Soil inoculum and its impact on plant and herbivore growth

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Abstract. A universal standard of soil inoculum of conditioned soil does not exist for plant-soil feedback experiments and soil microbiome experiments in general. This may cause discrepancies between laboratories or even experiments within laboratories. Additionally, experiments that do compare different levels of soil inoculum are few. Here, we will attempt to add to the discussion surrounding the standardization of soil inoculum. To do this, we compared three different types of soil inoculum provided by the conditioning of three different forbs (Tomato (*Solanum lycospersicum* 'Better Boy'), Collard Greens (*Brassica oleracea*), and Lettuce (*Lactuca sativa*)) at five different levels of soil inoculum (0, 1, 10, 50, and 100%). The results suggest that there may be a correlation between soil inoculum level and plant feedback, but more work needs to be done to further solidify this conclusion and a standard level of soil inoculum.

INTRODUCTION

The varied and complex ways in which plants interact with their soil microbiomes has been researched for centuries. For much of this time, the plant has been the focus of this research instead of these interactions (Berg 2009). This relatively new field of study has many implications to the broader goals of entomology and agriculture. It may impact crop yield and how effectively plants defend themselves against insects and pathogens among other important impacts. The plant microbiome also impacts plant-soil feedback loops (Kos et al 2014).

Plant-soil feedback loops is how plants react to and alter the biotic and abiotic qualities within the soil around them (Bever 1994). A negative plant-soil feedback occurs when the plants' fitness is negatively affected by the feedback loop while a positive plant-soil feedback loop is the opposite. The plant-soil feedback also has an insect direct effect on herbivore fitness.

This experiment is a part of the ever-expanding body of research associated with the soil microbiome and the plant-soil feedback loop. In terms of the level of inoculum used for plant-soil feedback loop experiments, every lab or even every experiment has their own level of soil inoculum that they use. This level is anywhere from 1% to 100%. This could impact results and an approach is needed to standardize results of these experiments. That was the main goal of this experiment; to create a universal standard of inoculum that can be used to the most optimal effect.

These types of experiments are few and far between. Tomato (*Solanum lycospersicum* 'Better Boy'), Collard Greens (*Brassica oleracea*), and Lettuce (*Lactuca sativa*) were the plants used in the experiment. They were used because they are relatively distantly related within forbs and they have shown some interesting interactions (Ingerslew, Kaplan 2018). For the feedback phase, the same species and cultivar of tomato was used because of the possible interactions between it and the conditioned soil created by the tomato during the first part of the experiment. As for the herbivore, the Potato Aphid (*Macrosiphum euphorbiae*) was used. This is due to their relative ease of access, insect feeding alters plant defenses, and Aphids have also shown interesting interactions with soil inoculum.

METHODS

This was a two phase experiment. Both phases were done within the same greenhouse during the months of January through June. The first phase was the conditioning phase. In this phase, each of the three plants mentioned above (*Solanum lycospersicum, Lactuca sativa,* and *Brassica oleracea*) were grown in pots with ten replicates each. Nine sterilized seeds were planted in each pot within three holes about one centimeter deep with three seeds in each of the holes and covered. These were later thinned to a single plant. They were then grown for 8 weeks with daily watering. The plants were also fertilized with one quarter cup of 250 ppm 20-20-20 NPK fertilizer. This mixture was achieved with one teaspoon of fertilizer powder mixed into one gallon of water from a hose. After the 8 week growing period, the plants and soil were harvested and the dry biomass of the above and below ground plant material were taken separately and recorded by placing the above and below ground parts into separate labeled bags and placed in an oven until completely dry. Then, they were placed in dishes and weighed with a digital scale. Until preparation for the next phase, the harvested soil was labelled and stored in a refrigerator.

Next, the feedback phase had to be prepared. To start, the inoculated soil needed to be prepared. To prepare this, 2 cups total of soil was mixed in individually marked plastic bags in different amounts of sterilized and inoculated soil. There were 5 different levels of inoculum which were 0 (control), 1, 10, 50, and 100% of conditioned soil. To form these the 0% was all sterilized soil, the 1% was 2 cups of sterilized soil and 1 teaspoon of conditioned soil, the 10% was 1 and ¾ cups of sterile soil and 9.5 teaspoons of conditioned soil, the 50% was 1 cup of sterilized soil and 1 cup of conditioned soil, and the 100% was 2 cups of conditioned soil. There were 10 replicates of each inoculated soil level for each of the different soil types provided by the three different plants in the conditioning phase. These different soil treatments were then put into two sets of 130 labeled conetainers, which are conical plastic pots, totaling 260 conetainers. Each of these conetainers then had 3 seeds of the same species and cultivar of tomato placed into a hole about 1 centimeter deep. These were watered daily and fertilized in almost the same manner as the conditioning, the only difference being that 1.5 Tablespoons of the fertilizer mixture was used instead of ¼ cup. After 7 days, the germination rate was taken. The plants were thinned approximately 3 days after the germination rate was taken. The plants were then grown for 6 weeks and the feedback phase began.

At the 6 week mark, 10 *Macrosiphum euphorbiae* were added to each of the first set of 130 plants. These were wrapped in a porous plastic cage to prevent the escape of the aphids, the tops and bottoms of which were wrapped with a rubber band. This began the herbivore feedback phase while the plant feedback phase began when the seeds were planted. After a week, the aphids were counted and recorded. As far as the tomato plants in the plant feedback phase, these were harvested after 8 total weeks of growth and the above and below ground dry biomass were taken in the same way as the plants in the conditioning phase.

RESULTS

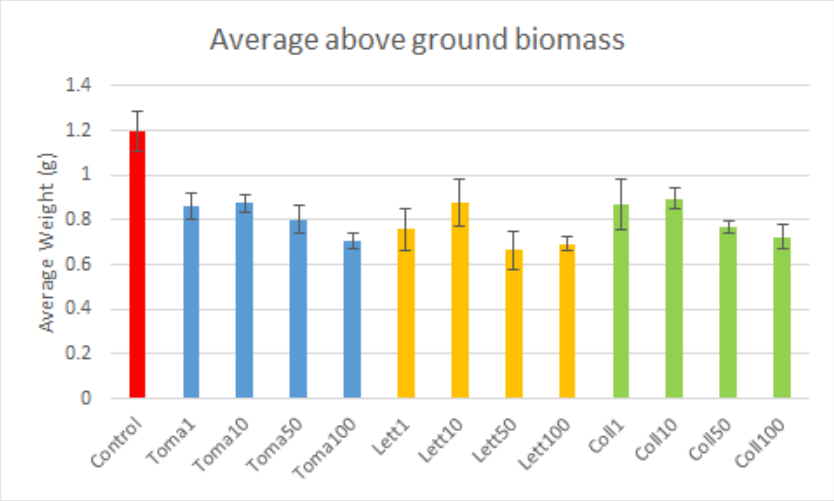

Fig 1. Bar graph comparing the different treatments for the average above ground biomass with error bars. The control is in red, the tomato, lettuce, and collard green treated soils are in blue, yellow, and green, respectively.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Above (g)	Between Groups	2.292	12	.191	3.471	.000
	Within Groups	6.438	117	.055		
	Total	8.730	129			
Below (g)	Between Groups	.213	12	.018	1.571	.110
	Within Groups	1.321	117	.011		
	Total	1.534	129			

Fig 1.1. This table shows the ANOVAs on above and below ground dry biomass.

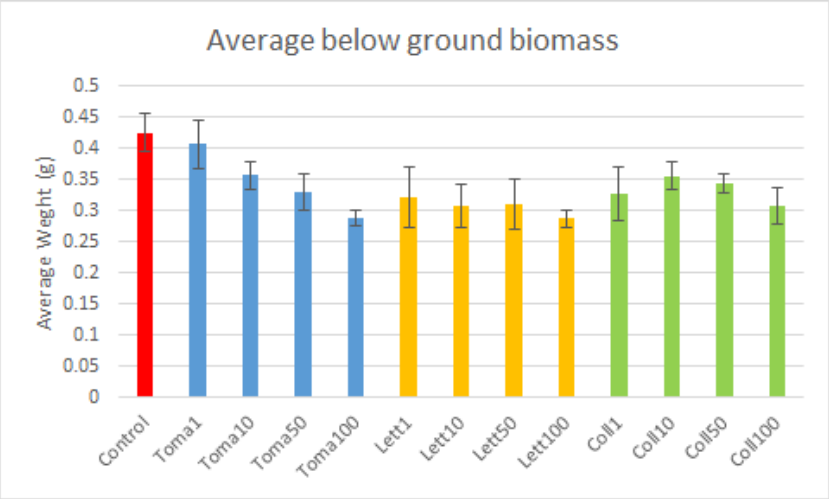

Fig 2. Bar graph comparing the different treatments for the average below ground biomass with error bars. The control is in red, the tomato, lettuce, and collard green treated soils are in blue, yellow, and green, respectively.

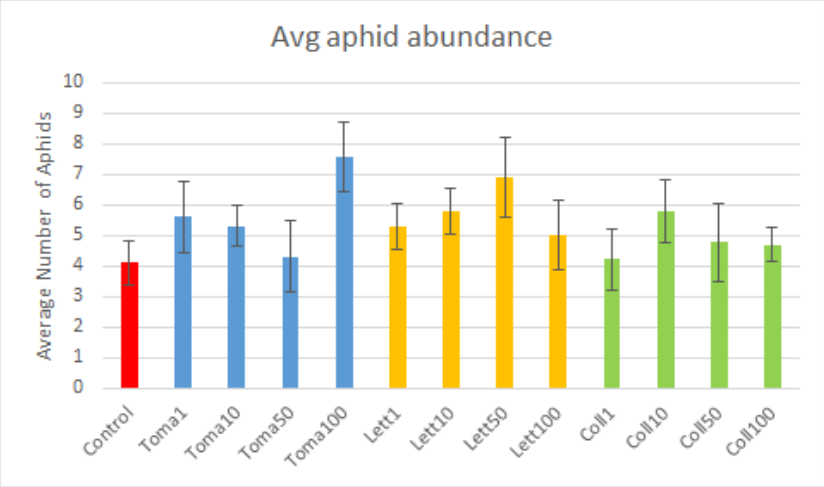

Fig 3. Bar graph showing average aphid abundance along with error bars. The control is in red, the tomato, lettuce, and collard green treated soils are in blue, yellow, and green, respectively.

ANOVA

Aphids found Dead on Plants

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	118.570	12	9.881	.910	.540
Within Groups	1227.089	113	10.859		
Total	1345.659	125			

Fig 3.1. This chart shows the ANOVA done on the average aphid abundance data.

DISCUSSION

In both of the above and below ground average biomasses the average of the control trials were the highest in weight. Though there were some graphical trends, it was not backed up by the statistics. The interesting relationships are exemplified by the tomato data labeled Toma 1-100, but they do exist in most of the different sets of data in the graphs. These trends show an inverse relationship between weight and level of soil inoculum, the highest being the control in both tests and the lowest generally being the 100% conditioned soil. This shows a negative plant-soil feedback loop, meaning that the soil microbiome formed during the conditioning phase has a negative impact on the growth of the plant. The container size will have also influenced the size of the above and below ground biomass because they were too small and may have impacted the fitness, but this would have been the same between all 130 conetainers. It is disappointing that this could not be correlated to the aphid data.

The aphid data is nearly impossible to interpret due to the mass death of the *Macrosiphum euphorbiae*. There remained only a few alive between all 130 plants. This could easily be attributed to many issues. The greenhouse getting too hot likely had a hand in the overstressing and eventual desiccation of the herbivore. The fitness of the tomatoes could have also influenced the *M. euphorbiae*. As aforementioned the conetainers were too small and it showed. Many of the tomato plants began to have issues at the beginning of the feedback experiment because they needed to be bent down to fit within the plastic cages and they had to be watered from below which reduced the ability to add water due to gravity. It was a combination of stressors and these would have influenced the tomatoes which could have impacted the aphids and likely did.

Finally, something that may have been an issue and impacted the soil microbiomes created by the inoculum is the fact that the *L. sativa* initially did not grow, so this had to be corrected which delayed the feedback phase by two weeks and the soil of the other two plants used in the conditioning phase sat in a refrigerator for two weeks longer than it should have.

There are many different new directions to take this experiment. For example, using multiple herbivores and comparing how herbivores of different sizes or herbivores from different classes, and instead of counting them their biomasses could be taken instead. This would be necessary for herbivores like *Manduca sexta* which is very commonly used in studies like this one. Expanding the plants used in both the conditioning phase and/or the feedback phase would also likely provide interesting results that would be important for understanding the legacy effects of the soil microbiome. This has near infinite possibilities and optimizing crop rotation is an important goal, as it is commonly used without much direction. In this experiment we used only forbs which were distantly related from one another. Anything outside of that group or within that group, but closely related are viable options.

Finally, field trials could be done to more accurately determine how these levels of soil inoculum would act within an open system. This could be more readily applied to farmers in terms of how much soil to inoculate their fields with, if any, and which plants genuinely have positive legacy effects on future crops planted in the same areas.

CONCLUSION

Currently, there is no standard between laboratories for soil microbiome and plant-soil feedback loop studies. It is important for future research to eliminate this as a possible discrepancy between studies done by different labs. It is important for the optimization of crop production and pest and pathogen management. If yields can be increased and instances of loss

decreased, that would be an important step in the right direction. Overall, this experiment shows a possible correlation, but more work in expanded trials needs to be done to determine the most optimum level of soil inoculum.

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