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Senior Project - Sehome Arboretum Soils

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COLLEGE OF THE ENVIRONMENT



Project Title: Characterizing Sehome Arboretum soils

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Project Dates: 03/28/2023 - 06/07/2023

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DATE: 06/07/2023

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Introduction

In some areas of the Arboretum, the canopy has been transitioning from coniferous Douglas fir (*Pseudotsuga menziesii*) to deciduous big-leaf maple (*Acer macrophyllum*). Dieback of mature Douglas fir trees may be due to the slow-growing root rot fungus, *Coniferiporia weirii*, or the story may be more complicated. Other soil characteristics like nutrient availability and soil biota communities may influence the susceptibility of mature Douglas fir to *C. weirii*. To better understand why this transition is happening, Rebecca Bunn is putting together a research class which will evaluate whether other soil characteristics may be contributing to the dieback of Douglas fir and will create a long-term study and database on the Sehome Arboretum. For my senior project my role was to test, edit, and create methods for the class to use for sampling and lab procedures.

Duties and Responsibilities

1. Sample Collection Methods: I first created sample sites in ArcGIS Pro to be able to locate potential sample sites. I used slope and dominant canopy cover as factors in the creation and selection of sample sites. All 10x10 meter sample sites had slope angles less than 50 degrees and dominant deciduous or conifer canopy covers greater than 60%. Five paired sample sites within 100 meters from each other were then selected. I took four samples from each 10x10 meter sample site and homogenized them into a single sample for each sample site. Samples were then stored in a fridge for later analysis. I then created methods for sample site selection and sample collection. Field maps used for sample collection can be accessed using the link or QR code below and can be used by the class in the future.



<https://fieldmaps.arcgis.app/?itemID=18bd2dcb9bdb4da790683ba22b70433a&referenceContext=open&portalURL=https%3A%2F%2Fwwu.maps.arcgis.com>

2. Arbuscular Mycorrhizal Infectivity Potential: With the samples I collected I then set up a MIP study in the WWU greenhouse. For each sample, three MIP samples were created. Corn was planted in each MIP sample and left to grow for four weeks. I came by three times a week to monitor environmental conditions by recording light readings. At the end of the four-week period I collected roots from each sample, cleared and stained them, and evaluated them underneath the dissection scope for colonization rates. Methods for MIP set up, light reading collection, root harvesting and MIP quantification were then created. Methods for clearing and staining already existed and just had to be edited.

3. Soil Water and Loss on Ignition: Using the samples from the arboretum, soil moisture and organic matter were analyzed using the methods provided by Rebecca Bunn. I then edited these methods to be used for the class.
4. Soil Biota Extractions: I extracted both nematodes and microarthropods for each sample site collected using methods provided by Rebecca Bunn. After performing extractions, I edited these to be used by the class. I quantified the extractions for both nematodes and microarthropods and created methods for both procedures for the class to use.
5. Soil pH: To analyze pH, I first air dried soil for each sample and created methods for air drying soil. I then analyzed the pH three ways with the methods provided by Rebecca Bunn. These methods were then edited and adapted to be used by the class.
6. Soil Texture: Soil texture for each sample site was analyzed following the methods provided by Rebecca Bunn. These methods were then edited and adapted to be used by the class.
7. Data Analysis: Once all parameters had been evaluated in the laboratory and all methods were finished, all of the data was analyzed using paired T-tests in R.

Assessment

The project is still on going as the class is still in development, but I believe I made a good contribution to the development of the class. I created many methods that needed to be created and edited the ones that did exist to be more specific and easier to follow for undergraduate students. All methods outlined in the contract were created except for the ectomycorrhizal slides. There was not enough time to analyze this parameter. We also determined that creating slides for the the AM and EM would not be possible in the time frame of a single school quarter, on top of everything else. We instead decided to count MIP results with the dissection scope to assess colonization rates. Although I ran out of time to assess all the sample sites using the dissection scope, I was able to test the methods I created for MIP quantification and was successful. I also created a system for students to locate sample sites and collect environmental data with. This will contribute to the long-term data storage and collection, as well as allow students to expand on the research by correlating other environmental factors with the soil characteristics. I also gained many skills in this project, including various laboratory skills for soil analysis outlined in the methods above, field sampling experience, project design and management, ArcGIS Field Map design and use, greenhouse experience, data management and analysis, and experience with the software R for data analysis.

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Appendices

Methods

ESCI 497 Arboretum Research

Laboratory Manual and Field Methods

Site Selection:

When selecting paired conifer and deciduous sample sites, environmental conditions at each site need to be taken into account. Some factors to consider are:

1. **Location:** within 100 meters of each other.
2. **Slope:** angle roughly within 15 degrees of each other
3. **Aspect:** Are the slopes facing the same direction?
4. **Natural and built boundaries:** Are they separated by any boundaries that would change environmental conditions?
5. **Neighboring vegetation:** Is the sample plot surrounded by the same canopy cover?

Site Selection Procedures:

1. Download the ArcGIS Field Maps app.
2. Scan the QR code or type in the link to access the field map.



<https://fieldmaps.arcgis.app/?itemID=18bd2dcb9bdb4da790683ba22b70433a&referenceContext=open&portalURL=https%3A%2F%2Fwww.maps.arcgis.com>

3. To ensure paired sample plots have similar environmental conditions, use ArcGIS Field maps to look at the factors listed above. Try and identify 5 to 10 potential paired plots. Once potential paired sample sites have been selected visit the sites in person to ensure that the environmental conditions are similar.
4. Location data may take a while to update to your location and may be inaccurate. It is a good idea to allow time for location data to update and if necessary, use the measure tool in Field Maps to gauge your location from another fixed location.

Sample Collection:

Materials:

1. Trowel
2. 10 – 1-gallon Ziploc bags
3. Pruners
4. Paper towels
5. Phone with ArcGIS-Field Maps

Procedures:

1. After locating your sample plot, determine the bounds of the 10x10 meter sample plot.
2. Click on the sample plot and click on the pencil icon in the lower left corner to edit the sample plot. Record the site conditions with images of both the canopy above the sample plot and the understory in ArcGIS Field Maps.
3. Record the dominant tree species, dominant understory species, presence of invasive species, dominant invasive species, presence of any dead conifers, any notes and the sample date in Field Maps.
4. Select four evenly spaced sample locations within the 10x10 meter plot and push the leaf litter aside from the selected sample locations.
5. Using the trowel, collect a 6-inch-deep and 2-inch-wide soil sample for each of the four selected sample locations within your 10x10 meter plot. Ensure that you collect soil evenly for the entire soil profile.
6. All four soil samples should be put into the 1-gallon Ziploc bag for your current sample plot and should be thoroughly mixed until homogenized.
7. Label the Ziploc bag with today's date, the Sample plot Grid ID (ArcGIS Field Maps), canopy cover type, and paired sample site number.
8. Before going to the next plot ensure that you clean your trowel with a paper towel to avoid contamination, fill in holes created by sampling and replace leaf litter.
9. Once all samples are collected, store in the fridge until needed.

Arbuscular Mycorrhizal Infectivity Potential

Setting up the Mycorrhizal Infectivity Potential

Materials:

1. Sterilized soil
2. Sand
3. Vermiculite
4. Arboretum soil samples
5. Corn seeds – 3 per sample
6. Cotton plugs – 3 per sample
7. Single cell cone trainers - 3 per sample
8. Support tray
9. Mixing tub
10. Large scooper

Procedures:

1. Label 3 single cell cone trainers for each soil sample with the sample ID and the sub sample letter. E.g., E-145-a, E-145-b, etc.
2. Place a cotton plug at the bottom of each single cell cone trainer to stop soil from falling out of the bottom.
3. In the mixing tub, place a scoop of sterilized soil, sand, and (3) so that there is a ratio of 1:1:1 of each medium. Continue adding one scoop of each until you have enough medium to fill all cone trainers.
4. Mix the combined sterilized medium thoroughly.
5. Fill each cone trainer with the combined sterilized medium until there is about 2 inches of space left.
6. Tap the cone trainer to settle the soil, you may need to add more after it settles.
7. Add 1 inch of the arboretum soil samples into their labeled cone trainers.
8. Place 2 corn seeds into each cone trainer making sure they are towards the center of the cone.
9. Fill the cone trainer to the top with the combined sterilized medium.



Figure 1. Completely filled cone trainers ready to be watered.

10. Evenly space the cone trainers in the rack and water thoroughly.
11. Come back every day or every other day to check progress and take light readings.
12. Once the corn sprout be sure to clip extra sprouts so there is only one corn plant growing per cone trainer.



Figure 2. Evenly spaced cone trainers with sprouts.

Light Readings

Materials:

1. LI-250A light meter

Procedures:

1. Turn on the LI-250A light meter and remove the sensor cap.
2. Check that the unit of measurement is set to μmol .
3. Place the sensor near the top of a cone trainer, ensure that it is flat, and you are not casting a shadow over it.
4. Push the average button and wait 15 seconds.
5. Record the light reading and repeat 2 more times in different areas of the tray.

Harvesting Plant Roots

Materials:

6. Ethyl alcohol or 10% bleach solution
7. Soil tray
8. Clean paper
9. 1 Clippy Box per MIP sample
10. Pruners or x-acto knife
11. Large beaker 500-1000 mL filled with distilled water.
12. Forceps
13. Compost bin

Procedures:

1. Clean the work area with ethyl alcohol or a 10% bleach solution. Place clean paper on surface for easy cleanup.
2. Select one of your samples and place it in the soil tray. Label a clippy box with the sample ID and the sub sample letter. E.g., E-145-a, E-145-b, etc.
3. Massage the cone trainer to work the plant material and soil out of the cone trainer as delicately as possible, but without spending excessive time. Plant roots will cooperate more readily if the soil is dryer.
4. Once removed from the cone trainer gently massage and shake the soil from the roots without breaking them.
5. Rinse the plant roots under flowing water, removing as much soil as possible. Be sure to use a sieve to catch soil to avoid clogging any drains. If necessary, remove any extra debris with forceps and rinse roots again.
6. Once the root system is satisfactorily clean, remove shoots at the root-shoot interface with a pair of pruners or an x-acto knife and toss shoots in compost.
7. Randomly select and cut fine root segments with an x-acto knife or pruners from the main root system. These roots should be small enough to fit into the clippy box, and yet not so small that they will escape. Cut roots from throughout the roots system to ensure a representative sample of mycorrhizal infectivity.
8. Place these segments into the labeled clippy box. Enough should be placed in the clippy box to make a slide of 12 fine root segments. Error on the side of more rather than less, although if the clippy box gets packed with roots, the stain solution will not reach the inner roots.
9. Store full clippy boxes in a large beaker of distilled water until ready to clear and stain.
10. Remaining plant material can be placed in a labeled envelope and dried in oven at 60°C for 48 hours. Store at room temperature. If more roots segments are needed for

mycorrhizal analysis, these dried roots can be re-hydrated in distilled water, and then cleared and stained.

11. Clean your work area, recycling paper, emptying compost, and wiping down surfaces and sanitizing with Ethyl alcohol or 10% bleach solution.

Clearing and Staining Roots

Materials:

1. Hot plate
2. 1 L stock 5% KOH
3. 1 L stock 2% HCl
4. 1 L stock 0.05% trypan blue + 1:1:1 lactoglycerol staining solution
5. 1 L stock lactoglycerol
6. 3 x 500 mL beakers
7. Oven gloves
8. Large forceps
9. Aluminum foil

Procedures:

1. Under the fume hood, add approximately 350 mL of 5% KOH to a 500 mL beaker. Place the beaker on a hot plate and bring to a boil. This can be done by setting the hot plate to 400°C until boiling.
2. Once the KOH is boiling, add the clippy boxes to the beaker and boil for 10 minutes. After adding the clippy boxes to the solution, wait for the solution to come back to a boil and reduce heat to 120°C.
3. Turn off the hot plate and remove the beaker using the oven gloves.
4. Let the solution cool and pour off some of the 5% KOH into the chemical waste container.
5. Remove the clippy boxes with the large forceps and place in the other 500 mL beaker. Then pour the rest of the 5% KOH into the chemical waste container.
6. Take out of the fume hood and rinse the clippy boxes 5 times with DI water.
7. Back in the fume hood, pour in 2% HCl until clippy boxes are fully immersed and soak for 15-20 minutes.
8. Pour the 2% HCl into the chemical waste container and add 0.05% trypan blue + 1:1:1 lactoglycerol staining solution to the beaker until the clippy boxes are fully submerged.
9. Place it on a hot plate and bring to a boil. Once the solution is boiling, turn the heat off. Allow to stain for 3-4 minutes for greenhouse roots, longer for field roots (This step can be performed without heat but will take approximately 12 hours).
10. Staining solution can be used 2 to 3 times. If this is the first or second time using the solution, pour the solution back into its original container for reuse. If this is the third use, then pour it into the chemical waste container.
11. Take out of the fume hood and rinse the clippy boxes 5 more times.

12. Once thoroughly rinsed, place the clippy boxes in a beaker with lactoglycerol. Make sure they are fully submerged, cover with aluminum foil and store them in a refrigerator for a week or longer to maximize the contrast between fungal and plant tissues.

Mycorrhizal Infectivity Potential Quantification

Materials:

1. Dissection scope
2. Petri dish
3. Sharpie
4. Ruler
5. Stained and cleared root samples

Gridline Intersect Procedures:

1. Pull the stained and cleared root samples from the fridge.
2. Mark your petri dish at 1 cm increments and draw straight lines using your ruler to make a grid.
3. Measure the diameter of the petri dish and calculate the area of the dish.
4. For each sample, take enough roots to conveniently cover the petri dish without any over lapping.
5. Place the petri dish and roots under the dissection scope.
6. Scan each horizontal line for root intersections and record the total number of gridline x root intersections for each line and the total number of gridline x root intersections that have the presence of arbuscular mycorrhizae for each gridline (Figure zinger).
7. Repeat this process for the vertical lines as well and record the results.
8. Rearrange the roots in the petri dish and repeat step 6.
9. Repeat the last step 5 to 10 times and take the average for the total number of intersections and the AM intersections.
10. Use the averages to calculate the proportion of root length colonized.

-
11. The total root length should then be calculated using the formula below and multiplied by the % root length colonized to calculate the length of colonized root.

$$\frac{\text{Total number of intersections} \times \text{Average root length}}{\text{Total number of gridlines}} \times \text{Proportion of root length colonized}$$

12. Repeat for all samples.

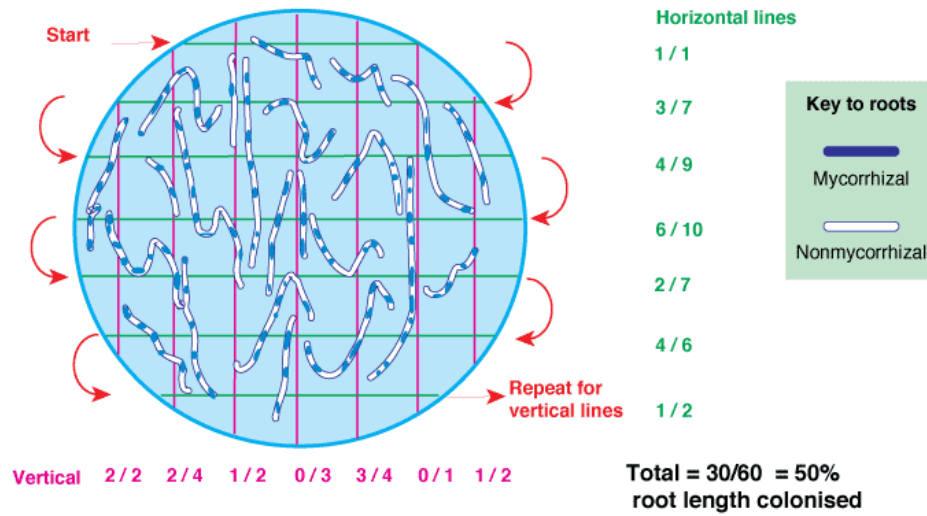


Figure 3. Methods for counting MIP colonization rates by the grid line intersection method (Mycorrhizal Associations).

Soil Water and Loss on Ignition

Materials:

1. Drying oven
2. Furnace
3. 3-place balance
4. Crucibles
5. Sharpie
6. Forceps
7. Gloves

Procedures:

1. Before using a set of crucibles, heat them in furnace at 500°C for 2 hours to remove any lingering organic matter. Let them cool overnight.
2. Put on gloves and label each crucible to correspond with its sample site.
3. Weigh the crucibles and record the **crucible weights**.
4. Add soil until about 1/3 full. Weigh the crucible with the soil and record the **wet weights**.
5. Place in the furnace and bake at 105°C for 24 hours.
6. Let the samples cool, weigh the crucibles with the oven dried soil record the **dry weights**.
7. Place samples in a furnace and bake at 500°C for 4 hours.
8. After 4 hours turn off the furnace and let them cool overnight before opening the furnace.
9. Remove crucibles, allow them to cool to room temperature, weigh crucibles with the ash and record the **ash weight**.
10. Clean your workspace and crucibles. Crucibles can be cleaned by pouring out the ash and wiping them with a kim wipe. Do not wash.
11. Calculate and record the % soil water and % loss on ignition.

Calculate:

Soil Biota Extractions

All soil biota extractions should be done as close to the sample date as possible to ensure the biota extractions are representative of the sample. Increased holding times can result in reduced abundance.

Nematode Extractions

Materials:

1. 3-place balance
2. Soil tray or clean paper
3. Aluminum foil
4. 50 mL beaker

For each sample, you will need:

1. 1 Baermann funnel with rubber tube
2. 1 pinch clamp
3. 2 pieces of cheese cloth
4. 1 binder clamp
5. 1 50 mL beaker
6. 1 Funnel stand

Procedures:

1. Set up Baermann funnel by placing one funnel in a stand for each sample site. Place a pinch clamp at the end of each of the rubber tubes.
2. Label each funnel with a sample ID. Paired sample sites can be placed on the same stand.
3. Fill funnels approximately $\frac{1}{4}$ full with water. Be sure to fill the funnels and rubber tubes slowly, squeezing the rubber tube periodically to remove all air bubbles.
4. Place a 50 mL beaker on the balance and tare it. Add in about 30 mL of sample soil and **record the wet weight.**
5. Lay out two pieces of cheese cloth on top of each other in the soil tray or on clean paper and place the 30 mL sample in the center of the cheesecloth. Wrap up the sample and secure the tops with binder clamps so no soil can escape.
6. Place the wrapped soil sample into the funnel and add water into the funnel **slowly** until samples are completely submerged. Be sure to pour or squirt water against the side of the funnel when adding water to reduce the amount of soil that escapes from the cheese cloth.
7. Repeat for each soil sample, ensuring to rinse the 50 mL beaker thoroughly between each sample.

8. Once all samples have been processed, place stands in a dark place for 48 hours and clean your work area.
9. After 48 hours, collect the water from each sample by placing a 50 mL beaker under each sample and removing the pinch clamps from the end of each tube.
10. Cover samples with aluminum foil and store in the refrigerator.



Figure 4. Nematode extraction set up using the Baerman funnel technique.

Microarthropod Extractions

Materials:

1. 3-place balance
2. Soil tray or clean paper
3. 91% isopropyl alcohol
4. 1 500 mL beaker
5. 1 50 mL beaker

For each soil sample, you will need:

1. 1 Tullgreen funnel with stand, light, and mesh screen
2. 1 collection jar

Procedures:

1. Set up one Tullgreen funnel for each soil sample by placing the mesh screen inside the funnel and labeling each funnel with the sample site ID.
2. Place a 500 mL beaker on the balance and tare it. Add about 250 mL of soil and **record the wet weight**.
3. Place the 250 mL soil sample onto the mesh screen inside the labeled Tullgreen funnel. Be sure to hold a paper towel at the bottom opening of the funnel to catch any soil particles that fall through the mesh and add them back into the funnel.
4. Label each collection jar with the sample site ID.
5. Pour 25 mL of water and 25 mL of 91% isopropyl alcohol into each collection jar.
6. Slowly place the labeled collection jar underneath its respective funnel. Be sure not to shake the funnel to reduce the amount of soil particles that fall into the collection jar.
7. Place the lid of the collection jar underneath the jar itself to make the funnel tight against the top of the jar. This will reduce the amount of liquid that will evaporate.
8. Lower the light until it is tight against the rim of the funnel and turn it on.
9. Once all samples have been processed, wait 48 hours and clean your workspace.
10. After 48 hours, check the collection jar for any signs of microarthropods.
11. Cover the collection jars and store them in the refrigerator.
12. Dispose of soil samples into a soil tray and dispose of the soil outside the ESCI building soil waste bin.
13. Clean your funnels and workspaces.



Figure 5. Microarthropod extraction set up using the Tullgren funnel technique.

Nematode Abundance

Materials:

1. Dissection Scope
2. Squirt bottle
3. 1 petri dish
4. Fine tip sharpie
5. Ruler

Procedure:

1. Pull nematode extraction from the fridge.
2. Mark your petri dish at 1 cm increments and draw straight lines using your ruler to make a grid.
3. Pour your nematode extraction into the petri dish and rinse the beaker with a small amount of water using the squirt bottle. Be careful not to overflow your petri dish.
4. If the sample is too large for the petri dish you can count the sample in multiple aliquots. Be sure to rinse your petri dish thoroughly between aliquots so no nematodes are double counted.
5. Turn on the dissection scope and place sample under the scope.
6. Adjust lighting and focus. Your frame of view should be able to see about $\frac{1}{4}$ of the 1x1 cm grid.
7. Count nematodes grid by grid until all nematodes are accounted for and **record the number**.
8. Pour out the sample and rinse petri dish thoroughly between samples and aliquots.
9. Repeat for all samples.

Nematode abundance is represented by the number of nematodes per dry gram of soil and is calculated by the following equation:

Microarthropod Abundance

Materials:

1. Dissection Scope
2. Squirt bottle
3. 1 petri dish
4. Fine tip sharpie
5. Ruler

Procedures:

1. Pull microarthropod extraction from the fridge.
2. Mark your petri dish at 1 cm increments and draw straight lines using your ruler to make a grid.
3. Extractions will be too large to analyze in one aliquot, so multiple will need to be done for each sample. Pour a portion of the microarthropod extraction into the petri dish.
4. Turn on the dissection scope and place your petri dish under the scope.
5. Adjust lighting and focus. Your frame of view should be able to see about $\frac{1}{4}$ of the 1x1 cm grid.
6. Count microarthropods grid by grid until all microarthropods are accounted for. Pour out the aliquot, rinse the petri dish thoroughly and repeat until the entire sample has been analyzed.
7. Record the total number of microarthropods and if possible, the number for each major group using the key:
https://keys.lucidcentral.org/keys/v3/soil_microarthropods/soil_microarthropods.html
8. Pour out the sample and rinse petri dish thoroughly between samples and aliquots.
9. Repeat for all samples.

Microarthropod abundance is represented by the number of microarthropods per dry gram of soil and is calculated by the following equation:

Soil pH

Air-Drying Soil

Materials:

1. Paper bags
2. 500 mL beaker

Procedures:

1. Label a paper bag for each sample with the sample ID and the date.
2. For each sample, measure approximately 300 mL of soil and place it in the labeled bag.
3. Close the paper bag and allow it to dry for at least 48 hours.

pH Measurements

Materials:

1. pH probe
2. 4, 7, 10 pH buffers
3. 3 x 30-50mL beakers
4. 1L stock solutions of 0.01M and
5. 4 mm sieve
6. 3-place balance
7. Air-dried soil
8. 200 mL beaker
9. 50 mL graduated cylinders
10. Waste container
11. DI and Tap water squirt bottles
12. Soil tray

Initial Procedures:

1. Pour the 4, 7, and 10 buffers into individual 30-50 mL beakers.
2. Calibrate the pH probe following the methods outlined by the model of pH probe you are using. Start with the 4 and continue to 7 and 10. Be sure to rinse the probe with a DI squirt bottle between buffers and blot dry with a kim wipe.
3. For each sample, pour the air-dried soil through the 4 mm sieve into a container or soil tray.
4. Dispose of the large debris and pour the sieved soil back into the paper bag.
5. Once all soil samples have been sieved, perform the soil pH measurements for each sample.

1:1 H₂O Procedures:

1. Place a 200 mL beaker on the balance, tare it and add 50 g of air-dry soil.
2. Measure 50 mL of distilled water in a graduated cylinder and add it to the beaker.
3. Stir vigorously for 30 seconds until thoroughly mixed.
4. Place pH probe into the slurry.
5. Wait until the pH reading stabilizes and record the pH reading.
6. Pour the solution through the sieve into the sink, be careful to retain as much soil as possible.
7. Dump the sieved soil into the waste container.
8. Rinse the pH probe with the DI squirt bottle and blot dry with a kim wipe.

1:2 0.01M CaCl₂ Procedures:

1. Place a 200 mL beaker on the balance, tare it and add 25 g of air-dry soil.
2. Measure 50 mL of _____ in a graduated cylinder and add it to the beaker.
3. Stir vigorously for 30 seconds until thoroughly mixed.
4. Place pH probe into the slurry.
5. Wait until the pH reading stabilizes and record the pH reading.
6. Pour the solution through the sieve into the sink, be careful to retain as much soil as possible.
7. Dump the sieved soil into the waste container.
8. Rinse the pH probe with the DI squirt bottle and blot dry with a kim wipe.

1:1 1N KCl Procedures:

1. Place a 200 mL beaker on the balance, tare it and add 50 g of air-dry soil.
2. Measure 50 mL of _____ in a graduated cylinder and add it to the beaker.
3. Stir vigorously for 30 seconds until thoroughly mixed.
4. Place pH probe into the slurry.
5. Wait until the pH reading stabilizes and record the pH reading.
6. Pour the solution through the sieve into the sink, be careful to retain as much soil as possible.
7. Dump the sieved soil into the waste container.
8. Rinse the pH probe with the DI squirt bottle and blot dry with a kim wipe.

Soil Texture

Materials:

1. Waste container
2. Ruler
3. Weighing spatula
4. 500 mL beaker

For each soil sample:

5. 500 mL glass container
6. 250 mL wet soil

Procedures:

1. Label the 500 mL glass container with the sample ID.
2. Measure 250 mL of wet soil and add it to the jar, avoiding the litter layer and large rocks.
3. Add 250 mL of tap water to the jar.
4. Wipe the rim of the jar to remove any debris from the threads and screw the cap tightly onto jar.
5. Shake vigorously for 30 seconds and check to make sure no large aggregates remain.
6. Place jar somewhere it will not be disturbed.
7. Repeat for all soil samples.
8. At the end of class, use a ruler to measure the depth of the different fractions, sand, silt, and clay. Measure the total depth of soil as well (Fig. 1).



Figure 6. Layers of clay, silt and sand in a jar test (Anna).

9. If time allows wait a full 24 hours before measuring depths.
10. Calculate the % depth of each layer.
11. Use a soil texture triangle to determine your soil texture.

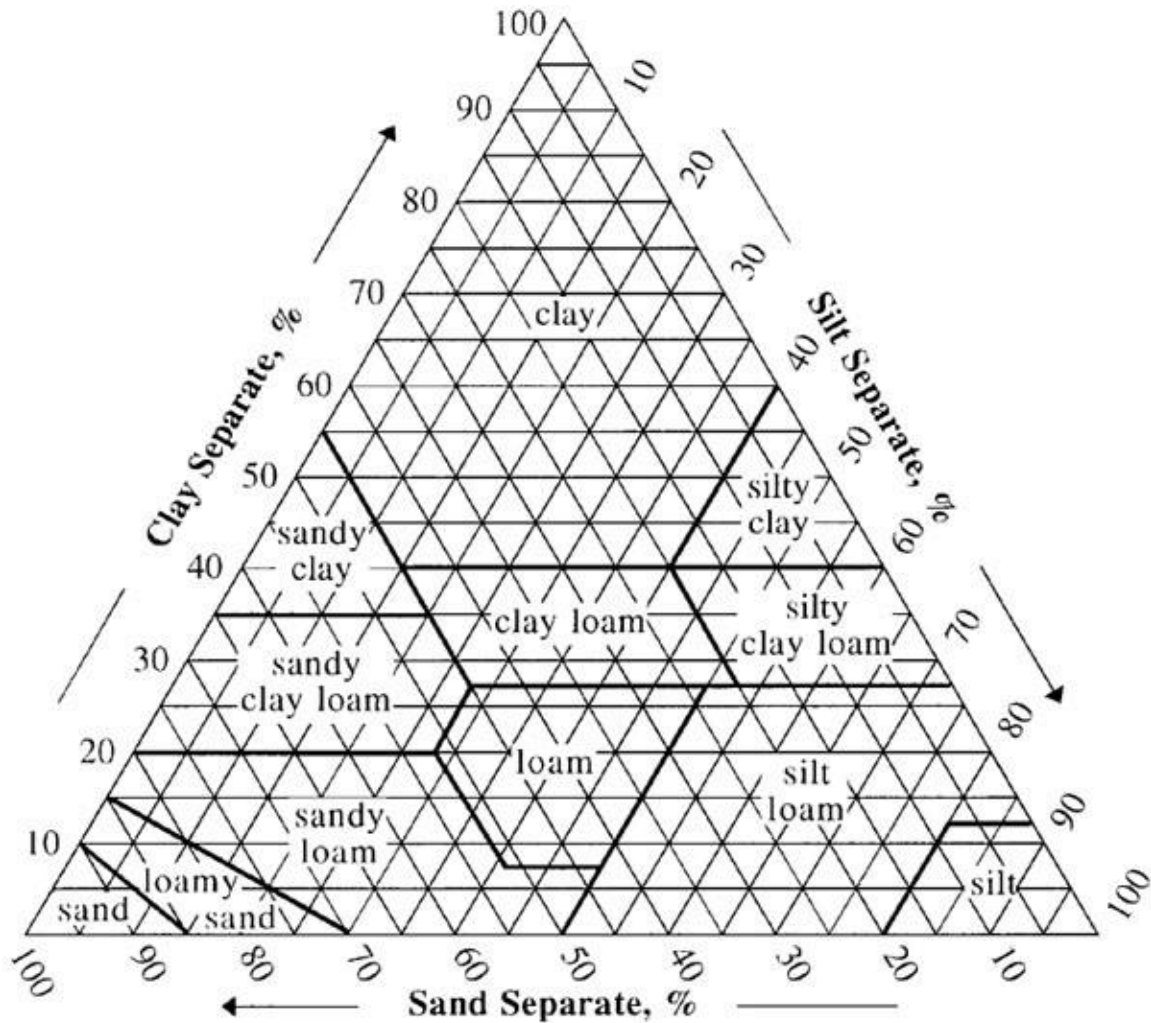


Figure 7. USDA soil texture calculator (NRCS).

T-Test Results and Graphs

Table 1. Paired T-Test results for all soil characteristics measured

Soil Characteristic	T-Value	P-Value	T-Critical	Confidence Interval	Significant?
Soil Moisture %	-0.43942	0.6831	2.776	95%	No
Organic Matter %	0.26191	0.8063	2.776	95%	No
Delta pH	3.7419	0.02009	2.776	95%	Yes
H2O pH	-0.68904	0.5287	2.776	95%	No
KCl pH	-3.0023	0.03985	2.776	95%	Yes
Sand %	0.8411	0.4451	2.776	95%	No
Silt %	-0.69161	0.5272	2.776	95%	No
Clay %	-0.39102	0.7157	2.776	95%	No
Nematode Abundance	1.4127	0.2306	2.776	95%	No
Microarthropod Abundance	-2.8303	0.4733	2.776	95%	Yes
MIP	N/A	N/A	N/A	N/A	N/A

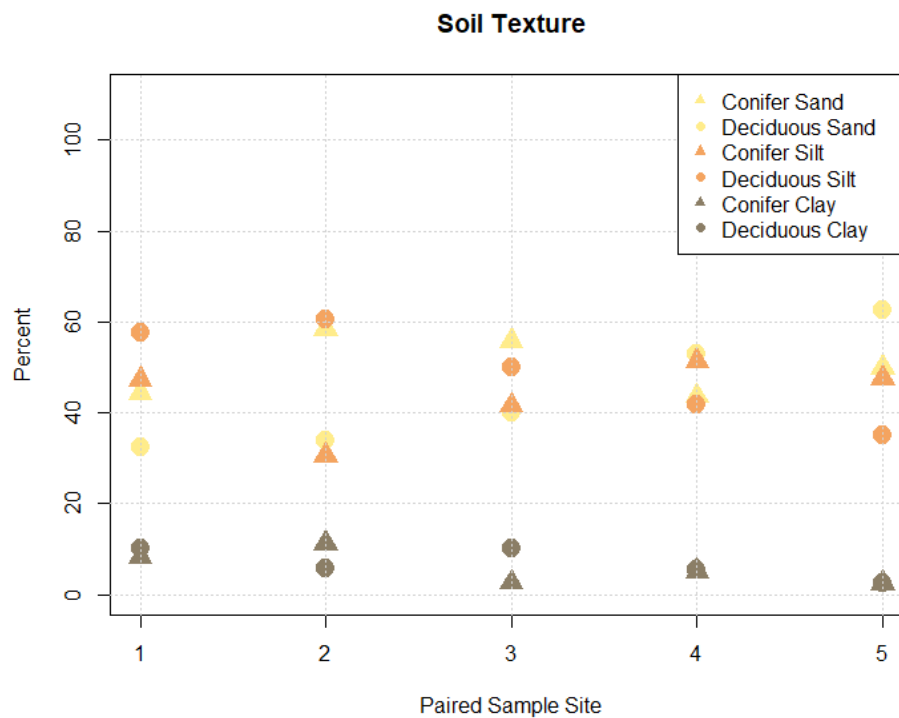


Figure 8. Soil Texture proportions of sand, silt and clay for each of the paired sample sites.

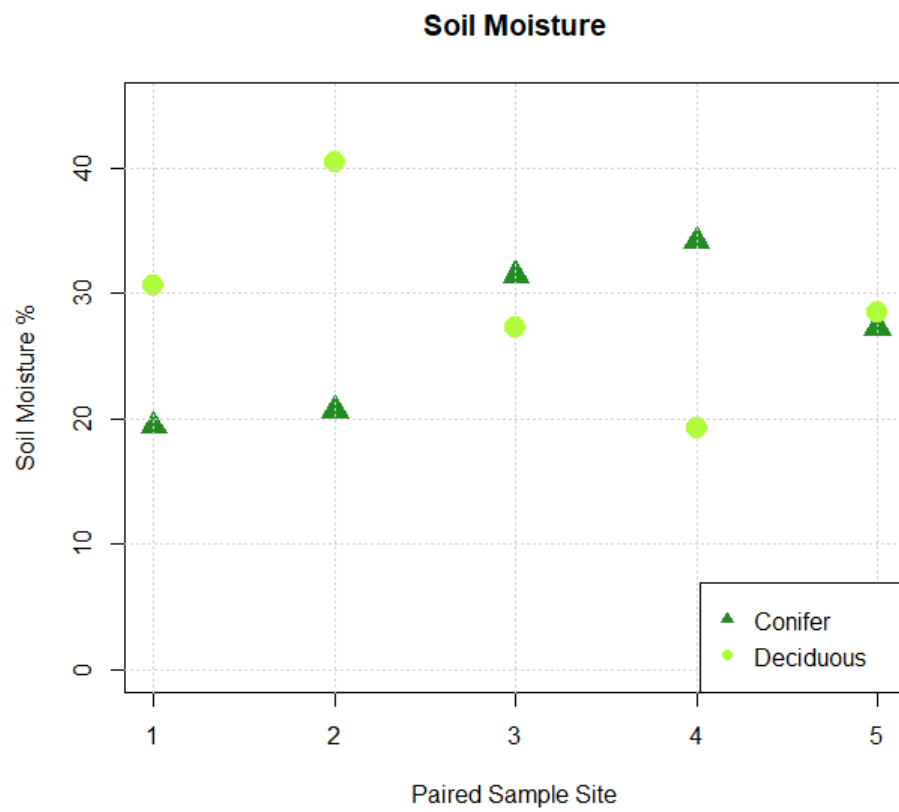


Figure 9. The soil moisture % for each of the paired sample sites.

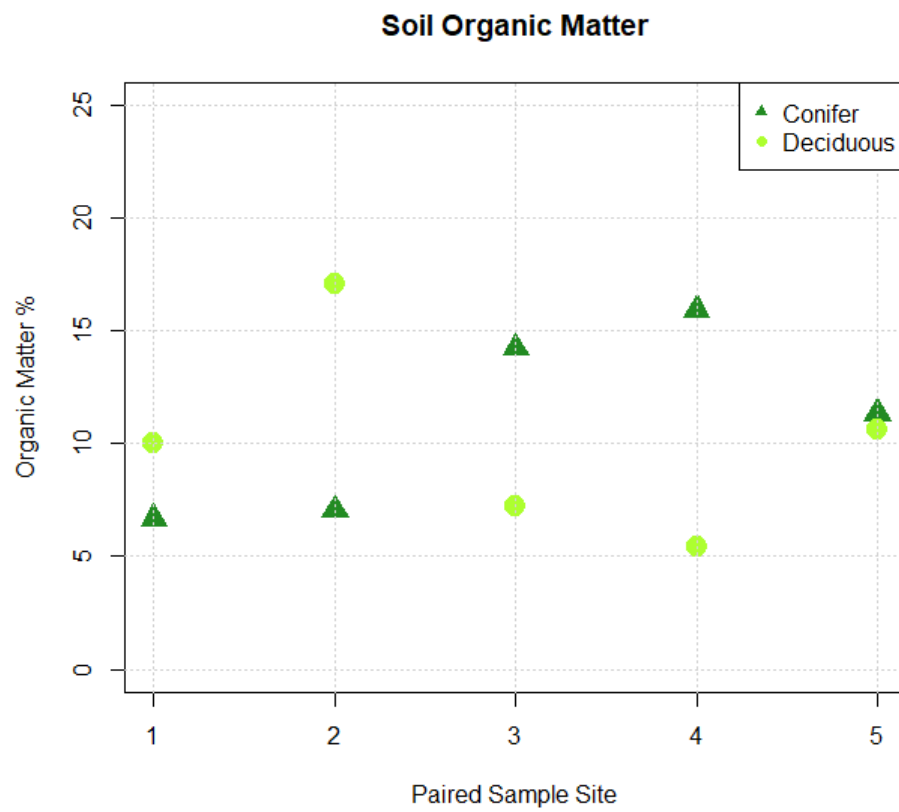


Figure 10. Soil organic matter % for each of the paired sample sites.

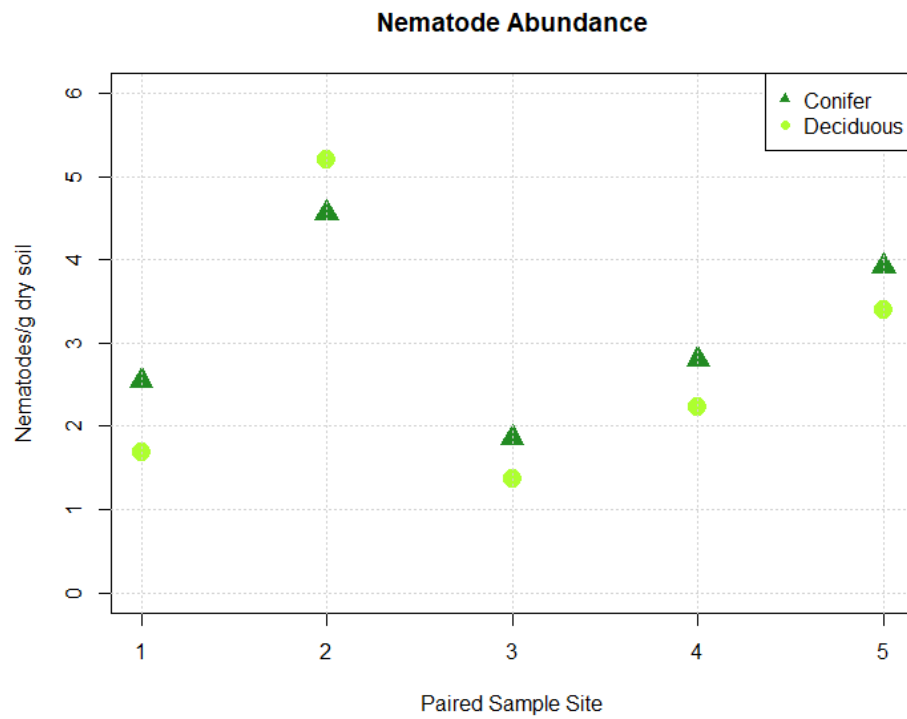


Figure 11. Nematode abundance expressed as number of nematodes per dry gram of soil for each of the paired sample sites.

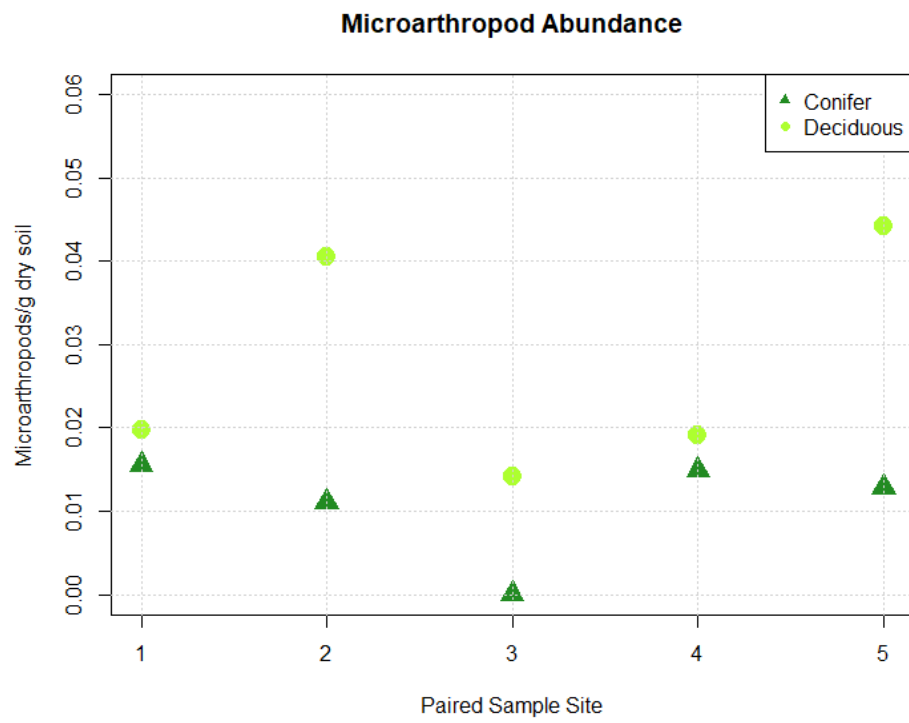


Figure 12. Microarthropod abundance expressed as number of microarthropods per gram of dry soil for each of the paired sample sites.

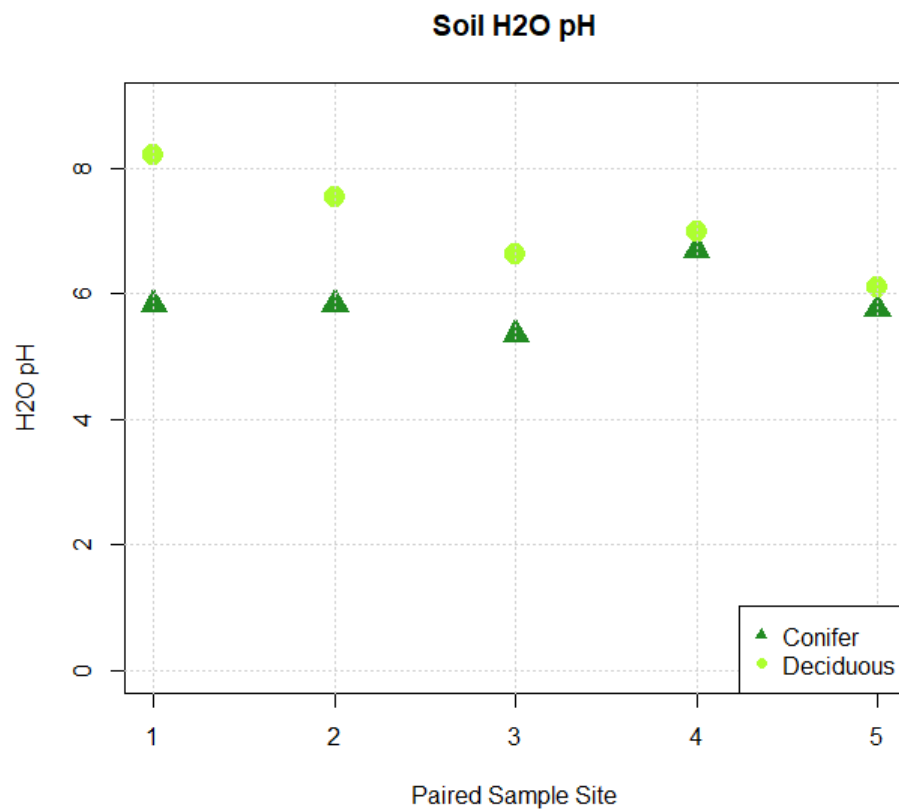


Figure 13. Soil pH for each of the paired sample sites.

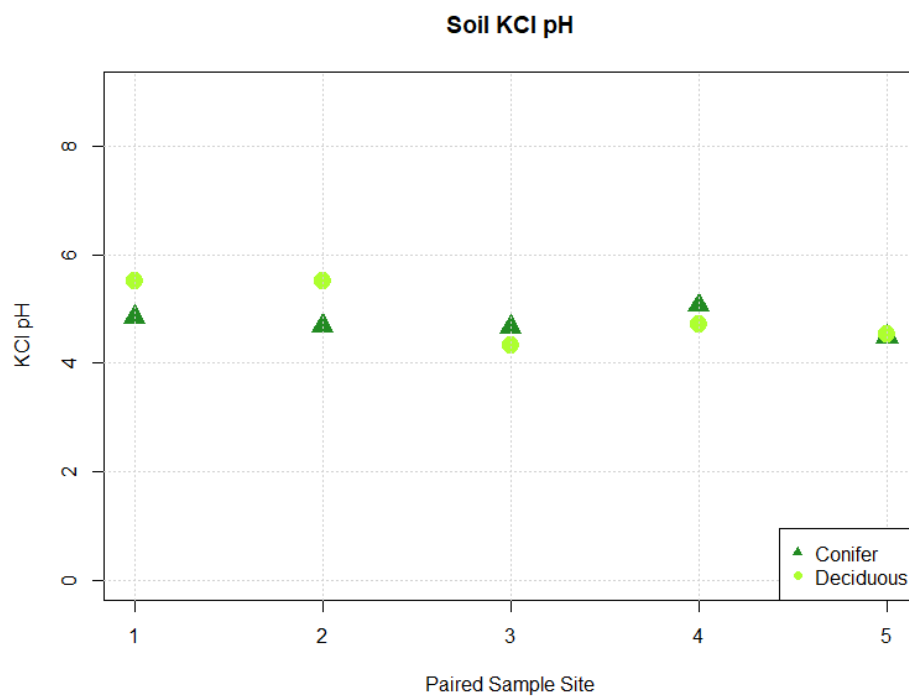


Figure 14. Soil KCl pH for each of the paired sample sites.

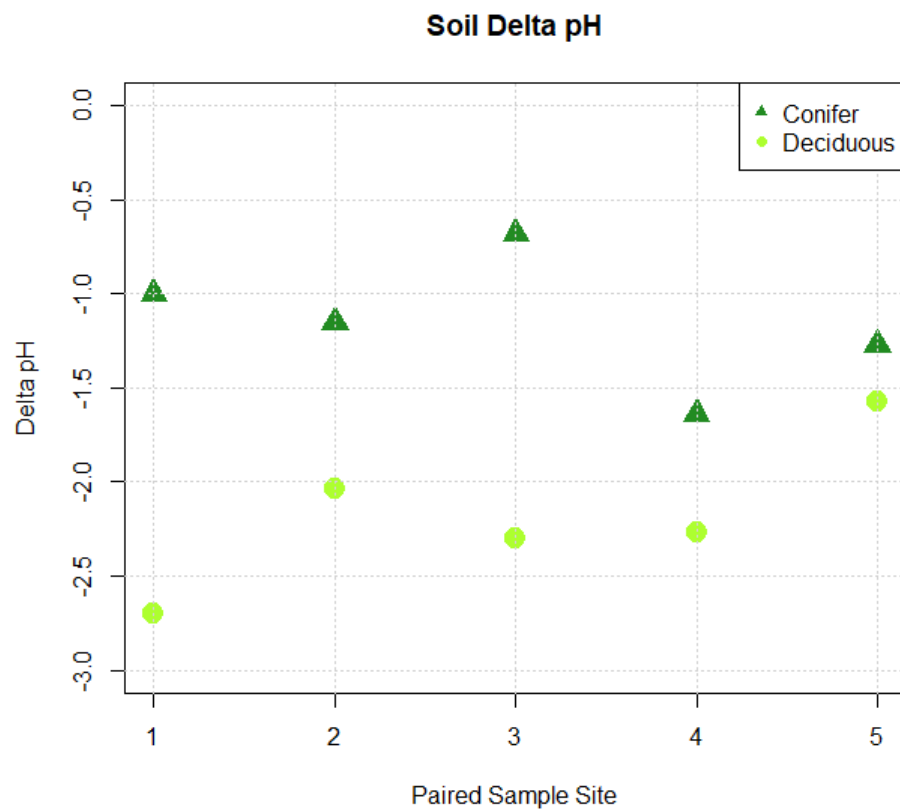


Figure 15. Soil Delta pH for each of the paired sample sites.

Weekly Hours		
Week	Hours	Description of Tasks
1	4	Preliminary research and setting up the project.
2	16.5	Preparing and creating sample sites in ArcGIS.
3	17.5	Editing sample sites in ArcGIS, creating a field map, collecting first trial samples, started trial runs for soil characteristics with trial samples.
4	24.5	Finished trial lab work for all soil characteristics, finished sample site selections, sampled all sample sites, started air drying soil for pH, started TLI, set up MIP.
5	17.5	Restarted TLI due to error, measured pH, worked on methods.
6	23	Finished TLI, measured soil texture, finished soil biota extractions.
7	34.5	Resampled, restarted soil biota extractions, worked on scholar's week poster.
8	25	Finished scholar's week poster, started soil moisture for new samples, collected soil biota extractions, started counting nematodes.
9	18.5	Finished nematode counting, edited and created methods, harvested corn roots for MIP.
10	24	Continued methods work, cleared and stained roots, collected environmental data for sample sites, microarthropod counting.

11	19	Finished editing methods, tested MIP methods, report writing, and data clean up.
Total	224	