2022

WWU Soil Ecology Lab Intern

Michael Ginster

COLLEGE OF THE ENVIRONMENT

Internship Title: Research Assistant (Soil Ecology Lab)

Student Name: Michael Ginster

Internship Dates: 9/30/2022 - 12/7/2022

STUDENT SIGNATURE: MICHAEL JOSEPH GINSTER
DATE: November 25th, 2022

Internship Report

Introduction: For my ENVS 498B internship, I worked as a Research Assistant on Dr. Rebecca Bunn's ongoing research project, which will eventually be published under the title *Establishment and cultivar-specific associations of indigenous mycorrhizal fungi in newly-turned raspberry fields*.The focus of the research is on how tilling in perennial raspberry fields impacts mycorrhizal fungi in those fields, which in turn increases the health of the raspberry plants in those fields. Fields are rotated every three years, meaning that raspberry plants can develop symbiotic relationships with mycorrhizal fungi without interruption for multiple years between tillings. This impact on mycorrhizal spores is measured through spore counts (the focus of my internship) and observations of mycorrhizal colonization in raspberry root samples, along with various greenhouse trials to compare the potential for mycorrhizal colonization in various soil types. Through this research, Dr. Bunn is trying to figure out how recency of tilling (just tilled vs. one year untilled vs. two years untilled) impacts the frequency of mycorrhizal fungi, which in turn has broader ramifications for the health and resilience of raspberry plants in the affected fields.

Description, Goals, & Outcomes: In this internship, my primary purpose was to count spores on filter paper and prepare soil samples for spore counts. The preparation process was conducted via sieving, weighing, and wetting a series of soil samples before placing them through two rounds of homogenization and centrifugation, the second of which involved replacing the water in each sample-filled test tube with sucrose. After the second round of centrifugation, the samples were filtered via a sieve before being poured onto a wetted piece of filter paper. Over the course of this internship, I completed 74 extractions out of 85 attempts, with three of the eleven unsuccessful extractions being cut off preemptively due to errors at the start. Prior to this, I conducted five practice extractions.

The counting process was more straightforward in theory, as the task largely involved identifying and counting spores. However, because of ambiguous particles in the sample, instances of user error, high debris counts in some samples, and other variables, counting could often be difficult to conduct precisely. Over the course of this internship, I conducted 37 counts, which were preceded by 35 practice counts. In addition to this, there was also a day where I collected field samples with Dr. Bunn at the sample site in Lynden. This process mostly involved packaging and labeling the samples Dr. Bunn collected, but I also got some experience with handling a soil drill.

When I had initially entered this internship, I had entered with six listed goals that fall more broadly into two subsets: practical knowledge and theoretical knowledge. For

Goals #2 and #3, I had hoped to gain hands-on research with the subject matter of the internship. Goal #2 involved getting hands-on experience with analyzing mycorrhizal spores under a microscope, while Goal #3 involved getting hands-on experience with handling soil samples in a lab setting. Meanwhile, Goal #4 involved getting broader experience with agricultural soil research. Meanwhile, the more theoretical goals involved gaining knowledge in various subjects related to the research. These subjects were plant-soil-fungi interactions (Goal #1), mycorrhizal processes (Goal #5), and increased knowledge about the impacts of soil ecology & health on agricultural processes (Goal #6).

Reflection: My experience in this internship was largely defined by both frequent difficulties and the general trial-and-error nature of the research process. In terms of difficulties, both of the main processes took lots of time and effort to learn, which was one of my main sources of frustration. The extraction process, while relatively routine to me now, used to feel incredibly long and convoluted, and it took a while for me to be able to conduct it without the instructions. Meanwhile, the counting process was more or less constantly refined for me, as my understanding of what did and didn't count as a spore had to be redefined at several points during the quarter. Even after several weeks of practice and improvement, my ability to count spores is still relatively poor. On top of these complications, the research process as a whole was largely defined by a constant trial-and-error process of seeing what worked and what didn't. This trial-and-error nature meant that I had to often add slight revisions to my auxiliary tasks just as I was beginning to get used to them.

Overall, in spite of the numerous frustrations that arose from these difficulties and trial-and-error processes, I think that the fact that I faced difficulty in most aspects of this internship was valuable in the long run. Because I have been in school for virtually all of my life, and because school is something I am generally good at, I don't have a lot of experience with repeatedly failing at and relearning things. While there were a few classes here and there that I struggled in, they didn't really acclimate me to failure in a significant way. In this position, I started to get used to being unsuccessful and to adapting when I am approaching something incorrectly. Furthermore, the trial-and-error aspects of the position are a direct product of science's messiness. If I end up doing graduate school at some point, this acclimation to the inherent trial-and-error nature of research is going to be very valuable, as few scientific research projects are completely smooth from start to finish.

Regarding the learning objectives established at the start of this quarter, I feel relatively mixed about how they turned out. On the one hand, I feel as if Goals #2, #3, and #4 were all met. Goals #2 and #3 involved gaining hands-on experience with

analyzing mycorrhizal spores under a microscope and handling soil samples in a lab, respectively. Both of these goals were directly met, as the vast majority of the tasks I completed through this position related to one or both of them. Furthermore, these tasks also helped me reach Goal #4, which was to gain hands-on agricultural soil research experience. The tasks outlined in Goals #2 and #3, along with some field sampling that was conducted near the start of the position, gave me skill sets that will likely build on a foundation of agricultural research skill sets. However, Goals #1, #5, and #6 were not met. This is because these goals all connected to theoretical knowledge that was not discussed during the position. My job largely was to carry out auxiliary tasks, rather than to gain knowledge on the scientific topics that these tasks were shining light on.

Appendix (Internship Log):

10/7/2022: So far, I have mostly been trained for lab work. There are two methods for preparing soil samples and we will have to choose between one of them. One of the methods involves sieving the soil before placing it into separate tubes and centrifuging it. The other method involves centrifuging the soil particles wholesale multiple times as a filtering mechanism. Microscope work has definitely been a trial and error process for me. Yesterday, I tried and failed to get consistent spore counts for each sample before Rebecca Bunn told me that the magnification I was using (.8x) was too zoomed out. After switching to 2x, I got a higher count on Sample 4.8, only to get a count five times higher on Sample 4.3 the next day with the same magnification. Overall, I have felt relatively incompetent, especially since my co-worker has far more experience with microscope work. I am not used to feeling so far out of my depth, and it's somewhat discouraging.

10/10/2022: Today, I went with Rebecca to the sampling sites in Lynden. My main task was to homogenize the soil samples before sorting them into bags, with the plastic bags being labeled by me and the paper bags already labeled by Rebecca. However, I also got experience with core drilling, which was our method of sample collection. I felt somewhat incompetent, especially since I lost one of Rebecca's Sharpies at one point, but I ultimately was able to complete the assigned tasks with few issues.

10/12/2022 (Written 10/13/2022): Yesterday, I went into the lab and counted spores in samples 4.8 and 4.3. This process was fairly difficult, as differentiating between spores and soil particles takes time to master. However, the counts that I got were more consistent with each other and with Max's results than the count I had gotten on 10/7/2022. I am making progress, albeit slowly and laboriously.

10/14/2022: Today, I got practice with centrifuging and I learned how to filter samples. The centrifuging process was somewhat hectic, as I accidentally altered a centrifuge setting and as a result could not get it up to the correct speed until multiple people had assisted me. However, the problem was eventually resolved after I had left to clean glassware and I was informed what had gone awry (changes to the bucket settings). Progress is once again tangible yet very slow.

10/17/2022: I spent about four hours counting spores today before entering my results in Excel. I feel more confident with spore identification, but my counts are still somewhat inconsistent. I found the samples on grid paper to be far easier to count than the samples on plain paper.
Hours: 12:00PM - 4:45PM

10/19/2022: I spent about an hour and a half today meeting with Rebecca, sorting bags of soil, and cleaning glassware. There isn't much else to report.
Hours: 12:00PM-1:30PM

10/20/22:
Hours Logged:
9/16/22-9/30/22 => 3
10/01/22-10/15-22 => 16.5
10/21/2022: Today was mostly spent refining counting technique with Max. We practiced on multiple trial samples.
Hours: 9:00AM-11:30AM

10/24/2022: Today, I prepared two samples for filtration and then worked with Rebecca on the filtration process. After this, I cleaned dishes and did one round of counting for each of three filter papers (Rebecca had used one of the two samples for two separate pieces of filter paper to test something).
Hours: 12:00PM-4:30PM

10/27/22: Yesterday, I ran two rounds of sample extractions and got trained on the microscopes upstairs. It was my first day completing the full cycle of extraction and filtering on my own. I felt daunted by something that day, but I cannot remember what.
Hours: 12:00PM-4:30PM

10/28/22: Today, I ran two rounds of sample extractions and washed some dishes between steps during the first cycle. I briefly spoke to Rebecca mid-shift and looked at an instructions list that she left me at the start, but this shift was the most independent that I have been in this position.
Hours: 12:00PM-4:00PM

10/31/22: Today, I worked with Rebecca on counts in the IWS lab. I initially felt confident with my identification abilities, but then ended up with wildly fluctuating counts later in the shift. The last two samples were filled with tiny rocks, which proved to be a massive hurdle to accurate spore identification. It took me the entirety of the shift to complete four counts. I will likely need to revise my counting technique and improve my spore identification, but I am also hoping that new filtration techniques will provide more consistent counts at a faster rate.
Hours: 1:00PM-4:45PM

Hours Logged 10/16/22-10/31/22 => 25.5

11/2/22: Today was spent on counts. The first sample was from 58, which had a ton of debris in it and was very difficult to work with. The debris and lack of grid lines caused me to spend an hour and a half on this sample. I then worked on three samples from the 7 batch. The first was very easy to work with, but the other two had some debris and were somewhat more difficult to navigate. My spore counts for these ranged from 94 to somewhere in the 130s (maybe 131?).
Hours: 12:00PM-4:30PM

11/4/22: Today was spent on counts. The counts on the 7 and 67 batches wildly fluctuated, but these fluctuations were more contained (albeit still bad) on the 4 batch. I looked at Max's counts afterwards and those also had interbatch variations, which made me feel a bit better about my own counts. However, I still think that there is something fundamentally off with how I am counting spores and I don't know what it is. In the future, I should really focus on identifying and omitting rocks from my counts.
Hours: 12:00PM-4:15PM

11/7/22: Today was spent on extractions. A missed or incorrectly executed step cost me sample 28A (I don't remember exactly what), but everything else went smoothly. Today was the first day where I ran two rounds of extractions with vials from two separate samples in each round. I did some dishes between steps of the extractions.
Hours: 12:00PM-5:45PM

11/9/22: Today was spent on counts. I logged counts from last week into a new spreadsheet before conducting five additional counts and logging those into the same spreadsheet. I felt a lot better on my counts today, taking a little over three hours to get five counts that were more internally consistent than last week's 4-5 hour sessions with 4 to 5 counts.
Hours: 12:00PM-3:45PM

11/13/22: On 11/11/22, I conducted a single extraction to create 28C. Because Max had done more extractions the previous day and because the lab was locked (Veteran's Day), I didn't do any further extraction or counting. I washed glassware and cleaned up the lab a bit during the extraction process.
Hours: 12:00PM-1:45PM

11/14/22: Today, I conducted 11 counts in about four hours, with my counts being far more internally consistent than before. However, my numbers were all extremely low, which could pose a problem if Max's counts on these samples are significantly higher. I am cautiously optimistic that today's counts represent an improvement, but I will have to wait and see what happens.
Hours: 12:00PM-4:00PM

11/16/22: Today, I conducted 8 counts in about four hours. Internal consistency was maintained, though a few counts took a very long time to complete and I wasn't as composed as I was two days ago. Furthermore, the numbers were very low again, which could be indicative of a larger

problem. However, the increased internal consistency and speed relative to my pre-11/14 counting is still a positive development.
Hours: 12:00PM-4:00PM

11/18/22: Today, I ran two rounds of double extractions (two pairs of samples per extraction). This process took me over five hours. Also, Rebecca has decided that it would be better for me to focus on extractions over counts.
Hours: 12:00PM-5:15PM

Hours Logged:
11/1/22-11/15/22 => 24

11/21/22: Today, I ran two rounds of double extractions. Due to a pair of mistakes and a faulty tube, I ended up losing three samples at various stages of extraction.
Hours: 12:00PM-5:00PM

11/23/22: Today, I ran two sets of double extractions. I accidentally lost a sample early on after it rolled off the table, but I created a C sample for that pair and had no other issues during the shift.
Hours: 12:00PM-5:00PM

11/28/22: Today, I conducted one set of double extractions with no issues. However, I was unable to conduct a second set due to a shortage of sucrose. I started the process of making a new batch, but eventually stopped early after being told by Rebecca that I didn't have to finish the process tonight if it ran for too long. I also sieved two samples for a second round of extractions, but did not conduct extractions because of the aforementioned sucrose shortage.
Hours: 12:00PM-5:15PM

Extractions & Counts:

10/5/22:
*One practice count

10/6/22:
*Seven practice counts

10/7/22:
*One practice count

10/12/22:
*Six practice counts

10/14/22:
*Two practice extractions => Two completed

10/17/22:
*18 practice counts

10/21/22:
*Two practice counts

10/24/22:
*Two practice extractions => Three filters created

10/26/22:
*One practice extraction => One completed
*Six extractions => Four completed

10/28/22:
*Four extractions => Four completed

10/31/22:
*Four counts completed

11/2/22:
*Four counts completed

11/4/22:
*Five counts completed

11/7/22:
*Eight extractions conducted => Seven completed

11/9/22:
*Five counts completed

11/11/22:
*One extraction => One completed

11/14/22:
*11 counts completed

11/16/22:
*Eight counts completed

11/18/22:
*Eight extractions conducted => Eight completed
*One extraction canceled due to error at start

11/21/22:
*Seven extractions conducted => Four completed

11/23/22:
*Eight extractions conducted => Eight completed
*One extraction canceled due to error at start

11/28/22:
*Four extractions conducted => Four completed

11/29/22: Today, I cleaned dishes before setting aside a small portion from each of today's soil samples (VD 1-20 11/29/22) for drying.
Hours: 2:15PM-4:30PM

11/30/22: Today, I ran two rounds of double extractions and emptied the drying rack. Eight extractions were successfully completed out of the eight that were attempted. A 9th was preemptively canceled due to leaking in the vial during the first homogenization stage.
Hours: 12:00PM-5:30PM

11/16/22-11/30/22 Hours: 32.25

12/02/22: Today, I ran two rounds of double extractions. Eight extractions were successfully completed out of the eight that were attempted.
Hours: 12:00PM-5:00PM

12/05/22: Today, I ran two rounds of double extractions and washed a few dishes. Eight extractions were completed out of eight attempted.
Hours: 12:00PM-5:15PM

12/07/22: Today, I ran one round of double extractions, washed the dishes, and cleaned off the countertops. Four extractions were successfully completed out of four that were attempted. The earliest portions of the shift saw some confusion, as both the notebook and the plastic bags were moved to the greenhouse, but the shift went fine after I retrieved the notebook and was given approval to use paper bags for sieved soil instead of plastic bags.
Hours: 12:00PM-4:45PM

Hours for 12/01/22 - 12/15/22 pay period: 15

Cumulative Hours: 116.5 hours