of soils and BAM as mean ± standard error across the five replicates at each depth.

Depth	0-10 cm			10-20 cm			20-30 cm		
Soil	Clay % (< 3.9 μm)	Fines % (<62.5 μm)	OMC (g/kg)	Clay % (< 3.9 μm)	Fines % (<62.5 μm)	OMC (g/kg)	Clay % (< 3.9 μm)	Fines % (<62.5 μm)	OMC (g/kg)
1[2]	--	--	930.3 ± 3.5	--	--	866.9 ± 12.7	--	--	861.3 ± 6.4
2	4.52 ± 0.27	41.79 ± 1.63	411.4 ± 42.3	4.66 ± 0.27	16.59 ± 1.37	275.7 ± 39.2	5.06 ±0.47	17.90 ± 2.12	106.8 ± 11.6
3	1.17 ± 0.07	4.46 ± 0.56	25.4 ± 2.2	0.99 ± 0.04	3.47 ± 0.22	10.2 ± 1.4	1.07 ± 0.03	3.75 ± 0.12	4.4 ± 0.5
4	8.48 ± 0.24	61.58 ± 0.47	366.1 ± 27.3	11.18 ± 1.47	86.4 ± 0.78	99.5 ± 17.5	10.83 ± 1.23	95.56 ± 0.47	29.9 ± 0.24
5	6.73 ± 0.13	48.11 ± 0.20	497.3 ± 41.8	7.68 ± 0.41	90.63 ± 0.58	67.8 ± 6.3	8.38 ± 0.41	66.69 ± 1.94	241.2 ± 34.9
6	13.99 ± 1.10	90.92 ± 0.02	90.5 ± 2.4	15.73 ± 0.39	90.84 ± 3.42	49.3 ± 2.3	16.63 ± 0.35	96.01 ± 0.82	21.7 ± 2.1
BAM[3]	2.62 ± 0.11	10.48 ± 0.34	9.1	--	--	--	--	--	--

[2] Laser testing was not possible for Soil 1 due to high OMC.
[3] BAM is an engineered media. Unlike a soil, properties of BAM do not vary with depth.

Soil 1 was characterized as an organic soil, composed of 86%–93% organic matter and little mineral material. The mineral fraction of Soil 1 was too low to accurately determine clay content. Soil 2 was a sandy loam that contained moderate amounts of organic matter (11%–41%) and small amounts of clay (~5%). Soil 3 was a sandy soil (95% sand) with little to no organic matter (< 2.5%) or clay (~1%). Soils 4 and 5 contained moderate amounts of organic matter (up to 37% and 50%, respectively, in surface soils layers) and moderate clay (7%–11%). Soil 6, comprising 91%–96% silt and clay, had the finest particle size distribution of any soil tested. OMC of Soil 6 was low (2%–9%) and clay content (14%–17%) was the greatest of any soil tested. As expected, bulk density tended to be lower in soils with high OMC, and field water content increased with OMC. Porosity increased with fraction of clay, silt, and OMC. Bulk density increased and porosity decreased as a function of soil depth. By comparison, BAM contained about 3% clay and negligible amounts of organic matter. The bulk density and porosity of BAM were most similar to Soil 3, the sandy soil, though BAM contained more silt-sized particles than Soil 3.

3.2 Metals

Metals have considerable impacts on soil biogeochemistry, including the transformation and speciation of nutrients, adsorption reactions via available surfaces sites and precipitation, and microbial diversity and function. Therefore, to understand the influence of metals on N and P cycling within these diverse soils, soil extractions were analyzed for magnesium (Mg), aluminum (Al), calcium (Ca), manganese (Mn), and iron (Fe). These particular metals were chosen because Ca, Mg, and Al commonly precipitate with P to form soil minerals and have influence on the hardness of groundwater in carbonate-rich soils. Fe and Mn are redox-active metals and form reduced mineral oxides in anoxic soils. P adsorbed onto Fe and Mn oxides can be released when these oxides dissolve during re-oxidation events.

Soil samples (0.2 g) collected from each of the core sections (0-10, 10-20, 20-30 cm) were acid-extracted (trace metal grade nitric acid) and analyzed by inductively coupled plasma mass spectrometry (ICP-MS) for Mg, Al, Ca, Mn, and Fe concentrations on a Thermo Fisher Scientific iCap Qc inductively coupled plasma mass spectrometer (ICP-MS) with QCell technology and operated in kinetic energy discrimination (KED) mode of analysis with helium as the collision gas. Calibration, internal, and quality control standards (Inorganic Ventures) were prepared in 2% trace metal grade nitric acid (Fisher Scientific). Holmium, bismuth, and yttrium were used as internal references in both standards and samples.

The soils from each site contained varying concentrations of Mg, Ca, Al, Mn, and Fe (Figure 3.2). Soil 3, which was sandy with no OMC or clay, had the lowest metals compared to the other soils. Soil 3 contained none of the typical cations found in Florida soils (Mg, Ca, and Al), as well as very low Mn and little Fe. Soils 4 and 5 had the highest concentrations of all metals. Soil 1, the organic soil, contained high concentrations of Mg, Al, and Mn, but little to no Ca and Fe. Soil 6 (greatest clay content) was primarily Al-based with little to none of the other metals. Metals tended to decrease with soil depth at most sites with a few exceptions such as Mg and Ca in Soils 4 and 5. Conversely, BAM contained very little metal content, with very small amounts (<277 mg/kg) of Al, Mn, and Fe.

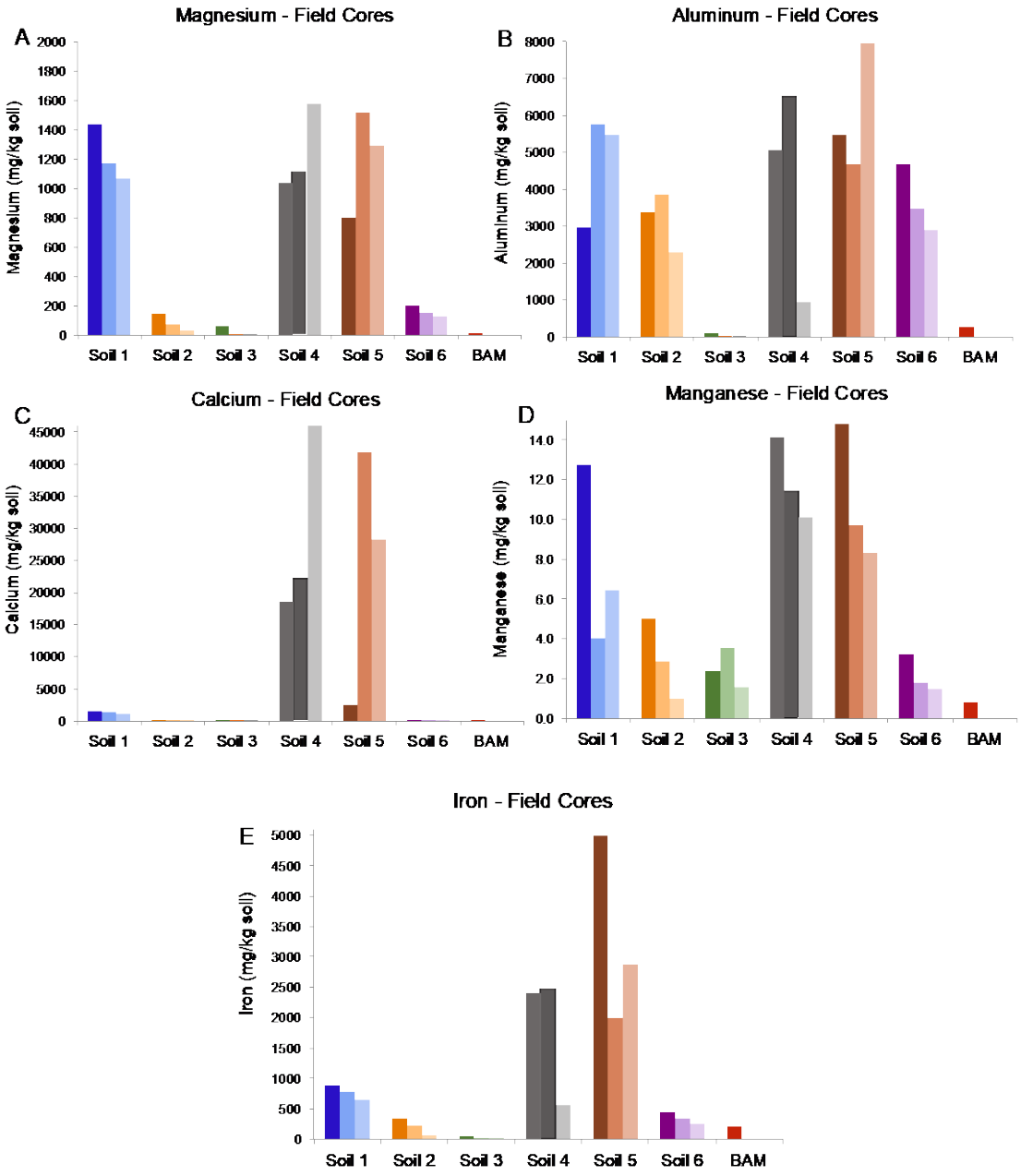

Figure 3.2. Initial field characterization of extracted metals (A) Mg, (B) Al, (C) Ca, (D) Mn, and (E) Fe in each soil type and BAM. Clustered bars represent soil depth in order of 0-10 cm, 10-20 cm, and 20-30 cm.

3.3 Soil Phosphorus Speciation

The speciation of phosphorus plays a major role in the availability and movement of P in soils. More labile forms of P move easily through groundwater and are available for microbial metabolism while less reactive P can be sequestered and eventually buried deep within soils. To better understand the processing of P in Florida soils, this study analyzed the different fractions of P chemical speciation in soils of different OMC, clay, and sand composition along with metal content to understand which soils provided the best systems for P retention. P fractions analyzed included *Exchangeable P* (loosely bound labile P), *Adsorbed* (P adsorbed to soil surfaces), *Precipitated* (P in mineral form), and *Organic* (P incorporated within organic complexes). To determine the different forms of P in the collected soils, sequential extractions were conducted on soils from each of the sites. Soil samples (0.2 g) collected from each of the core sections (0-10, 10-20, 20-30 cm) were chemically extracted according to the Standards Measurements and Testing Program (SMT) analytical protocol of Ruban *et al.*, (2001) for phosphorus analysis. First, ultrapure water was added to 0.2 g of wet sediment and rotated for 1 h at room temperature to obtain the *Exchangeable P* fraction, or the fraction of P loosely adsorbed. Second, a solution of 1 M sodium hydroxide (NaOH) was added to a 0.2 g dried aliquot of sediment and rotated for 16 h at room temperature. The supernatant was collected and treated with 3.5 M HCl for 16 h at room temperature to obtain the NaOH-extractable fraction, or the fraction of P bound (*Adsorbed P*) to metal oxides and other minerals within the sediment. The residual sediment was rinsed with 1 M sodium chloride (NaCl) and the supernatant discarded. A 1 M HCl solution was added to the pellet and rotated for 16 h at room temperature to obtain the fraction of *Precipitated P* as a mineral with calcium and/or other metals. The remaining residue was combusted at 550°C to remove organic matter. A 1 M HCl solution was added to the ashed residue and rotated 16 h at room temperature to obtain the fraction of *Organic P*. A separate sediment aliquot (0.2 g) was combusted at 550°C, treated with 3.5 M HCl, and rotated for 16 h at room temperature to obtain total P. Total P from each of the extracts was measured by inductively coupled plasma mass spectrometry (ICP-MS; see Section 3.2).

The soils contained varying concentrations of total P ranging from approximately 85 to 750 mg P/kg soil (Figure 3.3). The speciation of P within each soil type was diverse. P was primarily adsorbed to soil surfaces in Soils 1, 2, and 6, with smaller amounts in the organic P fraction. The lower concentration of P in the organic fraction of Soil 1 was surprising due to the high OMC of Soil 1. However, the high concentrations of Mg, Al and Mn (Figure 3.2) in Soil 1 provided the surfaces needed for P adsorption, suggesting that adsorption reactions out-competed the formation of organic P at this site. Higher P concentrations were found in the soils with moderate sand/silt/clay (Soil 4 and 5), where P was largely proportioned in the precipitated fraction. The high amounts of Ca, Mg, and Al in these soils would have made precipitation of phosphate minerals favorable particularly at the higher pH (~7.9; Table 3.4) at the sites. Soil 3 (mostly sand) contained the least amount of total P and it was found primarily in the adsorbed fraction. The low concentrations of Ca and Mg in Soil 3 explain the low amount of P in the precipitated fraction. Additionally, the pH ~5 of Soil 3 is not favorable for mineral phosphates formation, which typically occur around pH 7-8. BAM contained a moderate amount of total P (~270 mg/kg) that was primarily in the precipitated fraction, most likely formed with the small amounts of Al, Mn, and Fe in BAM.

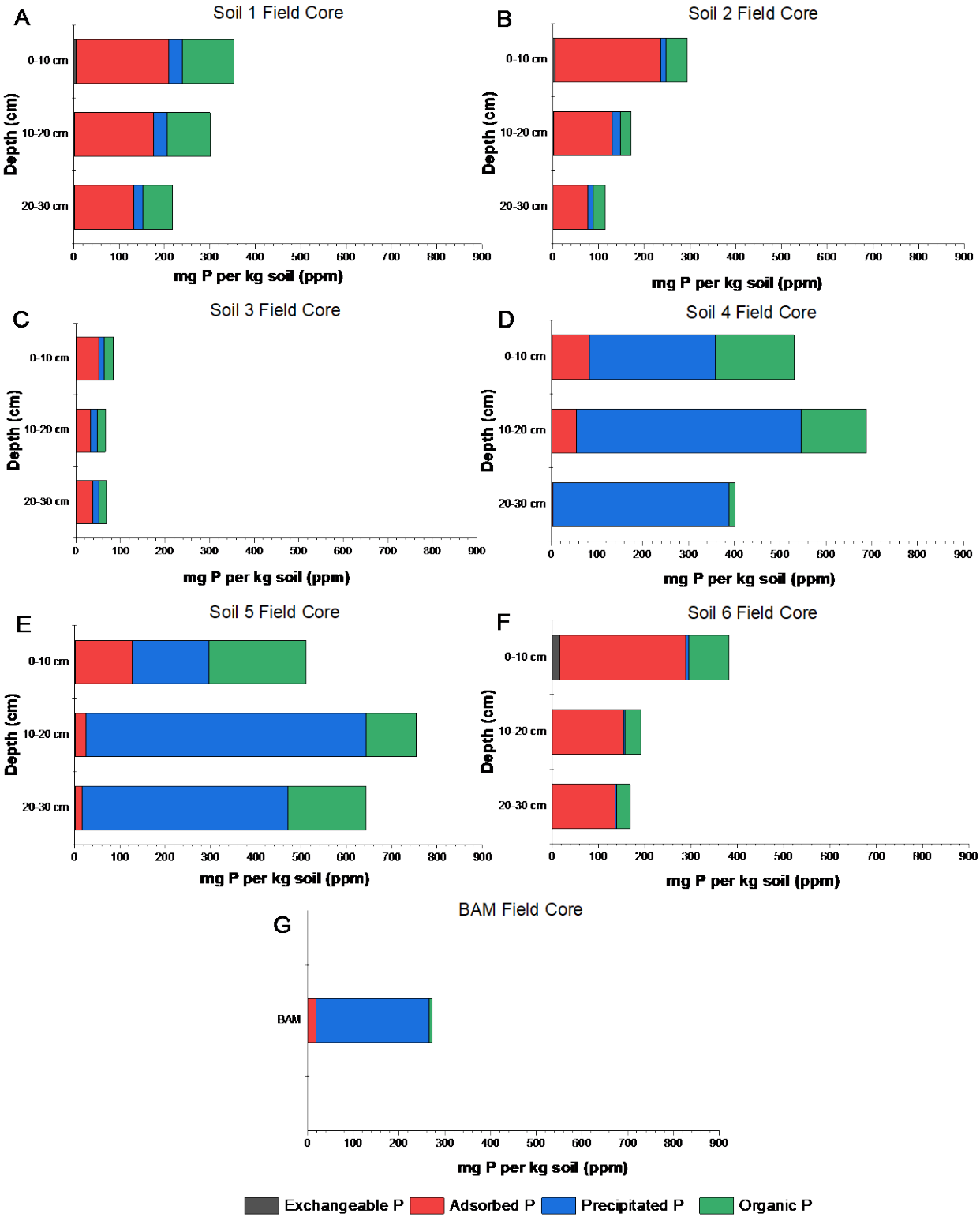

Figure 3.3. Initial field characterization of phosphorus concentration and speciation in each soil type (A-F) and BAM (G).

The speciation of P in soils influences its long-term susceptibility to uptake and transport as opposed to sequestration. While exchangeable P (P that is loosely sorbed to soil surfaces) is labile and easily used by microorganisms for metabolic reactions, including algal growth, other forms of P are not as readily available. Adsorbed P is susceptible to removal if redox conditions change, whereas precipitated P is easily dissolved if pH decreases. Organic P is the most stabile speciation. This fraction is grown by microbial metabolic processes and typically is not susceptible to changing pH and redox conditions.

3.4 Soil Microbiome

The soil microbiome directly facilitates processes related to nutrient transformation and sequestration within the soil profile. The diversity and abundance of microbes in each of the soils was determined in order to understand how the microbial community differs in each of the soil conditions and how the microbial activity changes in response to nutrient additions. Total genomic DNA was extracted from soil samples using the Qiagen® DNeasy PowerSoil Pro DNA Extraction Kit using an Omni Bead Ruptor 24 (Omni International, Inc.). Extracted DNA was quantified using the Qubit 3.0 dsDNA HS fluorescence assay. DNA extracts were analyzed by Illumina MiSeq 2 × 250 V2 amplicon sequencing using primer pairs 16S V4 515F-806R targeting the bacterial 16S V4 hypervariable region. Sequencing data was processed using a pipeline developed from the QIIME2 bioinformatics software package (Bolyen *et al.*, 2019). Figure 3.4 demonstrates the change in total microbial abundance at the various depths within each soil while Table 3.3 lists the abundance distribution by bacterial phylum.

The initial soil microbiome identified a highly diverse and varied microbial community among the different soils. The bacterial diversity represented (Table 3.3) is typical for soils, particularly with higher abundances in the *Actinobacteria*, *Firmicutes*, and *Proteobacteria*. Microbial abundance increased with soil depth in Soils 1 and 2 (Figure 3.4), where OMC, TP, and TN were higher at all depths compared to other soils. Interestingly, Soil 3 contained microbial abundances that were comparable to other soils and also increased at depth, though field moisture content, OMC, TP, and TN were low compared to other soils. Increase in abundance of *Deltaproteobacteria* (Table 3.3) in Soils 1-3 suggests that anaerobic iron and sulfate reducing bacteria were active, contributing to the higher abundance at depth. *Nitrospira* also increased with depth, which suggests that bacteria conducting nitrification were active.

The microbial abundances in Soils 4 and 5 decreased with soil depth, which is typical as less nutrients and water are usually available at depth. However, Soils 4 and 5 contained moderate amounts of OMC and had the highest TP even at depth, though P was primarily in the precipitated fraction (Figure 3.3) indicating it would not be available for bacterial respiration. These soils contained higher microbial abundances near the soil surface, where aerobic bacteria thrive, particularly the *Alphaproteobacteria*, which include many N-fixing bacteria and bacteria known to degrade OM. Soil 6 contained similar abundance across all depths; however, lower abundances of *Deltaproteobacteria* and *Gammaproteobacteria* indicate similarities to Soil 3 and a lower diversity of overall species.

In contrast to the soils, BAM contained very few bacterial species. This lack of microbial abundance is expected, as BAM itself initially does not contain microbes, but in situ acquires a donor microbiome from surrounding soils. The few bacteria detected were likely due to transfer from handling and exposure to air and container surfaces.

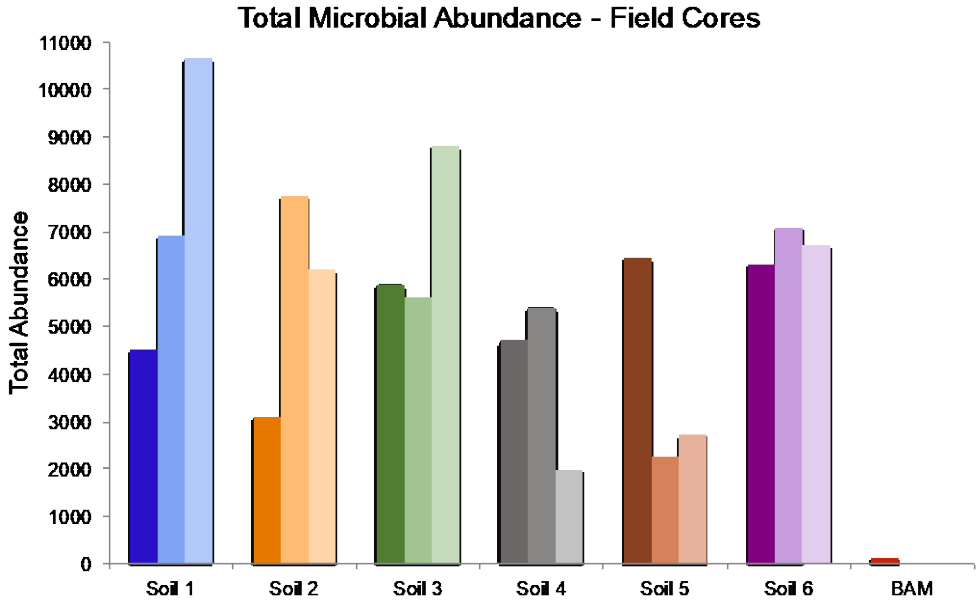

Figure 3.4. Initial field characterization of total microbial abundance in each soil type and BAM. Clustered bars represent depth range in order 0-10 cm, 10-20 cm, and 20-30 cm.

Table 3.3. Initial field characterization of total microbial abundance (counts per sample) distribution by phylum in each soil type.

Soil	Depth (cm)	*Acidobacteria*	*Actinobacteria*	*Bacteroidetes*	*Chloroflexi*	*Cyanobacteria*	*Firmicutes*	*Nitrospirae*	*Planctomycetes*	*Proteobacteria-Alpha*	*Proteobacteria-Delta*	*Proteobacteria-Gamma*	*Verrucomicrobia*	*Other*
1	0-10	438	1424	249	197	14	50	20	139	863	414	431	97	186
	10-20	510	1379	300	537	2	173	192	210	1159	1004	903	74	477
	20-30	3777	538	37	218	0	85	527	456	1906	1829	813	83	395
2	0-10	996	623	2	49	0	79	10	94	758	163	215	73	44
	10-20	3091	544	0	536	8	37	125	139	1908	922	231	34	191
	20-30	2366	262	0	341	0	69	154	53	1638	893	185	67	176
3	0-10	388	2788	23	100	28	481	0	45	1078	222	430	74	235
	10-20	1090	2032	6	82	0	252	0	79	1299	213	406	34	121
	20-30	2532	1845	13	296	20	290	17	532	1571	307	547	94	747
4	0-10	497	944	32	245	0	186	66	78	1147	475	795	45	211
	10-20	355	873	21	336	0	271	157	209	1378	706	672	62	367
	20-30	212	155	20	205	3	60	51	48	260	274	385	5	291
5	0-10	494	1507	138	352	0	262	69	196	1682	630	762	38	322
	10-20	179	325	12	216	0	71	91	76	379	308	346	16	242
	20-30	123	231	23	371	0	59	121	52	294	517	476	0	466
6	0-10	984	1898	17	394	9	768	7	501	1108	247	254	81	58
	10-20	2107	1160	0	552	4	270	98	550	1468	233	347	75	223
	20-30	1947	1160	0	815	12	249	110	204	1310	294	387	24	195
BAM		0	8	11	0	22	0	0	0	11	0	67	0	11

3.5 Soil Biogeochemical Processes

Soils taken straight out of the field were immediately analyzed for extractable nutrients and carbon to understand the natural chemical concentration and form of key nutrients important for supporting microbial processes, which in excess, are also responsible for nutrient pollution. Potential biotic processing was quantified for soil-only systems (no vegetation) using denitrification enzyme activity (DEA) and potentially mineralizable nitrogen (PMN) assays and by identifying microbes genes associated with denitrification pathways.

3.5.1 Soil Extractable Nutrients

Extractable pools of bioavailable nutrients encompass nutrients in the porewater and those adsorbed to the surface of soil particles that are displaced by the addition of salts. Extractable nutrients are considered the highly available form of soil nutrients for both plants and microbes. Once the soil samples returned from the field, they were weighed and homogenized by hand in the laboratory. Any live plants and rhizomes > 5 cm diameter were omitted from sample processing. All extractable nutrient pools were determined within 24 h of collection for nitrate + nitrite (herein referred to as NO_3^-), Dissolved Organic Carbon (DOC), and ammonium (NH_4^+). A solution of 2 M potassium chloride (KCl) was decanted into 40 mL centrifuge tubes containing 2–4 g of homogenized soil. The samples were agitated on an orbital shaker at 150 rpm at 25°C for 1 h, then centrifuged at 5000 rpm at 10°C for 10 min to separate the supernatant and the soil. The supernatant was then filtered through a Supor 0.45µm filter (Pall Corporation, Port Washington, NY, USA), acidified with distilled, deionized H_2SO_4 to a pH < 2 and stored at 4 °C. Concentrations of DOC, NO_3^- , and NH_4^+ were determined colorimetrically on a Seal AQ2 Automated Discrete Analyzer (Seal Analytical, Mequon, WI, USA) using EPA methods 353.2 Rev. 2.0, 350.1 Rev. 2.0, and 365.1 Rev. 2.0, respectively. All analytical runs included a 5-point calibration curve and were checked for quality assurance and quality control by including duplicates, spikes, internal blanks and standards, as well as external controls in a minimum of every 10 samples.

Results for initial field soil characterizations are listed below in Table 3.4. For all soils and BAM, most of the inorganic extractable N was in the form of NH_4^+, with low to undetectable concentrations of extractable NO_3^-. This is expected given that soil tends to be oxygen-limited (leading to an accumulation of the reduced forms of N) and the NO_3^- tends to be more mobile in the soil (due to the net negative charge). Soil 1 had the highest extractable N, which is likely a result of the high OMC of this soil. Extractable DOC was lowest in BAM and Soil 3, both of which had a very low OMC. However, the low concentrations of DOC in Soil 1 suggests that constant saturation limits decomposition and much of the OMC remains in particulate form.

3.5.2 Carbon and Nitrogen

Total carbon (TC) and total nitrogen (TN) includes all forms of these elements in the solid phase. They were quantified by folding 5 µg of dried, ground soil into a tin capsule and combusting using an Vario Micro Cube CN Analyzer (Elementar Americas Inc., Mount Laurel, NJ, USA). To quantify soil pH a 1:5 soil: deionized water slurry was mixed and allowed to equilibrate for 30 minutes as described by Thomas (1996) (with modifications). An Accumet XL200 benchtop pH probe (Thermo Fisher Scientific, Waltham, MA, USA) was placed in the slurry post-equilibration until a stable pH value was reached and recorded.

Table 3.4. Initial field characterization of all soils and BAM for extractable nutrients (NH_4^+, NO_3^-, DOC), TC, TN, and pH at each depth.

Depth	0-10 cm						10-20 cm						20-30 cm					
Soil	**NH_4^+ (mg/kg)**	**NO_x (mg/kg)**	**DOC (mg/kg)**	**TC (g/kg)**	**TN (g/kg)**	**pH**	**NH_4^+ (mg/kg)**	**NO_x (mg/kg)**	**DOC (mg/kg)**	**TC (g/kg)**	**TN (g/kg)**	**pH**	**NH_4^+ (mg/kg)**	**NO_x (mg/kg)**	**DOC (mg/kg)**	**TC (g/kg)**	**TN (g/kg)**	**pH**
1	27.4 ± 6.7	19.2 ± 0.8	660.7 ± 60.1	481.8 ± 2.6	14.9 ± 0.9	6.4 ± 0.1	38.0 ± 7.9	19.7 ± 0.9	529.9 ± 26.0	490.1 ± 11.0	15.7 ± 0.4	6.4 ± 0.1	31.9 ± 6.5	14.9 ± 0.8	455.1 ± 54.6	538.9 ± 3.4	13.2 ± 0.2	6.2 ± 0.1
2	1.0 ± 0.7	4.0 ± 0.9	1874.5 ± 196.3	355.3 ± 15.2	24.2 ± 1.1	4.7 ± 0.1	0.0 ± 0.0	1.0 ± 0.3	1209.3 ± 137.1	296.4 ± 24.8	17.2 ± 1.6	4.6 ± 0.0	0.0 ± 0.0	0.9 ± 0.7	522.5 ± 33.3	142.7 ± 33.0	6.5 ± 1.6	4.7 ± 0.1
3	0.3 ± 0.2	1.3 ± 0.3	492.0 ± 55.2	26.3 ± 4.3	0.99 ± 0.1	5.3 ± 0.1	0.0 ± 0.0	0.3 ± 0.1	726.3 ± 139.8	8.3 ± 1.4	0.48 ± 0.0	5.0 ± 0.1	0.0 ± 0.0	0.5 ± 0.3	410.3 ± 95.7	3.4 ± 0.3	0.4 ± 0.0	5.1 ± 0.1
4	12.3 ± 2.4	0.9 ± 0.2	1421.7 ± 197.4	231.3 ± 15.1	11.1 ± 0.8	7.9 ± 0.1	3.2 ± 0.9	0.6 ± 0.1	1414.4 ± 303.4	137.1 ± 6.5	3.8 ± 1.2	8.2 ± 0.1	1.4 ± 0.3	0.8 ± 0.3	533.9 ± 55.3	123.1 ± 0.5	0.7 ± 0.0	7.9 ± 0.2
5	13.7 ± 2.9	0.5 ± 0.3	1802.0 ± 708.7	298.2 ± 26.5	17.7 ± 2.1	7.2 ± 0.1	2.3 ± 0.5	0.4 ± 0.1	2681.3 ± 1186.3	138.3 ± 4.3	3.2 ± 0.6	7.8 ± 0.0	4.2 ± 2.1	0.5 ± 0.4	2643.6 ± 844.1	175.5 ± 11.6	7.4 ± 1.5	7.8 ± 0.1
6	133.8 ± 30.0	10.0 ± 3.3	1748.8 ± 460.0	43.5 ± 1.9	3.1 ± 0.1	5.2 ± 0.0	26.2 ± 5.4	7.0 ± 1.1	620.9 ± 55.2	22.3 ± 0.9	1.5 ± 0.1	4.6 ± 0.1	2.1 ± 0.6	2.1 ± 0.7	806.6 ± 500.5	10.2 ± 1.3	0.7 ± 0.1	4.8 ± 0.1
BAM	2.5 ± 0.3	0.0 ± 0.0	379.3 ± 11.3	217.7 ± 39.3	1.6 ± 0.4	5.5 ± 0.1	N/A						N/A					

Results for initial soil characterizations are listed in Table 3.4. As expected for soils with limited carbonate content, TC and TN concentrations mirror that of OMC, with TC comprising roughly 50% of the OMC pool and TN ~1-2% of the OMC pool.

3.5.3 Denitrifying Enzyme Activity

For each soil type at depth, the potential rate of denitrification (N removal) was measured utilizing a microcosm study. Soil denitrifying enzyme activity (DEA) is a short-term assay designed to quantify the in situ rate of denitrification by preventing soil microbes from synthesizing any new extracellular enzymes to catalyze the process due to the laboratory conditions. Instead, only the activity of presently existing (field-derived) enzyme activity is captured. DEA was determined at each depth (A, B, and C) using an acetylene inhibition method (Tiedje, 1982) with modifications by White and Reddy (1999). Briefly, 10 g of soil was placed in a 70-mL glass serum bottle, topped with a rubber septum, then sealed with an aluminum crimp cap. Bottles were vacuumed for 1 minute then purged with O_2-free N_2 gas for 3 min to create anaerobic conditions. Prior to shaking, 7.5 mL of N_2-purged nanopure water was added to each bottle, followed by 15 mL of acetylene gas (C_2H_2) added to each sealed bottle (Yoshinari and Knowles, 1976). Bottles were then placed on an orbital shaker at 150 rpm and 25°C for 40 min to evenly disperse the nanopure and the C_2H_2 gas. After 40 min, 12 mL of DEA solution (28 mg KNO_3-N L^{-1}, 144 mg dextrose C L^{-1}, and 1 mg chloramphenicol L^{-1}) was added to create a slight overpressure (Gardner and White, 2010). Bottles were continuously shaken at 150 rpm 25°C, 1 mL of headspace gas samples were extracted at 40, 80, and 120 min for analysis on a Shimadzu 2014 Gas Chromatograph equipped with an electron capture detector (ECD; Shimadzu Scientific Instruments, Kyoto, Japan). Headspace pressure was also measured and recorded at each time point. N_2O-N production rates were calculated for each bottle over time, per kilogram of soil, with consideration for the portion of gas in the aqueous phase using the Bunsen absorption coefficient (Tiedje, 1982).

Results of DEA activity for the field soils are presented in green as a negative (removal) rate in Figure 3.5. Soils 1 and 5 had on average higher rates of DEA than other soils, which can be attributed to the combination of high total carbon, extractable N, and flooded conditions. Looking across soils, TC content was well correlated with DEA, resulting in undetectable DEA in the low OMC/TC content samples (Soil 3 and BAM). All soils saw decreasing DEA with depth, as is typical due to decreasing microbial activity with soil depth.

3.5.4 Potentially Mineralizable Nitrogen

For each soil type at depth, the rate of N mineralization (N release) was measured utilizing a microcosm study. Potentially mineralizable nitrogen (PMN) activity measures the natural rate at which soil microbes are breaking down organic compounds and releasing N, which is first released as ammonium. PMN was determined at each depth via a 10-day bottle incubation study adapted from Roy and White (2013). After 10 days elapsed, the bottles underwent a KCl solution extraction with subsequent filtering for analysis of NH_4^+ determined colorimetrically on a Seal AQ2 Automated Discrete Analyzer (Seal Analytical, Mequon, WI, USA) using EPA method 350.1 Rev. 2.0. All analytical runs included a 5-point calibration curve and were checked for quality assurance and quality control by including duplicates, spikes, internal blanks and standards, as well as external controls in a minimum of every 10 samples.

Results of PMN Activity for the field soils are presented in red as a positive (release) rate in Figure 3.5. PMN activity across samples generally mirrored the results for DEA, with Soils 1

and 5 having the highest rates, and Soils 3 and BAM the lowest rates of PMN. Soil 6 also tended to have low PMN, attributable to low OMC/TC content.

3.5.5 Net Nitrogen Balance

Initial field rates of denitrification (N removal) and mineralization (N release) for all soil types were quantified and the net N effect rates were calculated as the difference between both rates with depth. Results of the net effect of N Activity for the field soils are presented in yellow in Figure 3.5. Yellow bars in the negative direction represent net N removal, while yellow bars in the positive direction represent N release. BAM and Soil 3 showed a net release of N, as indicated by higher PMN than DEA at all depths. Both of these samples were well-drained and low in OM, making it unlikely they will remain flooded long enough to develop the anaerobic conditions and/or microbial consortia required to stimulate denitrification (i.e., DEA). However, they do still experience mineralization (i.e., PMN), and possibly at even higher rates due to a greater availability of oxygen in the soil. In contrast, all other soils were generally neutral in regard to N cycling, with roughly equal N removal (DEA) and N release (PMN), as seen by yellow error bars overlapping zero. The only notable exception was Soil 6, which removed more N than it released in the top 0-10 cm. We suspect this may be due to the high concentrations of extractable inorganic N already in Soil 6 (Table 3.4), which would serve as a precursor to DEA, but may inhibit PMN due to the thermodynamic constrains of an existing accumulation of NH_4^+ (the product of PMN).

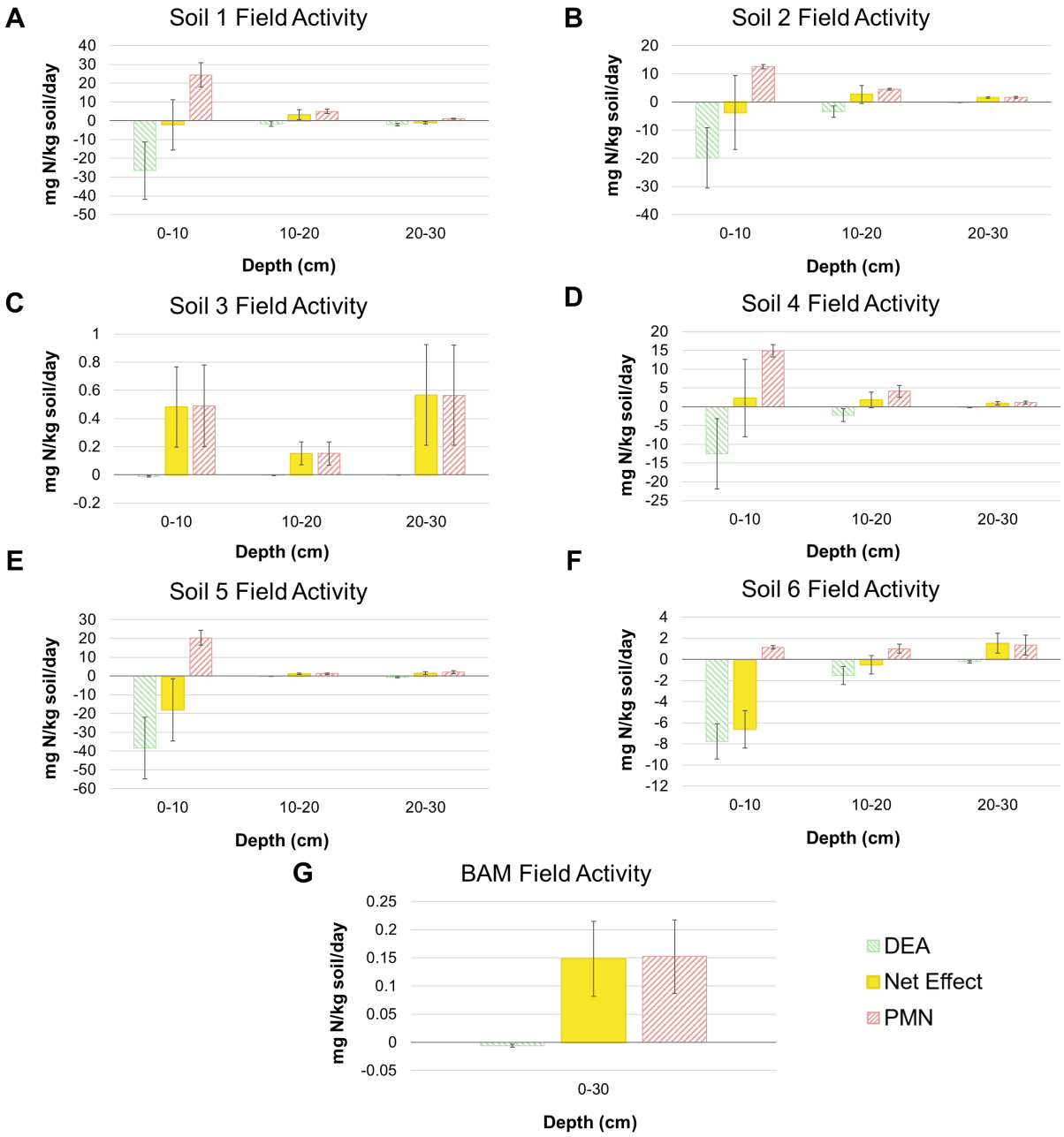

Figure 3.5. Initial field characterization of nitrogen transformation rate by depth. Nitrogen removal is represented as a negative rate (green) and is based on results of the DEA assay while nitrogen release is represented as a positive rate (red) and is based on the PMN assay. The net effect (removal-release) is represented in yellow.

3.5.6 Diversity of Nitrogen Cycling Genes Detected from DNA in Soils

Denitrification is driven by bacteria that use expressed enzymes to convert nitrate in a stepwise series to the final product of gaseous N_2. The diversity of key genes involved in bacterial denitrification and dissimilatory nitrate reduction to ammonium (DNRA) was determined by polymerase chain reaction (PCR) in soils from the PMN assays. Soil samples were collected at the conclusion of the PMN assays (Section 3.5.4) and the total genomic DNA was extracted as described in Section 3.4. DNA was amplified on a StepOne Plus™ Real-Time PCR System (Thermo Fisher Scientific) using the PowerUp SYBR Green Master Mix (Thermo Fisher Scientific). Five genes involved in N cycling were amplified by PCR. The genes analyzed included *napA* (nitrate reductase), *nirK* (nitrite reductase), *norB* (nitric oxide reductase), *nosZ* (nitrous oxide reductase), and *nrfA* (DNRA nitrite reductase) (Ma *et al.*, 2019; Papaspyrou *et al.*, 2014). Table 3.5 outlines the presence/absence of each of the genes in soils after completion of the PMN assay. The first step is the conversion of nitrate to nitrite, and the *napA* primer was designed to target the gene that encodes the nitrate reductase. Therefore, when analyzed by PCR if the *napA* primer test is positive, this suggests the bacteria in that sample were actively catalyzing nitrate to nitrite. The next steps in the denitrification pathway include the conversion of nitrite to nitric oxide via the nitrite reductase (*nirK*), nitric oxide to nitrous oxide via the nitric oxide reductase (*norB*), and then finally nitrous oxide to N_2 via the nitrous oxide reductase (*nosZ*). All soils were positive for these genes, indicating that denitrification was active throughout the soils. Soil 2, however, showed the least amount of positive amplification especially for *napA* suggesting that either denitrification was not very active in the assay or that nitrate was completely consumed and denitrification was nearing completion by the time the assay was complete and sampled. The low concentrations of both NH_4^+ and NO_x in the initial soil (Table 3.4), but high amounts of TN suggest that nitrogen was primarily in the organic fraction and not available for N reactions. An additional primer set was used to detect the *nrfA* gene, which encodes the DNRA nitrite reductase. Certain bacteria compete for nitrite to convert it to ammonium via the dissimilatory nitrate reduction to ammonium (DNRA) pathway. Interestingly, the *narfA* primer set was positive in all soils, suggesting that DNRA was an important metabolic pathway in these soils and the processing of stormwater nitrogen. The PMN assays conducted with BAM were not positive for any of the N-cycling genes indicating that denitrification and DNRA were not active in BAM. This is expected, as BAM did not contain an appreciable abundance of bacteria (Figure 3.4; Table 3.3).

Table 3.5. Diversity of nitrogen cycling genes as determined by PCR amplification in soils and BAM collected after the PMN assays. Confirmation of gene products in soils indicated by "+"; genes not detected are represented with "ND".

Soil	Horizon	PCR Amplification of nitrogen cycling genes in soil DNA				
		napA	***nirK***	***norB***	***nosZ***	***nrfA***
1	0-10 cm	+	+	+	+	+
	10-20 cm	ND	+	+	+	+
	20-30 cm	ND	+	+	+	+
2	0-10 cm	ND	ND	ND	ND	+
	10-20 cm	ND	ND	ND	ND	ND
	20-30 cm	ND	+	+	+	+
3	0-10 cm	ND	+	+	+	+
	10-20 cm	+	+	+	+	+
	20-30 cm	+	+	+	+	+
4	0-10 cm	+	+	+	+	+
	10-20 cm	+	+	+	+	+
	20-30 cm	ND	+	ND	+	ND
5	0-10 cm	+	+	+	+	+
	10-20 cm	ND	+	ND	+	+
	20-30 cm	+	+	+	+	+
6	0-10 cm	+	+	+	+	+
	10-20 cm	+	+	+	+	+
	20-30 cm	+	+	+	+	+
BAM	--	ND	ND	ND	ND	ND

CHAPTER 4. **Stormwater Flow-Through Experiment**

Each soil and BAM were tested in flow-through experiments using simulated stormwater runoff to measure the rate of change in nutrient concentrations as high-nutrient simulated stormwater moved through the soil profile. The 45-day flow-through experiments included periods of constant-head saturation with simulated stormwater runoff and periods of repeated wetting and drying, designed to mimic the hydrology that would result from irregular pulses of stormwater entering infiltration basins.

4.1 Experimental Setup

The five replicate 30-40 cm intact soil cores collected from each study site (as described in Chapter 2) were arranged as shown in Figure 4.1. Each flow-through experiment began with a four-day acclimation period to allow for saturation and settling of the cores (Figure 4.2). During the acclimation period, a low-concentration simulated runoff solution was pumped into the cores and maintained at a 20-cm depth using a multi-channel peristaltic pump (Ismatec 1PC 24 channel). The acclimation period was followed by 10 days of consistent saturation (Wet Season 1, WS1) with simulated runoff (Table 4.1) that contained a conservative bromide tracer.

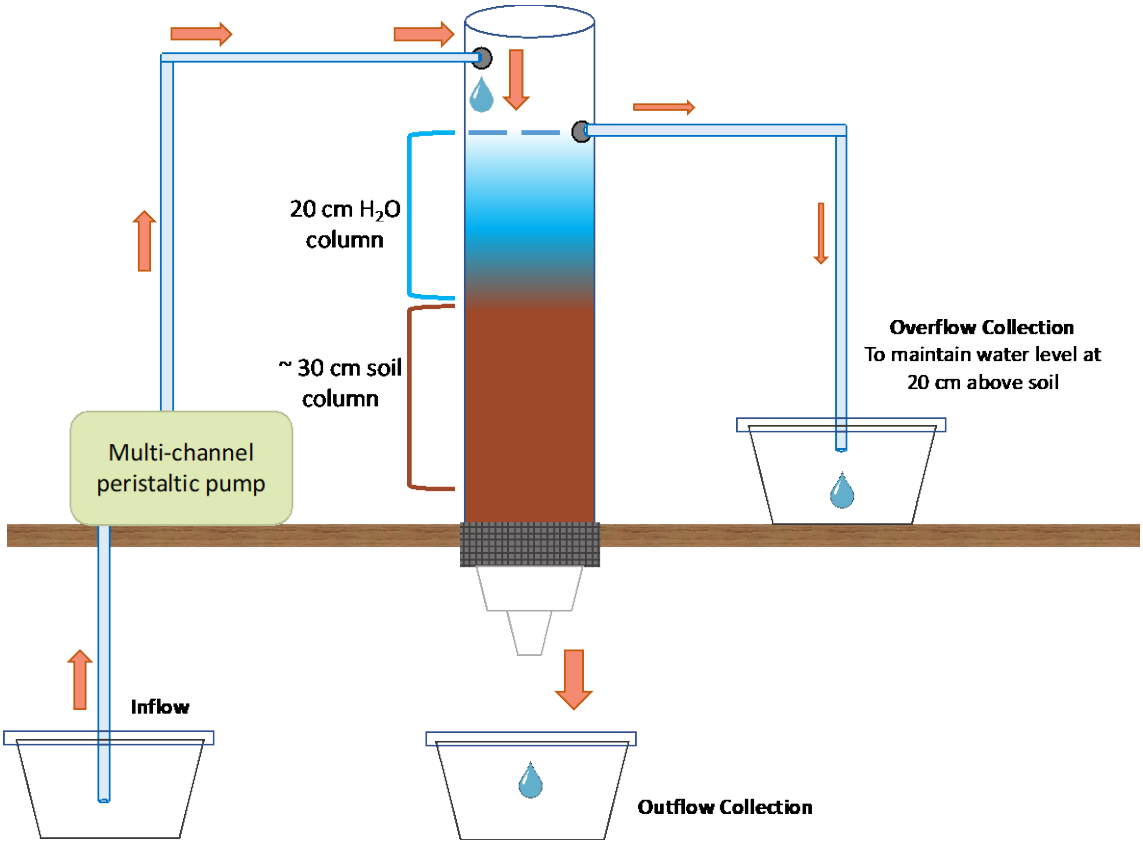

Figure 4.1. Schematic of flow-through study arrangement with pump inflow to outflow collection vessel.

Simulated runoff was prepared for the core flow studies, under sterile conditions, adapted from previously reported nutrient loads observed in roadway runoff deriving from review of published peer-reviewed literature (Shokri *et al.*, 2021). The simulated runoff was prepared in 5-gal

buckets by adding 1.81 mg/L $NaNO_3$, 0.492 mg/L K_3PO_4, 0.471 mg/L NH_4Cl, 9.27 mg/L glycine, 1.02 mg/L glucose-6-phosphate, and 1.29 mg/L NaBr to 15 L sterile deionized water adjusted to pH 6-6.5 with concentrated NaOH. The low concentration simulated runoff was prepared by diluting the simulated runoff recipe by 1:10 and removing the bromide (NaBr), which was a conservative tracer.

After 10 days of saturation, the flow of simulated runoff was stopped, and cores were allowed to dry down. Cores were subjected to a total of three dry-down periods (DD), each lasting 3 days. Dry periods were separated by pulse flows (P1 and P2), culminating in a second 10-day saturation period (Wet Season 2, WS2). On the day before a pulse or the initialization of the second wet season, any remaining standing water in the core was siphoned out. On pulse days, simulated runoff was added to each core to a depth of 20 cm and allowed to flow through the core. At the conclusion of the flow-through test, each core was destructively sampled for nutrient analysis, physicochemical properties, and DNA analysis, as described in Chapter 3.

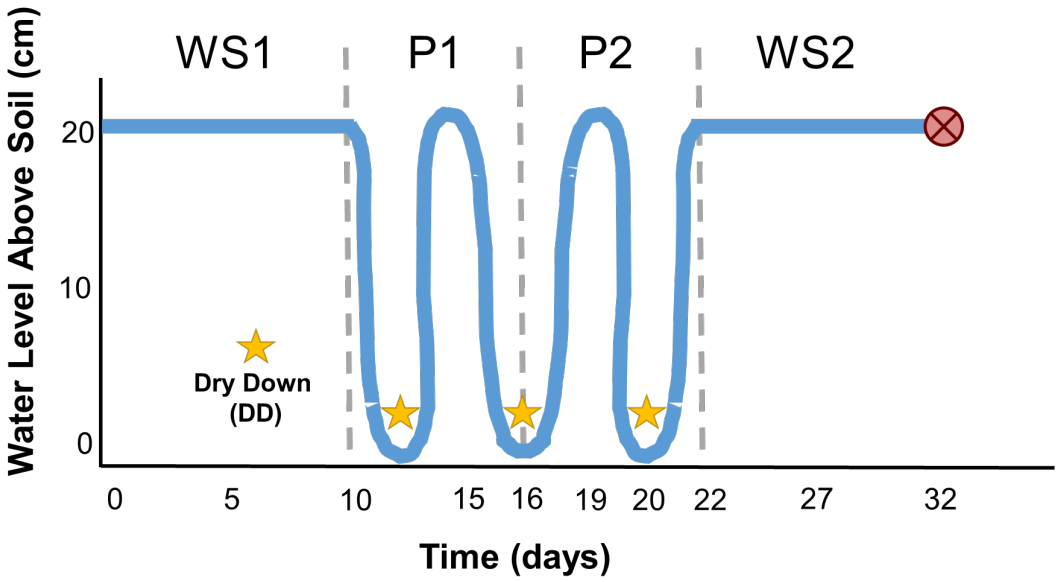

Figure 4.2. Hydrograph of flow-through experiments. After saturation, a ten-day wet season (WS1) was followed by three sequences of dry down (DD) and pulse flows (P1, P2) and a second ten-day wet season (WS2).

As the simulated runoff percolated through each flow-through core, the effluent, herein referred to as leachate, was collected in lidded plastic containers below each core. The volumetric flow rate of leachate from each core was continuously monitored. At least once a day, a 5 mL leachate sample was taken from the core outflow, with exact time and date recorded, and tested for bromide concentration. Flow-through water samples were filtered (0.22 µm or 0.45 µm pore size membrane) and analyzed by ICP-MS for TP (see Section 3.2) and for orthophosphate and bromide by ion chromatography (IC) using a Dionex ICS-1100 series integrated IC system (Thermo Fisher Scientific) and separated on a Dionex Ion Pac AS23 anion exchange column using carbonate/bicarbonate eluent and suppressed conductivity detection. Simulated runoff and leachate were collected for nutrient analysis at the start of each wet season, and after 5 and 10

days of saturation. Leachate samples were also collected 24 and 48 hours after each dry down commenced and 24 and 48 hours after each pulse event.

Five replicate cores of BAM were subjected to flow-through tests similarly as described for the soils above. Due to the lack of microbial abundance and diversity in BAM, the simulated runoff applied to BAM cores was created using non-sterile water from a stormwater retention pond. The acclimation period of BAM cores with the non-sterile stormwater was longer to allow sufficient time for development of the microbial community within the cores. The stormwater was analyzed for nutrient composition by IC and found to have nutrient concentrations below the detection limits. Therefore, the methods of creating the simulated runoff for the BAM cores were similar to the soil cores. However, due to the use of non-sterile water, reaction rates within vessels of simulated stormwater created for BAM flow-through experiments were different, leading to slightly different incoming nutrient concentrations for the BAM cores (Table 4.1).

Table 4.1. Mean and (range) of event-mean nutrient concentrations observed in roadway runoff (reported in peer-reviewed publications), and observed in simulated runoff, created with either sterile water (for soils) or stormwater runoff (for BAM).

	Roadway runoff ($\mu g/L$)	Simulated runoff ($\mu g/L$)	
		Sterile water (Soils 1-6)	Stormwater (BAM)
Nitrate	580 (230–1320)	310	360
Ammonium	110 (70–150)	770	380
Total Nitrogen	1750 (680–3200)	1770	1710
Total Phosphorus	210 (70–560)	220	73

4.2 Hydraulic Characterization

The hydraulics of water movement through the soil profile determines not only drainage, but also the contact time of infiltrated stormwater with the soil and microbes. Vadose zone hydraulics can therefore strongly influence reaction rates of stormwater nutrients and water quality in receiving waterbodies. Water movement in porous media is controlled by physical properties of the media (e.g. grain size distribution) as well as the soil structure, as macropores can create preferential flow pathways that greatly increase infiltration rates and decrease the hydraulic residence time of soil water. Hydraulic properties of soils have therefore been characterized in the context of the laboratory flow-through experiment (K_{sat}, hydraulic residence time), as well as in situ in the field sites (infiltration capacity).

4.2.1 Infiltration Capacity

Infiltration capacity was measured in triplicate at each field site using double-ring infiltrometers (Eijkelkamp standard set with measurements according to ASTM D3385-03). Following the ASTM D3385 methodology, each infiltrometer was placed each within 5 m of each other. The infiltrometer rings were driven into the soil and water was poured to an equal

level in the inner and outer rings. The water level was measured at the cross-section of the measurement float and the crossbar at 5-minute intervals (Figure 4.3). The infiltration rate ($f(t)$) was calculated by Eq. 4.1:

$$f(t) = \frac{h_1 - h_2}{t} \quad \text{(Eq. 4.1)}$$

where h_1 is the initial water level and h_2 is the water level after a time interval, t. Additional water was added to the rings as water infiltrated into the soil. Each infiltration test continued until a consistent infiltration rate was observed for at least consecutive 5 readings, at which time the infiltration capacity was confirmed (Table 4.2). As infiltration rates can be influenced by factors such as vegetation and initial soil moisture, vegetation at each infiltration site was characterized within three 1 m^2 quadrats. Percent cover of vegetation was categorized using a point-intercept grid within each 5 cm of the quadrat. Three core samples, each with a depth of 10 cm, were taken from each site on the day of testing to determine the moisture content of the soil at the sampling time (Eq 3.1). In situ infiltration testing is only possible when the vadose zone is unsaturated. Sites where Soil 1 and Soil 5 were collected were consistently saturated to the soil surface, such that collection of infiltration data was not possible at these sites. Infiltration data also could not be collected for BAM, since BAM was not studied in situ.

Figure 4.3. Collection of field infiltration data.

4.2.2 Saturated Hydraulic Conductivity

Saturated hydraulic conductivity (K_{sat}) of soil in each flow-through core was observed continuously while the soils were fully saturated during WS1 and WS2 of the flow-through experiments, through application of Darcy's law (Eq. 4.2):

$$q = -K_{sat} \times \frac{\mathrm{dH}}{\mathrm{dL}} \quad \text{(Eq. 4.2)}$$

where q (cm/day) is the specific discharge or Darcy velocity (volumetric flow rate Q per unit cross-sectional area A), K_{sat}(cm/day) is the saturated hydraulic conductivity of the media, dH is the change in hydraulic head within the core (cm), and dL is the soil core length (cm). While cores were saturated, volumetric flow rates of leachate were recorded one to two times per day, depending on the flow rate of the core. Leachate collected from each core was massed and transformed to a volumetric flow rate, assuming a density of water at 25°C (the laboratory temperature) of 0.997 g/cm^3. Mean K_{sat} was calculated from the time series following a period of acclimatization during the first wet season (Table 4.2, Figure 4.4).

Table 4.2. Hydraulic characteristics observed in soils and BAM.

Soil	In situ **Infiltration**			**Laboratory Flow Study**	**Laboratory Tracer**			
	Infiltration Capacity (cm/day)	**Field Moisture (%)**	**Vegetation Coverage (%)**	**Saturated Hydraulic Conductivity (cm/day)**	**Time of Initial Tracer Detection (hours)**	**Mean Hydraulic Residence Time (HRT) (hours)**	**Mean Soil Core Length (cm)**	**Normalized HRT (hours)**
1[4]	NA	NA	NA	5.70 ± 0.31	26	> 240	36.6	> 197
2	37	42.2	44	1.27 ± 0.03	49	> 240	38.7	> 186
3	150	14.8	32	5.96 ± 0.46	< 0.5	43	30.8	42
4	27	66	12	3.18 ± 0.31	51	241	36.4	199
5	NA	NA	NA	1.66 ± 0.07	39	>240	40.0	> 180
6	20	37.9	90	0.24 ± 0.01	65	>240	35.0	> 206
BAM	NA	NA	NA	41.67 ± 3.31	< 0.5	47	31.1	45

[4] In situ infiltration testing was not possible in Soils 1 and 5 due to consistent vadose zone saturation during the study period, or in BAM, which was not studied in situ.

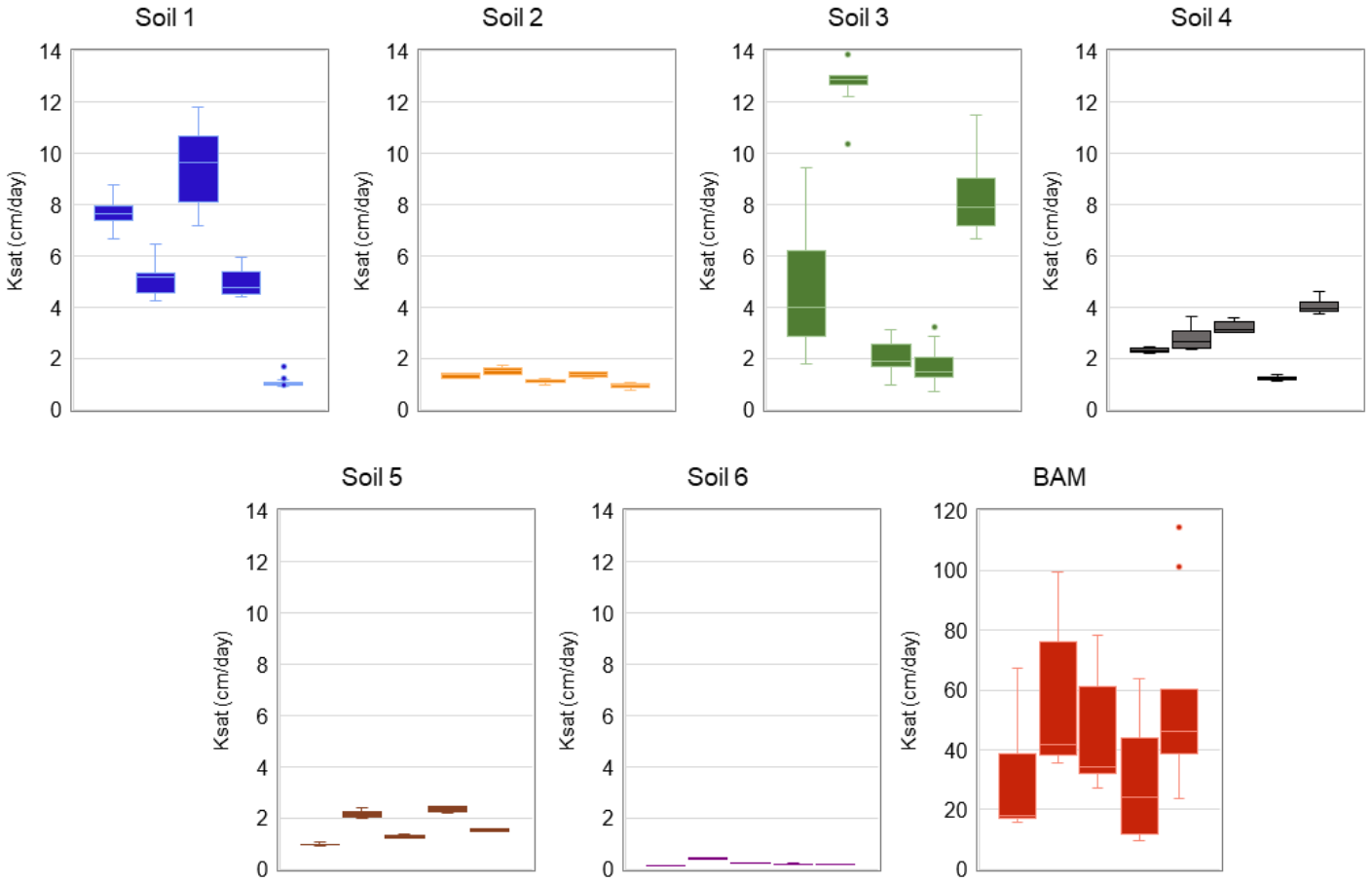

Figure 4.4. Distributions of hydraulic conductivity (K_{sat}) in each replicate core of each soil type and BAM. Midline is the median value, the ends of each box are the 1st and 3rd quartiles, and the ends of each whisker are the min and max observed K_{sat}. Note the different y-axis scale on the BAM figure.

4.2.3 Hydraulic Residence Time

Hydraulic residence time, a measure of the average time simulated runoff spent within the soil cores, was observed during the flow-through experiments. Steady-state breakthrough curves of bromide concentration in leachate samples (Figure 4.5) were used to estimate differences in hydraulic residence times of simulated runoff in the cores.

The three methods of characterizing flow through the soils and BAM provided a full range of information on soil profile hydraulics that largely corroborated one another. Soils with low saturated hydraulic conductivity (Soil 2, 5, 6) were also characterized by low infiltration capacity and high hydraulic residence times. Likewise, media with high saturated hydraulic conductivity (Soil 3 and BAM) were characterized by high infiltration capacity and lower hydraulic residence times. As expected, hydraulic parameters were largely correlated with the physical properties of the soils. Soils with the finest grain size distributions (Soils 5 and 6) were characterized by low conductivity and high residence time, while media with high infiltration capacity and lower hydraulic residence times (Soil 3 and BAM) were the ones with coarser grain size distributions.

As each hydraulic testing method offered insight into slightly different processes that influence nutrient transformations, comparison across methods can provide additional rationale as to why the different media performed differentially with regard to nutrient retention and removal. BAM had the highest observed saturated hydraulic conductivity and the lowest hydraulic residence time of any media tested. The mean K_{sat} of BAM was an order of magnitude greater than the highest K_{sat} of any soil tested, Soil 3. However, hydraulic parameters based on tracer data from BAM and Soil 3 cores were similar. The first tracer was detected in both media within one hour and the mean normalized hydraulic residence time of water in both media varied only slightly, from 42-45 hours. Similarly, Soil 1, the high-OMC soil, was characterized by a relatively high saturated hydraulic conductivity (5.70 ± 0.31 cm/day), which is similar to that observed in Soil 3 (5.96 ± 0.46 cm/day), yet the hydraulic residence time of water was greater in Soil 1 than in any other media tested. The large pore sizes of the organic matter comprising Soil 1 likely led to the fast flow rates observed through Soil 1 cores. Accordingly, the rising limb of bromide concentration in Soil 1 was somewhat steep, reflecting the relative speed that the tracer was able to move through the media due to its high hydraulic conductivity. However, after bromide concentrations reached steady state in Soil 1 within the first wet season, concentration stayed high throughout the flow-through experiment. Bromide concentrations did not decrease through the dry down and pulse flow cycles, as is seen in all other media. It is possible that the organic matter in Soil 1 absorbed large quantities of water, which was slowly released over time. Soils 2 and 5, which contained moderate amounts of organic matter, also are characterized by bromide release curves with relatively low slopes, suggesting that OMC held and slowly released water.

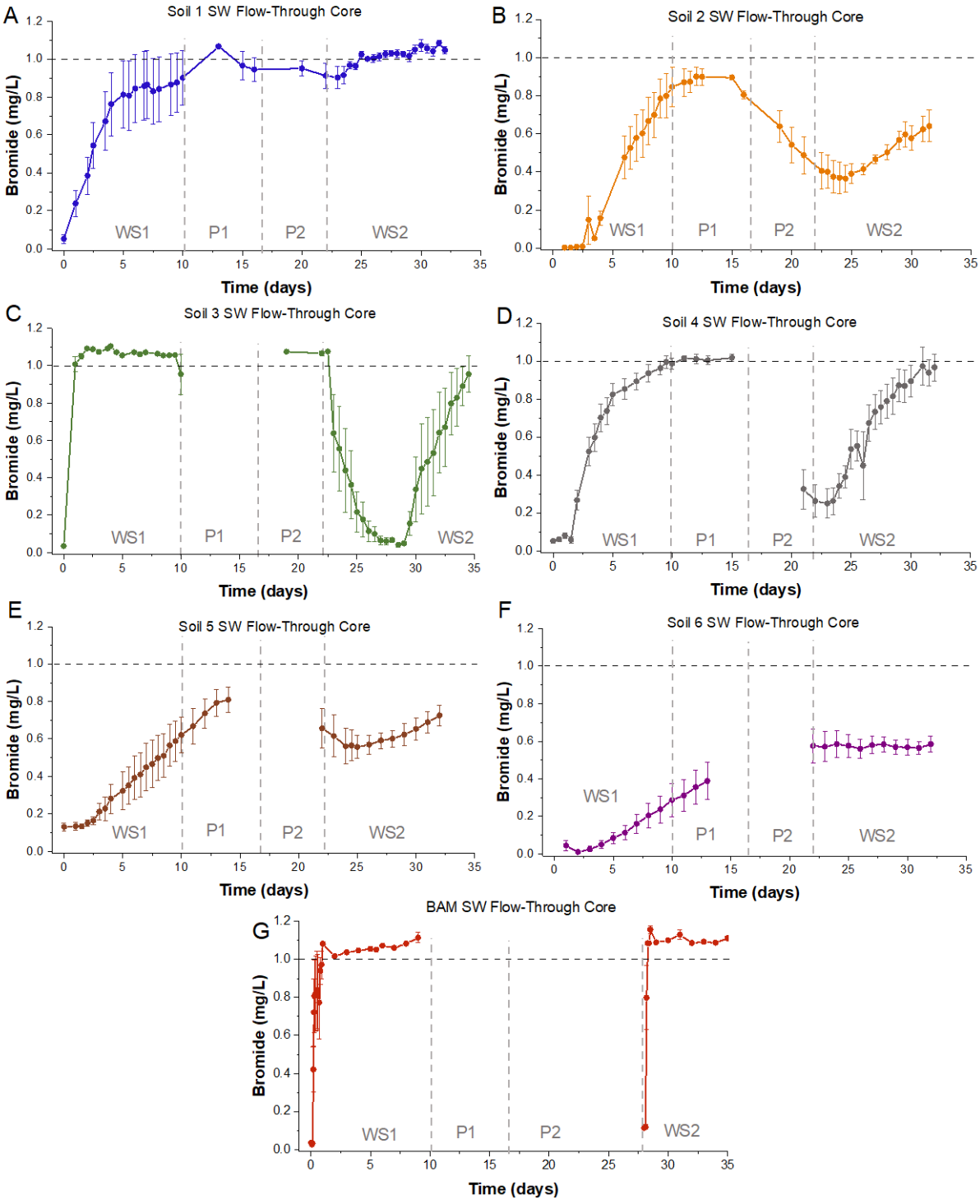

Figure 4.5. Dissolved bromide concentration as a function of time in leachate from soils (A-F) and BAM (G). Dotted line indicates inflow bromide concentration of 1.0 ppm. Error bars are the standard error of the five replicate cores. Gaps in data indicate dry down periods when some cores produced no leachate.

4.3 CO_2 Flux Rates

Fluxes of CO_2 were measured as an indicator of soil microbial activity. As microbes cycle and transform various nutrients and elements, they are also breaking-down C for energy, releasing the majority of it as CO_2 gas. Soil CO_2 fluxes were measured for each flow-through core using a LI-COR 8100 (LICOR Biosciences, Lincoln, NE) under flooded and exposed conditions. Measurements of the water level and distance from the top of the tube to the top of the water or the top of the soil during dry down events were used to calculate the offset distance in determining CO_2 flux rates.

Results of CO_2 flux are presented in Figure 4.6. Generally, average CO_2 flux was similar across all soil types and BAM. Dry-down conditions (represented by DD on the x-axis) typically had higher CO_2 flux than wet conditions because there was no water to restrict gas diffusion into the air and the microbial population is able to respire aerobically, which is faster and more efficient than anaerobic respiration.

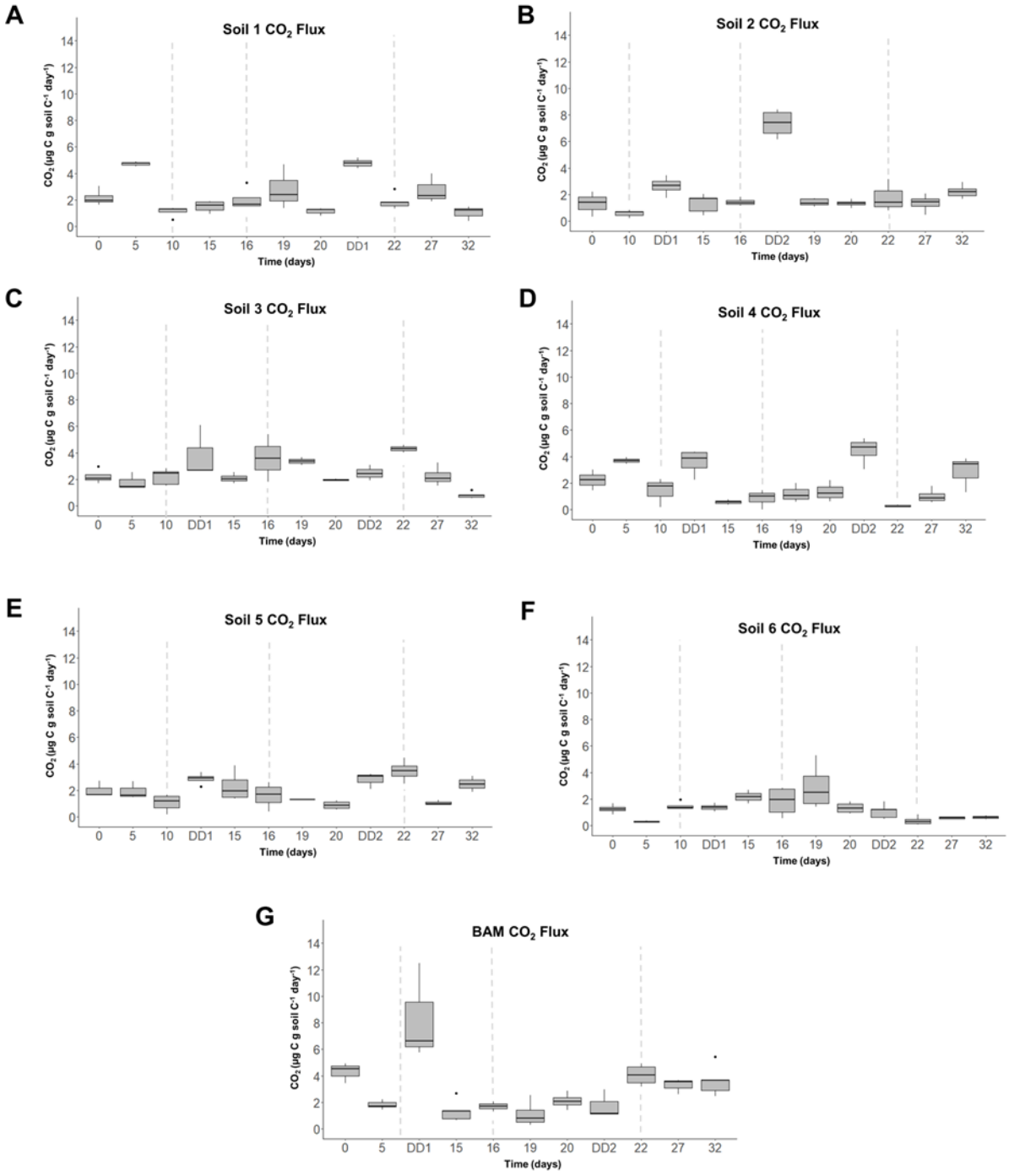

Figure 4.6. CO_2 flux rates for all soils and BAM during fluctuating wet and dry down (DD) events.

4.4 Stormwater Nutrient Retention and Removal

Simulated runoff and leachate from outflow of each core were collected at each sampling time to detect changes to nutrient concentrations as the simulated stormwater flowed through each soil and BAM. pH was tested to understand the acidity/alkalinity of the soil, which can affect the form and solubility of various elements and how ideal the environment is for microbes. Since all microbes require C for their processes, DOC was also tested to assess the availability of bioavailable C.

Leachate was collected in acid-washed Nalgene bottles and stored at 4 °C until processing. Within 24 hours of collection, pH was quantified, then the leachate was filtered through a Supor 0.45µm filter (Pall Corporation, Port Washington, NY, USA); acidified with distilled, deionized H_2SO_4 to a pH value < 2 and stored at 4 °C. Concentrations of Total Kjeldahl Nitrogen (TKN) was determined utilizing a copper catalyst digestion using a block digestion system (SEAL Analytical model BD-50, Seal Analytical, Mequon, WI, USA). The digested samples were subsequently quantified via AQ2 discrete analyzer for colorimetric ammonium determination using EPA methods 111-A Rev. 8.

4.4.1 Ammonium, Nitrate, Total N, and N speciation

Concentrations of NH_4^+ and NO_3^- and were determined by colorimetry on a Seal AQ2 Automated Discrete Analyzer (Seal Analytical, Mequon, WI, USA) using EPA methods 353.2 Rev. 2.0, 350.1 Rev. 2.0, respectively. All analytical runs included a 5-point calibration curve and were checked for quality assurance and quality control by including duplicates, spikes, internal blanks, and standards, as well as external controls in a minimum of every 10 samples. Relative change in concentration of each N species was calculated using Eq. 4.3, where concentration of nitrate is shown as an example:

$$\Delta[NO_3^-]_r = \frac{[[NO_3^-]_{leachate} - [NO_3^-]_{inflow}]}{[NO_3^-]_{inflow}} \quad \text{(Eq. 4.3)}$$

where $\Delta[NO_3^-]_r$ is relative change in concentration of NO_3^- through the soil core, $[NO_3^-]_{inflow}$ is the inflow concentration of NO_3^- in simulated runoff and $[NO_3^-]_{leachate}$ is the concentration of NO_3^- in leachate from the core. A positive change indicates that concentration has increased through the core (e.g. NO_3^- was released by the core) whereas a negative change indicates that concentration has decreased through the core (e.g. NO_3^- was removed within the core).

Removal rate (percent change in concentration from inflow to leachate) varied over time during the experiment. Minimum, mean and maximum observed rates are presented in Table 4.3 to represent the potential range of removal efficiency that could be expected. Concentrations of NH_4^+, NO_3^-, and total N in the leachate collected from the bottom of the intact soil cores over the course of the flow-through experiment are presented in Figures 4.7, 4.8, and 4.9, respectively. Dotted lines reflect inflow concentrations of simulated runoff. In Figure 4.10, total N speciation is presented over time.

Results indicated that all soils produced a net removal of NH_4^+, while BAM was a net source. NH_4^+ removal can result from either nitrification, or adsorption to the soil cation exchange complex. Soils 4, 5, and 6 all contained a notable amount of clay, which exhibits a net-negative charge and has a high capacity to hold cations like NH_4^+ to the soil. This likely contributed to Soils 4, 5, and 6's ability to consistently remove NH_4^+ with a high efficiency

throughout the experiment. In contrast, Soils 1, 2, and 3 contained very low, if any clay. Therefore, their cation exchange capacity is less, and may explain why the efficiency of NH_4^+ removal seemed to decrease over time. Removal of NO_3^- was high and consistent among all soil types, but fluctuated in BAM and exceeded the inflow concentration at the last sampling point. This may be due to the combination of a low clay content, a high hydraulic conductivity, and a lack of native denitrifiers to remove NO_3^- microbially. Soils 1, 4, 5 and 6 served in net removal of TN (Figure 4.9) while periodic TN release was observed from Soils 2, 3, and BAM. Soils 2 and 3 released TN as a result of very high organic N (ON) release (Figure 4.10). The ON in these soils was a result of site conditions that included large root masses that were severed during the collection of the cores and are therefore an experimental artifact.

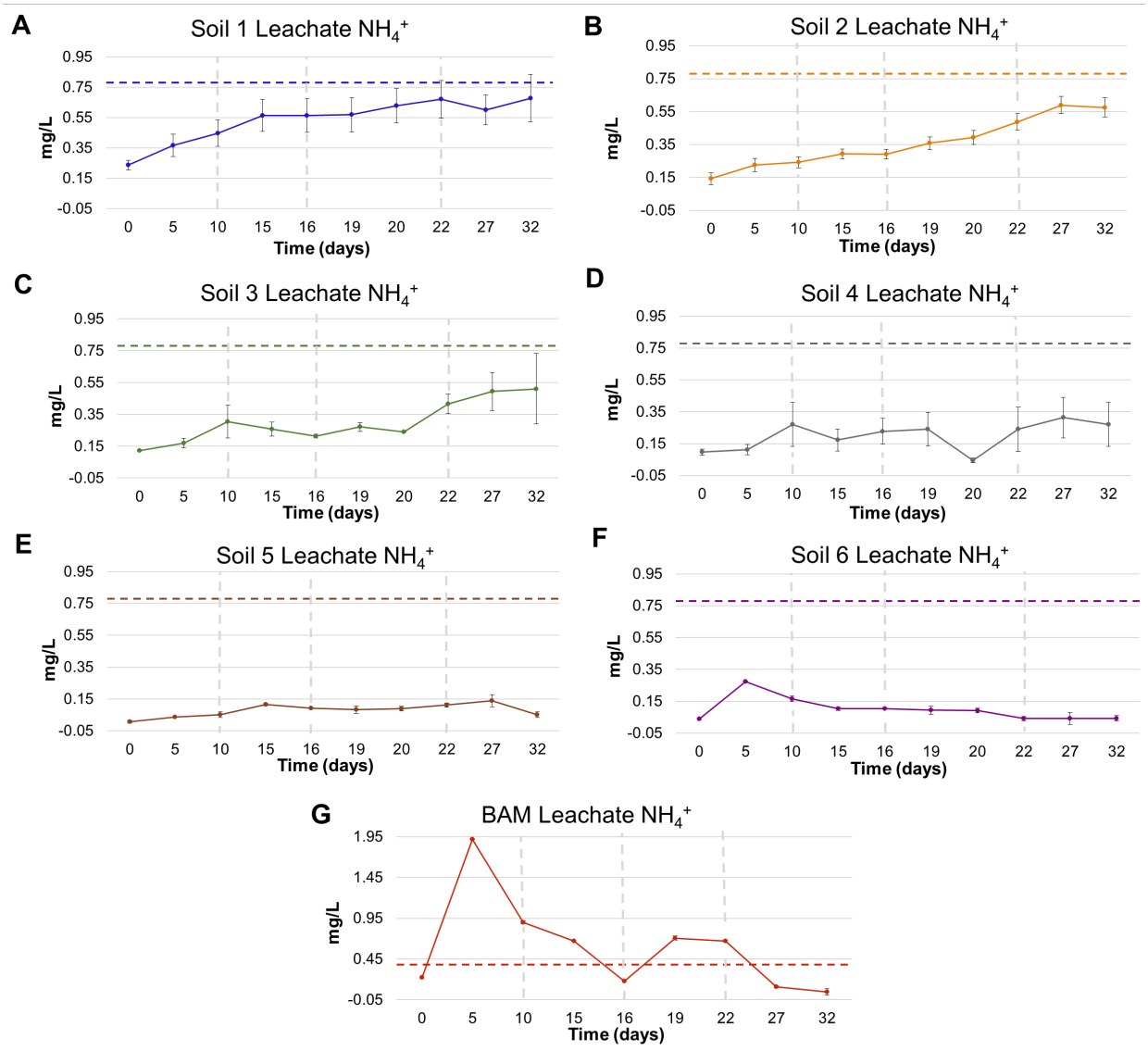

Figure 4.7. Concentration of NH_4^+ in leachate over time (solid line) for each soil type and BAM (A-G). Points represent mean; error bars represent standard error (n=5). Dashed line represents the inflow concentration in simulated runoff.

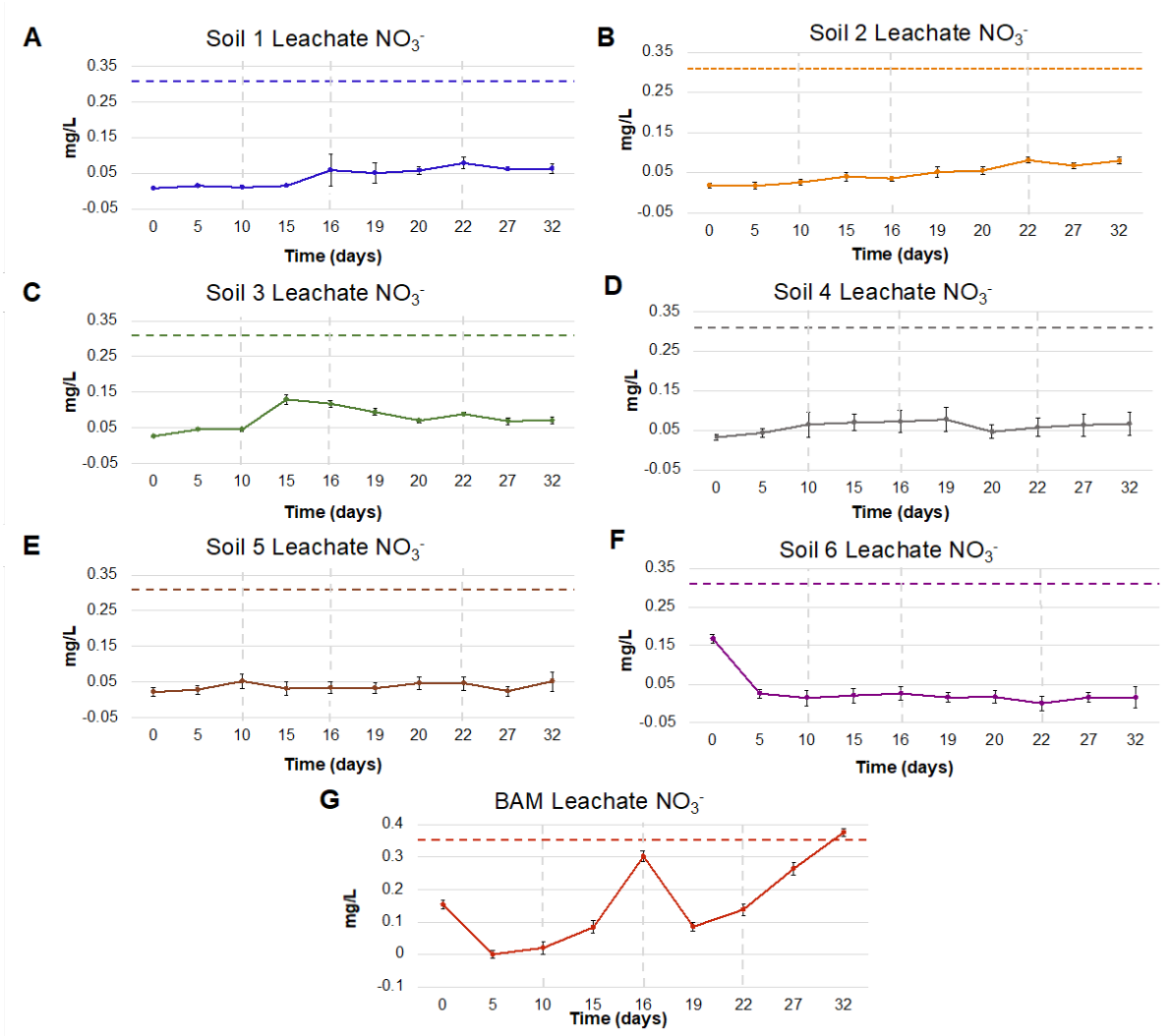

Figure 4.8. Concentration of NO_3^- in leachate over time (solid line) for each soil type and BAM (A-G). Points represent mean; error bars represent standard error (n=5). Dashed line represents the inflow concentration in simulated runoff.

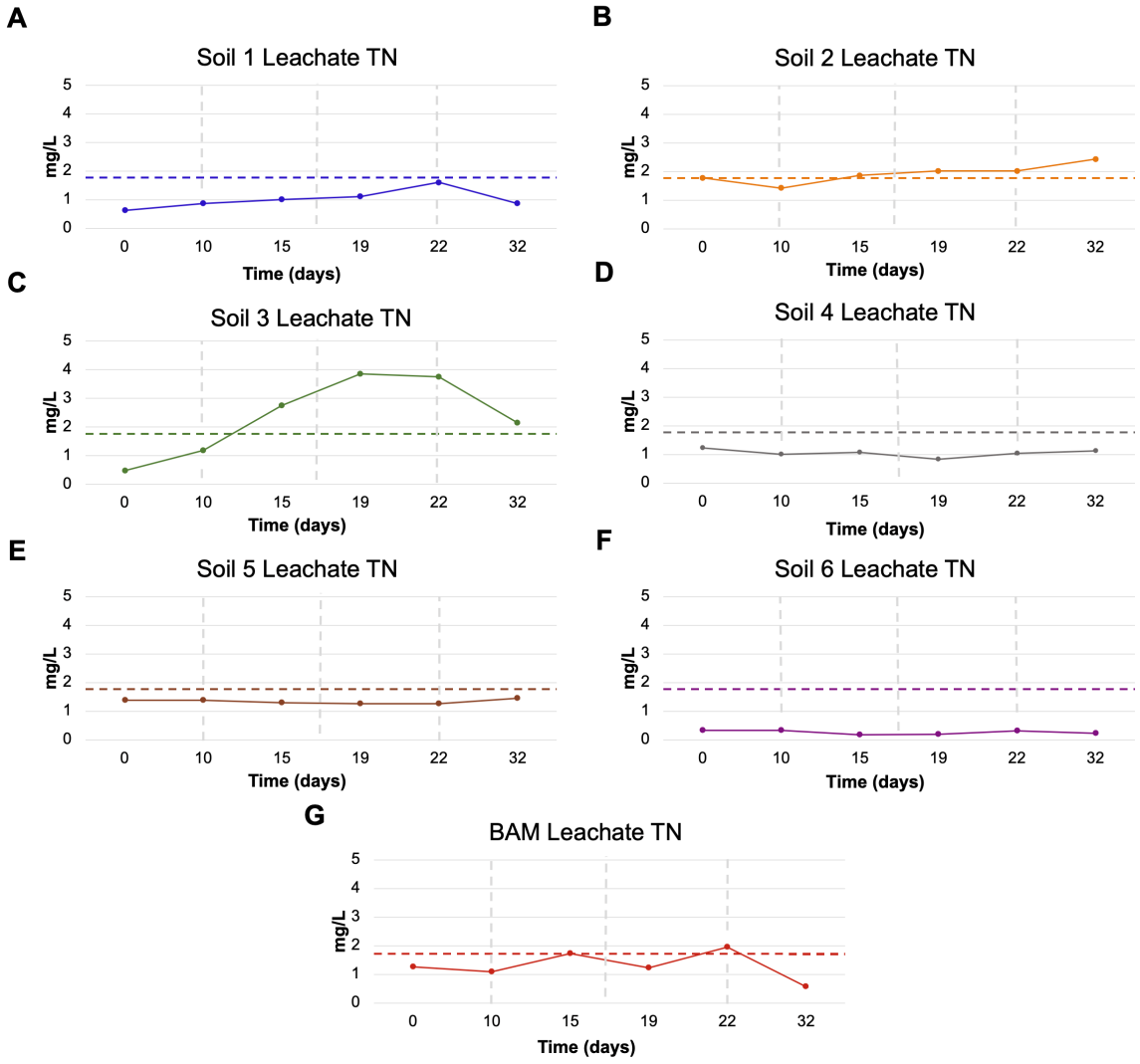

Figure 4.9. Concentration of total N in leachate over time (solid line) for each soil type and BAM (A-G). Points represent mean; error bars represent standard error (n=5). Dashed line represents the inflow concentration in simulated runoff.

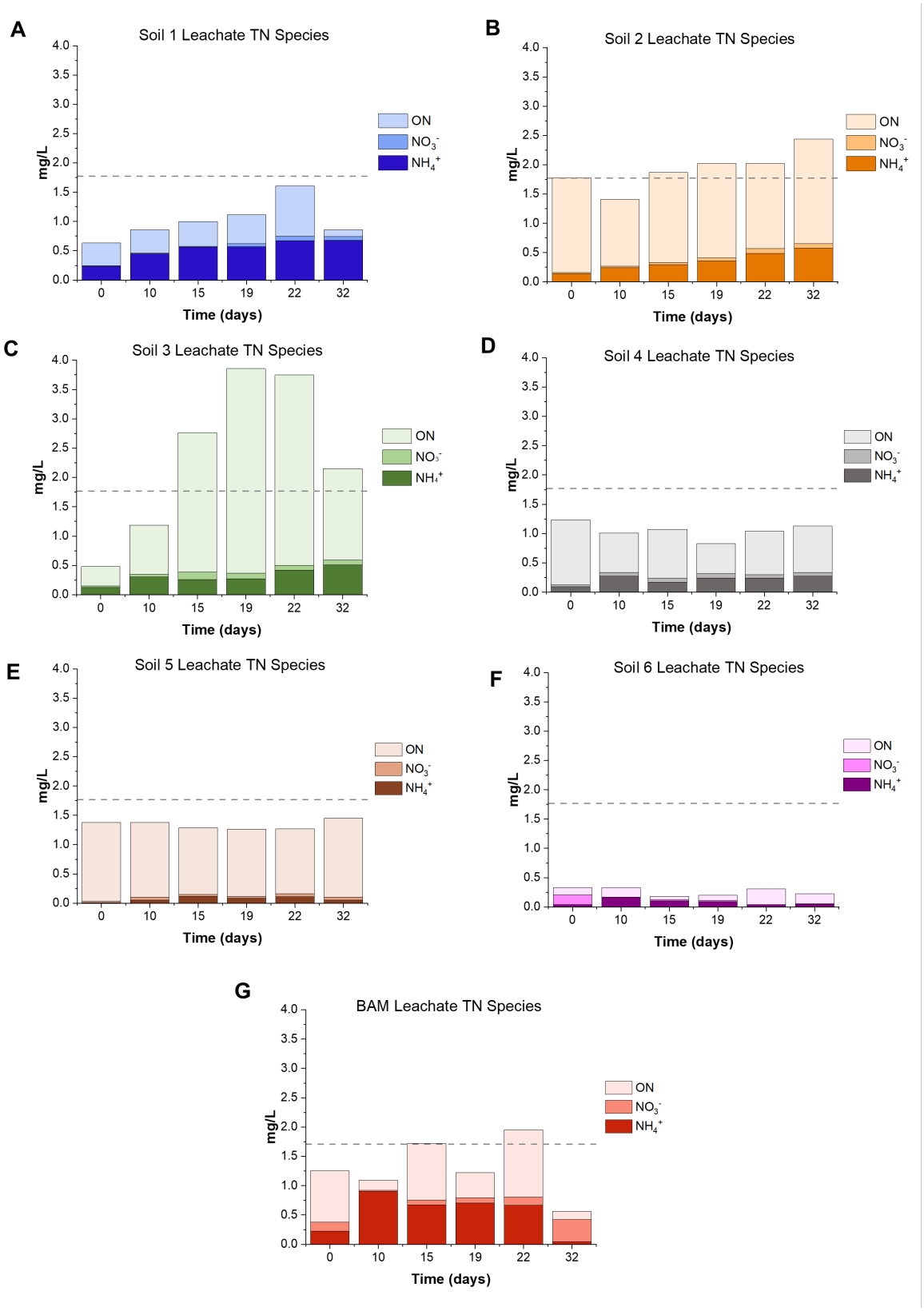

Figure 4.10. Speciation (form) of total N in leachate over time for each soil type and BAM (A-G). Dashed line represents the inflow concentration of total N in simulated runoff.

Table 4.3. Percent change of NH_4^+, NO_3^-, and total N concentration in leachate relative to inflow concentration for all soils and BAM. Negative values represent a decrease in N concentration (retention or removal of N within the soil) while positive values represent increase in concentration (release of N by the media). Minimum, maximum and mean change over the flow-through study is shown.

Soil	NH_4^+ Percent Change			NO_3^- Percent Change			TN Percent Change		
	Min %	Mean %	Max %	Min %	Mean %	Max %	Min %	Mean %	Max %
1	-12.43	-31.29	-69.51	-74.90	-86.66	-97.52	-8.91	-42.71	-64.25
2	-23.90	-53.59	-81.62	-74.46	-85.14	-94.60	38.13	8.88	-20.22
3	-34.12	-61.26	-84.16	-58.63	-75.88	-91.42	118.24	33.75	-72.81
4	-59.60	-74.30	-94.10	-75.35	-80.99	-89.71	-30.17	-40.43	-52.90
5	-82.07	-89.94	-98.92	-83.10	-88.13	-93.01	-17.86	-24.29	-28.54
6	-64.63	-87.17	-95.14	-46.88	-89.92	-100.00	-81.53	-85.24	-89.81
BAM	405.21	42.70	-100.00	4.67	-60.36	-100.00	14.11	-23.88	-66.84

4.4.2 Total Soluble Phosphorus

The retention of soluble P from stormwater during flow-through experiments is shown in Figure 4.11. The leachate was filtered (0.22 μm or 0.45 μm pore size membrane), acidified, and analyzed by ICP-MS for TP (see Section 3.2) and for orthophosphate by IC (see Section 4.1). Orthophosphate was below detection in all samples; therefore, Figure 4.11 represents soluble TP in the leachate. The graphs in Figure 4.11 represent the amount of soluble TP that was released from the soils in leachate. As shown, leachate P was well below the inflow concentration in all soils, indicating that more soluble P was retained within the soils than was released with the leachate. The relative change in concentration of soluble TP through the cores was calculated using Eq. 4.3 and outlined in Table 4.4. Soil 3 retained the least amount of soluble P as compared to the other soils (65% mean retention by Soil 3 as compared to 89%–99% in other soils), and leachate P concentration was similar to the inflow concentration by the second pulse. Soils 2, 4, and 6 demonstrated the highest retention of P during the first half of the experiments; however, breakthrough of P was observed during the second wet season. This suggests that soils had either reached P capacity or that the hydrology imposed upon the soils had caused more P to be released. Of note, Soil 2 contained high concentrations of soluble TP within the porewater after the soil was deconstructed. This high porewater TP may reflect decay of the vegetation root system that was severed during core collection. BAM did not demonstrate appreciable P retention (mean 21% retention), as the outflow leachate P concentration was similar to the inflow during the first wet season. BAM, however, retained more P during the second wet season.

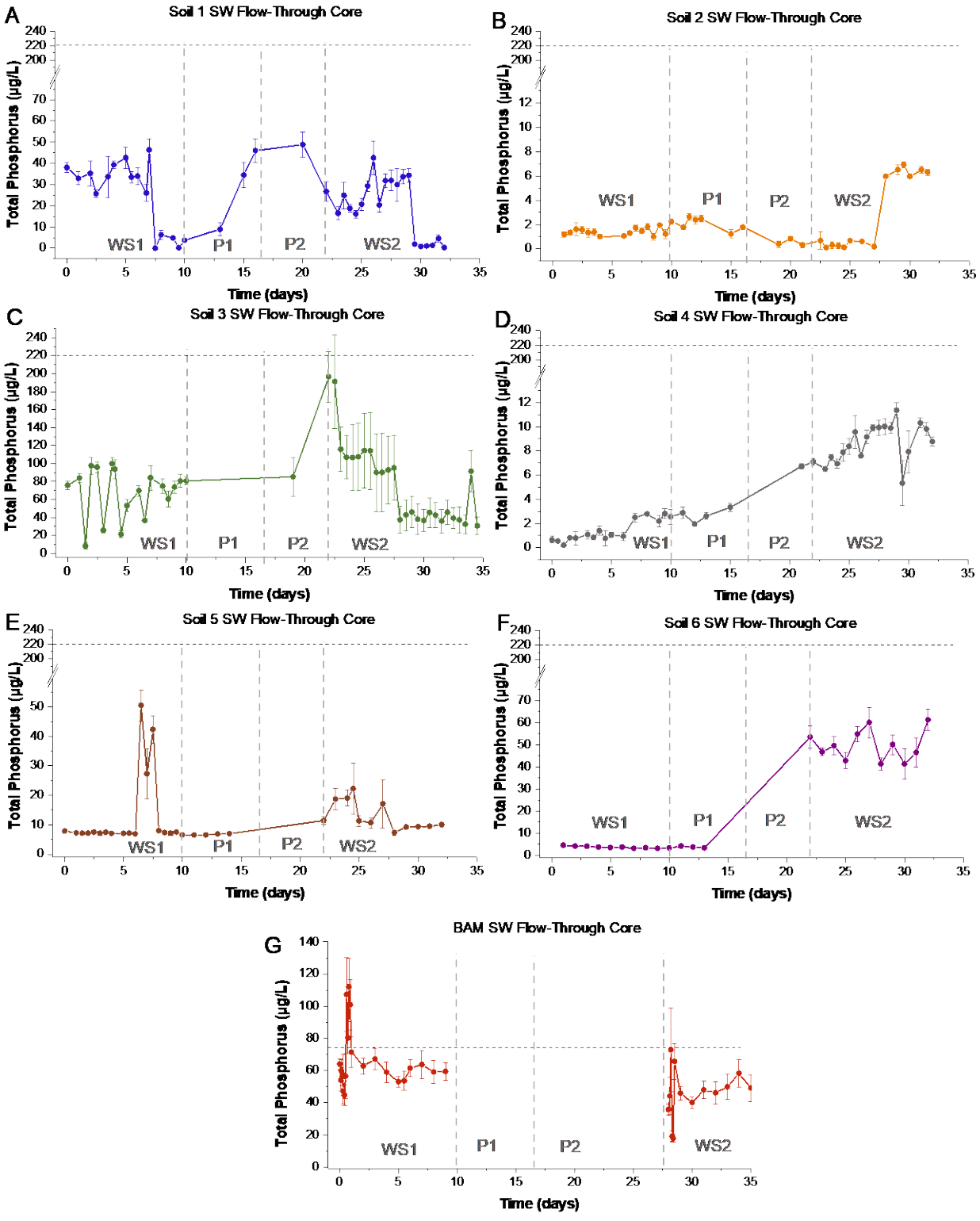

Figure 4.11. Concentration of total soluble phosphorus as a function of time in leachate for each soil type (A-F) and BAM (G). Dashed line indicates the inflow concentration in simulated runoff. Error bars represent the standard error of five replicate soil cores.

Table 4.4. Percent change of TP concentration in leachate relative to inflow concentration for all soils and BAM. Negative values represent a decrease in P concentration (retention of P within the core) while positive values represent increase in concentration (release of P by the core). Minimum, maximum and mean change over the flow-through study is shown.

Soil	TP Percent Change		
	Min %	Mean %	Max %
1	-77.80	-89.48	-100.0
2	-96.84	-99.09	-99.96
3	-13.08	-64.63	-96.30
4	-94.85	-97.71	-99.90
5	-77.08	-94.57	-97.06
6	-72.17	-88.70	-98.57
BAM	53.39	-21.00	-75.59

4.4.3 Leachate pH and Dissolved Organic Carbon

The pH of the leachate from the cores was measured with an Accumet XL200 benchtop pH probe (Thermo Fisher Scientific, Waltham, MA, USA) over time and results are presented in Figure 4.12. Extracted sample were also analyzed for DOC on a Shimadzu TOC-L Analyzer (Kyoto, Japan) and results are presented in Figure 4.13.

Soil pH stayed within the tolerances of most microbes for all soils and BAM. Soils with high calcium and magnesium (Soil 1, 4 and 5; Figure 3.2) tended to cause the water pH to increase as a result of flow-through. Soil 2 and 6 were high in Al and tended to cause pH to decrease. Otherwise, effects on water pH were minimal. All soils and BAM were a source of DOC as organic matter is leached from the material during flow-through. Released DOC could provide an energy source to microbes downstream.

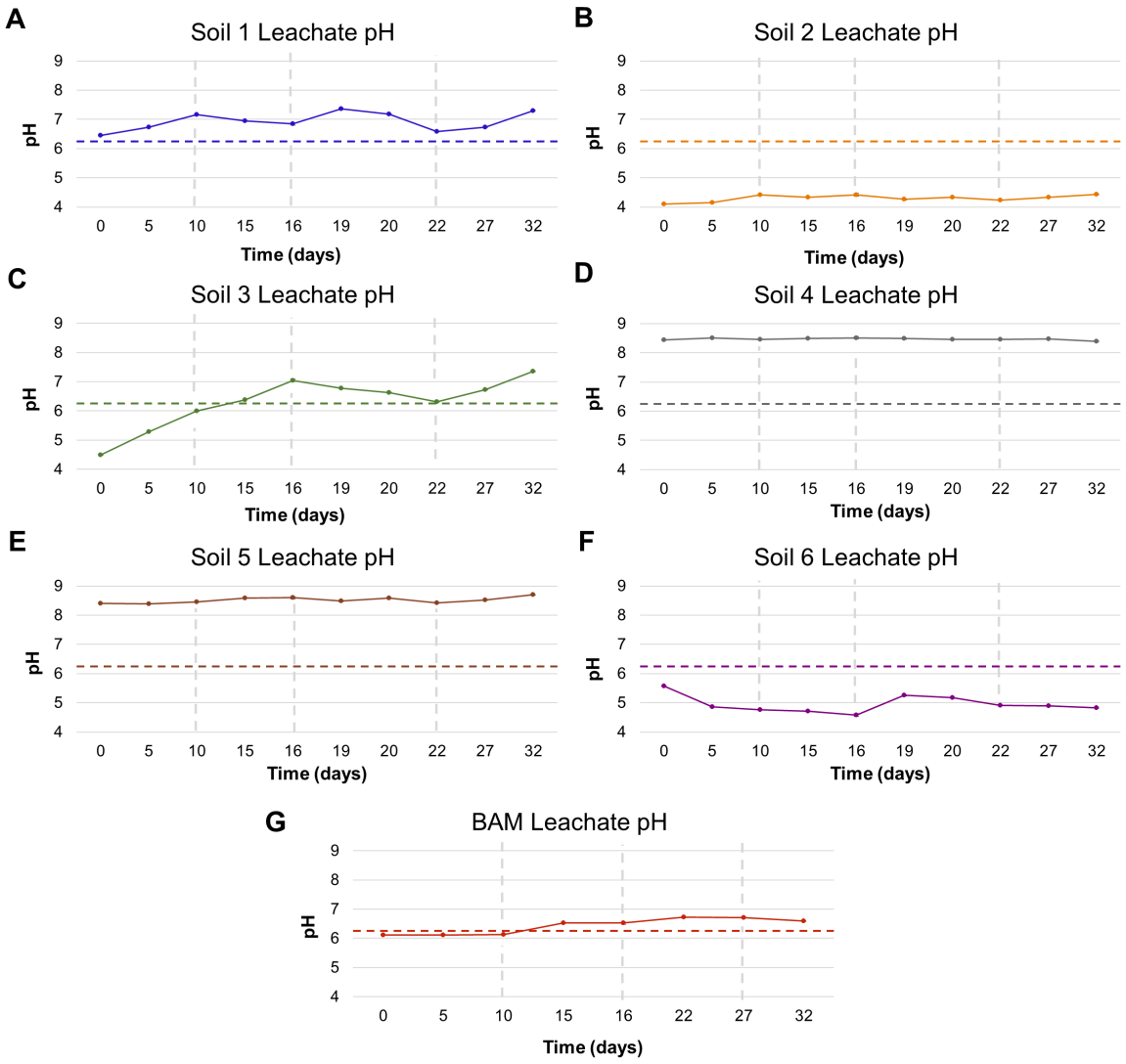

Figure 4.12. Water pH of leachate over time (solid line) for each soil type and BAM (A-G). Points represent mean; error bars represent standard error (n=5). Dashed line represents inflow pH of simulated runoff.

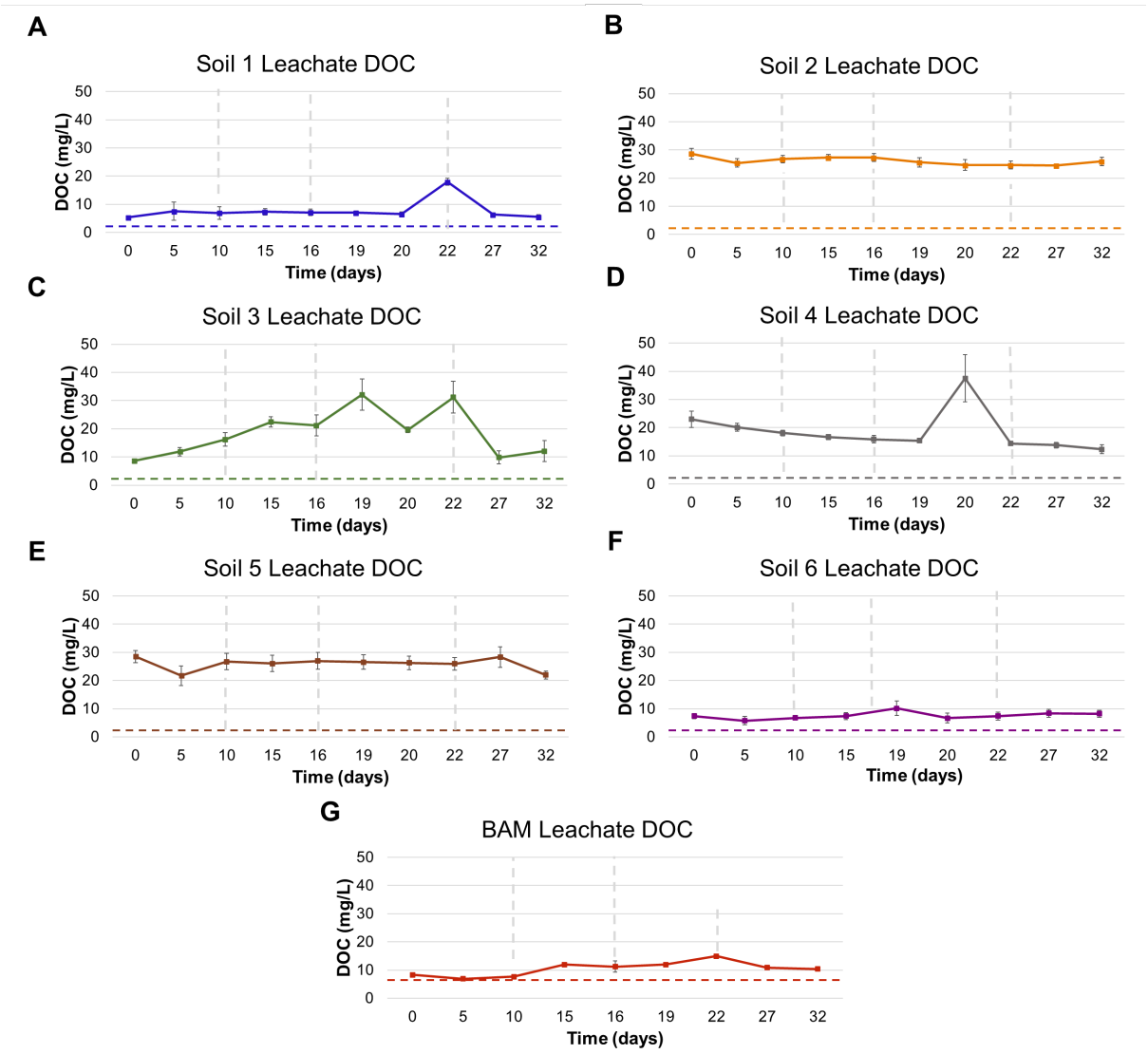

Figure 4.13. Concentration of dissolved organic carbon in leachate over time (solid line) for each soil type and BAM (A-G). Points represent mean; error bars represent standard error (n=5). Dashed line represents inflow concentration in simulated runoff.

CHAPTER 5. **Soil Changes After Stormwater Flow-through**

Following the flow-through experiment, soils and BAM were re-tested for phosphorus speciation, metals, microbial community, and soil biogeochemical processes using methods described in Ch. 3. This chapter assesses changes to these parameters that occurred during the flow-through experiment with simulated runoff. This analysis reveals how soil properties and processes may change after being exposed to the fluctuating hydrology and a constant load of external nutrients found in a stormwater retention basin. Once the flow-through study was completed, each soil core sampled by depth as described in section 2.2. The 20 cm head of water was kept on the cores during this time to maintain anaerobic conditions within the soil column. Samples were immediately weighed and stored at 4°C, while sub-samples were stored at -20°C. Each sample was processed as previously described in Sections 3.2 to 3.5.

5.1 Soil Phosphorus Speciation

After the flow-through experiments concluded each soil section was sequentially extracted for P as described in Section 3.3 to determine how the concentration and speciation of P changed as a result of the simulated runoff flow-through. The results of the speciation extracts are shown in Figure 5.1. In Soil 1, the stormwater P was retained primarily in the top 20 cm as evidenced by an increase in TP of about 50%, with the majority of that converted into organic P. The microbial community was highly active in this soil as the bacterial abundance was the highest at this depth (Figure 5.2) including *Actinobacteria* and *Proteobacteria* (Table 5.1). Soil 2 leached ~16% TP from the adsorbed P fraction in the top 0-10 cm soil. The low pH (~4.5) of the leachate (Figure 4.12) would have stripped adsorbed P from surfaces possibly near the end of the flow experiments as soluble P increased in the leachate (Figure 4.11). TP in Soil 3 decreased the most of all soil by ~ 85% from all fractions. The circumneutral pH would have made adsorptive reactions favorable; however, the low concentrations of metals indicate soil mineral surfaces were not available. Much of the P in Soil 3 was converted into labile exchangeable P. Soils 4 and 5 retained the highest amount of P of all the soils with increases of up to 2-fold in the 20-30 cm section of Soil 4. The retained P was primarily in the precipitated and organic fractions. The high concentrations of Ca and Mg along with leachate pH ~8.5 made conditions favorable for precipitation of phosphate minerals. Additionally, the high OMC as well as increases in microbial abundance indicated that conditions were favorable for organic P formation. Soil 6 behaved similarly to Soil 2, with a decrease in P retention within the adsorbed P fraction. The lower pH of ~5 would have stripped P from the soil surfaces. The distribution of P in BAM did not change during flow and was primarily in the adsorbed fraction. Even with the increase in microbial activity very little of P was converted into organic P.

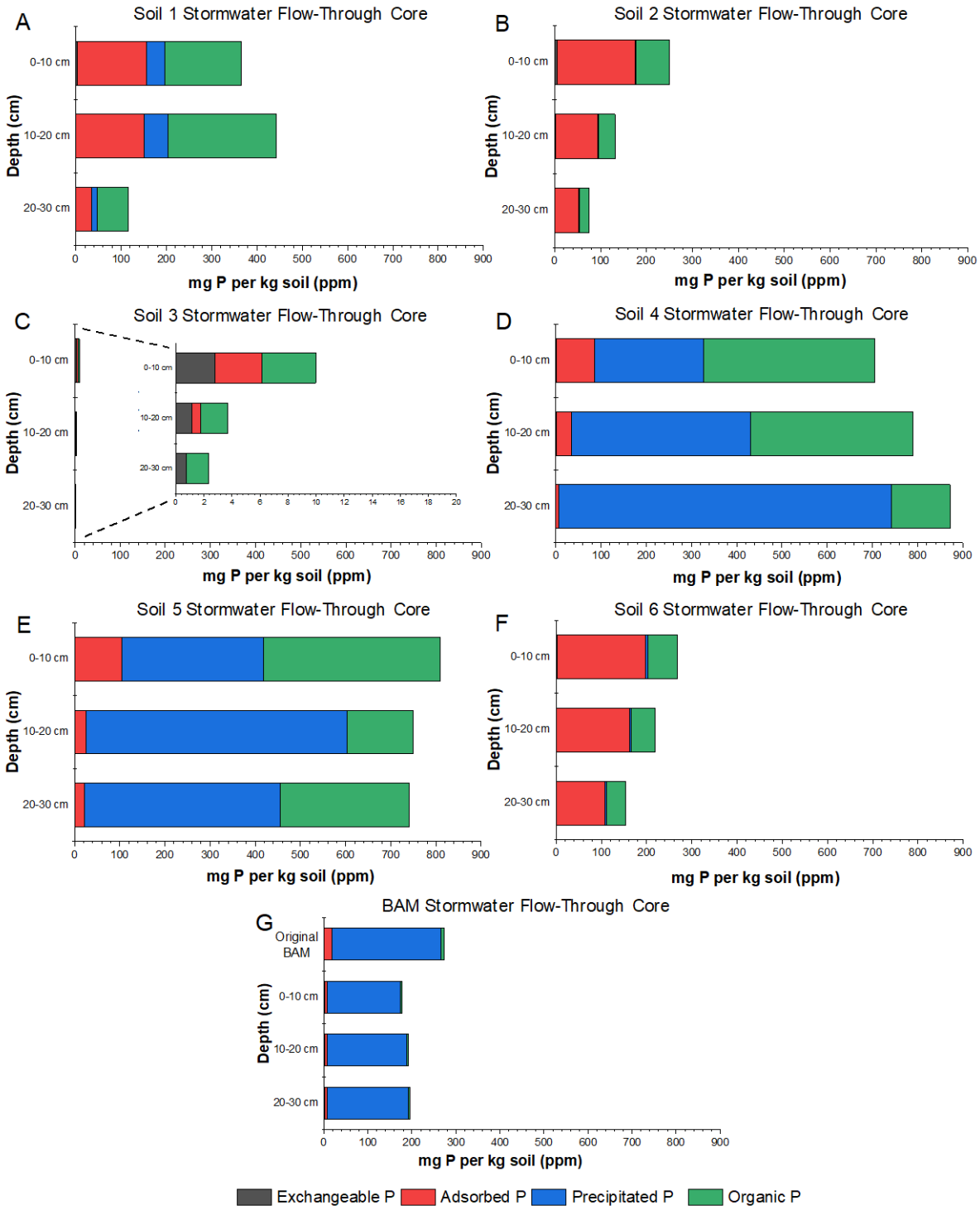

Figure 5.1. Post flow-through phosphorus speciation in each soil type (A-F) and BAM (G).

5.2 Microbial Community

Soils collected after the flow-through experiments were extracted for DNA and sequenced for microbial diversity and abundance as described in Section 3.4. Figure 5.2 demonstrates the abundance of total microbes at the different depths within each soil type. Table 5.2 lists microbial diversity and abundance by phylum and Table 5.3 outlines the % change in microbial abundance between the initial characterization and after the runoff flow-through. The % change indicates those soils where microbial abundance increased (positive values) or decreased (negative values) after stormwater runoff flow.

Microbial abundance primarily increased during the runoff flow-through experiment in all media, particularly in the surface (0-10 cm depth). This is expected, as the top of the core will have had more microbial activity due to better oxygen penetration (if any) into the top of soil and where many of the nutrients in the runoff are first accessed for respiration. The sterile simulated stormwater provided the necessary nutrients for the indigenous soil bacteria to respire and increase in abundance. In contrast, however, the microbial community was supplied to the BAM cores by the non-sterile natural pond water, and the considerable increase in microbial abundance in BAM is attributed to colonization by the stormwater microbes. Bacterial abundance in BAM increased mainly in the 0-10 cm depth and consisted primarily of *Actinobacteria*, *Alpha*- and *Gammaproteobacteria* (Table 5.2), which include typical natural water microbe species. Table 5.3 outlines the relative change (Eq. 4.3) in microbial abundance after stormwater flow. All soils except Soil 6 demonstrated an increase in total abundance after runoff flow-through.

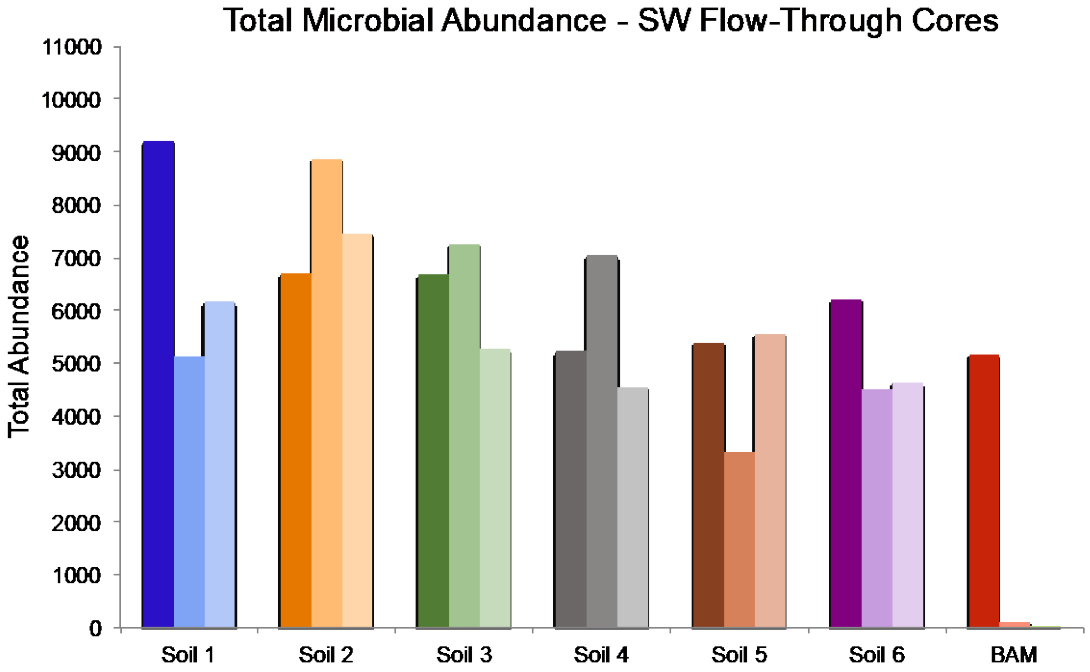

Figure 5.2. Total microbial abundance in soils and BAM following flow-through experiment. Clustered bars represent in order 0-10 cm, 10-20 cm, and 20-30 cm depth ranges.

Table 5.1. Total microbial abundance in soils and BAM following flow-through experiment. Distribution by phylum in counts per sample

Soil	Depth (cm)	*Acidobacteria*	*Actinobacteria*	*Bacteroidetes*	*Chloroflexi*	*Cyanobacteria*	*Firmicutes*	*Nitrospirae*	*Planctomycetes*	*Proteobacteria-Alpha*	*Proteobacteria-Delta*	*Proteobacteria-Gamma*	*Verrucomicrobia*	*Other*
1	0-10	557	1578	311	734	50	168	176	296	2183	1109	1574	193	285
	10-20	773	769	194	362	8	130	253	30	1053	748	325	184	303
	20-30	2081	230	77	86	0	109	267	182	1146	1182	435	113	267
2	0-10	1877	1058	9	132	20	163	29	533	1562	411	549	197	160
	10-20	2959	1084	0	621	12	110	170	392	1840	830	399	179	272
	20-30	2854	413	0	548	0	21	168	165	1747	1006	225	111	209
3	0-10	930	1901	81	0	0	211	0	190	1682	224	1225	145	95
	10-20	2019	1530	19	17	45	580	0	326	1369	447	518	118	274
	20-30	1984	768	8	26	105	380	3	212	767	195	470	189	172
4	0-10	244	1188	97	224	0	239	17	181	1465	627	690	91	185
	10-20	615	1302	0	360	0	292	151	235	1933	975	571	120	507
	20-30	395	334	26	762	0	153	169	88	630	597	627	24	751
5	0-10	429	753	95	365	0	292	185	138	1282	808	669	27	357
	10-20	351	394	37	195	0	160	170	54	629	498	401	22	420
	20-30	419	560	51	874	0	154	248	193	721	1013	511	0	812
6	0-10	1578	1513	7	368	3	258	55	528	1227	149	272	147	117
	10-20	1580	685	0	375	7	177	91	147	1088	103	116	71	79
	20-30	1495	533	5	819	0	77	98	154	905	179	117	79	192
BAM	0-10	31	1639	94	64	83	29	14	90	922	29	2142	2	32
	10-20	0	17	0	0	0	12	0	0	18	14	36	0	18
	20-30	0	0	0	0	0	0	0	0	6	0	4	9	0

Table 5.2. Percent change in total microbial abundance in soils and BAM after flow-through study. Positive values indicate increase in total abundance after exposure to simulated runoff; negative values indicate a decrease in total abundance.

Soil	0-10 cm	10-20 cm	20-30 cm
1	104%	-25.8%	-42.1%
2	116%	14.2%	20.4%
3	13.4%	29.4%	-40.1%
4	11.2%	30.6%	131%
5	-16.3%	47.3%	103%
6	-1.6%	-36.2%	-30.6%
BAM	3,878%	-11.5%	-85.4%

Figure 5.3 presents a principal components analysis (PCoA) based on a Bray-Curtis diversity metric, which calculates the statistical dissimilarity between samples. In this PCoA plot, if sample points are clustered close together that indicates those samples are biologically similar. The more different a sample is from another, the farther apart they are. As indicated in the analysis, similarities are observed between Soils 4 and 5 and Soils 3 and 6. Less similar are Soils 1 and 2 with points further apart but possibly clustering. The only BAM sample that could be included in the statistics was the 0-10 cm flow-through as all of the other BAM samples did not contain enough microbial abundance to be included in the analysis.

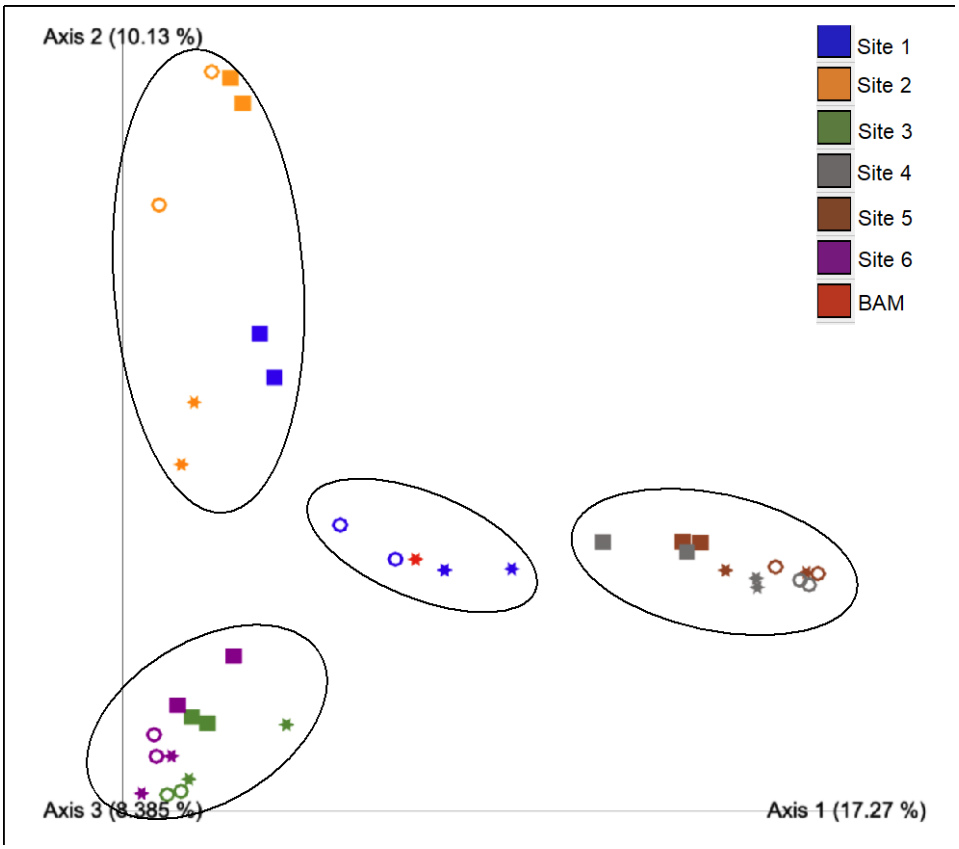

Figure 5.3. PCoA Emperor plots based on Bray-Curtis diversity metric. Soils (1-6 and BAM) and soil depth (0-10 cm: stars; 10-20 cm: circles; 20-30 cm: squares) for field cores and flow-through cores were compared based on microbial community abundance. Clusters were observed between Sites 3 and 6, Sites 4 and 5, and Sites 1 and 2. BAM is represented only by 0-10 cm depth as the other depths did not contain enough bacterial counts to be included in the PCoA analysis.

5.3 Soil Biogeochemical Properties

Extractable nutrients (NH_4^+, NO_3^-, DOC), total C and N, and soil pH in each depth segment after the flow-through experiment are presented in Table 5.3. Following exposure to simulated runoff, the extractable NH_4^+ data exemplifies the idea of soils adsorbing NH_4^+ onto the cation exchange complex. After the flow-through study, Soils 1-5 all exhibited a significant increase in extractable NH_4^+, while Soil 6 and BAM did not adsorb additional NH_4^+. Extractable NO_3^- also increased slightly in some soils, while extractable DOC, TC, and TN did not differ appreciably from field conditions (Table 3.4).

Table 5.3. Media properties by depth in each soil and BAM after the flow-through study.

Depth	0-10 cm						10-20 cm						20-30 cm					
Soil	NH_4^+ (mg/kg)	NO_x (mg/kg)	DOC (mg/kg)	TC (g/kg)	TN (g/kg)	pH	NH_4^+ (mg/kg)	NO_x (mg/kg)	DOC (mg/kg)	TC (g/kg)	TN (g/kg)	pH	NH_4^+ (mg/kg)	NO_x (mg/kg)	DOC (mg/kg)	TC (g/kg)	TN (g/kg)	pH
1	38.5 ± 8.5	27.4 ± 1.8	390.4 ± 104.0	477.7 ± 3.7	18.1 ± 0.7	6.8 ± 0.1	45.8 ± 9.6	21.7 ± 1.6	355.3 ± 12.3	463.0 ± 5.4	15.2 ± 0.5	7.0 ± 0.1	37.2 ± 6.9	17.7 ± 0.9	284.7 ± 24.7	542.5 ± 6.8	14.1 ± 0.4	6.8 ± 0.1
2	77.9 ± 16.7	0.8 ± 0.5	3116.5 ± 497.7	382.4 ± 16.7	25.5 ± 1.0	5.7 ± 0.3	16.5 ± 3.7	0.8 ± 0.4	3400.0 ± 483.2	290.6 ± 22.1	16.5 ± 1.9	4.9 ± 0.2	9.4 ± 2.1	0.3 ± 0.3	1943.2 ± 225.5	223.0 ± 37.9	11.0 ± 2.3	4.6 ± 0.1
3	11.6 ± 2.3	4.1 ± 0.2	483.0 ± 151.7	19.9 ± 2.9	0.76 ± 0.1	6.4 ± 0.1	4.7 ± 1.0	3.5 ± 0.2	326.0 ± 87.3	5.2 ± 0.2	0.3 ± 0.0	6.3 ± 0.1	3.0 ± 0.6	3.6 ± 0.2	166.6 ± 17.1	2.8 ± 0.2	0.26 ± 0.0	6.2 ± 0.1
4	48.4 ± 10.4	0.0 ± 0.0	331.1 ± 11.7	261.8 ± 28.9	15.4 ± 1.6	8.0 ± 0.1	17.1 ± 4.4	0.0 ± 0.0	333.8 ± 49.2	142.9 ± 14.4	6.6 ± 1.8	8.3 ± 0.1	3.3 ± 0.9	0.0 ± 0.0	281.1 ± 32.6	127.9 ± 7.9	3.0 ± 1.7	8.5 ± 0.1
5	63.7 ± 12.0	1.8 ± 0.1	450.6 ± 28.0	321.1 ± 13.3	18.6 ± 0.7	8.2 ± 0.0	7.6 ± 2.1	0.8 ± 0.1	472.4 ± 47.4	144.4 ± 4.3	3. 7 ± 0.6	8.3 ± 0.1	6.3 ± 4.0	1.1 ± 0.1	457.3 ± 29.1	172.5 ± 3.0	7.9 ± 0.7	8.2 ± 0.1
6	14.7 ± 3.1	0.9 ± 0.1	775.0 ± 74.6	51.6 ± 4.1	3.7 ± 0.3	5.1 ± 0.0	1.8 ± 0.7	0.5 ± 0.0	465.1 ± 49.2	26.2 ± 2.6	2.2 ± 0.1	5.1 ± 0.1	0.1 ± 0.1	0.5 ± 0.0	282.4 ± 48.8	10.1 ± 1.0	1.2 ± 0.1	5.1 ± 0.0
BAM	0.0 ± 0.0	3.2 ± 0.1	128.5 ± 14.0	146.2 ± 59.9	4.5 ± 3.7	7.3 ± 0.4	0.1 ± 0.1	2.4 ± 0.2	98.4 ± 18.4	256.7 ± 44.5	1.4 ± 0.2	7.2 ± 0.4	0.0 ± 0.0	2.3 ± 0.1	86.2 ± 9.5	141.4 ± 26.0	0.9 ± 0.1	7.4 ± 0.4

5.4 Net Nitrogen Balance

After the conclusion of the flow-through experiment and the destruction and sectioning of the soil cores, DEA and PMN incubations were repeated (as described in Sections 3.5.3 and 3.5.4) to determine if the simulated stormwater inflow conditions impacted the net balance of N biotic transformations in the soils. Following exposure to simulated runoff, rates of microbial N cycling tended to increase (particularly in Soils 2, 3, 4, and 5) and the net balance between N removal and release tended to be knocked off the relative equilibrium seen in the field activity samples (Figure 3.5). BAM continued to serve as a net source of N, but simulated runoff flow-through stimulated denitrification in the surface of Soil 3 and mineralization in Soils 1 and 6. Overall, Soils 2, 4 and 5 showed optimal conditions of net N removal via DEA (Figures 5.4 and 5.5).

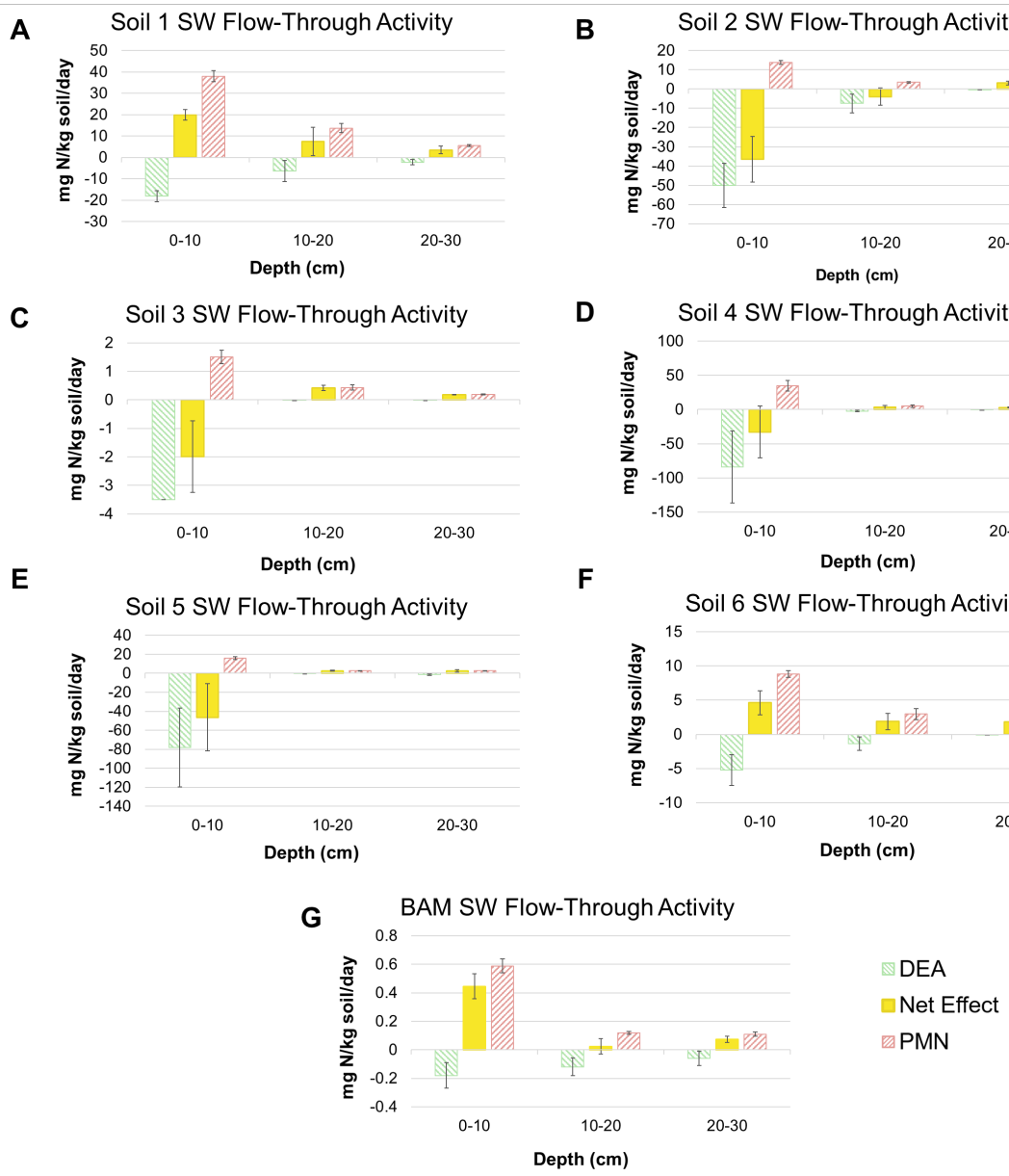

Figure 5.4. Rates of nitrogen transformation in soils and BAM post flow-through, by depth. Nitrogen removal is represented as a negative rate (green) and is based on results of the DEA assay while nitrogen release is represented as a positive rate (red) and is based on the PMN assay. The net effect (removal-release) is represented in yellow.

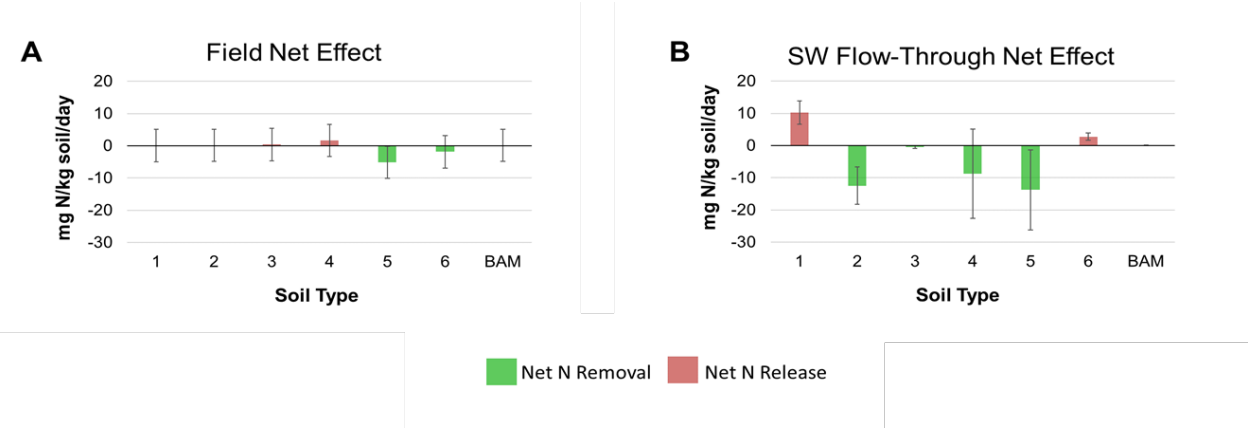

Figure 5.5. Depth-averaged net nitrogen balance for all soils and BAM before flow-through (field, A) and post flow-through (B).

5.5 Nitrogen-cycling Bacteria

Specific bacterial groups important to nitrogen cycling in soils were pulled from the sequencing data and their abundances compared between pre- and post-stormwater flow. Table 5.4 outlines the abundances of specific bacteria from each soil that are known to be involved in nitrification (ammonia oxidation to nitrate and nitrite), denitrification (nitrate to N_2), DNRA (nitrite to ammonium), along with other known important soil bacteria. This is not an exhaustive list but includes the more well known among the nitrogen-cycling community. In several examples, the abundances increased from before to after stormwater runoff. Soil bacteria are not always active and can remain in a dormant state, becoming active when water and nutrients are available, at which time bacteria begin respiring on carbon sources and increasing in cell counts. Therefore, during runoff events, it is expected that many bacteria will increase in numbers. However, several samples did not show much of a change. In these instances, it is possible that the specific groups were already active in situ at the time of collection. Site 1 specifically was inundated with water and high in OMC, providing bacteria with the needed water and nutrients to respire; as such the majority of listed bacteria did not change drastically between the pre- and post-flow. In other instances, if abundance decreases, it may be due to changing conditions (e.g., oxygen content, temperature, pH) that are inhospitable or bacteria out-competing for carbon and nutrients. It is interesting to note the differences between the soils in the presence or absence of individual bacteria. Other typical soil bacteria include those involved in plant root symbiosis such as N_2-fixation.

Table 5.4. Abundances of bacteria involved in N-cycling in soils. Numbers represent bacterial counts per sample

Bacteria	**Soil 1**		**Soil 2**		**Soil 3**		**Soil 4**		**Soil 5**		**Soil 6**		**BAM**	
Nitrification	Pre SW	Post SW	Pre SW	Post SW	Pre SW	Post SW	Pre SW	Post SW	Pre SW	Post SW	Pre SW	Post SW	Pre SW	Post SW
Nitrosomonadaceae	382	294	10	0	9	0	432	484	508	581	3	0	0	0
Nitrospira	739	696	289	367	17	3	274	337	281	603	215	244	0	14
Micrococcales	12	59	0	15	94	207	0	0	0	0	16	0	0	827
Denitrification														
Thiobacillus	0	32	0	0	0	0	0	13	64	16	0	0	0	0
Rhodocyclaceae	0	0	0	0	0	126	0	0	0	0	0	0	0	0
Burkholderiaceae	426	446	44	145	379	1008	74	25	113	54	194	18	6	120
Acintobacter	16	0	0	0	6	0	2	0	0	0	0	0	26	29
Rhodobacter	9	0	0	0	0	0	0	4	0	0	0	0	0	140
Flavobacteriales	20	0	0	0	0	0	0	0	0	0	0	0	2	0
DNRA														
Brocadia	0	0	0	0	0	0	0	3	0	0	0	0	0	0
Campylobacterales	0	5	0	0	0	0	0	0	0	0	0	0	0	0
Lachnospiraceae	6	3	0	0	0	0	12	15	25	13	0	0	0	0
Beggiatoa	0	8	14	7	0	0	0	0	0	0	0	0	0	0
Thiothrix	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Geobacter	202	402	8	16	12	886	0	64	48	120	16	0	0	0
Desulfobulbaceae	0	0	0	0	0	0	3	0	3	0	0	0	0	0
Other Soil Bacteria														
Streptomyces	0	0	0	0	25	0	87	169	48	42	0	15	0	0
Azospirillales	0	0	0	0	0	0	0	25	0	0	2	0	0	0
Rhizobiales	2987	3546	1833	2552	1684	1864	2280	3485	2118	2300	2266	2062	0	599
Mycobacterium	197	217	111	315	652	430	173	202	173	123	639	293	0	267

CHAPTER 6. **Conclusions**

Study conclusions are summarized by soil type (Figure 6.1, Table A.1) and according to the primary research questions addressed.

6.1 Nutrient Remediation Potential of Florida Soils

6.1.1 Initial Soil Characterization

Soils analyzed directly from the field all contained N and P in various forms and quantities. The amount of total N in native Florida soils was often positively related to OMC, and generally decreased with soil depth (Table 3.2). The rate of biotic processing of N (i.e., DEA and PMN) in native soil under natural field conditions was also positively related to OMC, such that high OMC soils tend to both release N (via PMN) and remove N (via DEA) at an overall greater rate than soils with less OMC (Figure 3.5). However, **under natural field conditions, N release and N removal were roughly equal in all native soils** (represented by the Net Effect yellow bars in Figure 3.5). Soil 5 showed a very slight tendency toward net N removal, but for all other soil types the net N balanced when averaged over the 30 cm soil depth and was not significantly different than zero (Figure 5.5A).

The concentration and speciation of P within each soil type was diverse (Figure 3.3). Whereas the characterization of N in native soils was driven by OMC, **P concentration and speciation was determined by pH and grain size** (Tables 3.2 and 3.4). Soils with higher pH (> 7) and moderate mixtures of sand/silt/clay contained greater amounts of TP that proportioned between mineral phosphate precipitates and organic P. Soils with high sand and low-to-moderate pH (~4.7–5.0) contained low TP adsorbed to particles with small amounts of organic P. Soils with the finest particle size and moderate pH (5–6) contained moderate amounts of TP that primarily was adsorbed and within the organic fraction in soils with higher OMC. Overall, TP concentration tended to decrease with soil depth.

The soil microbiome of the native soils contained a highly diverse microbial community typically found in soils with higher abundances of *Actinobacteria*, *Firmicutes*, and *Proteobacteria*. **Microbial abundance tended to trend with OMC, TP, and TN concentrations**, which is predictable as many soil microbes are decomposers of organic matter and require nutrients of N and P to respire.

6.1.2 Flow-through Experiment

Ammonium (NH_4^+): **All native soils consistently removed NH_4^+ from simulated runoff**, as shown by lower concentrations in the leachate as compared to the incoming simulated runoff solution. **Mean removal efficiency varied from 31%–90% across soils.** The Orange County soils (Soils 1-3) showed a decreasing NH_4^+ removal over time (Figure 4.4), suggesting the potential for NH_4^+ saturation over time on the cation exchange complex. *Longer-term experiments are suggested for Phase II to address the potential for NH_4^+ saturation over time under longer-term exposure to stormwater runoff.*

Nitrate (NO_3^-): **All native soils consistently removed NO_3^- from simulated runoff**, as shown by lower concentrations in the leachate as compared to the incoming simulated runoff solution. **Mean removal efficiency varied from 75%–90% across soils.** The capacity to remove NO_3^- remained relatively consistent over the course of the study (Figure 4.5).

Total N (TN): **Soils 1, 4, 5, and 6 consistently removed TN from simulated runoff**, as shown by lower concentrations in the leachate as compared to the incoming simulated runoff solution. Soil 6 (greatest clay content), in particular, served as the greatest sink for TN. Soils 2 and 3 were a source of TN, shown by higher concentrations in the leachate as compared to the simulated runoff solution (Figure 4.6). The release of TN observed in Soils 2 and 3 was driven by very high concentrations of organic N in the leachate (Figure 4.7). This was due to the severing of large root mats during the collection of soils from these vegetated sites. This release of ON is likely a temporary result of the disturbance caused by the experiment set-up. *Longer-term experiments at a mesocosm scale including live vegetation are suggested for Phase II to more realistically model in situ soil behaviors.*

Dissolved orthophosphate (PO_4^{3-}): **PO_4^{3-} the most labile form of phosphorus, was below detection in all leachate samples**.

Total Soluble P (TP)**: All native soils retained TP from simulated runoff**, as shown by lower concentrations in the leachate as compared to the incoming simulated runoff solution (Figure 4.11). However, mean retention efficiency varied across soils. **Soils with higher pH leachate, moderate sand/silt/clay proportions and at least moderate metal contents (Soils 2, 4, 5) removed the highest amounts of TP (95%–98%), while Soil 3, which contained low clay, few metals, and low OMC, removed the least amount of TP (65%)** [Table. 4.4]. The leachate of Soils 2, 4, and 6 contained low concentrations of soluble TP during the initial wet season, but the concentrations released increased after dry down periods. Soil 3 released almost all TP from simulated runoff solution after the dry downs, suggesting P may be remobilized after dry periods or the soil capacity for P was limited. *Longer-term experiments are suggested for Phase II to address the potential for P saturation over time under longer-term exposure to stormwater runoff.*

6.1.3 Soil Change After Flow-through Experiment

Nitrogen: Following the flow-through experiment, the repeat assessment of N biotic processing (DEA and PMN) revealed N release and N removal were no longer in balance for some soils, likely due to the implementation of artificial flooding and drainage regimes and a consistent external load of N in the simulated runoff. Specifically, **Soils 2 and 5 stood out as being significant sinks of N (high net N removal), while Soils 1 and 6 were sources of N (net N release)** (Figure 5.5b).

Phosphorus: Following the flow-through experiment, the repeat assessment of TP speciation fractions determined that **soils with moderate sand/silt/clay and higher pH (Soils 4 and 5) retained the highest concentrations of TP** that fractionated between the mineral phosphate precipitate phase and organic P (Figure 5.1). The pH increased during the stormwater flow (pH ~8.5; Figure 4.12) contributing to P precipitation, and high microbial activity converted P to biomass. Similarly, Soil 1, the soil with the highest OMC, converted much of the retained TP to organic biomass. Organic P is one of the most stable forms of P and eventually can become buried and permanently sequestered. Sandy soils (Soils 2 and 3) actually leached adsorbed P during stormwater flow and thus contained less P after the flow-through test.

Soil Microbiome: **The microbial abundance in all soils generally increased after stormwater flow** (Table 5.2). Microbes become active in hydrated conditions with added nutrients in the simulated stormwater. Microbial abundance increased the most in top layers of soil (0-10 and 10-20 cm layers). **Soils that converted the most TP to organic biomass (Soils 1,**

4, and 5) also contained a higher diversity of active N-cycling bacteria (Table 5.4). Likewise, soils that retained the least TP also contained the lowest diversity and abundance of nitrogen bacteria, suggesting that P sequestration in the organic fraction is also mediated by biological processes.

6.2 Nutrient Remediation Potential of BAM

6.2.1 Initial BAM Characterization

BAM had lower rates of biotic N processing as compared to native soils, with slightly higher rate of N release (PMN) than N removal (DEA) indicating that BAM is a net N source (Figure 3.5). This was expected because the **initial microbiome in BAM was basically nonexistent.** BAM contained very small concentrations of Al, Mn, and inappreciable amounts of Mg and Ca. BAM contained a moderate amount of TP in the form of precipitated P (possibly aluminum phosphates), comparable to concentrations found in Soil 2. DNA extracted from BAM collected after PMN assays did not amplify during PCR for the presence of any nitrogen cycling genes indicating that **BAM on its own did not have the ability to process N through denitrification or DNRA.** This result highlights the importance of combining BAM with native soils to leverage the donor microbiome of soils when BAM is applied within BMPs. *Longer-term experiments at a mesocosm scale with combinations of BAM, native soils and live vegetation are suggested for Phase II to model soil-BAM interactions that occur in BMPs.*

6.2.2 Flow-through Experiment

Like soils, BAM often decreased concentrations of N and P from simulated runoff. However, **mean efficiencies of nutrient removal were somewhat lower in BAM as compared to soils (TN: 24%; NO_3^-: 60%; TP: 21%)**. BAM was the only media tested that exhibited inorganic nitrogen release (increase in concentration relative to inflow). During the flow-through experiment, **BAM was often a source of NH_4^+**, as shown by higher concentrations of NH_4^+ in the leachate, as compared to the inflow of simulated runoff added to the cores (Figure 4.7); overall **mean concentrations were 43% greater after flowing through BAM**. BAM also became a slight source of NO_3^- by the end of the flow-through experiment (Figure 4.8). *Longer-term experiments are suggested for Phase II to confirm if BAM could serve as a source of NO_3^- via nitrification after long-term exposure to stormwater in an infiltration basin.* BAM leached low concentrations of ON, so through the lens of TN, **BAM was neutral or a slight sink for TN**. Stormwater leachate from BAM cores contained approximately the same amount of soluble P as the inflow, indicating that **BAM was not efficiently retaining P**, particularly during the first half of the experiment.

6.2.3 BAM Change After Flow-through Experiment

Prior to and during the flow-through experiments, BAM cores were exposed to stormwater collected from a retention pond to allow for development of the microbial community. While the **microbiome of BAM increased substantially after flow-through** (primarily in the 0-10 cm depth) **overall microbial diversity in BAM was low compared to soils** with assemblages from typical water and soil *Actinobacteria* and *Proteobacteria.*

Results of the DEA and PMN assays repeated after flow-through reflected the development of a microbial community (Figure 5.2). Although N rates did increase slightly after flow-through, **biotic N processing in BAM remained lower than all native soils** and had a

higher rate of N release than N removal **(BAM was a net source of inorganic N)** (Figure 5.4 and 5.5). Several of the nitrogen cycling bacteria were established in the BAM flow cores particularly *Micrococcales*, a group of bacteria known to conduct nitrification (ammonia oxidation to nitrate and nitrite), which could account for the source of nitrate observed near the end of the flow experiments. The speciation of P in BAM did not change during the flow experiment. *Longer-term experiments at a mesocosm scale with combinations of BAM, native soils and live vegetation are suggested for Phase II to allow for more complete microbial community development in BAM.*

6.3 Soil Properties Related to Nutrient Remediation

While all soils sequestered or removed nutrients from simulated stormwater, variations in performance across different soil types allow for preliminary conclusions about the soil properties associated with beneficial nutrient sequestration or transformation in the soil profile. Soils 2 and 5 performed the best at removing N through denitrification (DEA assay) more rapidly than N was released (PMN assay), resulting in net N removal when subjected to flow-through conditions (Figures 5.5, 6.1). Both Soils 2 and 5 contained surface layers with **moderately high concentrations of OMC** (i.e., 41%–50% by mass) and **moderate clay content** (i.e., 4.5%–6.7% by mass; Table 3.2). Of note, the soil with the highest OMC (Soil 1, 86%–93% OMC) and the soil with the highest clay content (Soil 6, 14%–17% clay) performed the worst at net biotic N removal. Similarly, the soil with little OMC or clay (Soil 3) did not perform well.

Soils 2, 4 and 5 performed the best at retaining P, though **all soils with at least moderate concentrations of metals (all soils except Soil 3) were associated with high P retention**. Soil 3 was characterized by low OMC, clay content, and metals and the coarsest grain-size distribution tested. Metal cations form oxyhydroxide minerals in soil that provide surfaces for P adsorption reactions. Conversely, the high proportion of quartz silica and lack of metals in Soil 3 did not provide favorable surfaces for P adsorption.

Overall study results are therefore congruent regarding the soil properties associated with both N and P remediation; **soils with moderate OMC and clay (Soils 2, 4, 5) were most effective at removing/sequestering N and P, while soils with the highest OMC (Soil 1), highest clay content (Soil 6) and lowest OMC and clay content (Soil 3) were not as effective** (Figure 6.1).

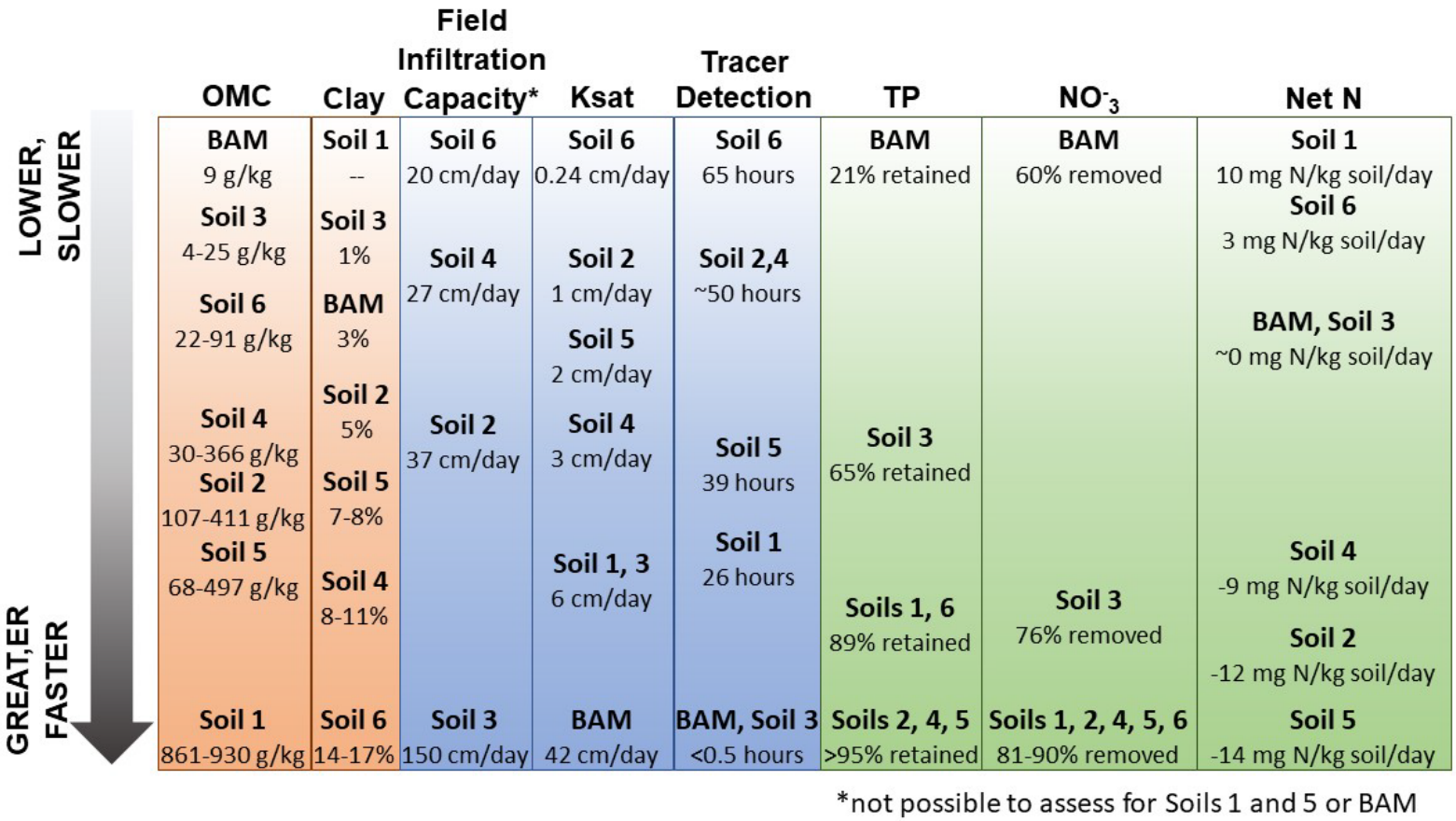

Figure 6.1. Summary of study findings. Soils 2, 4, and 5 retained the most P and had the greatest capacity to remove N (green columns). These soils all contained moderate OMC and clay content (orange columns) and water moved through these soils at low to moderate rates (blue columns).

Study results allow for preliminary exploration as to how and why soils with moderate OMC and clay content are effective at remediating nutrient loads. OMC and clay content can influence nutrient processing directly by affecting the soil composition, for instance through the cation exchange capacity on clay surfaces, or the availability of carbon as an energy source for microbes. The study highlighted the direct impact of soil composition to P retention, as soils with higher pH and at least moderate concentrations of metals were effective at retaining P. However, both OMC and clay content also enact indirect effects to nutrient transformations through hydraulic processing controls, which dictate contact time of stormwater within the soil profile and shape microbial communities. For instance, grain size distributions and OMC control the transmission rate (e.g. K_{sat}) and water-holding capacity (e.g. slope of falling tracer limb) of the soil. It is challenging to differentiate direct impacts to nutrient remediation arising from the soil material properties themselves from indirect impacts via hydrology. In this study, the flow-through experiment combined both biotic nutrient processing and soil storage capacity in the context of a simulated stormwater basin. All media were subjected to the same hydrology during flow-through experiments. All cores were fully saturated, saturation was maintained for the same length of time, and then each core was allowed to drain for the same length of time before simulated runoff was reintroduced in the same pulse flow rhythm. However, as the media properties controlled the rates of flow, drainage, and water retention in the cores (Table 4.2), **the same incident hydrology produced different soil profile hydraulics in each soil tested, which influenced microbial and nutrient dynamics**.

Soils with greater clay content (Soils 4, 5, 6) and slower hydraulic rates exhibited high removal rates for all species of N in the flow-through experiment. The high OMC soil (Soil 1) also exhibited high removal of nitrate during the flow-through study. In addition to the longer residence times allowing for more complete biotic processing of N, soils high in clays and fine minerals tend to have a high cation exchange capacity, which improves the ability of the soil to hold ions within the soil; this may have contributed to a higher N storage capacity in Soil 6. *Phase II studies should quantify the cation exchange capacity of the soil to address this hypothesis.*

Soils found to have the greatest biotic N processing potential (Soils 2 and 5) were commonly characterized by relatively **lower saturated hydraulic conductivity** (1.27–1.66 cm/day), **high hydraulic residence times, and a low gradient falling limb of tracer concentration**, suggesting that the range of OMC in these soils (107–411 g/kg and 68–497 g/kg in Soils 2 and 5, respectively) held and slowly released water. Notably, while Soils 2 and 5 contained comparable clay contents (5% and 7%–8%, respectively), their overall grain size distributions were different; Soil 2 was a sandy loam while Soil 5 contained high proportions of silt, especially at deeper layers. This suggests that **OMC may be as important, if not more important to N processing as mineral size class.**

Maximizing denitrification (the microbial conversion of NO_3^- to N_2 gas) is the most effective way to naturally remove excess N from stormwater. To maximize this pathway, soils must have, (1) an adequate supply of organic matter to serve as an energy source for the microbes, (2) slightly reduced (anaerobic) conditions so the denitrification pathway becomes energetically favorable, and (3) a sufficient supply of available NO_3^- to reduce. Requirement #3 was met with the flow-through conditions, allowing for a closer look at the ability of soil properties to meet requirements #1 and #2 for denitrification. As mentioned, **Soils 2 and 5 have sufficient organic matter for energy (#1). The water-absorbing capacity of this organic**

matter, combined with a moderate amount of clay, slowed the hydraulic conductivity and kept the soils slightly anaerobic (#2). These soil properties likely contributed to the high net N removal of Soils 2 and 5.

Altogether, study hypotheses (that OMC and clay content are associated with high rates of nutrient removal or retention) are supported; however, **soils with moderate amounts of both clay and OMC appear to be the most effective at remediating combined nitrogen and phosphorus loads**. This result suggests that replacement or augmentation of site soils with engineered media may not improve nutrient remediation from stormwater when site soils contain moderate contents of clay and organic matter. *Phase II studies should build upon this result to refine predictions of where it may be advantageous to amend site soils with engineered media in the context of nutrient remediation BMPs.*

6.4 Interpreting and Applying Study Results

This is a Phase I preliminary study of the complex relationship between soil properties and the hydrodynamic, chemical and biological processes that control how soils respond to nutrient load. In addition to the research being preliminary, there are limitations to what can be drawn from the results based on the experimental methods applied. These study limitations should be considered as results are applied. First, while attempts were made to study the soils as they occur in the real world (in situ), for example by preserving the structure of the soil profile, replicating the hydrology found in stormwater management ponds, ect., there are limitations to the extent that benchtop and column study laboratory methods can replicate critical zone functions. Soils in situ interact with surface vegetation, the effect of which was not considered in the laboratory portions of this study. In fact, severing and removal of the vegetative layer at the soil surface introduced confounding error into assessment of TN processing of soils, as organic nitrogen released from decaying root matter introduced nonnegligible influence. This influence was an artifact of the experimental method, not a true measure of how these soils might behave in situ, where living vegetation would influence infiltration and affect nutrient reactions in the rooting zone, for instance by creating macropores and supplying oxygen. The testing of BAM alone, without considering interaction with soils and vegetation, is another limitation of the experimental method. In a stormwater BMP, BAM is recommended to be used in close conjunction with soils, for instance layered between soils, and the surface soil layer is typically vegetated. This study confirms the vital importance of this approach, which facilitates the migration of a donor microbiome from the soils into the near-sterile BAM. Given more time for development of the microbial community and more interaction with native soils and vegetation, it is possible that especially nitrogen remediation potential in BAM may approach what was observed in some of the native soils tested in this study.

The simulated stormwater applied during the soil column study consisted primarily of salts of the minerals of interest, making the simulated runoff potentially more labile, chemically active and biologically available than actual stormwater runoff. Further, the simulated runoff used to test soils was made with a base of sterile water and did not contain other constituents typically found in actual stormwater runoff, such as suspended solids that often bind nutrients. This could cause the nutrient removal rates observed in the laboratory study to be either more or less than would be observed in situ. Runoff used to introduce microbial communities to BAM and to test BAM nutrient removals was created using actual stormwater runoff as opposed to the sterile water used as a base to the simulated runoff applied to test soils. While this

methodological difference was necessary to introduce a microbial community to the near-sterile BAM, it did introduce unanticipated experimental artifacts to the study, producing stormwater runoff with a slightly different mean concentration of nutrients. This was not due to lack of consideration for the nutrient content of the actual stormwater; this was tested, and adjustments were made as needed to accommodate the base nutrient level. However, the stormwater contained other constituents found in actual stormwater runoff, such as suspended solids and an active microbial community. These other constituents reacted with the added nutrients, such that nutrients were actively transformed at different rates before introduction to the BAM and soil cores. This activity produced a slightly different mean concentrations of runoff as compared to the simulated runoff used to test soils. Overall, the differences to the mean starting concentrations are minor, but use of different base water sources to test soils and BAM create an imperfect comparison.

Bearing in mind the caveats stated above, results of this preliminary study do suggest that the initial hypotheses of the research are correct: organic matter and clay content are indeed constituents related to the nutrient remediation capacity of soils. Furthermore, in this study, soils that contain both organics and clay within specific ranges had the greatest potential for nutrient remediation. Results of this study suggest that soils with clay content ranging from 5%–8% and OMC in the range of 400–500 g/kg in the surface 10 cm and 60–300 g/kg in 10-30 cm layers were associated with the greatest nutrient remediation potential. Furthermore, soils with pH over 7 and metal content in the range of 10^2–10^3 mg/kg were observed to retain phosphorus at high levels. To put this information into practice, it may be necessary to conduct site testing that is not usually performed in the context of roadway design. Soil classification methods as specified by the American Association of State Highway and Transportation Officials (AASHTO) such as grain size distributions and Atterberg limits relate to only the mineral fractions of soils and do not consider organics. Therefore, soils testing conducted for roadway design, including methods used in FDOT Standard Specifications, may not effectively distinguish potentially high- from low-performing site soils from the perspective of nutrient remediation potential.

Phase II studies should build upon these preliminary results to refine predictions of where it may be advantageous to amend site soils with engineered media for the purpose of nutrient remediation in stormwater BMPs. Longer-term experiments at a mesocosm scale would allow for testing more realistic combinations of BAM, native soils and live vegetation over timescales relevant to BMP construction and operation (e.g., in the months following disturbance after construction). Further study should also further explore the relationship between soil properties and the microbial communities. For example, testing soils of similar composition (e.g., within the optimum ranges of OMC and clay content observed in this study), but deriving from different places could confirm the role of physical properties as opposed to site-specific variables such as microbiome.

Results of this Phase I study suggests that nutrient remediation potential may be predictable based on soil properties. Overall, results underscore that properties of project site soils should be understood before soils are amended for the purpose of nutrient remediation. While this preliminary work offers a promising direction for identifying soils that require amendment, thus justifying the material and environmental costs of soil replacement, longer term study under more natural environmental conditions is needed to predict the nutrient remediation potential of heterogeneous soils.

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Appendix A: Soils and BAM Results Comparison

Table A.1. Soils and BAM results comparison.

SOIL	Physical properties	Hydraulics	Metals	Microbes	N	P
SOIL 1	High OMC, high porosity, high field moisture	High K_{sat} (similar to sand), slow release of water (low slope of tracer release); highest residence time	Mg, Mn, Al; low Fe; no Ca	High increasing natural abundance with depth; increased in top layer after flow; high anaerobe abundance; statistically similar to Soil 2	High N cycling; most removal and production of all soils; becomes a N source after loading	In the field soil P was balanced between adsorbed and organic fractions. Moderate soluble P removal during flow. Retained P converted to biomass.
SOIL 2	Moderate clay, moderate OMC; lots of sand	Moderate K_{sat} (1-2 cm/day); Gradual release of water (gradual slope of tracer release)	Al, Mn; low Fe; no Ca, Mg	High initial abundance that increased after flow; similar to Soil 1	High N removal in situ and in flow through; flow through did not change the potential denitrification; leached organic N (due to roots decomp)	Leached TP from adsorbed soil fraction due to low pH. Soil TP mostly in adsorbed fraction; very little conversion to organic P
SOIL 3	Pure sand, no OMC, no clay	High K_{sat} (6 cm/day); lowest initial tracer detection time, hydraulic residence time (similar BAM), steep release curve, highest infiltration capacity	Low Mn; no other metals	Moderate initial abundance that increased with flow; statistically similar to Soil 6	Low N removal; similar to Soil 6 in denitrification potential; leached organic N (due to roots decomp)	Lowest natural soil P. Soluble P leached during flow (due to sandy soil and roots decomp). Soil P became more labile after flow.

Table A.1. Soils and BAM results comparison (Continued).

SOIL	Physical properties	Hydraulics	Metals	Microbes	N	P
SOIL 4	Moderate OMC, moderate clay	Moderate K_{sat} (3 cm/day); Moderate slope on tracer release	Highest metals	Lower abundance initially particularly at depth; increased after flow; similar to Soil 5	Removed N through denitrification, in field and flow through	Highest natural P content, primarily precipitated with metal; high P removal during flow converted to biomass
SOIL 5	Moderate OMC, moderate clay	Moderate K_{sat} (1-2 cm/day); Gradual slope on tracer release; very slow movement of water despite similar texture and OMC to Soil 4	Highest metals	Lower abundance initially particularly at depth; increased after flow; similar to Soil 4	High N removal in situ and in flow through. Removed N through denitrification, in field and flow through; net N balance was higher in denitrification	Highest natural P content, primarily precipitated with metal; high P removal during flow converted to biomass
SOIL 6	Highest clay, lower OMC	Very slow movement of water; lowest K_{sat}, longest time of initial tracer detection, very gradual tracer release curve; lowest infiltration capacity	High Al; low Mg, Mn, Fe; no Ca	Moderate abundance and diversity; similar to Soil 3	Poor removal of N; similar to Soil 3 in denitrification potential; retained N in soil but not denitrifying	Moderate P adsorbed on Al; little organic P; small amount of soluble P removal by adsorption during flow
BAM	Low clay, no OMC	Very high K_{sat} (42 cm/day); lowest initial tracer detection time, lowest hydraulic residence time (similar to Soil 3), steep release curve	Very low Al, Mn, Fe; no Mg, Ca	No natural microbial community; increased in top layer after flow-through with retention pond water	Lowest, almost undetectable N cycling, even after flow-through. Only material that was a source of inorganic N in leachate.	Moderate natural P mainly precipitated with metals; very little soluble P removal during flow; speciation of BAM did not change after flow