

Development of an Inquiry-based Laboratory Lesson Focusing on the Usage and Benefits of Rain Gardens

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Runoff occurs during storm events when precipitation lands on impervious surfaces. The harmful effects of runoff include erosion, physical property damage, and the transportation of pollutants through stormwater drains into local waterways. One way to mitigate these effects is through rain gardens, which absorb runoff since they are placed in a depression in the ground. The garden allows runoff to infiltrate into the ground, includes native plants to promote biodiversity and filter any runoff pollutants. We have designed an inquiry-based laboratory activity for non-science majors taking an environmental science class to compare and contrast two gardens. Students examine the topography, soil, biodiversity, and pollution retention of the gardens. Based on these measurements and observations, the students then decide which rain garden they feel is more effective in mitigating stormwater runoff and pollution. This lab has been offered both in a face-to-face setting as well as online due to COVID-19.

Keywords: Rain garden, Climate change, Runoff, Soil analysis, Environmental science, Inquiry-based

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Introduction

As our climate changes, more frequent and intense precipitation events will occur (Trenberth 2011). This in turn can lead to increased stormwater runoff. In areas with impervious surfaces that do not allow for water to infiltrate, runoff can lead to flooding, environmental and infrastructure damage (Hassan et al. 2017). Runoff can also collect surface water pollutants. These pollutants are then able to enter various waterways. Possible pollutants can include ammonium, nitrate, phosphate, salt, and sediment. These pollutants can promote eutrophication (Smith et al. 1999), the spread of infectious diseases (Garfield et al. 2003) and can affect development, diversity, and species composition of aquatic organisms (Young et al. 2018).

For these reasons, it is important that runoff be properly mitigated. While most locations do have

stormwater drains, it is expected that these alone may not satisfactorily collect all stormwater runoff (Hassan et al. 2017). A concern with drains is that they do not filter pollutants. In addition to conveying nutrients, pollutants, and sediment to local waterways, stormwater drains change natural hydrology of receiving streams; all of which degrade them.

Green infrastructure technologies are designed to mitigate the effects of runoff. These include swales, constructed wetlands, green roofs, riparian buffer zones, and rain gardens (Green Infrastructure Ontario Coalition 2017, Sharma and Malaviya 2021, Shea et al. 2019). Rain gardens are shallow depressions with pervious surfaces that collect runoff and allow for infiltration to occur. They include a flow entrance or inlet for water to enter (DEP 2006). A ponding area provides surface storage and allows for some evaporation of the runoff. A domed riser can be added to allow for discharge of runoff that exceeds the capacity of the garden. The plants

placed in the rain garden are typically native to the area and are drought-resistant. Plants can reduce the volume of runoff in the garden through evapotranspiration. The presence of roots and rhizomes allow for water to enter the soil of the garden. These plants are able to help remove pollutants from runoff via phytoremediation (Syafriana and Afranin 2020). Mulch is added to protect the soil from drying and eroding. The soil contains various microorganisms that can remediate pollutants and/or decompose organic material to provide nutrients for organisms living in the garden. The soil composition of a rain garden is 20-30% compost and 70-80% soil. Typical rain gardens can have a depth of approximately 45 cm (18 inches). Previous studies have shown that rain gardens are effective in runoff retention and pollution reduction (Dietz and Clausen 2005; Hunt et al. 2006; Zhang and Guo 2014; Tang et al. 2016).

To allow students to see the functional importance of rain gardens, we have designed a laboratory-based lesson where students collect data in order to compare and contrast two rain gardens. Saint Joseph's University campus has two rain gardens (Science Center & Merion Hall) (Figure 1). Students measure the species richness and

evenness of organisms using the rain garden as a habitat, measure the elevations of each area, and analyze the soil porosity and permeability. Students are then challenged to determine which rain garden they feel is more effective. A more effective rain garden should have more biodiversity present, have a topography such that runoff can run into the garden, and have soil that promotes infiltration and the collection of water pollutants.

This lesson covers various topics in environmental science, including the culturing of microorganisms, community ecology, basic geology, urban hydrology, and water quality analysis. In this paper, we present the lesson and show how such a laboratory can be adapted. Instructors can analyze rain gardens in their local neighborhoods, where available. Data is also provided for institutions that do not have access to rain gardens and wish to use data from the Saint Joseph's University rain gardens.

This lesson can be adapted for both non-science and science majors (Table 1). In addition, this laboratory can be taught in both on-ground and online learning environments.



Figure 1. Merion Hall rain garden (A) and Science Center rain garden (B).

Table 1. Variations on rain garden lesson.

Activity	For non-science majors (on-ground)	For non-science majors (online)	For science majors (on-ground)	For science majors (online)
Bacterial Diversity*	Have students swab soil samples onto CHROM-Agar plates.	Provide pictures of inoculated CHROM-Agar plates.	Have students swab soil samples onto CHROM-Agar plates. OR Isolate DNA from soil and submit for metagenome sequencing. Analyze results via BLAST.	Provide pictures of inoculated CHROM-Agar plates. OR Provide raw data from metagenome sequencing for students to analyze via BLAST.
Plant Diversity*	Use architectural drawings of the rain garden (if available). OR Have students use a Plant Guide or app using their smartphone.	Use architectural drawings of the rain garden (if available). OR Provide pictures of the plants for students to ID.	Use architectural drawings of the rain garden (if available). OR Have students use a Plant Guide or app using their smartphone.	Use architectural drawings of the rain garden (if available). OR Provide pictures of the plants for students to ID.
Invertebrate Diversity*	Have prepared pinned insects available for students.	Provide images of pinned insects.	Students prepare their own pit-fall traps. OR Have prepared pinned insects available for students.	Provide images of pinned insects.
Slope/ Topography	Use Google Maps with predetermined markers.	Use Google Maps with predetermined markers.	Use Google Maps with predetermined markers. OR Use a sight level (if available).	Use Google Maps with predetermined markers.

Soil Slide	Have pre-made soil slides available.	Provide a micrograph image of wet-mount.	Have pre-made soil slides available. OR Have students prepare their own wet-mount.	Provide a micrograph image of wet-mount.
Soil Porosity	Students can complete this activity as written.	Provide raw data for students to work on.	Students can complete this activity as written.	Provide raw data for students to work on.
Soil Permeability	Students can complete this activity as written.	Provide raw data for students to work on.	Students can complete this activity as written.	Provide raw data for students to work on.
Pollutant Detection	Students can complete this activity as written. OR Instructor can choose any pollutant test kit of their choice.	Provide raw data for students to work on. OR Have images of each pollutant test result so students can interpret results.	Students can complete this activity as written. OR Instructor can choose any pollutant test kit of their choice. (For majors, you can ask for more precise measurements using either a plate reader or a standard curve).	Provide raw data for students to work on. OR Have images of each pollutant test result so students can interpret results.

(* for majors level classes students can use their species richness and evenness data to calculate diversity (e.g. Shannon-Weiner) for each rain garden).

Student Outline

Objectives

By the end of this lab, students will be able to:

- Describe the function of a rain garden.
- Define biodiversity in terms of species richness and evenness.
- Determine the topography of an area and predict the direction of runoff.
- Learn how soil microorganisms can be observed.
- Identify water pollutants that are trapped by rain gardens.

Introduction

One of the ways we can mitigate the effects of runoff is by the use of **rain gardens** (Figure 1). Some properties of rain gardens include:

- A rain garden can store runoff since they are placed in a depression in the ground.
- They are also made up of a mixture of soil that allows the runoff to permeate easily.
- They are filled with native plants to help promote biodiversity and help filter any pollutants the runoff might have collected along the way.

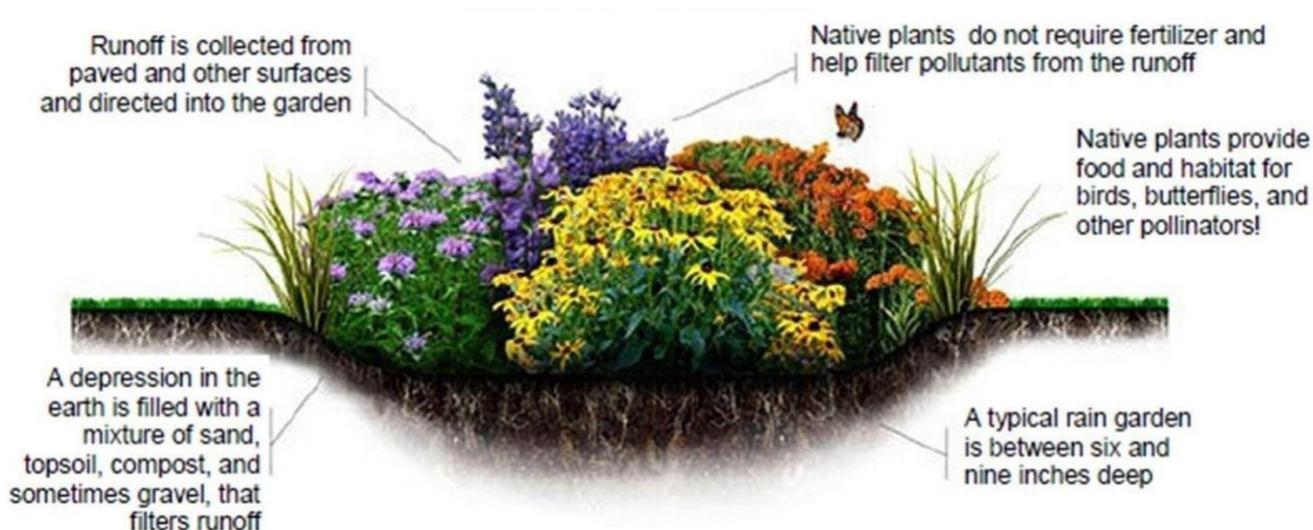


Figure 1. Function of a Rain Garden (<https://rurallivingtoday.com/gardens/rain-garden-how-to-build-tips/>)

In today's lab, you will be comparing and contrasting two gardens to see which one you feel is the more effective garden.

Methods and Data Collection

Part A: Biodiversity Measurements

Rain gardens should promote local biodiversity.

Bacteria:

Your lab instructor will inform you of what procedure you will use to identify the soil bacteria present in the gardens. Record the names of the bacteria you have identified.

Rain Garden #1	Rain Garden #2

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Soil Invertebrates:

Your lab instructor will inform you of what procedure you will use to identify any soil invertebrates in the rain garden. Record the names of the invertebrates you have identified. To assist you, a guide is provided below.

Rain Garden #1	Rain Garden #2

Table 1. Insect/invertebrate guide for rain gardens.

Ant	
Bee	
Beetle	
Butterfly	
Caddisfly	

Centipede	 A photograph of a centipede, showing its segmented body and numerous pairs of jointed legs.
Cicada	 A photograph of a cicada, showing its dark body and large, transparent wings.
Cricket <i>(long antennae)</i>	 A photograph of a cricket, showing its dark body, long antennae, and powerful hind legs.
Dragonfly	 A photograph of a dragonfly, showing its blue body and large, transparent wings.
Earthworm <i>(segmented worm)</i>	 A photograph of an earthworm, showing its segmented, pinkish-brown body.
Earwig	 A photograph of an earwig, showing its brown body and long, pincered abdomen.
Fly	 A photograph of a fly, showing its dark body and large, transparent wings.
Grasshopper <i>(short antennae)</i>	 A photograph of a grasshopper, showing its green and yellow body and long hind legs.

<p>Millipede</p>	
<p>Mite</p>	
<p>Moth</p>	
<p>Nematode <i>(non-segmented worm)</i></p>	
<p>Pillbug</p>	
<p>Roach</p>	
<p>Spider</p>	
<p>Squash Bug</p>	

Stink bug <i>(wider and rounder than squash bug)</i>	
Termite	
Tick	

Plants:

Your lab instructor will inform you of what procedure you will use to identify the plants in the rain garden. Record the names of the plants you have identified. Be sure to keep track of how many of each plant you observe.

Rain Garden #1	Rain Garden #2

1. Species evenness refers to the relative abundance of each species in an area. Species evenness is highest when there are equal numbers of each species in an area. Based on your observations, which garden has greater species evenness with regards to the plants? How did you arrive at this answer?

2. Species richness refers to the number of different species in an area. Based on your observations, which garden has greater species richness with regards to the plants, insects and bacteria? How did you arrive at this answer?

Part B: Slope Measurements

Topography refers to the physical features of an area. This includes the elevations of the area. Rain gardens should be situated in a location such that runoff drains into them.

Your lab instructor will inform you of what procedure you will use to measure the topography of the area. Record your data and any calculations in the space below.

Rain Garden #1	Rain Garden #2

3. Which garden has greater slopes and would more easily direct water into their garden?

Part C: Soil Observations

Soil is defined as the unconsolidated mineral or organic material on the immediate surface of the Earth that serves as a natural medium for the growth of land plants. Soil contains solids, liquids, and gases. Microorganisms also grow in the soil and contribute to keeping the soil fertile by cycling nutrients.

The type of surface an area has can influence the amount of runoff or infiltration that occurs in a given area. **Pervious** surfaces allow for infiltration to occur. Two characteristics of soil that regulate this includes (Figure 2):

- **Porosity** – the amount of space in the soil. Porosity is how much water a substance can hold and is reported as a percentage of the material’s total volume.
- **Permeability** –how well water flows through the soil.

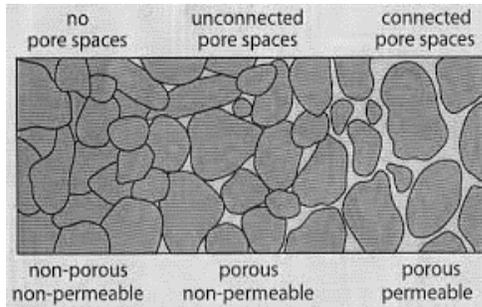


Figure 2. Porosity and Permeability (<http://arnwine.weebly.com/porosity-permeability-percolation.html>)

Wet-Mounts:

Procedure adapted from JoVE (2021).

Day 1:

- Collect a cup full of soil from each garden.
- In each cup, insert a microscope slide vertically.
- Cover the cups with plastic wrap and secure with rubber bands.

- Poke a few holes on the plastic wrap.
- Incubate the slides at room temperature until the next class period.

Day 2:

- Remove all the glass slides from the soil by gently pushing them against the soil to tilt them so that the upper side of the slide (the one with the label) is not in contact with the soil. Gently pull the slides out so that the surface with the label is not scratched by the soil.
- Gently tap the slide against a paper towel on the bench to remove the loose particles. Wipe clean the "back" of the slide.
- Let the slide dry on the bench for a couple of minutes.
- In the chemical hood, immerse the slides in 40% acetic acid for 1 to 3 minutes (record the fixing time). This is the equivalent of heat fixing when you do smears. It will fix the cells to the glass.
- Place the slides on the staining rack and carefully and very gently, rinse off the excess acetic acid with water.
- Immerse the slides in the Rose Bengal dye for 5 to 10 minutes (use tongs). Record the staining time.
- Place the slides on the staining rack and carefully and very gently, rinse off the excess dye with water.
- Allow the slides to dry on a paper towel on your bench before observing under the microscope. Observe under the 40X and under the 100x oil immersion objectives.

Rose Bengal stains bacteria or fungi cells (bacteria will appear as tiny spheres/rods, fungi appear as long filaments). Soil particles will have irregular shapes. Record your observations below. Be sure to label your diagram and record the objective/magnification.

Rain Garden #1	Rain Garden #2
Objective used: _____. Magnification: _____.	Objective used: _____. Magnification: _____.

Porosity:

- Fill a porosity cup with the 100 mL of soil from rain garden #1 (the mark on the side of the cup indicates 100 mL).
- Measure out 100 mL of water in a graduated cylinder.
- Pour 100 mL of water slowly into the soil. Stop when the water level just reaches the top of the soil.
- Complete the table below for rain garden #1.
- Clean your cup and repeat for the soil from rain garden #2.

	Rain Garden #1	Rain Garden #2
Total Volume	100 mL	100 mL
Volume Left in Cylinder (mL)		
Pore Space Volume (= total volume – volume left) (mL)		
% porosity = $\frac{\text{pore space volume}}{\text{total volume}} \times 100$		

Permeability:

- Fill a permeability cup with 100 mL of soil from rain garden #1 (the mark on the side of the cup indicates 100 mL).
- Get a timer ready. Hold the cup over a beaker to catch the water.
- Pour 100 mL of water quickly into the cup of soil. Start recording as soon as the water hits the soil.
- Stop timing as soon as the first drop of water comes out of the hole in the bottom. Record the time below.
- Repeat the procedure for soil from rain garden #2.

	Rain Garden #1	Rain Garden #2
Permeability (seconds for water to pass)		

4. Based on your calculations and observations of each rain garden’s soil, which rain garden do you believe has more pervious surfaces?

Do you believe the definition of a pervious surface is based more on the measurement of porosity or permeability?

Part D: Pollutant Measurements

We will now see if any water pollutants are present in the rain garden soil.

- Take approximately 10 mL of soil from the first rain garden and add it to 50 mL of distilled water in a beaker (Beaker “1”). Use a stirring rod to mix. Pass the soil/water mixture through a filter and funnel. Collect the filtrate into a clean beaker (Beaker “2”) (Figure 3).

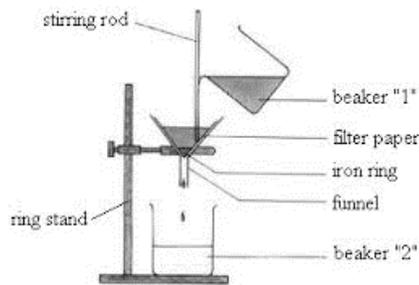


Figure 3. Filter set-up for rain garden water pollutant measurements.

- Test the water to see if any water pollutants are present in the soil using the directions provided by your lab instructor. Record your data in the space below.

Pollutant	Distilled Water (Before being added to Soil)	Rain Garden #1	Rain Garden #2

5. Based on your data, what water pollutants were present in the soil of the rain gardens? What would be the sources of these pollutants?

Discussion

6. Based on all of the observations and measurements, which rain garden do you think is more effective in reducing runoff and promoting biodiversity? Be sure to support your answer from the data you collected in this lab.

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Materials

All materials and procedures for each activity in this lesson can be found in Appendix A.

Notes for the Instructor

Preparation

Instructors should begin preparing for the lab at least two weeks ahead of time (Table 2).

Table 2. Timeline of preparation.

2 weeks prior to the lab activity	<ul style="list-style-type: none"> Preparation of pit-fall traps if you are having students catch their own soil invertebrates.
1 week prior to the lab activity	<ul style="list-style-type: none"> Collect soil from rain garden and bring to lab (or alternatively, bring your students to the rain garden). Have students insert slides into soil if you are having students prepare their own wet-mount slides. Have students collect soil samples to plate onto CHROM-Agar (if you are using this method to determine bacterial community).
Day of lab activity	<ul style="list-style-type: none"> Have students observe their bacteria plates (If you are using CHROM-Agar), otherwise have students isolate DNA for metagenome sequencing. Have students observe insects (either prepared ahead of time or from their pit-fall traps). Have students observe plants in the gardens. Have students calculate topography of the gardens (either by Google Earth or sight level). Have students collect soil data (wet-mount, porosity, permeability, pollutant detection).

Science Center and Merion garden measurements:

For institutions that do not have access to rain gardens but wish to use this lesson, we have

provided sample data about our rain gardens at our university in Appendix B.

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About the Authors

Jacquelyn Lomino received her Bachelor of Science in Environmental Science from Saint Joseph's University in May 2020. She is currently a Juris Doctorate Candidate at New England Law | Boston, interested in pursuing Environmental Law.

Brian M. Forster received his Ph.D. from Cornell University. In 2011, he joined the faculty of Saint Joseph's University. Dr. Forster is the laboratory coordinator for laboratory-based classes designed for students who are not science majors. He teaches courses in general biology, heredity, environmental science, and a course in microbiology designed for students wishing to enter nursing or allied health programs.

Appendix A

The list below describes the materials and procedures for each lab activity described in the lesson. We list the materials for 26 students or 13 groups (2 per group).

General notes about rain gardens:

- If using an off-site rain garden, owner's permission may be required before proceeding.
- Try to disturb the gardens as little as possible.
- Any leftover soil should be returned to the garden after experimentation.

Safety:

- When working with microorganisms, the authors recommend all instructors and students review the American Society for Microbiology's Guidelines for Biosafety in Teaching Laboratories. They can be found at: <https://asm.org/Guideline/ASM-Guidelines-for-Biosafety-in-Teaching-Laborator>.

Soil:

Materials:

- If students are working in the lab, then approximately 2 liters of soil from each garden is needed (210 mL of soil needed for each group, for each garden). Soil is kept in front of the lab for groups to come up and collect as needed. If working in the field, then just have students use soil in the garden.
- Handheld shovels (2 per group – 26 total)

Biodiversity Measurements (Bacteria):

Procedure #1: CHROM-agar

Materials:

- 20 mL Sterile water samples (1 per group, 13 bottles total).
- Sterile swabs (2 per group, 26 swabs total).
- CHROM-Agar Orientation Plates (2 per group, 26 plates total). Plates can be purchased from <https://www.chromagar.com/>.
- Incubator set to 37°C.

Procedure:

- Take a sterile test tube with 20 mL sterile water. Add 10 mL of rain garden #1 soil.
- Vortex to thoroughly mix.
- Take a sterile swab and swab 2 CHROM-Agar plates.
- One plate will be incubated at room temperature (25°C). The other plate will be incubated at 37°C.
- Repeat procedure for rain garden #2 soil.
- After 24 hours, examine plates. Plates can be stored in refrigerator (4°C) until next lab period.
- Different bacterial species will appear as different colors.
- CHROM-agar key can be found at: https://www.chromagar.com/produit.php?PARAM_NAVIGATION=clinical

Instructor Notes:

- Sterile water samples – use 50 mL graduated conical tubes, filled up with 20 mL sterile water. This way, the students can easily add 10 mL of soil without using a graduated cylinder.
- We ask students to incubate the plates at room temperature and at 37°C in case there are any microbes that cannot grow at one temperature, they can be seen at the other temperature.

Procedure #2: Sequencing of 16S rRNA gene from soil bacteria (since some soil bacteria are not culturable)

Materials:

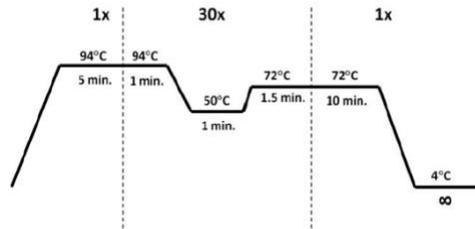
- PowerSoil® DNA Isolation Kit.
- 0.25 grams of soil from each garden.

Procedure:

- Follow procedure from PowerSoil® DNA Isolation Kit.
- Submit isolated DNA for DNA sequencing, following sequencing company instructions. We recommend barcoded amplicon sequencing of the 16S rRNA gene. The 16S rRNA gene, found in the 30S small ribosomal subunit, is highly conserved amongst bacteria and is commonly used for identification purposes.

Instructor Notes:

- Before submitting for sequencing, you can test your DNA isolation for the presence of the 16S rRNA gene using PCR.
 - PCR Product size should be approximately 1600 bp.
 - Primers used: 16S For: (AGAGTTTGATCCTGGCTCAG), 16S Rev: (ACGGCTACCTTGTTACGACTT).
 - PCR Directions:



Biodiversity Measurements (Invertebrates):

Procedure #1: Preparation of pit-fall traps

Materials:

- Medicine cups.
- Shovel.
- Propylene glycol (or anti-freeze).
- Styrofoam trays (1 per garden for each group. 26 trays total).
- Insect pins (black steel).

Procedure:

- Go into each rain garden and dig small holes throughout the garden. Insert medicine cup into the hole. Make sure top of cup is level with the ground.
- Add a small amount of propylene glycol (or antifreeze) into each cup. (*You may want to do this in the lab ahead of time.*)
- Check cups every few days to see what insects have been caught.
- Pin recovered invertebrates to appropriate Styrofoam trays using insect pins.

Procedure #2: Preparation of pinned invertebrates (if access to rain gardens or setting up pit-fall traps is not possible)

Materials:

- Various preserved soil invertebrates (available from various laboratory supply companies).
- Styrofoam trays (1 per garden for each group. 26 trays total).
- Insect pins (black steel).

Procedure:

- Instructor can take various insects and pin them to the Styrofoam trays.
- Instructor can choose which insects appear in each garden.

Biodiversity Measurements (Plants):

Procedure:

- Students can use various methods to identify plants in the garden.
 - iNaturalist. Free on Android and iOS
 - Leafsnap. Free on iOS
 - Plantifier. Free on Android and iOS
 - iPflanzen. Free on Android and iOS
 - SmartPlant. Free on Android and iOS
 - Source: (<https://better.net/homes/garden-landscaping/7-top-apps-to-identify-plants/>)
 - Plant ID guide books
 - Architectural plans of the rain gardens (if available).
- Students should take note of the relative abundance of each plant in each garden.

Topography/Slope Measurements:

Procedure #1: Use of Google Maps

Materials:

- Google Maps.
- Calculator.

Procedure:

- Students can use Google Maps to get an aerial view of the rain gardens you are investigating.
- Have students pick marker locations near and within the garden. Have students determine the elevation and distances between each marker (Google Maps will provide this information for you).
- Students should then calculate the slopes within the area. $\text{Slope} = (\text{Change in Elevation}/\text{Change in Distance}) \times 100$.

Procedure #2: Use of sight level

Materials:

- Sight level.
- Tape measure

Procedure:

- Students can use a sight level and record elevations near and within the garden.
- Students should then calculate the slopes within the area. $\text{Slope} = (\text{Change in Elevation}/\text{Change in Distance}) \times 100$.

Soil Wet-Mount:

Materials:

- Clean glass slides (two slides per group, 1 for each garden – 26 slides total).
- 40% v/v Acetic Acid (needed for stain).
- 2% w/v Rose Bengal stain (needed for stain).

Procedure:

- Procedure for preparing and staining the wet-mount can be found in the Student Outline.

Instructor Notes:

- Instructor can prepare the slides ahead of time for students, if they wish.

Soil Porosity:

Materials:

- Access to water (tap water will work).
- 100 mL graduated cylinder (1 per group – 13 total).
- Plastic drinking cup (with 100 mL mark noted on the cup). Label cup “POROSITY” (2 per group – 26 total).

Procedure:

- Procedure for porosity measurements can be found in the Student Outline.

Instructor Notes on how to prepare POROSITY cups:

- These can be any cup or beaker that can hold 100 mL of soil. If you are not using a graduated beaker, make sure you mark on the beaker/cup where 100 mL would be so students can fill up the beaker/cup appropriately.

Soil Permeability:

Materials:

- Access to water (tap water will work).
- Timer (1 per group – 13 total).
- 100 mL graduated cylinder (1 per group – 13 total).
- Plastic drinking cup with hole in bottom (with 100 mL mark noted on the cup). Label cup “PERMEABILITY” (2 per group – 26 total).

Procedure:

- Procedure for permeability measurements can be found in the Student Outline.

Instructor Notes on how to prepare PERMEABILITY cups:

- Take a plastic cup and drill a small hole (approximately 0.5 cm) at the bottom of the cup. Cover the hole with a small piece of cheesecloth. The cheesecloth prevents any soil from falling out of the hole when it is filled.
- Have a larger cup or beaker with this permeability cup to catch the water as it passes through the soil in the cup.

Pollutant Detection:

Instructor can use alternative pollutant indicators. Be sure to follow all manufacturer directions.

Materials:

- 50 mL aliquot of distilled water (2 per group – 26 aliquots total).
- Ring Stand (one per group – 13 total).
- Filter/Funnel/filter paper Setup (one per group – 13 total).
- Clean beaker to collect filtrate (2 per group – 26 total).
- Test Tubes (5 per group – 65 total).
- Pipet or graduated cylinder to measure 5 mL aliquots (2 per group – 26 total).
- Refractometer (1 per group – 13 total).
- 5 mL aliquot of 0.2% sodium rhodizonate solution (1 per group – 13 aliquots total).
- Ammonium API Kit (1 per group – 13 total).
- Nitrate API Kit (1 per group – 13 total).

- Phosphate API Kit (1 per group – 13 total).

Procedures:

- **Salinity**

- To test for salt, obtain a refractometer and a bulb-head pipet, and draw some liquid into the pipet.
- Open the refractometer, and squeeze just **ONE** drop from the pipet onto the face of the refractometer. Close the top, and determine the salinity (amount of salt) in the sample by reading the value in the view on the right hand side where the line that separates the blue from the white falls (o/oo).
 - (saltwater will be approximately 35-40 ppt)



- **Ammonium (use API KIT)**

- Use a clean pipette and put 5 mL of water in the test tube.
- Squeeze 8 drops from BOTTLE #1 and then 8 drops from BOTTLE #2 into the test tube.
- Cover the test tube with parafilm, and invert the test tube 2 to 3 times.
- Wait 5 minutes for the color to develop. Let the sample sit for 5 minutes and allow the color to develop. Refer to the color indication card to see how much phosphate (if any) is present in your sample. This is measured in parts per million (ppm).

- **Lead**

- Use a clean pipette and put 5 mL of water in the test tube.
- Add a few drops of 0.2% sodium rhodizonate.
- If blood red color is observed, then the soil contains lead.

- **Nitrate (use API KIT)**

- Use a clean pipette and put 5 mL of water in the test tube.
- Squeeze 10 drops of nitrate solution from BOTTLE #1 into the test tube that contains the 5 mL of sample. Cover the test tube with parafilm, and invert the test tube 2 to 3 times.
- Shake the contents of nitrate solution BOTTLE #2 for at least 30 seconds, and add 10 drops into the test tube sample. Re-cover with parafilm and vortex for 1 minute.
- Let the sample sit for 5 minutes and allow the color to develop. Refer to the color indication card to see how much phosphate (if any) is present in your sample. This is measured in parts per million (ppm).

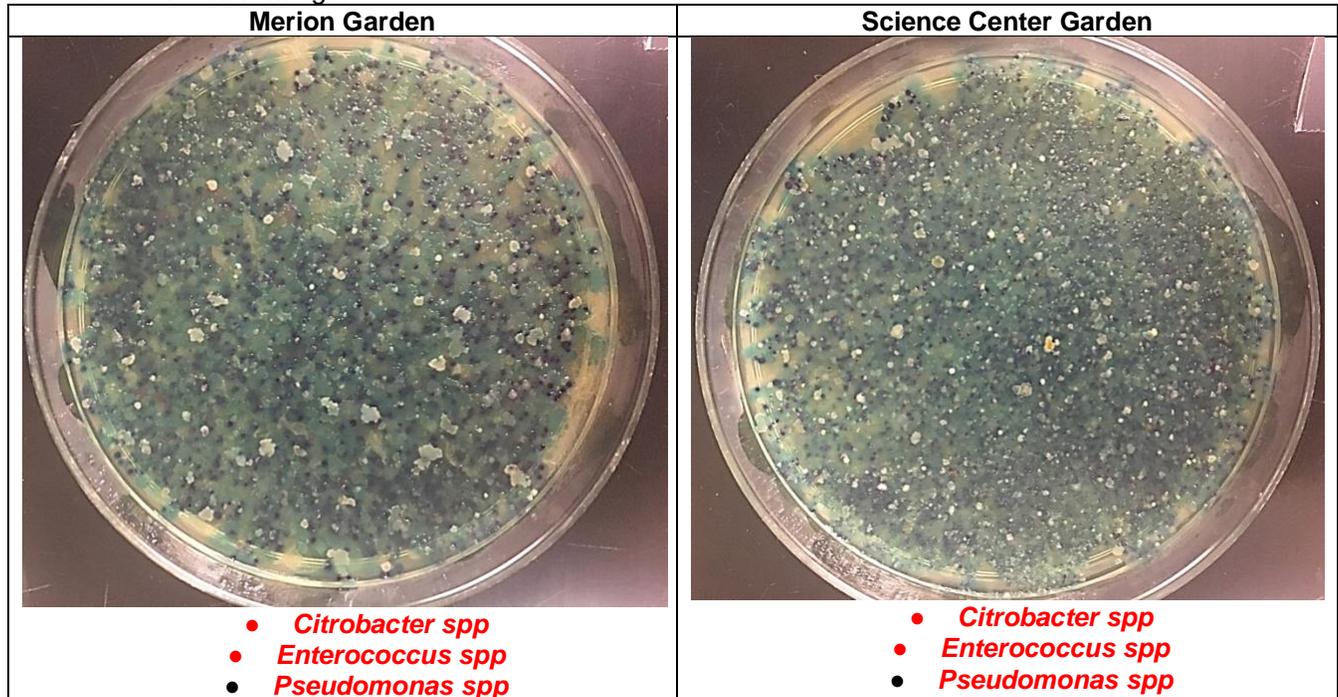
- **Phosphate (use API KIT)**

- Use a clean pipette and put 5 mL of water in the test tube.
- Squeeze 6 drops of the phosphate solution from BOTTLE #1 into the test tube you have designated for the phosphate test that also contains 5 mL of sample.
- Cover with parafilm and vortex for 5 seconds.
- Squeeze 6 drops of phosphate solution BOTTLE #2 into the test tube and re-cover and vortex for another 5 seconds.
- Let the sample sit for 3 minutes while the color develops, and refer to the color indication card to see how much phosphate (if any) is present in your sample. This is measured in parts per million (ppm).

Appendix B
Sample Data of Science Center and Merion Hall Rain Gardens

Biodiversity Measurements (Bacteria):

Procedure #1: CHROM-agar



Procedure #2: 16S rRNA gene sequencing of soil bacteria

Given that many bacteria are not culturable, we additionally utilized molecular methods of identification. We isolated DNA from the soil of both gardens. As controls, we isolated DNA from soil located near each garden. Barcoded amplicon sequencing of the 16S rRNA gene was performed. Sequencing primers used were 27F (AGAGTTTGATCMTGGCTCAG) and 519R (GWATTACCGCGGCKGCTG). The results of our sequencing are listed below:

- Phyla most common in both the gardens and control areas was *Proteobacteria* (Gram-negative bacteria).
- Phyla unique to Merion Garden (vs. control):
 - Lentisphaerae* (similar to *Chlamydiae* & *Verrucomicrobia* phyla- common soil inhabitant).
 - Thermodesulfobacteria* (Sulfate reducer from waterlogged soil)
- Phyla unique to Science Center Garden (vs. control):
 - Aquificae* (Gram-negative, autotroph, carbon fixer)
 - Tenericutes* (lack cell walls)
- Phyla Unique to both gardens (vs. control):
 - Synergistetes* (Gram-negative, anaerobic bacteria found in soil and water-treatment plants)

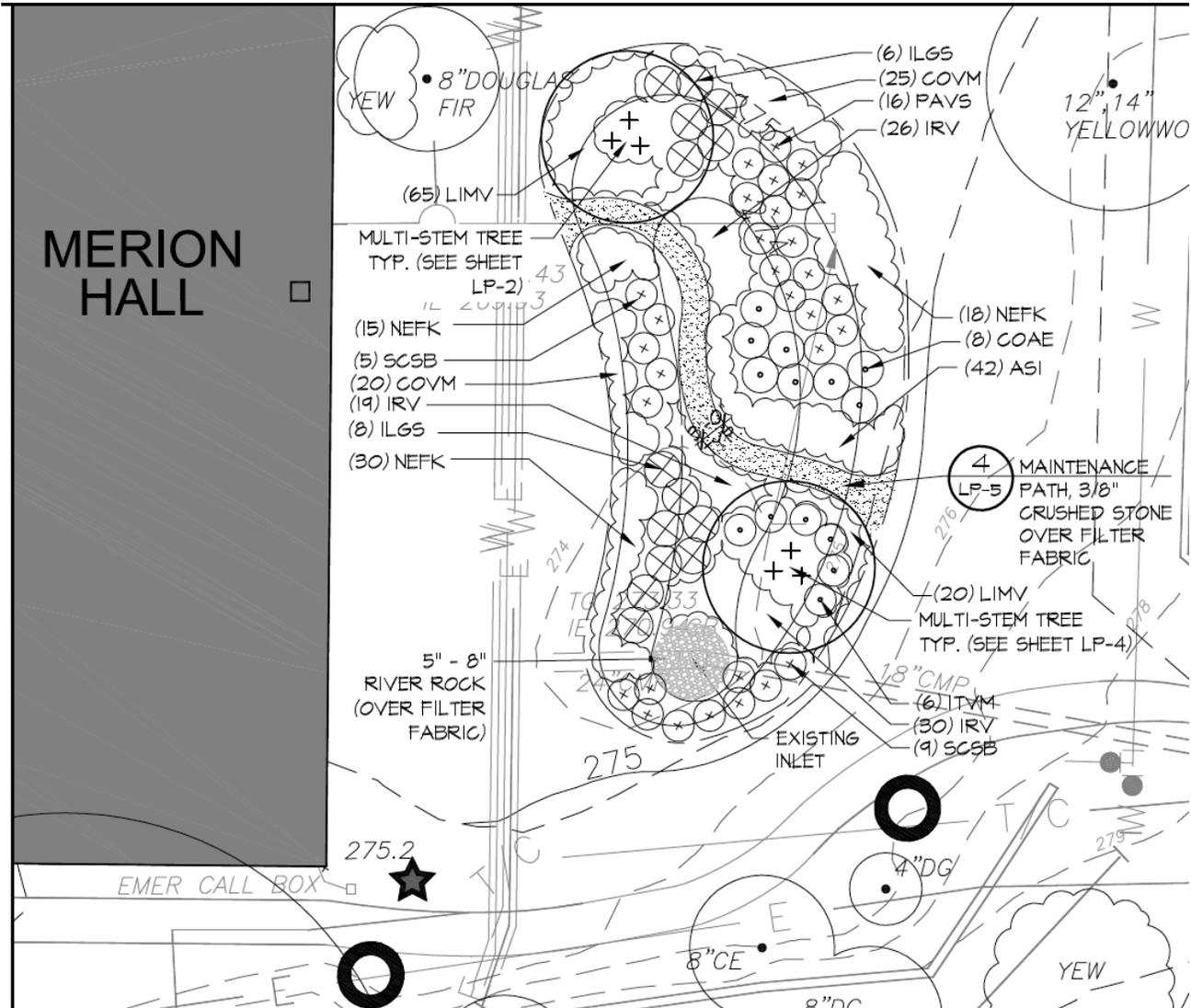
Biodiversity Measurements (Invertebrates):

Pit fall traps were set in each garden. As controls, we also set up traps near each garden. Our results are shown below. Both gardens contained invertebrates not found in the nearby control areas.

	Merion Garden	Control area near Merion Garden	Science Center Garden	Control area near Science Center Garden
Spider	X	X	X	X
Ants	X	X	X	X
Beetle		X	X	X
Pill bug	X		X	X
Worm	X		X	X
Centipede		X	X	
Millipede	X	X		
Snail	X			

Biodiversity Measurements (Plants):

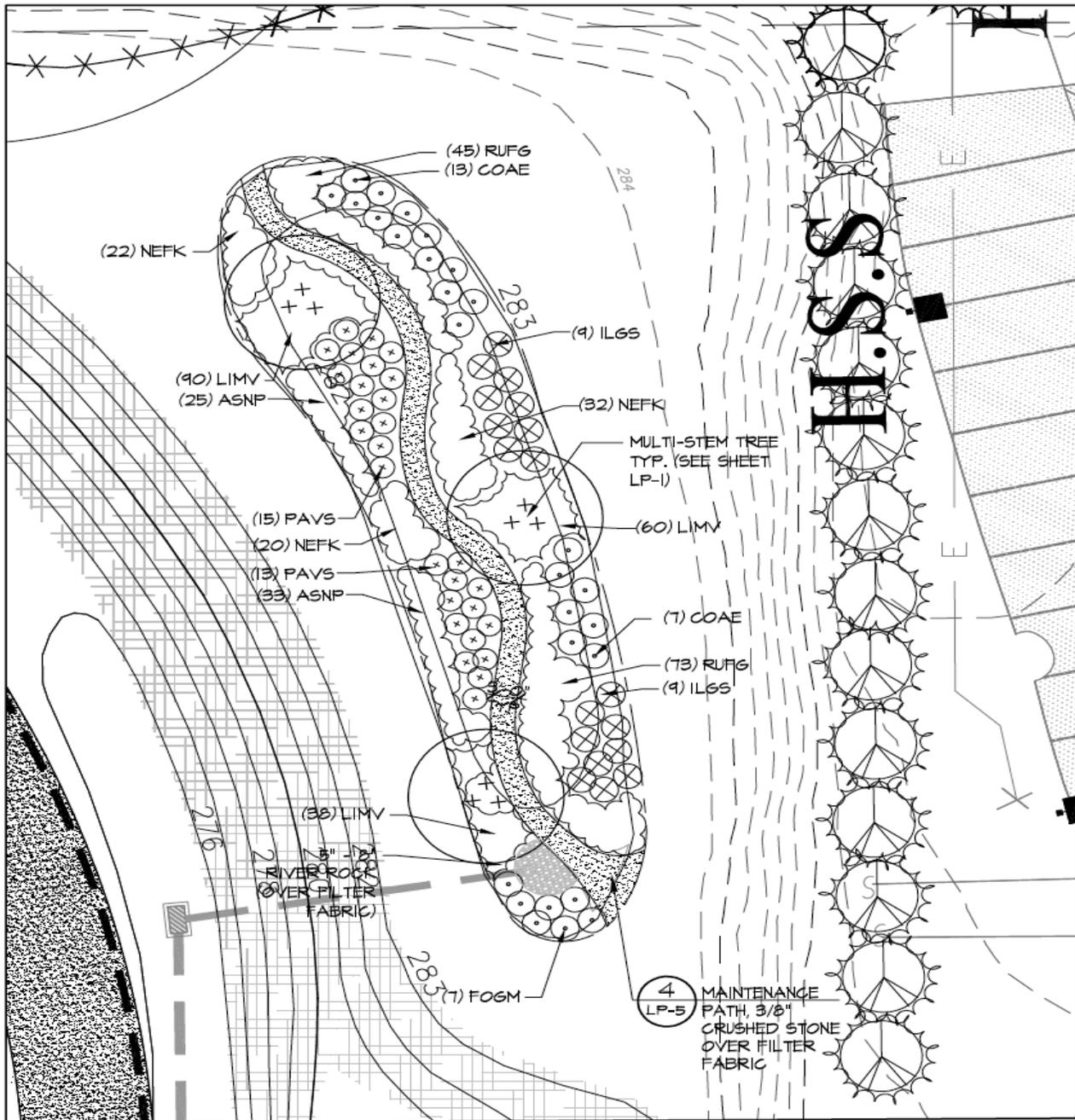
The next two pages show the architectural plans to the two rain gardens. The number in parenthesis indicates the number of each plant in that location. The codes for each plant are listed below each map.



PLANT LIST:

KEY	QTY	BOTANICAL NAME	COMMON NAME	SIZE	REMARKS
SHRUBS					
COAE	8	<i>Comus alba</i> 'Elegantissima'	Variagted Red Twig Dogwood	24"-30" ht.	Cont.
ILGS	14	<i>Ilex glabra</i> 'Shamrock'	Shamrock Inkberry Holly	24"-30" ht.	Cont.
ITVM	6	<i>Itea virginica</i> 'Merlot'	Merlot Virginia Sweetspire	24"-30" ht.	Cont.
PERENNIALS & GROUNDCOVERS					
ASI	42	<i>Asclepias incarnata</i>	Swamp Milkweed	1 gal.	Cont.
COVM	45	<i>Coreopsis verticillata</i> 'Moonbeam'	Tickseed	1 gal.	Cont.
IRV	75	<i>Iris versicolor</i>	Blue Flag Iris	1 gal.	Cont.
LIMV	85	<i>Liriope muscari</i> 'Variegata'	Variegated Lilyturf	1 gal.	Cont.
NEFK	63	<i>Nepeta faassenii</i> 'Kit Kat'	Kit Kat Catmint	1 gal.	Cont.
PAVS	16	<i>Panicum virgatum</i> 'Shenandoah'	Shenandoah Switch Grass	3 gal.	Cont.
SCSB	14	<i>Schizachyrium scoparium</i> 'The Blues'	The Blues Little Bluestem	3 gal.	Cont.

Science Center Garden:



PLANT LIST:

KEY	QTY	BOTANICAL NAME	COMMON NAME	SIZE	REMARKS
SHRUBS					
COAE	20	<i>Cornus alba</i> 'Elegantissima'	Variegated Red Twig Dogwood	24"-30" ht.	Cont.
FOGM	7	<i>Fothergilla gardenii</i> 'Mt. Airy'	Mt. Airy Fothergilla	24"-30" ht.	Cont.
ILGS	18	<i>Ilex glabra</i> 'Shamrock'	Shamrock Inkberry Holly	24"-30" ht.	Cont.
PERENNIALS & GROUNDCOVERS					
ASNP	58	<i>Aster novae-angliae</i> 'Purple Dome'	Purple Dome Aster	1 gal.	Cont.
LIMV	188	<i>Liriope muscari</i> 'Variegata'	Variegated Lilyturf	1 gal.	Cont.
NEFK	74	<i>Nepeta faassenii</i> 'Kit Kat'	Kit Kat Catmint	1 gal.	Cont.
PAVS	28	<i>Panicum virgatum</i> 'Shenandoah'	Shenandoah Switch Grass	3 gal.	Cont.
RUGF	118	<i>Rudbeckia fulgida</i> 'Goldsturm'	Blackeyed Susan	1 gal.	Cont.

NOTES:

Topography/Slope Measurements:

We utilized Google Maps to determine the topography of each garden. Individuals interested in obtaining the Google Map files of each garden should contact the corresponding author, Dr. Brian Forster (bforster@sju.edu).

Merion Garden	Science Center Garden
Transect AB: 1.7% Transect AC: 4.1% Transect AD: 3.4% Transect AE: 3%	Transect AC: 2.8% Transect AD: 2.9% Transect AE: 2.2% Transect AF: 1.8%

Soil Porosity:

	Merion Garden	Science Center Garden
Total Volume	100 mL	100 mL
Volume Left in Cylinder (mL)	20 mL	50 mL
Pore Space Volume (= total volume – volume left) (mL)	80 mL	50 mL
% porosity = $\frac{\text{pore space volume}}{\text{total volume}} \times 100$	80%	50%

Soil Permeability:

	Merion Garden	Science Center Garden
Permeability (seconds for water to pass)	5 seconds	15 seconds

Pollutant Detection:

Pollutant	Before being added to the soil	Merion Garden Soil Analysis	Science Center Garden Soil Analysis
Salt (ppt) (refractometer)	0	3 ppt	1 ppt
Ammonia (ppm) (measure with kit)	0	0 ppm	0 ppm
Lead (sodium rhodizonate) (yes/no)	No	No	No
Nitrate (ppm) (measure with kit)	0	5 ppm	0 ppm
Phosphate (ppm) (measure with kit)	0	10 ppm	10 ppm

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