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WWU Soil Ecology Lab Intern - Mycorrhizal Relationships in Raspberry Roots and Soil Analysis of Sehome Arboretum

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COLLEGE OF THE ENVIRONMENT



Internship Title: WWU Soil Ecology Lab Intern
Student Name: Brigid Wills
Internship Dates: April 2022 - June 2022

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STUDENT SIGNATURE

A handwritten signature in black ink, appearing to read "Brigid Wills", written over a horizontal line.

DATE:

6/8/22

Brigid Wills
Internship Report, Spring 2022

Mycorrhizal Relationships in Raspberry Roots and Soil Analysis of Sehome Arboretum

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INTRODUCTION

In Spring Quarter 2022, I worked in the WWU Soil Ecology Lab. I assisted Dr. Rebecca Bunn on course method development, as well as Amber Kelley and her raspberry graduate research. I assisted with research, method development, and lab organization. The [WWU Soil Ecology Lab](#) is committed to researching mycorrhizal relationships, with forestry and agricultural applications.

Acknowledgements: Gratitude to Dr. Rebecca Bunn for giving me independence in the lab to make mistakes and learn. Thank you also to Amber Kelley for mentoring me in lab techniques.

DESCRIPTION OF DUTIES AND RESPONSIBILITIES

Assisting Dr. Rebecca Bunn with Lab Organization

A basic component of my internship in the lab was being available to assist with lab operations, as well as helping Dr. Rebecca Bunn. Before some of my other projects got started, one task I worked on was organizing Dr. Bunn's Mendeley Citation Library. I assigned tags to specific articles to make them more easily findable. Within the lab, I was also responsible throughout the quarter for washing dishes and keeping the lab organized.

Lab Assistant to Amberose Kelley, Graduate Researcher

One aspect of my work within the Soil Ecology Lab was being available to assist Amber Kelley, who is working on her graduate research on AMF (arbuscular mycorrhizal fungi) in raspberry plants. In Western Washington, the two main culprits of raspberry disease are *Phytophthora rubi*, a fungal disease, and *Pratylenchus penetrans*, a root lesion nematode (Weiland et al, 2018). AMF has been hypothesized to be integral in allowing raspberry plants to develop resistance to diseases. Amber's project builds off the past work of Erika Whitney in the Soil Lab, whose main finding was that AMF did create more tolerance (in the 'Meeker' raspberry cultivar) against *P. rubi* (Whitney, 2020, p. 49). The secondary conclusion was that AMF from cultivated fields was the most effective inoculum when compared to commercial and created AMF inoculums (ibid).

I was involved with Amber's project throughout her work, though I did not assist in field collections. I helped pot up raspberry plants, which were planted in different inoculations of soils from different agricultural fields. This process included putting poly-fill stuffing into pots, adding in soil, and planting raspberry plugs in them. Later in her project I worked on creating root slides from the raspberries, as well as corn plants. Pictures of this process can be seen in Figure 1. These slides serve as visual identifiers for AMF, since vesicles and hyphae can be seen within stained roots. Roots were bleached with KOH (potassium hydroxide) and acidified with HCl (hydrochloric acid) before being stained with Trypan blue. After staining, small sections of roots were isolated and added to a slide, which would later be observed for presence of AMF vesicles. These were all part of a pilot study to determine best in-practice methods that Amber will continue to use in her research.

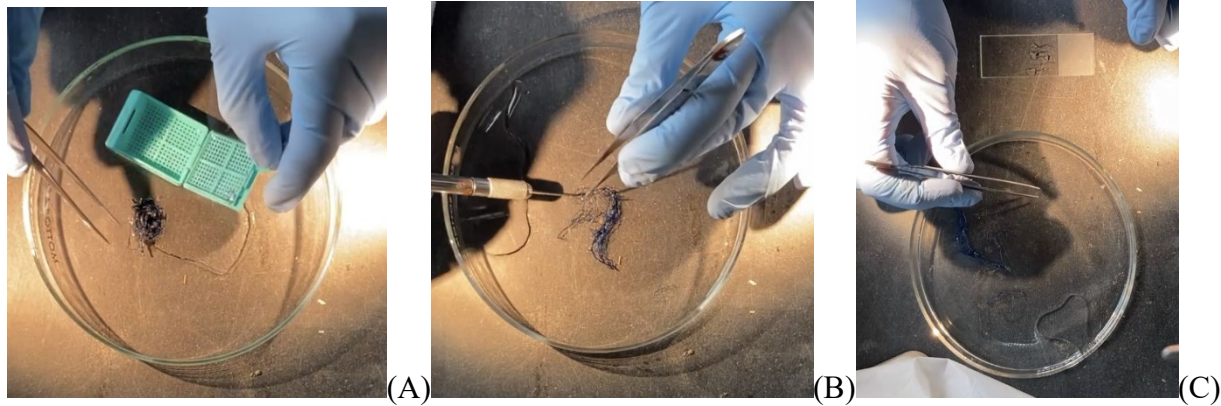


Fig. 1: Overhead pictures of removing stained roots from a cassette (A), selecting and cutting off sections of roots (B), and placing slide selections onto a glass slide (C).

Collaboration with Biology Students (Dr. Marion Brodhagen, Dr. Rebecca Bunn, Dr. John Tuxhill collaborative Sehome Project)

The final project that I worked on was assisting in the development of a course that Dr. Rebecca Bunn, Dr. Marion Brodhagen, and Dr. John Tuxhill are collaborating on. The hope is that this course will end up being a way to create a survey study of Sehome Arboretum that includes biology aspects, ecological soil aspects, and a humanities aspect. There is also a fungal pathogen that has been present in the Pacific Northwest that infects Douglas Fir trees. This class could be used to monitor the impact of this slow-moving fungal disease.

As part of creating this course, Dr. Bunn had me test and develop some methods for sampling and taking measurements of the soil. Working from methods that she had written, I collected samples from an area in the Arboretum near the Fairhaven dorms. I collected three replicates of mineral soil and three replicates of leaf litter in a primarily deciduous area, and then repeated this process in a primarily coniferous area. During this process, I also wrote up sampling methods (see Appendix 1) that could be used by incoming students to this class. These methods were expanded versions of what Dr. Bunn had laid out.

After collecting samples of litter and soil, I ran several tests on them. Berlese-Tullgren funnels were used immediately after collection to begin collecting soil biota. These funnels work on the principle that soil arthropods will move away from the heat and light. After 48 hours, biota experiments were stopped, and biota were counted and identified from the ethanol containers (see Figure 2 for setup).

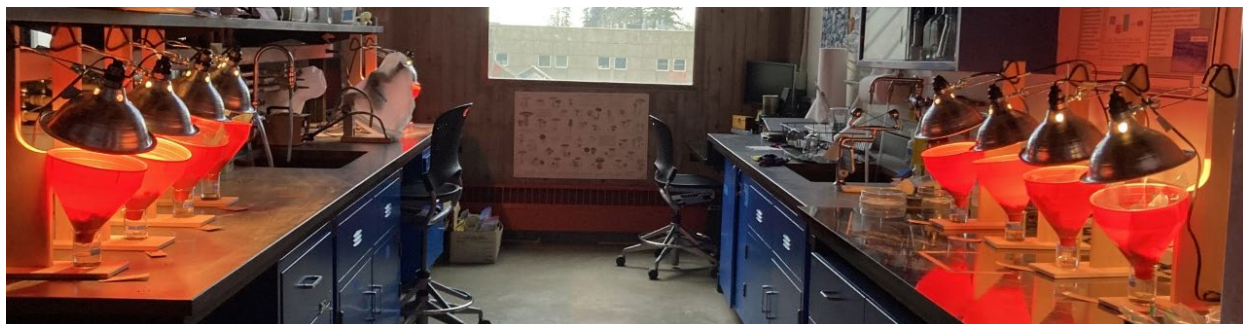


Fig. 2: Picture of Berlese-Tullgren funnel set ups. Soil was placed at the bottom of the red funnels, and biota fell (away from the heat and light coming from the heat lamp) into the beaker with ethanol.

Next, I ran physical tests on the mineral soil. Acidity (pH) tests were done with three different solutions: KCl, CaCl₂, and H₂O. Any of these three solutions could be used, but since I was testing methods, I used all the solutions. One precursor to this was mixing KCl and CaCl₂, which involved using analytical chemistry techniques, including calculating molarities and weighing out powdered KCl and CaCl₂ before dissolving it in water. After mixing these solutions, I air-dried soil (in paper bags on the counter) and then mixed them with the solutions at different ratios. I used a pH probe to measure the pH of the soil slurry after the solutions were mixed and had sat for 10 minutes (see Figure 3).

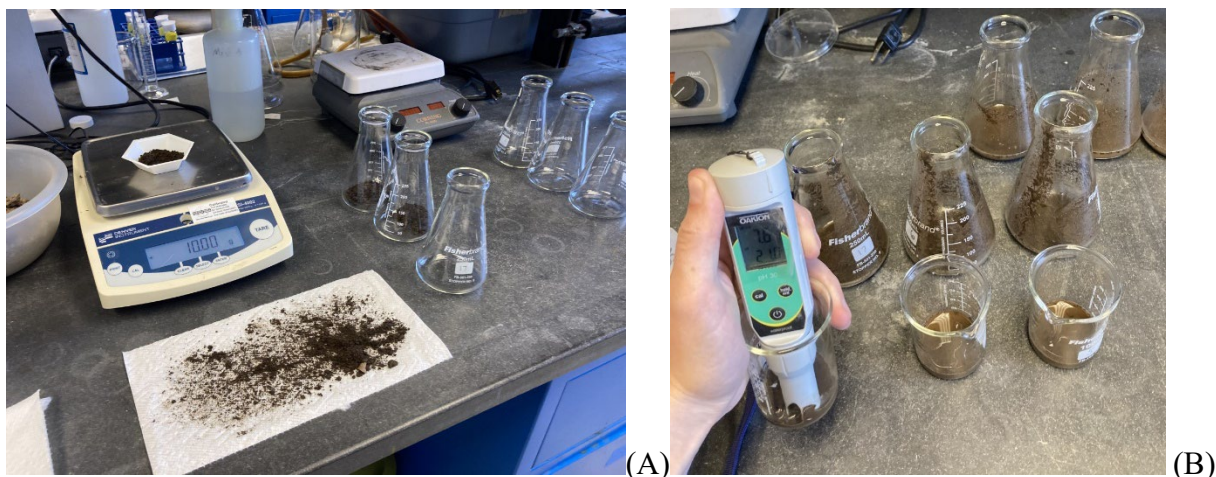


Fig. 3: Weighing out 10.00g of soil for each solution test (A). (B) shows the pH probe being used to measure pH of each soil slurry solution.

Another test I did was measuring soil moisture content and organic matter content of the soils. Using mineral soil that had been stored in the refrigerator, I used a 3-balance scale to weigh soil from each replicate. Next, the soil was placed into crucibles (pre-weighed). For soil moisture content, the crucibles were placed in an oven for 24 hours at 105 degrees C, which caused the water to evaporate. To measure organic matter, the crucibles were then placed in a 500 degree C muffle furnace, which causes organic matter to combust. After weighing, calculations were performed to determine the percent soil moisture and percentage loss on ignition.

Lastly, I ran simple soil texture test, using a jar test that combined half part soil and half part water (see Figure 4 for setup). These settled for an hour before measurements were taken. The depths of each level of soil were measured, and then the USDA soil chart was used to categorize the type of soil.



Fig. 4: Jar test setup, showing the 50/50 soil and water ratio for each jar.

After running all these tests, I collated the raw data into a metadata csv file. I then ran the data through R (RStudio Team, 2020) to do calculations (e.g. means) and create graphs with ggplot (Wickham, 2016). Graphs are displayed in the Outcomes section. This RStudio analysis also involved using piping to extract data and create new datasets to create graphs. Working with this small, real-life dataset I had collected, I was able to develop more data processing skills in RStudio, including dplyr tidyverse piping (Wickham, 2022) as a data wrangling method and ggplot (Wickham, 2016) as a method for data visualization.

As another aspect of developing methods, I also instructed biology students working with Dr. Brodhagen on soil sampling techniques. Communicating methods was a key aspect of this internship, as I met with Dr. Brodhagen and her team to explain what I had been working on and how our projects could be combined. The goal for collaboration with her team is to eventually be able to sample soils from the same plots that biology students are doing native species identification from, as well as fungal samples. The collaboration for this sampling did not end up happening within the timeline of this quarter, but had there been time for this to happen, similar Berlese-Tullgren extractions, pH tests, soil texture tests, soil moisture levels, and organic matter percentage.

OUTCOMES

Lab Assistant to Amberrose Kelley, Graduate Researcher

The experiments that Amber is running are still ongoing, so final results for her project are not yet available. However, below are the completed results from my work on her project. Figure 5 shows a completed set of root slides, which were made from different field samples of raspberry roots.

Additionally, I collected several pictures of the root slides to begin assessing the presence of AMF. These were taken on my iPhone through the viewfinders of a compound microscope. Figure 6 shows an example of the setup of the scope, as well as a side-by-side comparison of root walls. Figure 6b shows root walls with the presence of AMF, and Figure 6c shows root walls without AMF. Figures 7 and 8 show more examples of AMF within the roots, both at 100x and 400x magnification. Since this was a pilot study, these pictures also were designed to determine the optimal amount of KOH needed for roots to soak in before staining. Figure 7 is after 72 hours in KOH, and Figure 8 is after 96 hours in KOH. To be consistent with AMF, I looked for absent or irregular cell walls, and dimorphic hyphae (having two different sizes of hyphae: one fat and one thin).

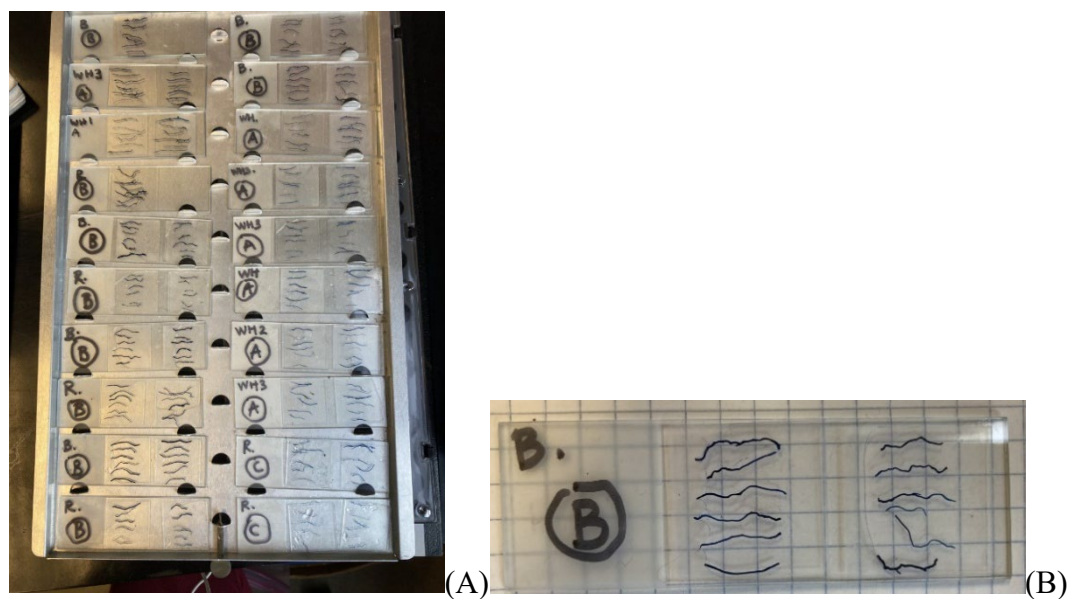


Fig. 5: A completed set of root slides (A) and an example of what a completed slide looks like (B).

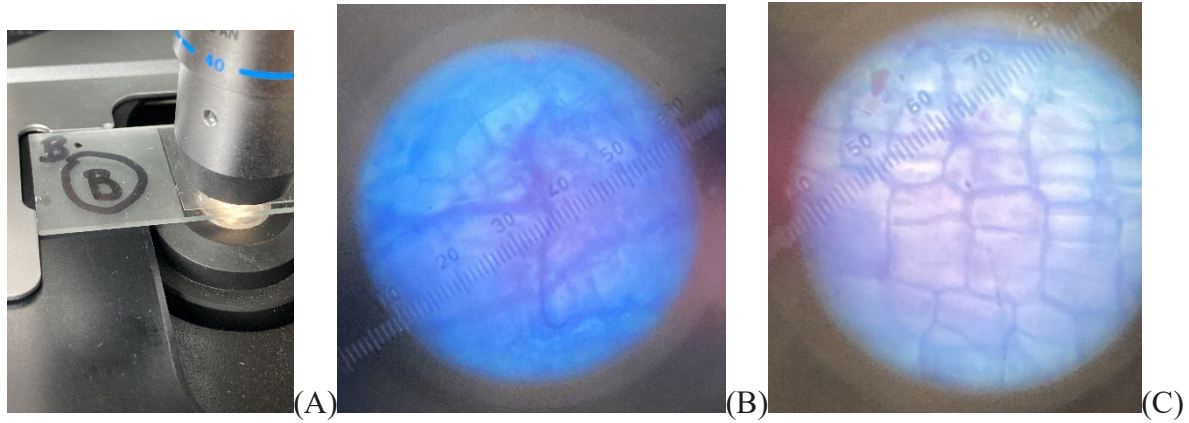


Fig. 6: Pilot study root samples from Bartlett fields. Picture A shows set-up for using the compound scope to assess presence of AMF in root slides. Picture B shows the presence of AMF at 400x magnification, in contrast with Picture C showing root without AMF (also at 400x magnification).

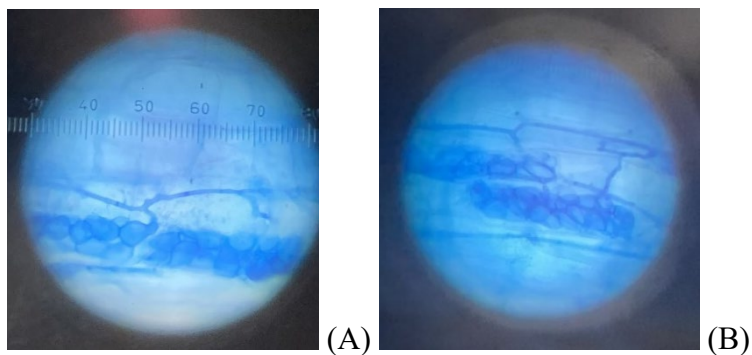


Fig. 7: Pilot study root samples from Rammerman fields after treatment in KOH for 72 hours. AMF-consistent vesicles can be seen in A and B, at 400x magnification. Hyphae consistent with AMF hyphae can be seen at 400x in picture A and 400x in picture B. These may be AMF or may be more consistent with dark septate endophytic fungi (Knapp, 2018)

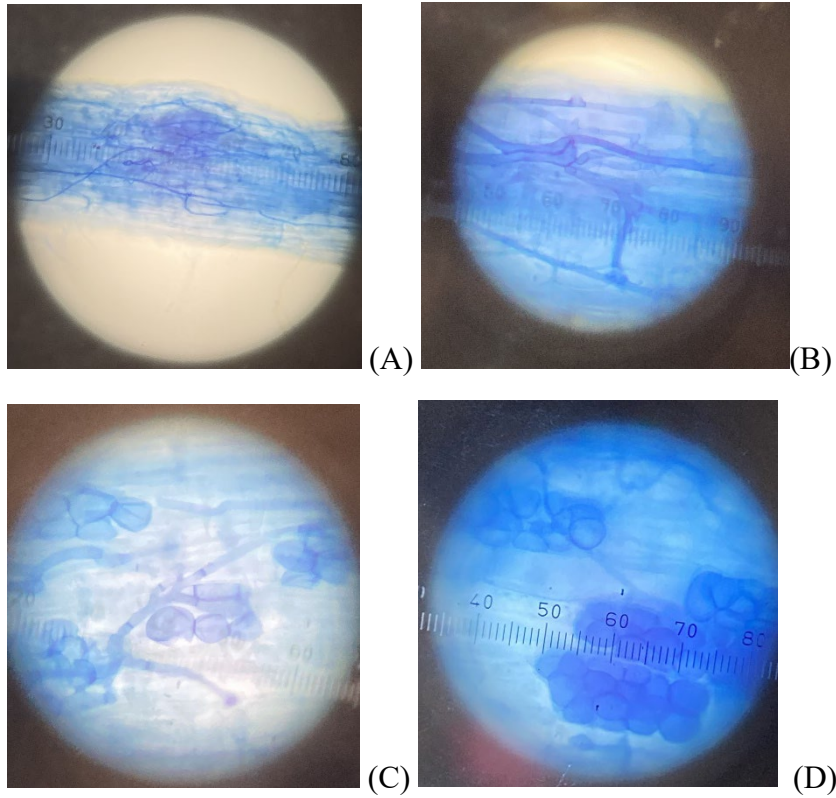


Fig. 8: Pilot study root samples from Rammerman fields after treatment in KOH for 96 hours. AMF-consistent vesicles can be seen in pictures C and D, at 40x magnification. Hyphae consistent with AMF hyphae can be seen at 100x in picture A and 400x in picture B. These may be AMF or may be more consistent with dark septate endophytic fungi (Knapp, 2018).

Collaboration with Biology Students (Dr. Marion Brodhagen, Dr. Rebecca Bunn, Dr. John Tuxhill collaborative Sehome Project)

Below are results from my soil assessments of Sehome Arboretum samples. Figure 9 shows bar graphs (with standard error bars) of the percentage of soil moisture lost after being heated at 105 degrees C, and the loss on ignition after being burned at 500 degrees C. Despite both soil moisture loss and loss on ignition being lower for coniferous stands than deciduous stands, there is not a significant difference between the values (SW p-value = 0.26, LOI p-value = 0.67; see Figure 10 for two-sample t-tests).

The pH values are lower for coniferous stands for every pH solution. This could be in part due to pine needles falling in the soil underneath coniferous stands. The only statistically significant difference between coniferous and deciduous stands is from the CaCl_2 data ($p = 0.02$); pH tests run with H_2O and KCl did not return significantly different data ($p = 0.24$; $p = 0.77$).

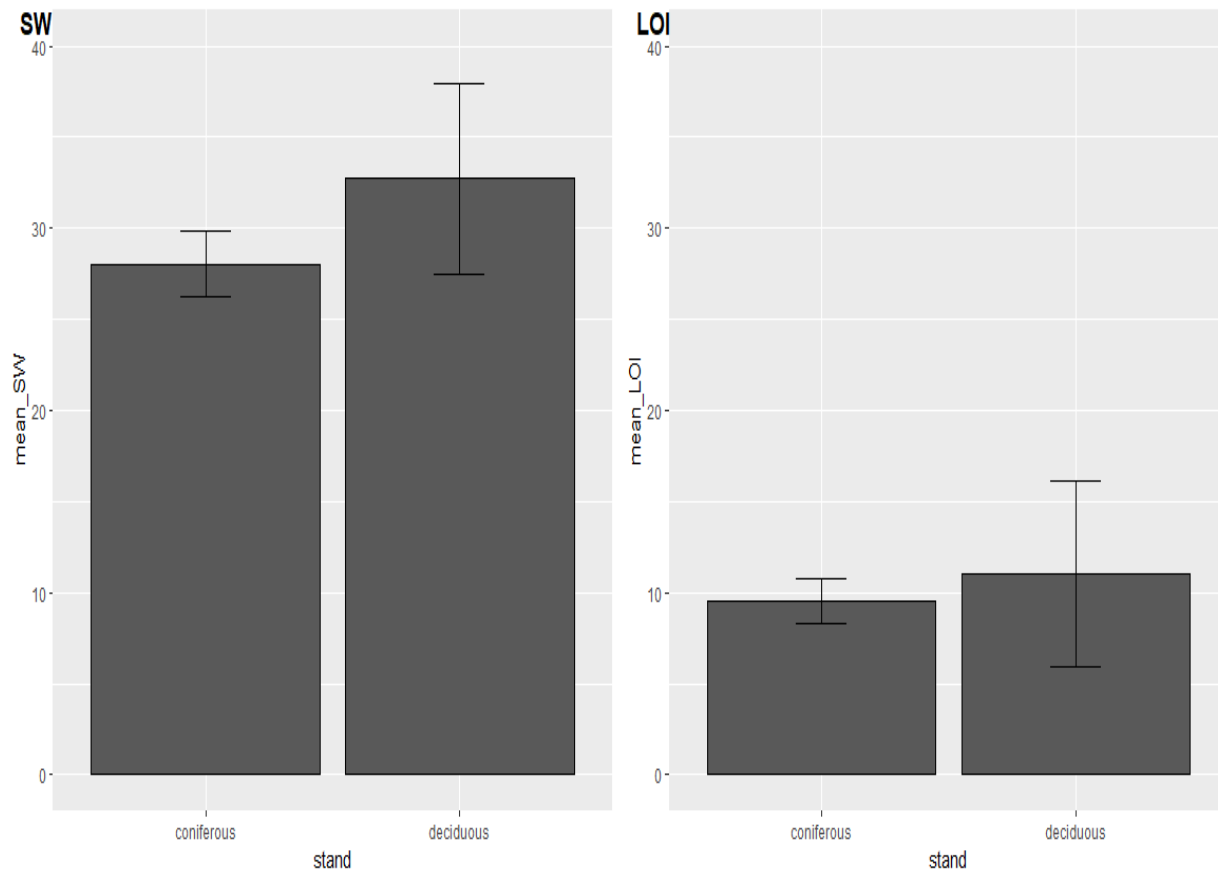


Fig. 9: Percentage mean soil moisture bar graph (SW) and percentage mean loss on ignition (LOI), with standard error bars.

```

welch Two Sample t-test

data: x and y
t = -1.4629, df = 2.4844, p-value = 0.2574
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -16.163604  6.805606
sample estimates:
mean of x mean of y
 28.03710  32.71609

```

(A)

```

welch Two Sample t-test

data: q and r
t = -0.48059, df = 2.2332, p-value = 0.6738
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -13.34923  10.41987
sample estimates:
mean of x mean of y
  9.560183 11.024864

```

(B)

Fig. 10: Screenshots from two-sample t-tests of SW (A) and LOI (B). $P > 0.05$ in both cases.

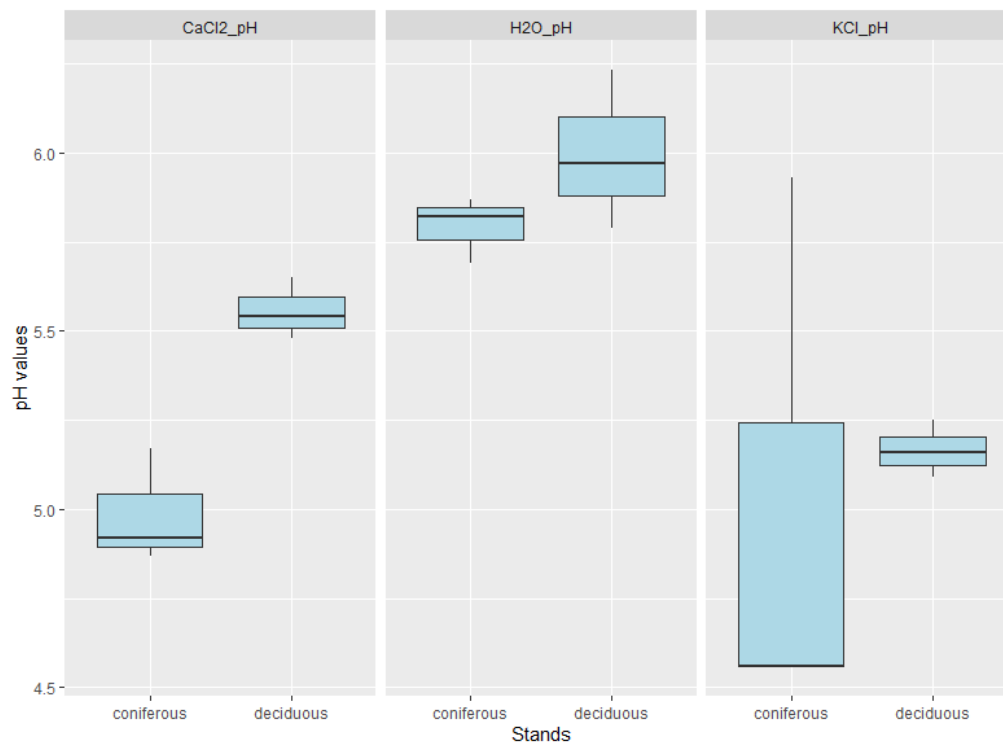


Fig. 11: pH values for each treatment (CaCl₂, H₂O, and KCl).

```

welch Two Sample t-test

data: x and y
t = -5.4694, df = 3.0041, p-value = 0.01197
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.9045691 -0.2394309
sample estimates:
mean of x mean of y
 4.988333  5.560333

```

(A)

```

welch Two Sample t-test

data: c and d
t = -1.4964, df = 2.7245, p-value = 0.2403
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.6681107  0.2574440
sample estimates:
mean of x mean of y
 5.795000  6.000333

```

(B)

```

welch Two Sample t-test

data: e and f
t = -0.33295, df = 2.0384, p-value = 0.7703
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -2.094918  1.788918
sample estimates:
mean of x mean of y
 5.017333  5.170333

```

(C)

Fig. 12: Screenshots of t-test results for pH values. CaCl₂ (A), H₂O (B), and KCl (C) results.

ASSESSMENT

Amber's project recently received more funding, so it is likely to continue to return results about raspberry pathogens. These results may be relevant to agriculture in Whatcom County and throughout Washington. Helping to develop these methods seemed to be helpful for both Amber, as well as for me in looking at past methods from the lab and building off of those. I also learned about sanitization of soil in autoclaves to sterilize the soil that we were using in the raspberry pots. As I was only working in the lab for one quarter, I wasn't able to develop skills in post-harvest destructive harvesting techniques, but that is something that I would be interested in learning in the future.

The start of the Sehome Arboretum Project this quarter seemed very successful. Since Dr. Bunn is aiming to offer the class in Summer 2024, the beginning collaboration with Dr. Brodhagen was ultimately successful for the timeline. Given that this project will continue being developed, there is now a starting point, as well as some tested and standardized methods for soil sampling.

Working in the Soil Ecology Lab this quarter gave me an opportunity to have a self-directed experience in a lab setting. I was able to practice analytical chemistry techniques, as well as learning soil sampling techniques and plant processing techniques. These included mixing solutions at different molarities, soil pH testing methods, muffle furnace use, and root staining processes. I also gained skills and experience working in RStudio, learning more about piping as a data wrangling technique, and using ggplot to develop data visualization.

Additionally, I learned more about collaborating as part of a scientific team, and making methods communicable and replicable. Working with the other biology students from Dr. Brodhagen's team was good practice for communicating methods with my peers. Additionally, meeting with them was helpful for learning more about the process that comes from developing methods.

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Appendix I**Sehome Data User Guide**

Brigid Wills

Associated files:

Master:

sehome_metadata.csv

Accessory:

*Master_Doc_Data_Collection_Methods+Notes.docx**Collection_Methods_BIO.docx**Collections_Location.docx**ZoomIn2FH.JPG**ZoomInFH.JPG**ZoomOutFH.JPG**Mass_preBerlese.xlsx**Observed_Biota.xlsx**pH_values.xlsx**Soil_Texture_Measurements.xlsx**SW and LOI table.docx**SW_and_LOI_measurements.xlsx*

Collection Dates: Spring 2022 (Samples: April 5th, 2022. Tests run: April 5th, 2022 – April 11th, 2022)

About this data:

Preliminary data was collected by undergraduate Brigid Wills in Spring 2022, in an effort to assess and develop replicable methods for Sehome Arboretum sampling data, to assist Dr. Rebecca Bunn in developing a course monitoring Sehome Arboretum. Methods are outlined in detail in *Master_Doc_Data_Collection_Methods+Notes.docx* and *Collection_Methods_BIO.docx*. In brief, samples were collected from the south of the Arboretum, with three leaf litter samples and three mineral soil samples from a coniferous and a deciduous stands. Locations are reflected in *Collections_Location.docx*, *ZoomIn2FH.JPG*, *ZoomInFH.JPG*, and *ZoomOutFH.JPG*.

Berlese extractions were performed, and biota were loosely identified and counted (see *Observed_Biota.xlsx*). A jar test was used to determine texture measurements (*Soil_Texture_Measurements.xlsx*). Additionally, pH values were taken with a probe (*pH_values.xlsx*). Lastly, soil moisture and organic matter percentages were collected and calculated using oven and loss on ignition (*SW_and_LOI_measurements.xlsx*).

Specifics of sehome_metadata.csv:

id: unique id for each collected sample

e.g. CL2_22_4_5_SEF ==> meaning: coniferous litter sample 2; 22_4_5 = collection date (year_month_day); SEF = specific Arboretum location (Southeast Fairhaven)

stand: coniferous or deciduous stand

litt_min: litter or mineral sample

replicate: sample number (1, 2, or 3 from each location)

code: code that corresponds to stand, litter and replicate (unique for each collected sample but for future could be used across multiple years or collection dates/locations)

coll_year, coll_month, coll_date: collection year (e.g. 2022), month (e.g. 4 = April) and day (e.g. 5 = 5th)

country, state, county, arb_location: location details: country, state, county, and location within the Arboretum. In this document SE_fairhaven corresponds to attached map locations.

mass_pre_berlese_inc_beaker: mass of soil and beaker (grams) going into each Berlese experiment. Could be used later to calculate soil moisture of soil going into Berlese funnels.

mass_pre_berlese_minus_beaker: mass of soil (grams) going into each experiment. Calculated in Excel.

quant_observed_biota: number of soil biota (arthropods and worms) collected from the Berlese extractions. Could be used later or in more specificity (e.g. which types of biota) as a soil biota biodiversity proxy.

H2O_pH, CaCl2_pH, KCl_pH: measured pH value (using probe) for each sample. Three solutions (H₂O, CaCl₂, and KCl) were used to compare pH measurements. In future, one or all of these solutions could be used to determine pH values. In future, this should be done only with mineral soil.

depth_clay, depth_silt, depth_sand, depth_total: measured (centimeters) depth of each respective soil layer and the total combined depth when using the jar soil texture test. In future, this should be done only with mineral soil.

USDA_texture: calculated from the USDA soil texture pyramid chart. In the future this potentially could be calculated in R.

crucible: mass (grams) of crucible after initial cleaning

crucible+soil: mass (grams) of crucible after initial cleaning plus initial fridge sample soil mass

oven_dried+soil: mass (grams) of crucible and soil after initial oven-drying at 100 degrees C

crucible+ash: mass (grams) of crucible and soil/ash after burning at 500 degrees C

Appendix III**Master Methods Document
Brigid Wills Spring 2022**

Overall Goal: Extracting soil biota, taking soil texture, pH, and water content measurements by running different tests

Test	CL1	CL2	CL3	CM1	CM2	CM3	DL1	DL2	DL3	DM1	DM2	DM3
Berlese-Tullgren	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Soil Texture (Jar Test)	X	X	X	Yes	Yes	Yes	X	X	X	Yes	Yes	Yes
PH measurements	X	X	X	Yes	Yes	Yes	X	X	X	Yes	Yes	Yes
SW & LOI	X	X	X	Yes	Yes	Yes	X	X	X	Yes	Yes	Yes

Written Methods

Collecting Soil Samples

Location: Sehome Arboretum behind Fairhaven residence halls

1. Find two areas close to each other, one that is primarily deciduous and one that is primarily coniferous
 - a. NOTE: for replicable year after year research, maybe locations could be marked with stakes to ensure consistent sampling area, or GPS coordinates
2. Sample six replicates (three leaf litter samples, three mineral soil samples) from the coniferous location and six replicates (three leaf litter samples, three mineral soil samples) from the deciduous location
 - a. NOTE: for replicable year after year, standardize the distance between each replicate and/or place three flagged stakes to ensure same location. I triangulated locations (equilateral triangle with sides ~1.5 feet apart)
 - b. For each mineral replicate (e.g. coniferous mineral 1, etc.), fill a gallon Ziploc bag about half full
 - c. For each leaf litter replicate (e.g. coniferous litter 3, etc), fill a gallon Ziploc bag about ¼ full.
 - d. *Leaf Litter*: Collect leaf litter, duff and a small amount of the loose layer of larger soil (O horizon). This is the top ~2inches of leaves and soil
 - e. *Mineral Soil*: Digging deeper into the soil, collect the A and E horizons (~10 cm total, after clearing the top ~2inches of litter and soil)
 - f. NOTE: homogenize this sample in the Ziploc bag, since there will be distinct layers of mineral textures.
3. Label each Ziploc bag on collection (Coniferous Litter 1, Coniferous Litter 2, Coniferous Litter 3, Coniferous Mineral 1, Coniferous Mineral 2, Coniferous Mineral 3, Deciduous Litter 1, Deciduous Litter 2, Deciduous Litter 3, Deciduous Mineral 1, Deciduous Mineral 2, Deciduous Mineral 3)
4. Upon returning to the lab, split each sample into two and refrigerate a bag of each sample. Ensure a large amount is kept refrigerated to have enough soil for the next several experiments.

Berlese-Tullgren Funnel Biota Extractions

Goal: Extracting biota (primarily arthropods) from collected soil samples

- 1) Immediately following sampling, Berlese extraction funnels should be set up (12 funnels total; Scott Wilkinson has exactly 12). Berlese extractions will be done on leaf litter and not mineral replicates.
- 2) Label each funnel with its associated replicate (e.g., coniferous litter 2)
- 3) Weigh an empty 100 mL beaker and record mass
- 4) Fill same (pre-massed) 100mL beaker loosely with litter, weigh it, and record (mass of soil put into extraction funnel = total mass – mass of empty beaker)
- 5) After weighing, add litter to Berlese funnels. After adding the soil to the sieve, place a 100mL beaker filled with 25mL isopropyl alcohol underneath the bottom of the funnel. Position heat lamp ~6inches (~15 cm) away from the top of the funnel and turn on.
- 6) Depending on availability of Tullgren Funnels, biota extract can happen with mineral soil, though past results have not been very successful for captured arthropods. If doing this:
 - a. *Leaf Litter*: samples can be placed directly into the funnels
 - b. *Mineral*: samples should be placed into black hardware cloth squares inside of the funnels
- 7) Leftover soil should be refrigerated at this point.
- 8) Extraction funnels/setups should be left in an undisturbed place
- 9) Extractions can be left on for up to 48 hours (lamps can and should be left on overnight). They can be left for longer if needed but most extraction should happen within the first 48 hours.
- 10) After 48 hours: pour contents of beakers (from under the funnels) into a petri dish; rinse beaker with distilled water if needed to ensure all substrate goes into the petri dish
- 11) Observe and document biota seen under a dissecting microscope
- 12) Counting method: Upon finding an arthropod, document type and pipette it into a smaller vial to save if verification is needed later and avoid double-counting.

Soil Texture (Jar Test)

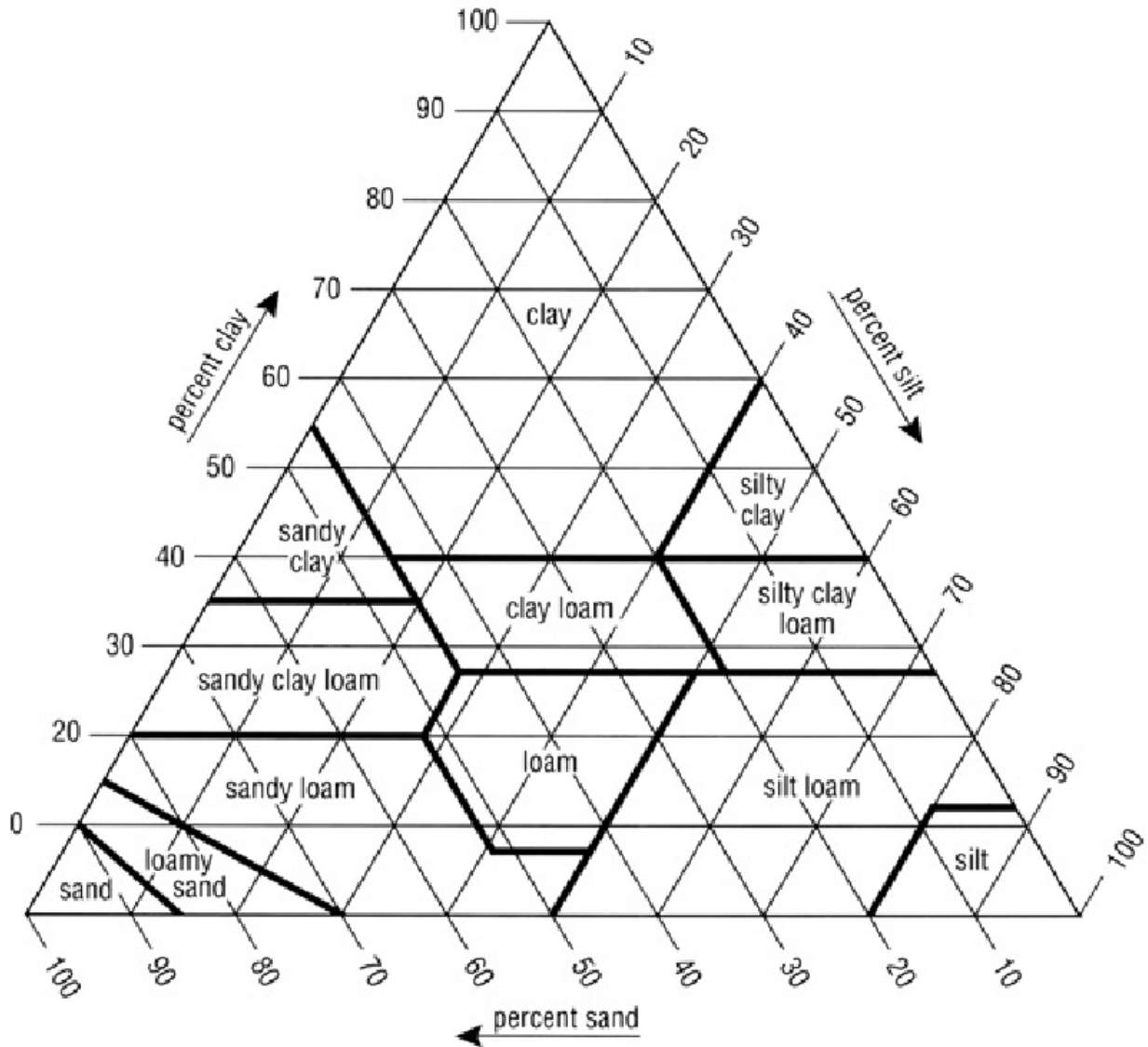
- 1) Soil texture will be done on mineral replicates only (6 samples total, three from each location)
- 2) For each replicate: in a large jar, combine equal volumes of moist soil (straight from the fridge) and distilled water.
 - a. I did 650mL distilled water and 650 mL soil, to fit in the big (2qt?) mason jars in lab
- 3) Shake vigorously for 30 seconds (time it)
- 4) Let the jar sit, undisturbed, for 24-48 hours
- 5) After 24-48 hours (standard for all replicates), measure the depth of clay, silt, and sand layers and record.
- 6) Record the total depth of combined clay (top), silt (middle), and sand (bottom).
- 7) To calculate percentage: e.g., coniferous mineral 3: $(\text{clay depth}/\text{total depth}) * 100$. These percentages can then be used to classify soil type according to the soil texture chart

Picture from Rebecca's instructions:



Figure 1. Layers of clay, silt and sand in a jar test photo credit:
http://www.waldeneffect.org/blog/Jar_test_to_measure_soil_texture/

Figure 1. USDA soil textural triangle (Soil Survey Division Staff 1993).



pH Measurements

(adapted from

https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/ref/?cid=nrcs142p2_053575)

1. Allow soil from each replicate to air dry (put soil in labeled paper bags on the counter for ~72 hours)

Note: pH measurements can be done with any/all the solutions described (Water, CaCl₂, or KCl). KCl is primarily used to look for [Al³⁺] ions, whereas H₂O and CaCl₂ are more broad-spectrum. Additionally, measurements from the H₂O method will likely vary across seasons, whereas CaCl₂ results will standardize for seasonal variation. Because of the different strengths of each test, it is good to run all of them if time and materials allow.

Note: When taking pH probe measurements, record the pH number the probe settles on and doesn't change for 10 seconds

Water measurements

- 1) Once soil is air-dried, combine 10g air dry soil of each replicate with 10 mL of distilled water in an Erlenmeyer flask. Swirl for ~20 seconds to mix.
- 2) Let flasks sit for 10 minutes, undisturbed.
- 3) After 10 minutes, pour contents of flask into a beaker and use a pH probe (check out waterproof ones from Scott Wilkinson) to take measurements of the acidity of each slurry. Stick the probe into the soil and rinse the probe with water between different replicates to avoid contamination. Record measurements.

CaCl₂ Measurements

- 1) Once soil is air-dried, combine 10g air dry soil of each replicate with 20 mL of 0.01M CaCl₂ in an Erlenmeyer flask. Swirl for ~20 seconds to mix.
- 2) Let flasks sit for 10 minutes, undisturbed.
- 3) After 10 minutes, pour contents of flask into a beaker and use a pH probe (check out waterproof ones from Scott Wilkinson) to take measurements of the acidity of each slurry. Stick the probe into the soil and rinse the probe with water between different replicates to avoid contamination. Record measurements.

KCl Measurements

- 1) Once soil is air-dried, combine 10g air dry soil of each replicate with 10 mL of 1M KCl in an Erlenmeyer flask. Swirl for ~20 seconds to mix.
- 2) Let flasks sit for 10 minutes, undisturbed.
- 3) After 10 minutes, pour contents of flask into a beaker and use a pH probe (check out waterproof ones from Scott Wilkinson) to take measurements of the acidity of each slurry. Stick the probe into the soil and rinse the probe with water between different replicates to avoid contamination. Record measurements.

SW and LOI (Soil Water and Loss on Ignition)

Goal: Determining soil moisture and organic matter content

Note: Do on mineral soil only, use replicates from the fridge in order to measure soil moisture from the field

- 1) Use 6 crucibles: one for each replicate of deciduous and coniferous.
- 2) Use a 3-scale balance to mass the crucible (record)
- 3) Fill each crucible ~1/3 full
- 4) Use a 3-scale balance to mass the crucible + soil mass (record).
- 5) NOTE: Labeling the crucibles is tricky, since tape will burn/melt in the ovens. I used tape *after* massing to identify replicates, and then recorded locations after putting them in the oven (e.g., all coniferous on the top rack, 1-3 left to right)
- 6) Put the crucibles in a 105 C oven (you can use muffle furnace in ES405 or scientific oven in ES306) overnight
 - a. I ran it for 21 hours
- 7) Let crucibles cool in the oven
- 8) Take out and weigh on the 3-scale balance
- 9) Put samples in cold muffle furnace
- 10) Furnace in ES405: Set furnace to 250C. Return after half an hour (temperature of oven should be at 250C) and up to 500C. Total time in oven from cold will be 4 hours.
- 11) Let muffle furnace cool overnight
- 12) I let it cool over a weekend and on the next Monday the crucibles were cold to the touch inside the closed furnace
- 13) Let crucibles cool and weigh mass of ash+crucible. Record.

Appendix IV

Bio students' methods

Collecting Soil Samples

Locations: same areas as plant species ID cataloging is happening

1. Sample six replicates total (three leaf litter replicates and three mineral soil replicates) from each 4'x4' plot, from three different (randomly) picked places within each plot. The three replicates will be homogenized (in the field). Homogenizing for soil samples looks like mixing the soil thoroughly within the gallon Ziploc bag. For leaf litter, putting the three replicates in the same bag will suffice.

a. For each leaf litter collection (I.e., three random replicates homogenized together), fill a sandwich bag completely

i. *Leaf Litter*: Collect leaf litter.

a. For total mineral collection (I.e., three random replicates together), fill a gallon Ziploc bag about half full.

i. *Mineral Soil*: Put a ruler 10cm down into the soil, where the top of the measurement starts at the bottom of the leaf litter layer. With a shovel scoop out this 10cm deep sample. Repeat twice more in two more locations

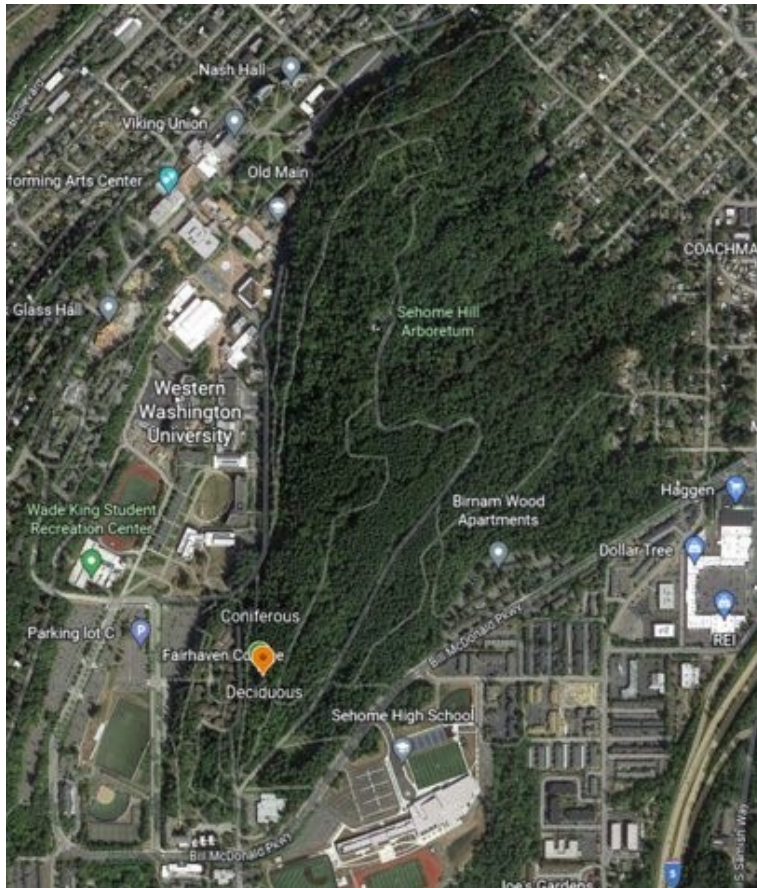
a. Label each Ziploc bag and sandwich bag on collection: include name (initials are fine) of sampler, date collected, Arb location (latitude and longitude if that's what you're using to specify location), which 4'x'4 plot (healthy, diseased, in between healthy and diseased, etc.)

1. Communicate w/ Brigid before and after collecting. Mineral soil samples can be refrigerated and processed later, but soil biota collection (which comes from leaf litter samples) needs to happen on the same day as collection. I'm around every weekday, but let's just make sure to touch base so I know when to expect samples and so we can facilitate drop-off. Thanks!

a. For reference the soil lab is ES306

Appendix V

Collection Locations (SE Fairhaven)



Appendix IX

Soil Texture Measurements

	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
1	Coniferous	Date	4/18/2022	4/18/2022	4/18/2022	4/18/2022	4/18/2022	4/18/2022							
2			CL1	CL2	CL3		CM1	CM2	CM3	method: 650 mL water (distilled) to 650 mL wet soil (from fridge)					
3		Clay	0.75	1		1	0.01	0.5	0.01						
4		Silt	2.5	1.5		1.25	0.5	1	0.5						
5		Sand	1.25	2		2.5	3.75	2.5	5						
6		Total Dept	4.5	4.5		4.75	4.26	4	5.51						
7															
8		Total perc	100	100		100	100	100	100						
9		%clay	16.666667	22.222222		21.05263158	0.2347418	12.5	0.1814882						
10		%silt	55.555556	33.333333		26.31578947	11.737089	25	9.0744102						
11		%sand	27.777778	44.444444		52.63157895	88.028169	62.5	90.744102						
12		%total	100	100		100	100	100	100						
13															
14			silt loam	loam		sandy clay loam	sand	sandy loam	sand						

Appendix X

SW and LOI table

Stand	% SW (calculated in R)	% LOI (calculated in R)
Coniferous	29.99920	10.937698
Coniferous	27.74347	9.213042
Coniferous	26.36861	8.529810
mean	28.03709	9.560183
Deciduous	30.79980	8.294343
Deciduous	38.63160	16.943507
Deciduous	28.71687	7.836740
mean	32.71609	11.02486

Appendix XI

SW and LOI measurements

Excel SW_and_LOI_measurements - Saved							Search (Alt + Q)
File Home Insert Draw Page Layout Formulas Data Review Accessibility Reminder View Automate Help							
Calibri		11	B	<i>I</i>			General
P12							
	A	B	C	D	E	F	G
1			masses in grams	collected on 3-place balance		Mass after 20 hours in 105C oven	Mass after 4 hours at 500C
2			Crucible only	crucible+soil	soil mass pre-test (crucible+soil - crucible)	oven-dried soil + crucible	crucible+ash
3	Coniferous	M1	45.4615	57.8915	12.43	54.1626	53.2109
4		M2	41.4501	52.2357	10.7856	49.2434	48.5254
5		M3	41.659	55.8869	14.2279	52.1352	51.2416
6		AVERAGE	42.85686667	55.33803333	12.48116667	51.84706667	53.2109
7							
8	Deciduous	M1	28.2726	40.5032	12.2306	36.7362	36.0342
9		M2	43.9039	55.3523	11.4484	50.9296	49.7392
10		M3	46.148	59.1334	12.9854	55.4044	54.679
11		AVERAGE	39.4415	51.66296667	12.22146667	47.69006667	46.81746667
12							
13		Oven:					
14		Coniferous (Top Rack)					
15		1	2	3 (ordered Left to Right)			
16		Deciduous (Bottom Rack)					
17		1	2	3 (ordered Left to Right)			
18							

Appendix XII

Internship Hours Log

Excel InternHoursLog - Saved											Search (Alt + Q)															
File Home Insert Draw Page Layout Formulas Data Review Accessibility Reminder View Automate Help											Editing															
Calibri 11 B I											Merge General \$ 0.00															
fx																										
Description and Hours of Internship Responsibilities																										
Date	Start	End	Start	End	Start	End	Total Hour	Running Total	Brief Description																	
3/30/2022	13:00	15:00					2	2	Meeting with Rebecca to outline duties and start on Mendeleev organization (Biodiversity file done (tag: biodiversity); Drought file done (tag: biodiversity); Questions (tag: question no pdf file); A-Ba- authors checked for repeats)																	
3/31/2022	8:00	9:30	14:00	14:30			2	4	Emailing Liz Zimmerman about GIS project, picked up densitometers from Scott Wilkinson, trouble-shooting Mendeleev sync issue, meeting with Liz about truing maps																	
4/1/2022	7:45	9:45					2	6	Mendeleev working with Amber in the greenhouse (bleaching pots and getting ready for planting; 9:30 check in meeting w/ Rebecca																	
4/4/2022	7:45	9:45					2	8	Emailing Liz about GIS, Mendeleev (leaf litter, leaf litter/biodiversity/decomposition, light																	
4/5/2022	7:45	9:45	13:00	16:15			5.25	13.25	AM: Mendeleev (some connectivity issues), Emailing Rebecca and Scott; collecting Berlese extraction funnels from Scott; setting up extractions and bags /// PM: collected soil samples for Berlese extractions, started extractions																	
4/6/2022	9:00	11:30	13:00	15:00	16:00	17:45	6.25	19.5	Continued Berlese extractions; running into some issues w/ Mendeleev; potted up raspberries w/ Amber in the greenhouse; ground truing with Liz in the Arb for GIS map																	
4/7/2022	8:00	9:45					1.75	21.25	Continued Berlese extractions, emailed scott to check in about leaving on overnight (okay to do), uploaded GIS truing pics																	
4/8/2022	7:45	9:45					2	23.25	Berlese extractions; finished mendeleev folders-> tags; weekly meeting w/ Rebecca																	
4/11/2022	7:45	11:30					3.75	27	cleaning Berlese extraction set ups; setting up soil to airy for pH; returning supplies to Scott; looking at soil biota																	
4/12/2022	7:45	9:45					2	29	making dilutions, looking at soil biota																	
4/13/2022	13:00	15:00					2	31	setting up soil texture jar tests; more biota observations																	
4/15/2022	12:00	13:00	14:00	16:30			3.5	34.5	biota observations, cleaning out first round of soil texture																	
4/18/2022	7:45	11:45					4	38.5	washed a lot of dishes, measured second round of texture jars, took pH measurements with KCl and CaCl2																	
4/19/2022	7:45	9:45					2	40.5	washed more dishes, categorized soil types, pH data analysis																	
4/21/2022	7:30	9:30					2	42.5	(working from home) writing up methods for what I've been doing the past couple weeks																	
4/22/2022	9:00	10:00					1	43.5	meeting with Rebecca, brief work with writing up methods																	
4/25/2022	9:00	10:30					1.5	45	(working out of the lab) editing methods after notes from Rebecca, categorizing soil types from texture tests																	
4/26/2022	7:45	9:45					2	47	dishes, sending emails to set up DOI access with Scott																	
4/27/2022	9:00	11:15	13:00	14:00			3.25	50.25	Talking to Scott about muffle furnaces; getting crucibles and masses of initial masses; set up and started 105C oven; working on writing up methods and assessing replicability																	
4/29/2022	7:45	12:45					5	55.25	Running muffle furnace LOL, R data analysis, meeting w/ Rebecca, combining data into csv file and starting to work with it in R																	
5/2/2022	9:00	11:30					2.5	57.75	Learning slides from Amber, R data analysis (tidyverse)																	
5/3/2022	7:45	10:00					2.25	60	Slides and R Studio																	
5/4/2022	11:30	15:00					2.5	62.5	Slides and R studio, dishes																	
5/5/2022	7:00	8:00	13:00	3:30			3.5	66	R Studio, checking slides for mycorrhizae, taking pics of biota, trying to figure out GIS for sample map																	
5/6/2022	7:45	10:00					2.25	68.25	Meeting w/ Rebecca, R data																	
5/9/2022	7:45	12:15					4.5	72.75	R Data analysis, meeting w/ Marion et al.																	
5/10/2022	7:45	8:45					1	73.75	Meeting w/ Marion et al. re plot transects																	
5/11/2022	16:00	15:00					1	74.75	Processing roots w/ Amber																	
5/12/2022	8:00	10:00					2	77.75	Inventory for Amber																	
5/16/2022	7:45	11:45					4	81.75	Writing up methods for Marion's team, showing Marion's team how to do soil samples, Processing roots for Amber, Air drying soil for Amber, cleaning up lab																	
5/19/2022	13:00	16:15					3.25	85	Sea thrift, corn root wash, sort KOH clippy boxes raspberry roots																	
5/20/2022	9:00	10					1	86	Restarted computer																	
5/23/2022	7:30	8:45	13:00	15:45			4	90	Started working on report, R data processing, checked in w/ Rebecca																	
5/24/2022	9:00	10:00					2	92	Helped Amber w/ some root processing, more R																	
5/25/2022	8:00	9:30					1.5	93.5	Working on report																	
5/26/2022	13:00	15:45					2.75	96.25	Writing up user guide for metadata file																	
5/27/2022	8:00	10:00	14:00	15:00			3	99.25	Data recovery; dishes																	
5/31/2022	7:30	10:00					2.5	101.75	Data recovery; dishes; microscope work																	
6/1/2022	9:00	10:00	13:00	15:00	16:00	18:00	5	106.75	Internship Report Work; R data work																	
6/2/2022	8:00	10:00	13:00	16:00			4	110.75	microscope work; Report work																	
6/3/2022	7:30	10:00					2.5	113.25	R work (error bars); report work; meeting w/ Rebecca																	
6/6/2022	9:00	15:00					6	119.25	Writing up internship report																	
6/8/2022	11:00	17:00					6	125.25	Editing internship report																	

Appendix XIII**R Script**

```

#Brigid Wills
#Internship spring 2022 Dr. Rebecca Bunn
#Sehome data analysis

#to update: Help -> Check for Updates
sessionInfo() #make sure version of R is above 4.0.0
install.packages(c("tidyverse", "hexbin", "patchwork", "RSQLite",
"ggplot2", "cowplot", "dplyr", "gt"))
library(tidyverse)
library(hexbin)
library(patchwork)
library(RSQLite)
library(ggplot2)
library(cowplot)
library(dplyr)
library(gt)
#import dataset
sehome <- read.csv(file.choose())

#rebecca's facetwrap code for pH
seh2 <- sehome %>%
  filter(litt_min == "mineral") %>%
  select(stand,code,H2O_pH:KCl_pH) %>%
  pivot_longer(cols = H2O_pH:KCl_pH)
p1 <- seh2 %>%
  ggplot() +
  geom_boxplot(aes(x=stand,y=value), fill = "lightblue") +
  facet_wrap(vars(name))
p1 + xlab("Stands")+ylab("pH values")

#calculating and plotting Sw and LOI
seh5 <- sehome %>%
  filter(litt_min == 'mineral') %>%
  select(stand, code, crucible:crucible.ash)%>%
  mutate(SW = ((crucible.soil - oven_dried.soil)/(crucible.soil -
crucible))*100) %>% #SW
  mutate(LOI = ((oven_dried.soil - crucible.ash)/(oven_dried.soil -
crucible))*100) #LOI
means <- seh5 %>%
  group_by(stand) %>%
  summarize (mean_SW = mean(SW, na.rm = T), mean_LOI = mean (LOI,
na.rm = T))
#SW plot
pSW <- ggplot(data = means, aes(stand, mean_SW)) +
  geom_line(color = "steelblue", size = 1) +
  geom_point(color="steelblue") + geom_bar(stat="identity") +ylim(0,
35)
#LOI plot
pLOI <- ggplot(data = means, aes(stand, mean_LOI)) +
  geom_line(color = "steelblue", size = 1) +
  geom_point(color="steelblue") + geom_bar(stat="identity") +ylim(0,
35)
plot_grid(pSW,pLOI, labels = c('SW', 'LOI'))

```