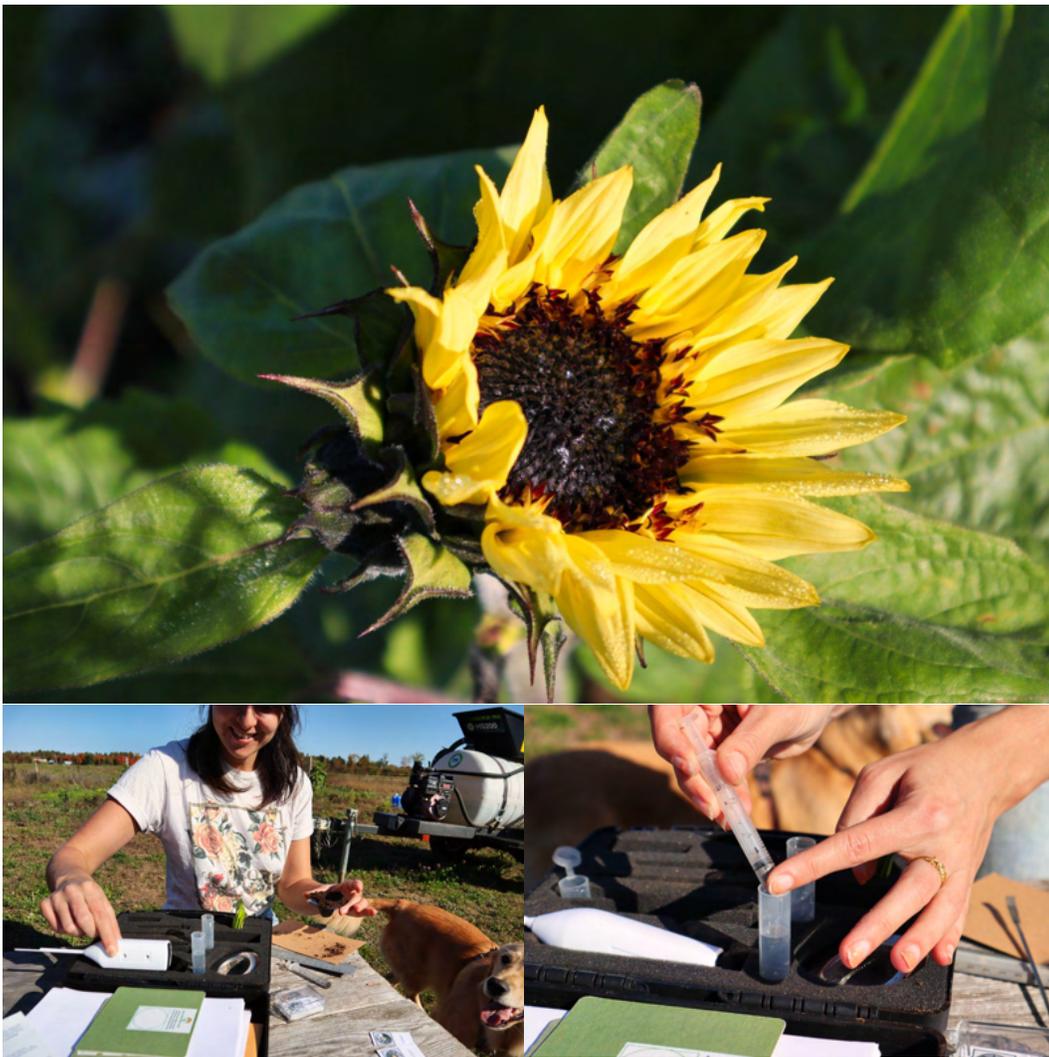


The Regenerative Effects of Fungal Dominant Compost: Pilot Study

By: Emma Roy, Scott Hortop, Hailee Turry



December 1st 2021

*Images by Jessie Domanski 2021

Abstract

Research into Mycorrhizal fungi is vast and far-reaching, with many topics particularly regarding soil health being studied by various teams around the globe. There is a need in our current agriculture practices to switch to more sustainable forms of soil maintenance and plant growing methods which could increase yields while reducing pollution from fertilizers. Many methods including no-till, crop rotation, and home composting have become very popular in recent years as even home horticulturists see the switch to sustainable practices as important with oncoming climate changes. This study is a pilot study intended to explore the use of a product created by the non-profit ONfungi which is said to have a high microbial biomass of AMF fungi, similar to soils in forest floors. By creating a simple growth experiment with various species in a farming area, we were able to determine that ONfungi's fungal inoculant is statistically significant and effective at increasing the growth rate and health of the most common garden plants. In this case the treatment had a p-value of $P=0.01651$ after running an ANOVA on growth data from week 6 to week 9. In addition to statistical analysis of the growth rate, we used the microbiometer tool to determine that the soil on the effected plot held +236 ugC/g more carbon when sampled compared to before the experiment, and the control actually had a loss of carbon in the soil by 30ugC/g.

Introduction

As fewer direct studies have been completed, we are hoping this small pilot project could help jump start further research in this area. Johnson-su over a 4.5 year management of agricultural soils managed to measure an increase of 20-50 times the amount of carbon captured by the soil upon the use of his own created BEAM in management practices (Johnson et al., 2015). These practices are intended to mimic how detritus naturally degrades in forests, and increases the natural biodiversity of the microbial community within the soil (Johnson et al., 2015). This in turn can help agricultural soils mimic natural ecosystems (Johnson et al., 2015). It is likely that the fungal dominant compost will help with moisture retention and overall soil health. The purpose of our study is to attempt to evaluate the use of FDC on private gardens, city lawn/park applications, and regenerative agriculture practices. Although much of the research has been created on a wide scale agricultural level over longer periods of time, we believe individual action is also extremely beneficial and there may be ways to apply FDC in smaller scale projects in order to store more carbon and improve soil health (Shi et al., 2012). City parks, home lawns and gardens, and environmental remediation projects may see a benefit in use of FDC even with only one application (Shi et al., 2012). The fungal compost is expected to improve the soil over time, and with each additional generation of plants and lack of tilling (Johnson et al., 2015). We hope that the use of FDC compost can increase plant health, reduce the use of pesticides on lawns and gardens, and regenerate the soil in a relatively simple way similar to how our earth naturally operates.

Research has shown that there is a possibility of various benefits to the addition of AMF fungi into agricultural soils or in other environmental remediation projects. AMF fungi perform many functions within the soil which are integral for the growth of most land plants. In particular, AMF fungi are especially good at collecting and transferring phosphorus to plant roots (Ellis, 1998). Upon the loss of AMF fungi in soil due to major disturbances such as tilling or flooding, less spores are able to establish themselves in the soil, leading to less diversity and a phosphorus deficiency in the plants, stunting growth (Ellis, 1998). In one study outlining the current knowledge of fungal dominance on soil dynamics stated that “Crop rotations, reduced or no-tillage practices, organic farming, and cover crops increase total microbial biomass and shift the community structure toward a more fungal-dominated community, thereby enhancing the accumulation of MOM” (De Vries et al., 2006). There is also evidence to suggest that AMF fungi diversity in soil is related to the ability of plants to fight off pathogens (Griffiths & Philippot, 2013). The relationship between plants and fungi is strong, as plant diversity can also improve fungal populations and diversity (Griffiths & Philippot, 2013). According to the insurance hypothesis, it is less likely for any one organism, pathogenic or otherwise, to overtake the colony if there is significant diversity present (Loreau et al., 2001). Following this, adding a diverse fungal inoculant should in theory help re-establish lost fungal colonies, strengthening diversity and the flexibility and adaptability of the environment (Loreau et al., 2001).

Common critiques in this area of research stem primarily from our fundamental lack of understanding of complex fungal networks and the exact mechanisms of which nutrients are stored or shared in the field as a whole. In addition, since many of these experiments cannot be done in a lab, additional conflicting variables could explain some of the effects of the product's effects or call into question the effectiveness of the inoculant. One researcher proposed that increased plant health could be due to an increased phosphorus or nitrogen content within the compost, adding nutrients to the soil. Although possible, this would be unlikely as very little nutritionally dense components, and nearly zero high nitrogen components were added in the composting process. Rather than composting typical sugary and nutrient rich grocery store fruits and vegetables, the ONfungi compost is made primarily from fallen leaves, wood, or other detritus high in cellulose and typically covered in fungal spores. In this case, the nutrition added to the compost is intended to support and feed the fungi rather than the plant. Typically a very small amount of the inoculant needs to be used, and larger volumes can have a decreased effect on plant growth (Johnson-Su 2010). Other studies have also proven that adding chemical fertilizers can increase acidity of the soil and decrease the fungal network as high nitrogen environments are not hospitable to them (Tate 1991). Adding chemical fertilizers actually ends up depleting nitrogen in most cases (Mulvaney et al., 2009), and our goal is not to add nutrition to the soil, but to increase the soil's capacity to maintain its own nutritional stores through AMF fungi.

Adding chemical Nitrogen fertilizers also decrease the amount of carbon stored within the soil (Mulvaney et al., 2009). We managed to see a dramatic increase not only in fungal biomass, but in the carbon captured in the soil as well, so this directly contradicts what would be expected if a large nutrient compost was added without diverse fungal colonies (Khan et al., 2007). In addition to the critiques about possible nutritional components which would need to be explored quantitatively, one study had also found that various fungal dominant compost/inoculants from different producers had negative effects on the germination of lettuce seeds (Samuelson et al., 2019). This was confirmed by our experiment, as a few species did become affected by delayed germination due to the compost, but contrary to the author's assumption, this was abundantly not true for all species and many species had an increased rate of germination compared to control, contrary to the prior studies results. Further germination studies will need to be completed to quantitatively measure this divergence and how exactly the inoculant affects germination across species.

Materials and Methods

The experiment was completed at an agricultural property near White Lake Ontario. The initial soil quality was quite sandy and had proven difficult to grow plants prior to the experiment. There are cattle on sight to assist with regenerating the farm soil in other areas over a longer term, but these cows were fenced away from the experimental plot area to avoid destruction. Each plot was created by tilling 1ft into undisturbed soil in order to reduce the weeds and grasses which were already present. Any weeds remaining were removed by hand.

Each plot is 8 feet x 4 feet and contains seeds from 9 different species and various plant types. Out of these 9 species, only 7 of which germinated and began a successful growth cycle. The seeds were planted in each plot in the same pattern to make it easier to assess the plants the same way. The plots were about 10ft away from each other, close enough to to ensure nearly identical environmental conditions whilst also far enough to prevent the possible benefit of the compost plot from leaching into the control. There were no significant trees or buildings nearby to provide shade, so both plots experienced full sun. The seeds were planted on August 1st and had been receiving a weekly check up and allowed to grow fairly undisturbed for 9 weeks. The primary difference between the two plots are that one was planted with 0 additions for control, whilst the other was planted with an inoculation of FDC compost from ONfungi. The null hypothesis is that the FDC has no significant effect on any plant growth or health related factors. In particular, we were measuring variables such as shoot length, number of leaves or buds, and the presence of pests or disease.

Plant Type	# of Seeds/plot	Planting Depth	Inoculant/Plot grams
Sunflower (<i>Helianthus annuus</i> L.)	10	2cm	50
Zinnia (<i>Zinnia Elegans</i>)	50	0.5cm	50
Aster (Mixed Species)	50	0.5cm	50
oregano (<i>Origanum vulgare</i>)	50	1cm	50

dill (<i>Anethum graveolens</i>)	50	1cm	50
peas (<i>Pisum sativum</i>):	14	2cm	50
spinach (<i>Spinacia oleracea</i>)	50	1cm	50
lettuce (<i>Lactuca sativa</i>)	50	1cm	50
cucumber (<i>Cucumis sativus</i>)	20	3cm	50
Grass	150	0.5cm	200

Table 1: Each plant was planted according to seed directions for each species, seeds acquired at home hardware on August 1st 2021. Some seeds like the cucumbers and sunflowers had 6-8 seeds per planting hole whereas others had a single or double seed per hole, and inoculant quantities were identical for all plants. n=9

Results:

Material/ Plot	n	Microbiometer Reading (ugC/g) Start	F:B START	Microbiometer Reading End	F:B END	Mean Difference Carbon (ugC/g)
Compost	1	730	1.4:1	730	1.4:1	0
Control Soil	3	464	0.7:1	434	0.6:1	-30
FDC Soil	3	464	0.7:1	700	1.2:1	+236

Table 2: Displays the change in soil carbon after 9 weeks following a single application of compost. Final F:B ratios are the average of multiple samples taken in different areas of each experimental plot. n=7.

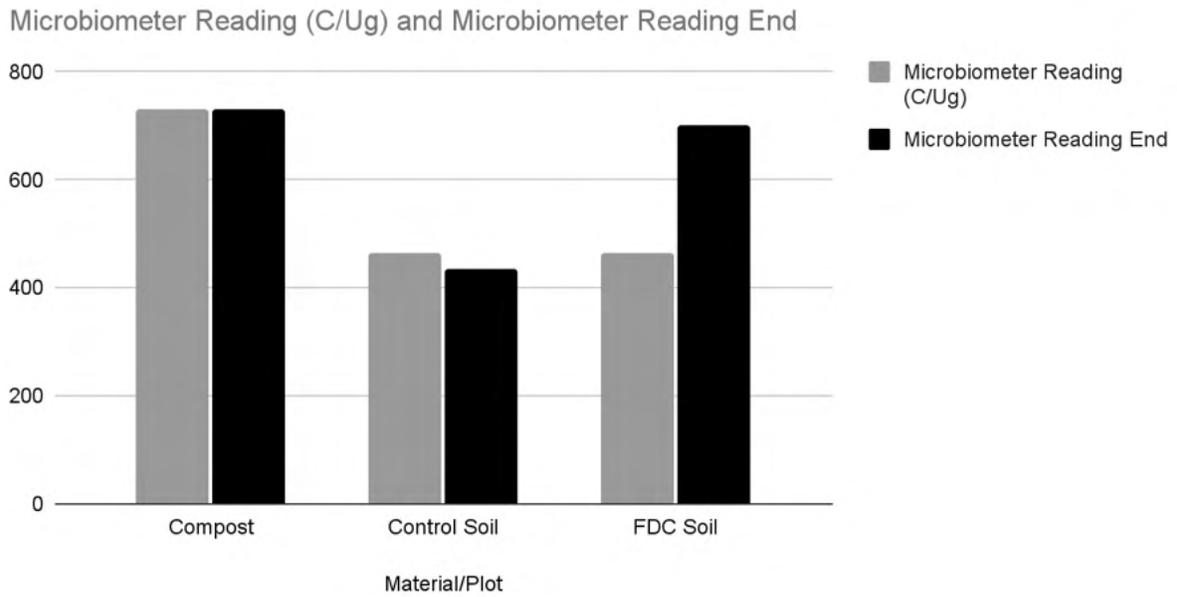


Figure 1: Displays the change in MicroBiometer readings before and after a 9 week growing period. The lighter grey bar is the starting reading and the black bar on the right is the final reading. n=7



Figure 2: The affected plot was visually darker by a few shades, indicating a higher water and carbon content. Approximately 8 weeks had passed since the initial application of the compost, with no further additions.



Figure 3: Grass from control (Left) grass from affected (Center). (Right) is spinach upon being pulled from the plots, with the control on the right and the affected on the left.

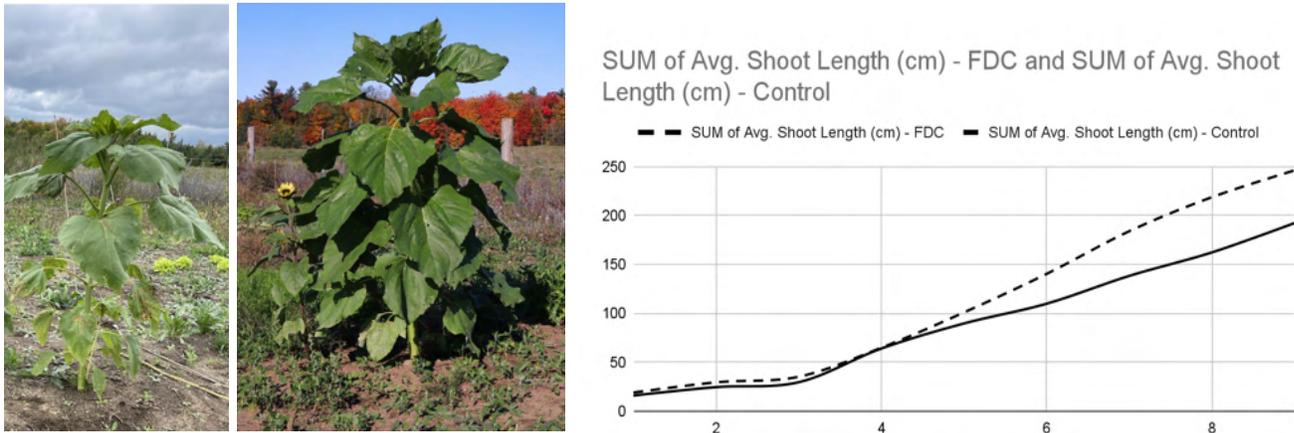


Figure 4: Pictured on the left is the largest of the sunflower plants, with control on the left and the affected on the right. A graph visualizing the growth of all of the plants over time. n=7

```
Anova Model Code Used: model1 <- lm(height ~ species + Treatment + Week +
species:Treatment,
data = data)
Anova(model1, type = 3)
```

Anova Table (Type III tests)

Response: height	Sum Sq	Df	F-Value	Pr(>F)
(Intercept)	165.0	1	4.2609	0.04537 *
species	5779.8	6	24.8821	3.295e-12 ***
Treatment	242.0	1	6.2509	0.01651 *
Week	1930.2	1	49.8567	1.352e-08 ***
species:Treatment	474.5	6	2.0430	0.08158
Residuals	1587.3	41		

Table 2: Upon loading the data for species including Sunflower, African Daisy, Zinnia, Grass, Peas, lettuce, and Spinach, from week 6 to week 9 into R. $p < 0.05$

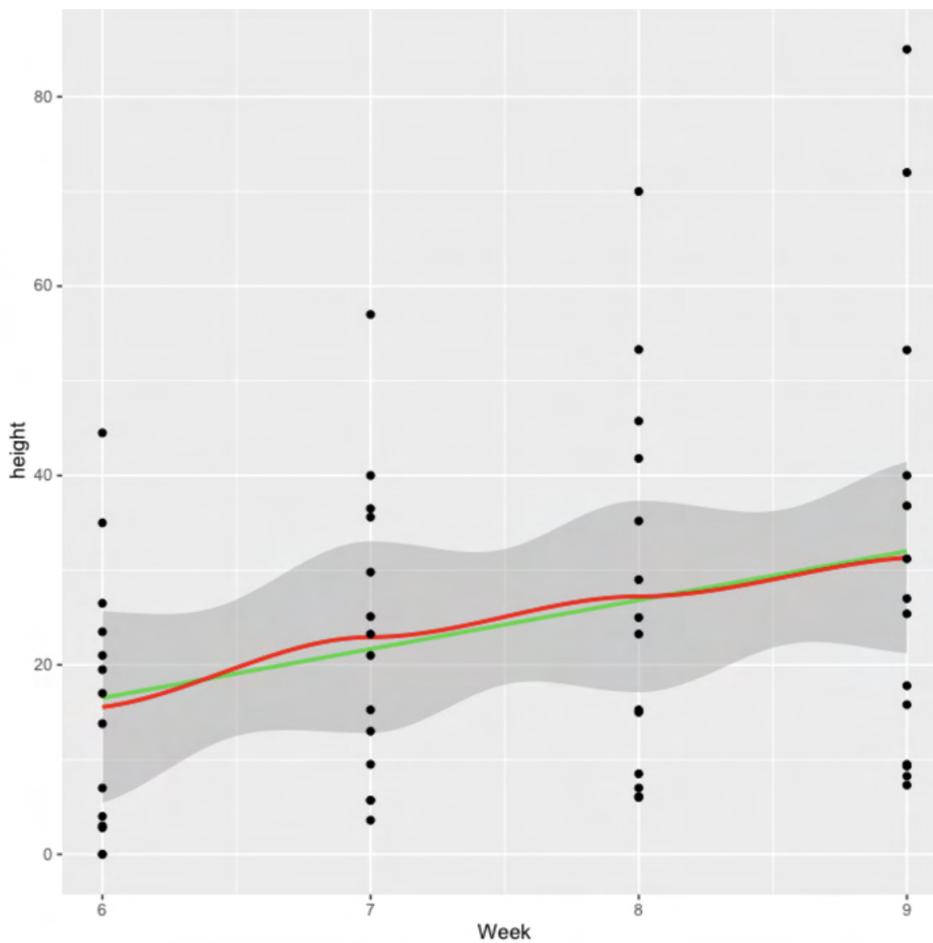


Figure 5: Visual representation of the Anova Model from R, in which a positive linear correlation is clear. $P < 0.05$.

Discussion:

Figure 1: The micro-biometer reading had a 95% accuracy rating according to internal tests, and over 8 weeks the quantity of micro-biomass rose from 464ugC/g to over 700ugC/g. As we took two samples per plot upon completing the experiment, we noticed that samples taken from different areas in the plot had different readings often related to species type. For example the sample taken within the grass patch had a lower reading of 590ugC/g but this is in line with prior research which suggests certain species prefer higher fungal soil ratios such as native species. This was confirmed as the other sample taken near the sunflower plant, which is a native species, had a much higher F1.4:1B ratio.

Figure 2: After only 5 weeks of the experiment, the soil on the FDC plot was visually different when prior to this week they were identical. The control plot had lighter, sandier soil which seemed to be more prone to drought. Both plots received identical amounts of water and in fact the affected plot was slightly elevated in comparison to the control, so it is unlikely that the control plot was not receiving as much water and likely received slightly more than the affected, which would have promoted the opposite effect than measured. There were also less weeds on the FDC plot as the higher fungal content in the soil is much less hospitable to them, and weed growth decreased over time on the fungal plot while increasing in the control plot.

Figure 3: After 9 weeks of growth, the grass patches were visually much different. The grass in the control was quite patchy, pale, and not as long or bushy as the grass which grew in the control. On average after 9 weeks, the grass in the affected plot was approximately 10cm longer than the control.

Figure 4: The sunflowers in the affected plot had a much faster growth rate of 8.9cm/week, whereas the control only had a rate of 7.4cm/week. In addition as is visible in the images, the sunflower plant on the control plot was suffering from an invasion of cucumber beetles. These beetles were already present on the farm and are the reason a few of our plants were unable to successfully complete a growth cycle, in particular the cucumbers. Although beetles were visually seen on the affected sunflower plants as well, it seems as though the disease resistance in the affected was stronger as none of the affected plants became victims of pathogenic plant illness despite obvious signs of beetles eating leaves. The control plants on the other hand were much less lush, had less leaves, and had smaller leaves on average with many signs of disease such as wilting/brown leaves, white fungal powdery patches on the lower leaves, stunted growth, and brown stems with canker-like sores. The buds on the affected sunflower plants also bloomed about 1-2 weeks prior to the control. Further experiments would need to be completed to determine if the inoculant increases reproductive rate or yield quantitatively.

Figure 5: Once we collected the data and organized it based on species and the week collected, we used the statistical program R to analyze the data. Initially, we analyzed the data with the entire run of the growth cycle of the plants, presuming these results would improve throughout the course of the experiment. The initial growth period from week 1 to week 5 did not achieve a significant difference in plant height, although week 6 to week 9 proved the plants in the affected plot were significantly taller. A positive correlation can be seen in Figure 5 which illustrates that our null hypothesis can be definitively denied. Height was our strongest predictor of health, with bud and leaf formation not achieving statistically significant results, though this may change if the experiment is repeated with a larger sample size. There is in fact a significant difference in plant health after applying a single application of ONfungi's compost/inoculant.

Now that we have established that the compost does in fact have a positive effect on most common garden plant species, future experiments could be done to flesh out additional questions brought forward by this experiment. A full germination study will have to be completed, as certain species seemed to have an increased rate of germination whereas others had significantly delayed germination (lettuce, spinach). Data on germination was not collected accurately enough to provide analysis for this aspect of plant health, so we focused more on height, buds, and leaf development. We only chose to apply a single application of compost, so further applications throughout the growth cycle in different methods (compost tea, regular watering, etc) could also be analyzed. We have current plans to repeat this experiment in a slightly larger scale come spring, as this summer we were limited by beginning late in the growing season and we did not have comprehensive funding, which meant most of the work has been done by volunteers or in the most cost effective way possible. We would also like to return to the same plots in order to determine if a winter cycle and growth cycle can improve the soil over time with one application or if further applications will be needed to maintain higher levels of AMF fungi.

Conclusion

The purpose of this experiment was to explore our product created by Scott Hortop in order to determine if there was any degree of effectiveness. As we assumed the treatment would have no effect, we determined that this null hypothesis was not correct. After 6 weeks the treatment allowed for significantly faster and healthier growth in the affected plot. Further experiments will need to be done in order to quantitatively analyze the effects that were touched on qualitatively in this report.



Additional Images:

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