Mycorrhizal Symbiosis Unlocking the Potential of Soil

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Today, more than 99 per cent of the world's food supply comes from the land. More than six billion people rely on food that is grown on just 11 percent of the global land surface. Of this land, just 3 percent offers inherently fertile soil.

Widespread development, mono-cropping, over tillage, and excessive use of pesticides and fertilizers have been shown in countless studies to be destructive to the health of our soil. Now, after decades of these practices, we have rendered unproductive and abandoned nearly one-third of the world's usable cropland. Human induced soil degradation is transforming productive agricultural areas into wastelands at tragic speeds.

As our usable land is shrinking, our population is growing at an alarming rate. It is estimated that in 2011 the world's population will reach 7 billion. The US Census bureau estimates that by the year 2050, more than 9 billion people will inhabit the earth and to feed this many people, agricultural yields must double. A recent United Nations report states farmers will need to grow as much food in the next 50 years as we have produced in the last 10, 000 years.

Healthy soil is critical for the production of food. Therefore, soil remediation/restoration is crucial for our survival. There are many viable solutions to our current soil crisis and as with rebuilding anything, you have to start from the ground up. Rebuilding a healthy rhizosphere by reintroducing a healthy population of fungi and other microorganisms plays an essential part in restoring our soil.

An important member of a healthy rhizosphere is mycorrhizal fungi. Unknown to most, mycorrhizal fungi colonize the roots of over 90% of plant species, forming a mutualistic

symbiotic relationship with their plant hosts. Many plants cannot grow without this relationship, which is one of the longest and most successful relationships in nature.

With the help of mycorrhizal fungi, a plant can take up many times more nutrients, particularly phosphorous, than would be possible without mycorrhizal fungi. Because they enhance the plant's ability to take up nutrients and water, mycorrhizal fungi can help plants compensate for low nutrient availability, poor soil structure, and low water holding capacity often found at harsh soil sites.

Mycorrhizal fungi are able to free up insoluble nutrients by secreting powerful organic acids that lower the soil pH making it more acidic which helps to solulize bound nutrients. The fungi then transfer these nutrients to the associated host plants that it is associated with. Networks of Mycorrhizal Fungi can be associated with multiple plants.

In addition to solulizing nutrients, mycorrhizal fungi also solulizes heavy metals. It then traps and immobilizes the heavy metals by storing them in its extraradical mycelium and preventing them from being taken up by the plant. In this way the fungus provides protection to the plant from contaminants and assists the plant to grow in hazardous areas. Another positive aspect of this process is that heavy metals are removed from the soil as well.

The sequestration and binding of heavy metals and toxins in extraradical mycelium has been suggested to be the method by which mycorrhizal fungi develops a tolerance to these otherwise harmful elements.

Mycorrhizal fungi also help plants resist pests. Most experts in pest management say that plant health is the most important aspect of pest management. Healthy plants have much fewer pest problems. Better nutrition and water uptake through mycorrhizae helps plants stay healthy.

There are two primary types of mycorrhizal fungi: Endomycorrhizae and Ectomycorrhizae. Endomycorrhizal fungi grow inside the cells of roots. They form vesicles and arbuscles on the plant roots. Arbuscles are the sites of exchange of nutrients between the plant

and fungus, and vesicles are the storage organs. Endomycorrhizal fungi colonize the plant's roots and grow outward into the surrounding soil. The wide dispersion of the fungal hyphae into the soil enables the plant to access soil volumes up to 1500% larger than the roots alone. The fungus acts as an extension of the root which gives a greater surface area for absorbing water and nutrients.

Ectomycorrhizal fungi grows both between the cells of roots and on the outside of the root rather than inside the cells. These fungi have a thick network of hyphae that cover the roots. This covering protects the roots in a sheath of ectomycorrhizal cells.

In order to have healthy plants, we must also have healthy soil and in order to have healthy soil, essential soil microorganisms must be present. The reintroduction of mycorrhizal fungi into our soil is a critical step in process of remediating damaged, depleted and contaminated soil.

Over the past four years, my indoor mycorrhizal research and experimentation has shown very promising results. The results of my past experiments have shown that inoculating plants with mycorrhizal fungi dramatically improves their health and productivity in not only optimal, but also depleted and extreme soil conditions.

This year I wanted to test my hypothesis in the natural world.

The purpose of my Science Fair project this year was to see if mycorrhizal fungi can help increase the viability and yield of Zea mays (corn) crops grown outdoors in high pH, depleted, compacted soil that is not ordinarily suitable for agricultural purposes.

In addition, I wanted to determine if the soil properties of the plots treated with mycorrhizal fungi will change making it more suitable for agricultural purposes.

I also wanted to see if Zea mays kernels from plants grown in soil treated with mycorrhizal fungi would have higher nutritional values than those grown without. (Malnourishment is another topic of great global concern.)

- I hypothesized 1st that Zea mays plants grown with mycorrhizal fungi in high pH, depleted, compact soil will be more viable and have higher yields than plants grown in the same soil without the assistance of mycorrhizal fungi.
- I hypothesized 2nd that the soil properties of the plots treated with mycorrhizal fungi will change making the land more suitable for agricultural purposes.
- I hypothesized 3rd that Zea mays kernels grown in soil treated with mycorrhizal fungi would have higher nutritional values than those grown without.

The procedure I followed was:

I acquired the following materials:

- 1) Mycorrhizal fungi suitable for coating seeds
- 2) 210 Organic, Non GMO Zea mays seeds
- 3) Tools for digging and planting Gloves, shovels, hoes etc.
- 4) Materials for documentation: Digital camera, microscope, ruler, Schaeffer brand black fountain pen ink (for root staining), white household vinegar
- 5) Food Chemistry Test Kit
- 6) Miscellaneous water, mixing containers, paper/pencils/pens

I then followed the steps below:

Step#1: I found a crop test site

Step#2: I tested soil and document pH / nutrient values (N-P-K), SAR and general soil properties.

Step#3: I dug and prepared six separate 6' X 8' land plots 2' apart (three designated as 'Myco' plots and three were designated as 'Control' plots) with an 8' X 8' barrier plot in-between to prevent mycorrhizal fungi from contaminating the control plots.

Step#4: I soaked 210 Zea mays seeds for two hours then separated out 105 seeds and coated them with mycorrhizal fungi powder leaving 105 untreated for the Control Group.

Step#5: I planted five rows, seven seeds per row, of uncoated Zea mays seeds (1 inch deep) every two feet in each of the three 'Control' plots and planted five rows of mycorrhizal fungi coated Zea mays seeds (1 inch down) every two feet in each of the three 'Myco' plots.

Example Plot Plan:

| Plot Plan | Control 1 | Control 2 | Control 3 | Barrier | Myco 3 | Myco 2 | Myco 1 |
|------------|-----------|-----------|-----------|---------|--------|--------|--------|
| Seed Row 1 | | | | | | | |
| Seed Row 2 | | | | | | | |
| Seed Row 3 | | | | | | | |
| Seed Row 4 | | | | | | | |
| Seed Row 5 | | | | | | | |

Step#6: I watered plots at timed durations and continue to water every four days or as needed.

Step#7: I documented successful germination rate.

Step#8: I documented plant growth and changes after first sprout emergence.

Step#9: When substantial had growth occurred, I conducted a seven day drought stress test documenting overall plant heath and mortality rate.

Step#10: At harvest time I documented crop yield and overall crop health and harvested only ears with a minimum 2" diameter and 7" length.

10a: I was unable to get into the CSU Pueblo lab at this time so I waited to uproot plants until 2nd harvest. I ran Food Chemistry / Nutritional Value Tests at this point using a solution of corn kernels randomly taken from multiple cobs in each group crushed and mixed with distilled water. This solution was used with the test kit reagents to determine the presence and general amount of Sugar, Protein, Fat and Vitamin C.

Step#11: 2nd Harvest – I documented crop yield and overall crop health and harvested only ears with a minimum 2" diameter and 7" length.

11a: I carefully dug and uprooted the healthiest plant from each plot in both groups.

11b: I examined and compared overall plant health and biomass

11c: I examined and compared primary root lengths and overall root masses using the grid intersection technique and noted irregularities and obvious differences.

Step #12: I took the root balls to the CSU Pueblo lab and stained a section of each root in each group for the presence of mycorrhizal fungi using Lactophenol Cotton Blue Stain under the supervision of my Qualified Scientist. I then examined the root sections under a microscope.

Step #13: At the CSU Pueblo lab I prepared soil and tissue samples for analysis in the ICP-MS (*Inductively coupled plasma mass spectrometer*)

Step #14: I re-ran Food Chemistry / Nutritional Value Tests again with the newly harvested corn using the same method that was used during the first harvest.

Step #15: I charted and assembled all data for presentation.

Step #16: Growing plots were cleaned.

The results of my experimentation showed the following:

June 4th 2010:

Soil samples were taken at growing site. Three soil samples were taken from different locations in the planned garden plot area and were mixed together. Initial soil sample tests indicated a pH of 8.5 and depleted nitrogen (10ppm) and phosphorus (10ppm) levels. Potassium levels were acceptable (900ppm). Soil structure was very hard/compact. Sodium absorption ratio test indicated a SAR of 15.5.

On June 14th 2010 I planted the Zea mays seeds as stated in my research plan.

June 29th 2010 - Day 16:

Control 1 (C1): 5 seedlings Control 2 (C2): 8 seedlings Control 3 (C3): 11 seedlings

Total = 24

Myco 1 (M1): 16 seedlings Myco 2 (M2): 18 seedlings Myco 3 (M3): 27 seedlings Total = 61

July 10th 2010 – Day 27:

Control 1 (C1): 10 plants Control 2 (C2): 11 plants Control 3 (C3): 18 plants

Total = 39

Myco 1 (M1): 20 plants Myco 2 (M2): 24 plants Myco 3 (M3): 33 plants Total = 77

Heavy/monsoonal rains began in late July / early August and supplemental watering was not needed from 7 /25 /2010 to 8 / 14 / 2010

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August 1<sup>st</sup> 2010 – Day 49
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Control 1 (C1): 11 plants Tallest Plant Height = 20" Control 2 (C2): 15 plants Tallest Plant Height = 26" Tallest Plant Height = 28" Control 3 (C3): 21 plants Total = 47

Tallest Plant Height = 37" **Myco 1 (M1)**: 20 plants Myco 2 (M2): 26 plants Tallest Plant Height = 34" Tallest Plant Height = 39" Myco 3 (M3): 33 plants

Total = 79

Observations: Myco group is growing at a much more consistent rate than the control group. Plants are on average taller and darker in color. Small tassels are developing in the Myco groups. No tassels in the Control group yet.

Due to the slope of the meadow, the upper (west) rows get less water and have fewer plants. The outer beds in both groups have fewer plants than the inner beds for reasons unknown.

August 22nd 2010 – Day 70

Heavy rains sprouted seeds I thought would not sprout so there are now new plants in each group. Overall consistent growth / height in the Myco group except for the new sprouts. Plant size in the Control group varies dramatically.

All plants in the Myco Group except the ten new sprouts have tasseled and each plant is developing ears. Two plants in Myco 1, three plants in Myco two and six plants in Myco 3 each have two ears.

The Control group's tassel – silk – ear production is noticeably behind the Myco Group. All plants have tasseled but only twelve plants have silk and only five plants have developing ears.

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Tallest Plant Height = 34" Germination % = 40%
Control 1 (C1): 14 plants
Control 2 (C2): 16 plants
                           Tallest Plant Height = 33" Germination % = 46%
Control 3 (C3): 23 plants
                           Tallest Plant Height = 30" Germination % = 66%
                           Total Plants = 53
                           Total Germination % = 50 %
                           Tallest Plant Height = 45" Germination % = 69%
Myco 1 (M1): 24 plants
Myco 2 (M2): 31 plants
                            Tallest Plant Height = 41" Germination % = 88%
Myco 3 (M3): 34 plants
                           Tallest Plant Height = 42" Germination % = 97 %
                           Total Plants = 89
                           Total Germination % = 85 %
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September 4th 2010 – Watering duration increased to 20min per bed every four days due to the hot weather.

September 7th 2010 –

Seven day drought stress test begins. (No water / No Rain)

September 14th 2010 – Day 93

Seven day drought stress test ends. (No water / No Rain)

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Control 1 (C1): 14 plants / 5 Developed Ears / 7 Dead-Dry / Height = 36"
Mortality Rate = 50%
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Control 2 (C2): 16 plants / 8 Developed Ears / 9 Dead-Dry / Height = 42"

Mortality Rate = 56%
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Control 3 (C3): 23 plants / 10 Developed Ears / 10 Dead-Dry / Height = 44"

Mortality Rate = 39%

Total Plants = 53
Total Dry/Dead plants = 25
Total living plants = 27
Total Drought Mortality Rate = 47%

Myco 1 (M1): 24 plants / 18 Developed Ears / 1 Dead-Dry / Height = 47"

Mortality Rate = 4%

Myco 2 (M2): 31 plants / 25 Developed Ears / 0 Dead-Dry / Height = 45"
Mortality Rate = 0%

Myco 3 (M3): 34 plants / 29 Developed Ears / 3 Dead-Dry / Height = 50"
Mortality Rate = 9%

Total Plants = 89
Total Dry/Dead plants = 4
Total living plants = 85
Total Drought Mortality Rate = 5%

Control Group showed more visible signs of drought stress than the Myco Group. The new plants in both groups are growing tassels / silk / ears.

Four day watering cycles were resumed.



September 21st 2010 – Day 100

Harvest

Harvest criteria were: Ears with a minimum 2" diameter and 7" length.

Mature stalk diameters were also taken from the tallest plant in each group using a notched out ruler that would slip onto stalk.

Control 1 (C1): 7 plants / 2 Ears Harvested / Height = 36" / Stalk = ½"
Control 2 (C2): 7 plants / 2 Ears Harvested / Height = 42" / Stalk = ½"
Control 3 (C3): 13 plants / 5 Ears Harvested / Height = 44" / Stalk = 5/8"
Total standing plants = 27
Total Ears Harvested = 9

Myco 1 (M1): 23 plants / 10 Ears Harvested / 1 Dead-Dry / Height = 47" / Stalk = 7/8"

Myco 2 (M2): 31 plants / 14 Ears Harvested / 0 Dead-Dry / Height = 45" / Stalk = 1"

Myco 3 (M3): 31 plants / 17 Ears Harvested / 3 Dead-Dry / Height = 50" / Stalk = 1 1/4"

Total standing plants = 85 Total Ears Harvested = 41



Observations: More than four times more ears were harvested from the Myco Groups than the Control Groups. All ears had ear worms which upon further investigation is quite normal unless corn is treated with pesticides.

I had hoped to take this corn and the soil to CSU at this point to analyze but was unable to get into the lab.

I was able to run simple food chemistry tests at home with my designated supervisor.

Simple Food Chemistry tests:

Two slurries were made, one Control slurry and one Myco slurry, using 35 grams of corn (taken equally from multiple random cobs in each respective group) which was than blended with 20ml of distilled water, then strained through a fine mesh screen to make a Zea mays juice solution. This solution was used in the following tests:

Test 1: Sugar test - Benedict's solution

Benedict's solution is used to test for simple sugars, such as glucose. It is a clear blue solution of sodium and copper salts. In the presence of simple sugars, the blue solution changes color to green, yellow, and brick-red, depending on the amount of sugar.

- 1. I labeled two test tubes, one C and one M for each group and added 40 drops of liquid Zea mays solution.
- 2. I added 10 drops of Benedict's solution to each test tube and heated the test tubes by suspending in a hot water bath at 45 degrees Celsius for five minutes.
- 3. Instructions say "Note any color change. If sugar is present solution will turn green, yellow, or brick-red, depending on sugar concentration."

RESULTS: C tube stayed blue M tube turned a yellowy green



*This test shows a higher sugar concentration but I think the solution might be too diluted to turn a full red. I thought about making a more concentrated solution but I think it would be too thick and I do not have an actual juicer available.

Test 2: Protein - Biuret solution

Biuret solution is used to identify the presence of protein. Biuret reagent is a blue solution that, when it reacts with protein, will change color to pink-purple.

- 1. I labeled two test tubes, one C and one M for each group and added 40 drops of liquid Zea mays solution.
- 2. I added 3 drops of Biuret reagent solution to each test tube and shook gently to mix.
- 3. Instructions say: "Note any color change. Proteins will turn solution pink or purple."
- 4. RESULTS: C tube turned blue

M tube turned tan

*Obvious color differences but results are inconclusive

Test 3: Fat - Sudan III stain

Sudan III is used to identify the presence of lipids in liquids. It will stain fat cells red.

- 1. I labeled two test tubes, one C and one M for each group and added equal parts of Zea mays solution and water to fill tubes half full.
- 2. I added 3 drops of Sudan III stain to each test tube and shook gently to mix.
- 3. Instructions say: "A red-stained oil layer will separate out and float on the water surface if fat is present."

4. **RESULTS:**

C tube no change / layer on top M tube – an orangey red layer appeared on top



*This test shows a higher fat concentration

Test 4: Vitamin C

Vitamin C Reagent indicator solution is blue. A colorless end point will be reached when a solution containing vitamin C (such as orange juice) is added to this indicator.

- 1. I labeled two test tubes, one C and one M for each group and added 50 drops of blue vitamin C indicator solution.
- 2. One drop at a time in ten drop increments, I added the Zea mays solution one to the indicator solution in the test tubes.

Instructions say: "Count drops until dark blue color disappears. Compare different juices. Those that require more drops to reach the clear end point are LOWER in vitamin C."

RESULTS: C tube - 100 drops to clear end point M tube - 50 drops to clear end point



^{*}This test shows double the Vitamin C concentration!

Simple food chemistry tests show that the Myco group in general produced more nutritious corn.

October 5th 2010 - Day 114

CSU Pueblo Lab time confirmed. 2nd harvest. Fortunately, the weather was very warm and more ears had developed.

Control 1 (C1): 0 additional ears harvested Control 2 (C2): 0 additional ears harvested Control 3 (C3): 2 additional ears harvested

Total New Ears Harvested: 2

Grand Total Combined Control Ears Harvested = 11

Myco 1 (M1): 4 additional ears harvested Myco 2 (M2): 3 additional ears harvested Myco 3 (M3): 4 additional ears harvested **Total New Ears Harvested: 11**

Grand Total Combined Myco Ears Harvested = 52

The healthiest plant from each bed in each group was carefully dug up and the root mass was placed in a bag for transport. Soil from each group was collected for testing as well.

At home, Root masses were laid out on a 1.5" grid and measured using the grid intersection technique.



Results:

Control 1 (C1): 11 intersections Control 2 (C2): 14 intersections Control 3 (C3): 15 intersections

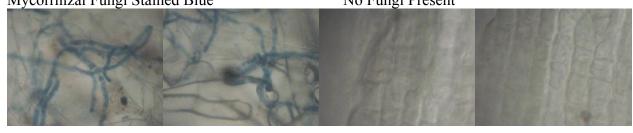
Myco 1 (M1): 30 intersections Myco 2 (M2): 33 intersections Myco 3 (M3): 29 intersections

Myco root masses were visible larger and more developed.

At the CSU Pueblo lab I prepared soil and tissue samples for analysis in the ICP-MS and my qualified scientist helped me stain root samples and prepare slides for viewing under the microscope. A root sample from each root mass in each group was taken and prepared.

At 500X magnification, blue stained mycorrhizal fungi were clearly visible in the Myco Group samples while none were visible in the Control Group Samples at all.

Myco Slides Mycorrhizal Fungi Stained Blue **Control Slides** No Fungi Present



Soil tests were carried over to the following day due to time.

October 6th 2010 -

Root balls were kept in bags over-night and when root balls were removed, there was obvious new root growth and what appeared to be fungal hyphae growing from the Myco groups root balls. No new root growth was present in the Control group.



Out of curiosity, I stained fungi at home using the simple ink and vinegar technique and prepared slides for viewing. Again, mycorrhizal fungi were clearly visible in the Myco group's new root growth. No fungi were visible in the control group.

Secondary soil tests:

Original Sample tested on June 4th 2010

pH = 8.5

N=10ppm, Deficient / P=10ppm, Deficient / K= Adequate, 900ppm

SAR = 15.5

Original Sample re-tested on October 5th 2010

nH = 8.3

N= 10ppm, Deficient / P=10ppm, Deficient / K= Adequate, 900ppm

SAR = 15.5

Control Group: (One cup soil from all three Control beds was gathered and mixed together in a bag.)

Results:

pH = 7.9

N= 10ppm, Deficient / P=10ppm, Deficient / K=600ppm, Deficient

SAR = 13.38

Myco Group: (One cup soil from all three Myco beds was gathered and mixed together in a bag.)

Results:

pH = 6.5

N= 40ppm, Sufficient / P=50ppm, Sufficient / K= Above 900ppm, Sufficient to surplus SAR = 10.65

I also decided to re-run the simple food tests with this new harvest of corn and the results were identical with a higher overall nutritional content in the Myco Group.

*Original sample results are identical to initial test with the exception of pH dropping from 8.5 to 8.3. Sample was kept in a sealed bag for the duration of the experiment.

*Control group's results show a decrease in pH and overall deficient nutrient levels. Potassium levels dropped when compared to original samples. SAR decreased noticeably, most likely due to leaching from waterings.

*Myco group's results show a decrease in pH and surprisingly show an increase in available nutrients. SAR decreased dramatically.

ICP-MS Soil data on next page

ICP-MS Soil Data (PPM)

Original Soil = (OS) Control Soil = (CS) Myco Soil = (MS) Compared to the Original Sample: ↑=raised ↓lowered

Purple= Beneficial Change Blue= Detrimental Change Black= Negligible Change

| (OS) Be = .8428 | (OS) Mn= 445.5 | (OS) Cd = .8625 |
|--|---|----------------------------------|
| (CS) Be = .7267 ↓ | (CS) Mn = 419.2 \downarrow | (CS) Cd = 3.487 ↑ |
| (MS) Be = .9332 ↑ | (MS) Mn = 533.5 \uparrow | (MS) Cd = 2.942 ↑ |
| (OS) Na = 994.6 | (OS) Fe = 19930 | (OS) Sb = .1475 |
| (CS) Na = 114.9 \downarrow | (CS) Fe = 16880 \downarrow | (CS) Sb = .1251 \downarrow |
| (MS) Na = 115.7 \downarrow | (MS) Fe = 21180 \uparrow | (MS) Sb = .1641 \uparrow |
| (OS) $Mg = 4654$ | (OS) $Co = 8.022$ | (OS) $Cs = 1.507$ |
| (CS) $Mg = 2849 \downarrow$ | (CS) $Co = 7.590 \downarrow$ | (CS) $Cs = 1.11 \downarrow$ |
| (MS) $Mg = 3761 \downarrow$ | (MS) $Co = 10.63 \uparrow$ | (MS) $Cs = 1.464 \downarrow$ |
| (OS) Al = 21750 | (OS) Ni = 11.87 | (OS) Ba = 122.2 |
| (CS) Al = 21240 \downarrow | (CS) Ni = 14.83 ↑ | (CS) Ba = $105.5 \downarrow$ |
| (MS) Al = 15220 \downarrow | (MS) Ni = 10.88 ↓ | (MS) Ba = $138.8 \uparrow$ |
| (OS) $P = 443.5$ | (OS) $Cu = 16.25$ | (OS) Hg 201 = .01310 |
| (CS) $P = 372 \downarrow$ | (CS) $Cu = 20.21 \uparrow$ | (CS) Hg 201 = .01922 ↑ |
| (MS) $P = 420.9 \downarrow$ | (MS) $Cu = 15.36 \downarrow$ | (MS) Hg 201 = .02191 ↑ |
| (OS) $K = 4502$ | (OS) $Zn = 49.43$ | (OS) Hg 202 = .02209 |
| (CS) $K = 3339 \downarrow$ | (CS) $Zn = 58.23 \uparrow$ | (CS) Hg 202 = .005447 ↓ |
| (MS) $K = 4188 \downarrow$ | (MS) $Zn = 47.41 \downarrow$ | (MS) Hg 202 = .01639 ↓ |
| (OS) Ca $40 = 4444$ | (OS) $As = 2.43$ | (OS) Pb 206 = 10.33 |
| (CS) Ca $40 = 2875 \downarrow$ | (CS) $As = 3.541 \uparrow$ | (CS) Pb 206 = 12.38 ↑ |
| (MS) Ca $40 = 3315 \downarrow$ | (MS) $As = 2.833 \uparrow$ | (MS) Pb 206 = 9.804 ↓ |
| (OS) Ca $44 = 4491$ | (OS) Se $78 = .1983$ | (OS) Pb 207 = 9.852 |
| (CS) Ca $44 = 2998 \downarrow$ | (CS) Se $78 = .1795 \downarrow$ | (CS) Pb 207 = $11.78 \uparrow$ |
| (MS) Ca $44 = 3767 \downarrow$ | (MS) Se $78 = .2642 \uparrow$ | (MS) Pb 207 = $9.422 \downarrow$ |
| (OS) Ti = 40.72 | (OS) Se $82 = 1.041$ | (OS) Th = 4.472 |
| (CS) Ti = $34.44 \downarrow$ | (CS) Se $82 = .8878 \downarrow$ | (CS) Th = $3.981 \downarrow$ |
| (MS) Ti = $39.86 \downarrow$ | (MS) Se $82 = 1.743 \uparrow$ | (MS) Th = $4.535 \uparrow$ |
| (OS) $V = 43.350$ | (OS) Mo = 6.868 | (OS) $U = 126.9$ |
| (CS) $V = 46.09 \uparrow$ | (CS) Mo = $1.353 \downarrow$ | (CS) $U = .8858 \downarrow$ |
| (MS) $V = 35.23 \downarrow$ | (MS) Mo = $1.480 \downarrow$ | (MS) $U = .7336 \downarrow$ |
| (OS) $Cr = 21.27$ (CS) $Cr = 16.55 \downarrow$ (MS) $Cr = 22.1 \uparrow$ | (OS) $Ag = .05631$ (CS) $Ag = .07874 \uparrow$ (MS) $Ag = .1478 \downarrow$ | |

Total Beneficial Changes: Control = 4 Myco = 14 Total Detrimental Changes: Control = 14 Myco = 2

-Beneficial/detrimental based on CSU Extension office's provided baseline data

ICP-MS Corn Data (PPM)

Control Corn = (CC) Myco Corn = (MC) Control Group element levels were generally normal

Myco Group Compared to the Control Group: ↑=raised **↓=lowered**

Blue= Detrimental Change Black= Negligible Change Purple= Beneficial Change

| (CC) Na = 51.85 (MC) Na = $22.55 \downarrow$ | (CC) Fe = 19.940 (MC) Fe = $23.54\uparrow$ | (CC) $Sb = .006026$ (MC) $Sb = .005572 \downarrow$ |
|--|---|---|
| (CC) $Mg = 964.5$ (MC) $Mg = 1490 \uparrow$ | (CC) $Co = .0175$ (MC) $Co = .02184\uparrow$ | (CC) Ba = $.631$ (MC) Ba = $.3294$ |
| (CC) Al = 4.307 (MC) Al = $4.17\downarrow$ | (CC) Ni = .5945 (MC) Ni = .6404↑ | (CC) Hg 202 = .004699 (MC) Hg 202 = .003626↓ |
| (CC) $P = 2842$ (MC) $P = 4149\uparrow$ | (CC) $Cu = 4.705$ (MC) $Cu = 3.709 \downarrow$ | (CC) Pb 206 = .03967 (MC) Pb 206 = .02453↓ |
| (CC) $K = 7746$ (MC) $K = 13220 \uparrow$ | (CC) $Zn = 28.050$ (MC) $Zn = 38.66\uparrow$ | (CC) Pb 207 = .04631 (MC) Pb 207 = .02974↓ |
| (CC) Ca 40= 110.3 (MC) Ca 40= 126.8↑ | (CC) $As = .03319$ (MC) $As = 0 \downarrow$ | (CC) $U = .05889$ (MC) $U = .02094 \downarrow$ |
| (CC) Ca $44 = 107.4$ (MC) Ca $44 = 153.1$ ↑ | (CC) Se $78 = .05770$ (MC) Se $78 = .03506 \downarrow$ | |
| (CC) $Ti = .0656$ (MC) $Ti = .01329 \downarrow$ | (CC) Se $82 = .3071$ (MC) Se $82 = .4243\uparrow$ | |
| (CC) $V = .2681$ (MC) $V = .2019 \downarrow$ | (CC) Mo = 1.099 (MC) Mo = $2.342\uparrow$ | |
| (CC) $Cr = .4813$ (MC) $Cr = .667\uparrow$ | (CC) $Ag = .05832$ (MC) $Ag = .2123\uparrow$ | |
| (CC) $Mn = 6.292$ (MC) $Mn = 8.298\uparrow$ | (CC) $Cd = 2.234$ (MC) $Cd = 2.098 \downarrow$ | |

Total Beneficial Changes: 13 Total Detrimental Changes: 0

-Beneficial/detrimental based on CSU Extension office's provided baseline data-

In general, the ICP-MS data showed the test site soil is typical for southern Colorado. The uranium levels are a bit high at 126.9ppm. What is interesting is that the uranium level decreased only slightly in the control group down to 88.58 ppm, which is still high but the Myco group decreased dramatically down to .7336ppm.

In general, the beneficial elements increased in the Myco group while the dangerous elements/heavy metals decreased. This was not the case in the control soil.

Interestingly, the copper, zinc, arsenic, and cadmium levels rose in both the control and Myco groups to higher levels than in the original soil sample. I hypothesize that the increase in these elements came from the water I was irrigating with. However, the levels of these elements were lower in the Myco group than in the Control group.

ICP-MS Zea mays/Corn Data:

In general both groups of corn are normal but dramatic differences can be seen between both groups. The magnesium levels in the Myco corn are beneficially high at 1490ppm while the control group borders on low at 964.5ppm. The same is true for phosphorous, potassium, iron, and manganese. Arsenic, cadmium, lead, and uranium all decreased noticeably more in the myco group than in the control group.

These are the most notable ICP-MS results. These results support my simple soil and food chemistry tests.

Cost analysis: The application of mycorrhizal fungi to agricultural lands is very cost effective. According to Mycorrhizal Applications (a current leader in mycorrhizal product development), a medium sized farm (40 acres) would need 100,000 propagules or 1lb of mycorrhizal fungi and should expect to pay \$10 per acre. A home gardener could expect to pay about the same for a 20 X 20 plot. Mycorrhizal fungi do not need to be reapplied and farmers report an average of 30% less water and nutrients needed. This means a 3 to 5 X cost return.

Fertilizer prices have been increasing rapidly and the process of determining which and applying the proper nutrients can sometimes cost as much as \$90 per acre. (Ball)

Variables: The independent variable in this experiment was the addition of mycorrhizal fungi to the soil. The dependent variable was plant growth, plant health and plant yield, end result soil quality and plant nutritional value. The controls were the location and duration / amount of water.

Conclusion: After observing and documenting the growth and health of the plants in both groups over the course of 114 days, I can confidently say that the plants inoculated with mycorrhizal fungi were the healthiest and most productive.

In the Myco group, the germination rate was 85% whereas in the control group the germination rate was 50%. The drought stress test showed a 47% mortality rate in the Control group versus a 5% mortality rate in the Myco group. Total ears harvested from the Myco group were 52 versus 11 in the Control group. The Myco groups root masses were substantially larger and more developed.

Nutritionally, the Myco group's corn surpassed the control groups as well as having lower heavy metal concentrations.

The soil itself was improved in the Myco group having more available nutrients at the end of the experiment and lower heavy metal concentrations than the control group. The Myco soil ended up better in quality at the end of the experiment than when the experiment began.

Based on my experimentation and research to date, I conclude that my hypothesis were correct. My experimentation showed that the Zea mays plants grown in mycorrhizal fungi inoculated soil were healthier and more productive.

The soil quality was dramatically improved in the Myco group and the Myco group corn had lower heavy metal concentrations, and higher nutritional values.

I plan to continue my mycorrhizal research, investigating in more detail the use of mycorrhizal fungi in remediating contaminated soil and increasing the nutritional values of edible plants.

Mycorrhizal fungi play many important rolls in our ecosystem and I look forward to learning more and continuing my research in this field. Franklin D. Roosevelt said, "A nation that destroys its soils destroys itself." I believe this is true and that now more than ever we need to develop and implement sustainable agricultural practices.

Acknowledgements

My greatest thanks and appreciation to Mr. Bruce and Mrs. Joan Cantrell for allowing me to use their land for my experimentation. Thanks as well to Mr. Bruce and Mark Marquez for sharing their knowledge and helping me tend to my growing plots. I am grateful to Dr. Brian Vanden Heuvel for acting as my Qualified Scientist, reviewing my work and for helping me prepare/run soil tests and root sample slides. Thanks to Annie aaaaa for serving as my Designated Supervisor in this project and for helping me edit my photos and graphs. I would like to thank Paul Stamets and everyone at Fungi Perfecti in Olympia, WA for sharing their information and resources with me. Thanks to Dr. Self at the CSU Extension office, Dr. Auge and Dr. Toler at the University of Tennessee for answering my questions. I would especially like to thank Patricia Keeling for patiently reviewing my project and sharing her knowledge and experience with me.

Mycorrhizal Symbiosis – Unlocking the Potential of Soil

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