

# The Effect of Biochar on the Root Development of Corn and Soybeans in Minnesota Soil and Sand

By: Teresa Howard

## Abstract

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Biochar is the product of organic material, like wood, that is burned in a low oxygen environment. This results in a charcoal that acts as a carbon sink for high concentrations of environmental carbon dioxide. It is thought to serve a myriad of functions that aid in plant growth and development such as housing microorganisms and fostering their growth, and assisting in water and nutrient absorption and retention.

The effect of three concentrations of biochar (1.5%, 3%, and 6% by weight) on the root growth of corn and soybean plants in Minnesota soil and sand was investigated. The dry root biomass of the corn and soybean plants was measured at 27 days of growth. Soil pH and electrical conductivity was measured in the soil and sand samples.

Of the four trials conducted for each percentage of biochar, the corn in sand at 3% biochar resulted in the greatest dry root biomass weight. The trend for corn was an increase in root biomass with an increase in biochar up to 3.0%, followed by a decrease in root biomass with a further increase in biochar to 6.0%.

The 1.5% biochar trials in sand had the greatest dry root biomass in soybeans. The trend for soybeans was similar to corn in MN soil, but differed in sand with the peak root biomass occurring at 1.5% biochar, followed by a decline with 3.0 and 6.0% biochar to below control root biomass.

Soil and sand pH levels increased with biochar due to its basic nature. Root biomass for both soybeans and corn increased in accordance with pH up to a level of 3.0% biochar then decreased with an increase in biochar and pH levels.

As the electrical conductivity in the soil and sand increased with an increase in biochar, root biomass increased up to a biochar level of 3.0% and then decreased as the biochar level increased to 6.0% and electrical conductivity increased as well. The exception was corn in MN soil where the electrical conductivity decreased in tandem with the decrease in root biomass peaking at 3.0% biochar.

The data supports the idea that biochar does improve the root growth of corn and soybeans up to a certain concentration. It is possible that the increased pH and the increased amount of ions (as measured by electrical conductivity) available in the soil and sand due to the presence of biochar benefited the plants with increased uptake of nutrients, better water retention and increased microbial activity. However, at a certain point, the amount of biochar appears to have become a negative factor on plant growth. This could be a result of too many ions, increasing basicity of the sand or soil, too much retention of nutrients by biochar or potentially toxic ions or microbes present in biochar.

I would recommend repeating this experiment with a greater number of samples to increase the statistical strength of the results, as well as increasing the grow time. Tests could also be conducted to determine the types of ions and microbes present in the soil and sand with biochar addition.

## **Purpose**

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Biochar is a type of soil amendment that scientists are investigating today. It is made from biomass that is burned in an enclosed environment with little or no oxygen. This carbon rich product is a type of charcoal, which is being used to improve soil conditions and properties, and as a carbon sequester.

Corn and soybeans are key crops in the Mid-West. Scientists have been studying the effect of biochar with corn and some soybean plants, however their studies have concentrated on leaf and stem growth and not the root growth of the plant. A positive effect of biochar has been noted on leaf and stem growth, and a similar finding for root growth would be expected. Studying corn and soybean plants will also offer a look at how biochar affects two different root systems, tap root and fibrous.

Because data supports the idea that biochar improves the quality of soil conditions and since sand seems to be lacking in important microbes and does not retain water well, biochar may be able to improve its quality allowing farmers to plant crops on some sandy soil. Soil is also being used in this experiment to see if biochar can improve on its quality as well.

## **Hypothesis**

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If the addition of biochar to corn and soybeans in MN soil and sand will result in an increase in the root biomass of the plants in proportion to the percent increase of biochar, then 3% biochar will have more of the increase as 1.5% biochar because the more biochar in the soil, the more absorption of water, microbes, and other nutrients in the MN soil and sand in which would increase the plants growth.

If the plants are grown in the sand, then their root biomass will remain lower than that of the plants grown in MN soil with or without biochar because the MN soil contains more nutrients and microorganisms than sand.

## Background Research

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Biochar is an ancient idea used in crop production that has only been recently rediscovered. Biochar was uncovered in the Amazon just this past century, and only in the 1990's was it declared by scientists to be manmade. Scientists determined that beginning around 450 A.D., natives in the Amazon Basin were using a method of burning organic materials in covered pits with little oxygen supply, similar to pyrolysis. Unfortunately the knowledge of biochar died with the natives following the arrival of the Europeans and disease with them.

Biochar is made through a process called pyrolysis. Feedstock, which is a form of biomass, is heated with little or no oxygen to temperatures of 350° C to 700° C (662° F- 1,292° F). The type of biochar made depends on two variables: the type of biomass being used and the temperature and speed at which it is being heated. For example, a higher, faster heating of the biomass (fast pyrolysis) will result in biochar with less microorganisms, smaller pore size, and more liquid and gas components. Biochar made at a slower and lower temperature (slow pyrolysis) will have more microorganisms, a bigger pore size, and more solids, thus more biochar produced. Biochar can be made by people using homemade pyrolysis devices however it is also being made on a large-scale basis by industries.

Biochar contains pores that can house microorganisms (i.e. like a sponge). Soil microbial communities can change upon the addition of organic matter, in this case, biochar. It is thought that the pores in biochar could protect beneficial microorganisms from their predators, therefore allowing those good microorganisms to live longer, increase reproduction, and help the plant grow better. However, biochar could also house bacteria or toxins harmful to the plant, which could then have a negative result. Pore size can promote a positive environment for different microbes. The overall effect of biochar may be dependent on what the specific microbes are in the soil or sand and which kind of plant is beginning treated. An idea that is being studied right now is that biochar may be effective as an inoculate carrier for microorganisms that can destroy or neutralize toxins in the ground.

Biochar can retain water and important nutrients in the soil, so there is less loss of nutrients and more uptake by plants, including nitrogen. The smaller the pores on biochar, the longer they can retain capillary soil water. It has been shown to remove soil constraints that limit plant growth, and neutralize acidic soil due to its basic nature. Carbon dioxide and oxygen occupy the air-filled spaces on pores of biochar or can be chemisorbed onto the surface. Since biochar can hold nutrients, microorganisms, bacteria, and syngases, it may also be able to hold fertilizer in the soil longer than other soils, and prevent it from leaching into water sources, such as rivers, and lakes. Another major reason that biochar helps the environment is that it

is a carbon sink, in other words it can capture carbon that has been released into the atmosphere in the ground, therefore helping to prevent global warming. However, one big question to be answered is whether biochar will stay stable. If not, while its use as a soil amendment may still be beneficial, it would not be a practical carbon sink.

A question that is being discussed about biochar is where and how it should be mixed into the soil. It may be better to put it underground to collect leaching fertilizers or methane. It could also be put on the top part of the soil to provide nutrients for the plants, however it could also cause a negative effect by absorbing the nutrients the plant needs instead of provide it. Though it has many benefits, biochar may sometimes do too good of a job, by reducing the level of pesticides that are applied to crops because of its ability to absorb those nutrients.

Corn and soybeans are key crops in the Mid-West. Scientists have been studying the effect of biochar with corn and some soybean plants, however their studies have concentrated on leaf and stem growth and not the root growth of the plant. A positive effect of biochar has been noted on leaf and stem growth, and a similar finding for root growth would be expected. Studying corn and soybean plants will also offer a look at how biochar affects two different root systems.

Corn is classified as a monocot and has a fibrous root system, that is many roots with a numerous amount of smaller root hairs on it, spread out that takes up the nutrients and moisture for the plant. The root growth of corn starts off with a seminal, or seed root system, where the first root comes from the seed. While this root anchors the plant and provides water, the nutrients come from the corn seed during this process. The second root that comes from the seed is called the nodal, or crown root system. The nodal starts to form at the crown part of the stem and is near the ground when the new seedling sprouts. From this point the nodal seed takes over for the seminal seed and gets the water and nutrients for the plant.

The soybean seed, unlike the corn seed, is classified as a dicot with one main root called the taproot. The root hairs on the taproot collect the key nutrients and are the main water absorber for the soybean plant. Over their lifetime, these plants develop root nodules that supply the plant with nitrogen, however in order to make these nodules the plant needs mycorrhizal fungi from the soil. This is a characteristic of soybeans that is different from corn: the need for a microbe to help it grow more effectively. Nodules are created by the infection of the root hairs by the fungi. Studies show that biochar may be able to protect the sensitive fungi.

Seeds treated with chemical fertilizer are becoming commonly used in the Midwest. Should biochar display a positive effect on root growth it could decrease or eliminate the need to use treated seeds in the future. Biochar could be used instead of fertilizer on the seeds to make them grow better. This project will be using treated seeds to be as close to what farmers use today.

Data supports the idea that biochar improves the quality of soil conditions. Since sand seems to be lacking in important microbes and does not retain water well, biochar may be able to improve its quality allowing farmers to plant crops in some sandy soil. The effect of biochar on sand will be investigated and compared with MN soil in this experiment to see if biochar can improve its quality as well as that of soil.

## Materials

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Washed Sand 0.6kg per pot, 38.4kg in all  
Minnesota Soil (taken from a soybean field, Brown County, MN) 0.4kg per pot (12.8kg in all)  
64 pots 9.5x8.6 cm  
6 Grow lights (123.19x10.16cm) with 12, 40-watt multi-use Do It bulbs  
32 201-16VT3 Channel Corn seeds (treated)  
32 2300R2 Channel Soybean seeds (treated)  
Wood biochar  
Water  
Heavy duty tin foil  
Thermometer - La Crosse Technology  
Measuring tape  
Clock - La Crosse Technology  
Notebook, and pencil  
11 black, Plastic trays 53.98x26.67cm  
A 241.3x74.93cm table, and 85.73x85.73cm table  
A Konnie scale (0-500g) and a small kitchen scale (0-25g)  
String  
Labels  
Markers of different colors  
Thermo Scientific, MaxQ2506 - reciprocating shaker  
Accumet Basic 4B15 pH Meter by Fisher Scientific- pH Meter  
Thermo Scientific, Orion 3 Star Conductivity Benchtop Conductivity meter  
Symmetry, Cole-Parmer, PA 220 220x0.0001g, Fisher Scientific- desiccator  
Fisher Scientific Isotemp Oven Drying oven  
64 metal boats  
Squeeze bottle  
Graduated cylinder  
Analytical balance (scale)  
Measuring cup

## Method

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Two tables were set up in a basement room to place the samples on. Six grow lights were hung side-by-side at a height of 15.4cm above the rim of the pots (Figure 1.7). Two heaters were placed on the floor on either side of the room. Eleven black liner trays were set up on the tables according to Figure 7. Pots with similar percentages of biochar were placed close together to ensure decreased variability in light between samples. 64 pots were set up and labeled in 16 treatment groups. The pots were filled with 400 grams Minnesota soil (MN soil) or 600 grams sand according to their label. Each set of four samples was emptied into a sterile bowl and the correct percentage of biochar was added. Biochar was measured as a percentage of total dry weight of sand or soil. Since the MN soil was dry and the sand was relatively wet, 200mL of water was added to the soil mixtures to make sure that there was not an experimental advantage given to the sand mixtures. The bowl was washed between mixing different trials so that none of the microbial bacteria in the MN soil could mix with the sand and therefore change the outcome of the experiment. Since the sand was still considerably wet after mixing in the biochar it was left over night, along with the MN soil, with the heaters on to try to dry it out a bit. The seeds were planted 3.8cm deep, covered over with the soil or sand, and 25mL of water was applied over the soil and sand (gloves were worn when handling the treated seeds). A temperature of 18.4-23.9 C (65-75°F) was maintained, with the heaters being turned off at the same time as the grow lights to simulate nighttime cooling in the summer. A thermometer was placed in the room to monitor the temperature throughout the experiment. The grow lights were turned on for 16 hours and off for 8 hours to simulate the daytime hours in the growing season for corn and soybean crops. The height of the grow lights was adjusted from 15.4cm to 22.86cm (day 18) to 33.02cm (day 21) to 40.64cm (day 24) while the plants grew. The plants were watered every other day (25mL/pot) from day 1. The plants grew for 27 days. When the growing period was over the plants were carefully removed from the pots and shaken gently to dispose of the majority of sand and soil. The roots of the plants were dipped in water to further remove the sand and soil residue on the roots, and were pressed gently with a paper towel to dry. A ½ cup of the 16 sand and soil treatments (including plain MN soil) were collected at this time for analysis at the lab. The plants were laid on a paper towel in a warm room at 29.5 C for 12 hours to encourage drying without any mold prior to analysis at the lab.

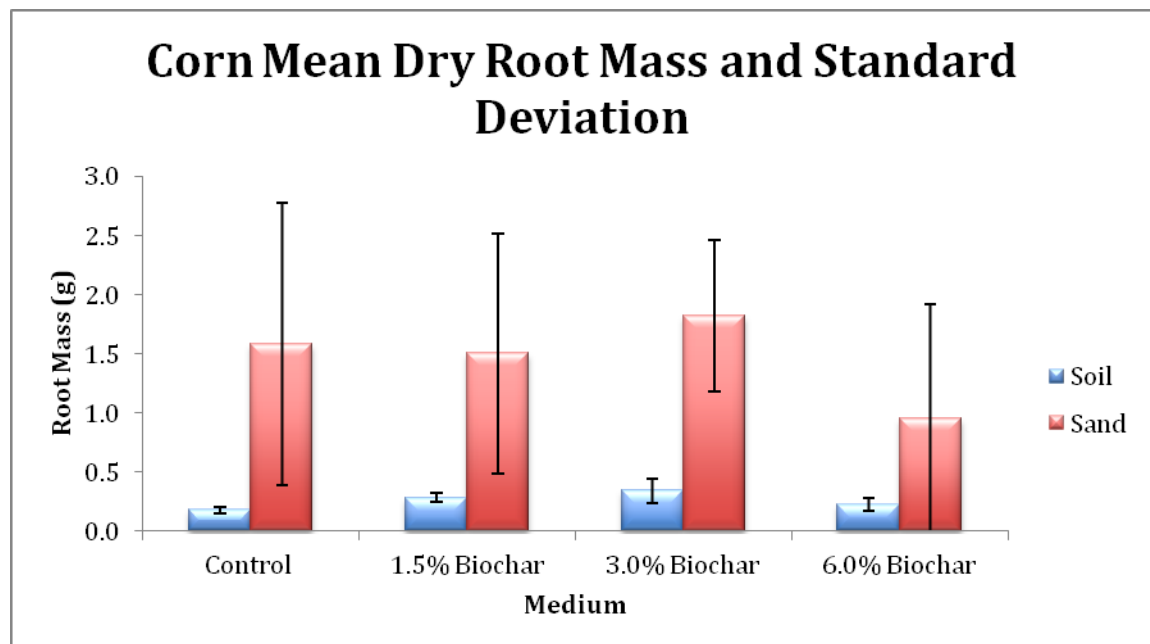
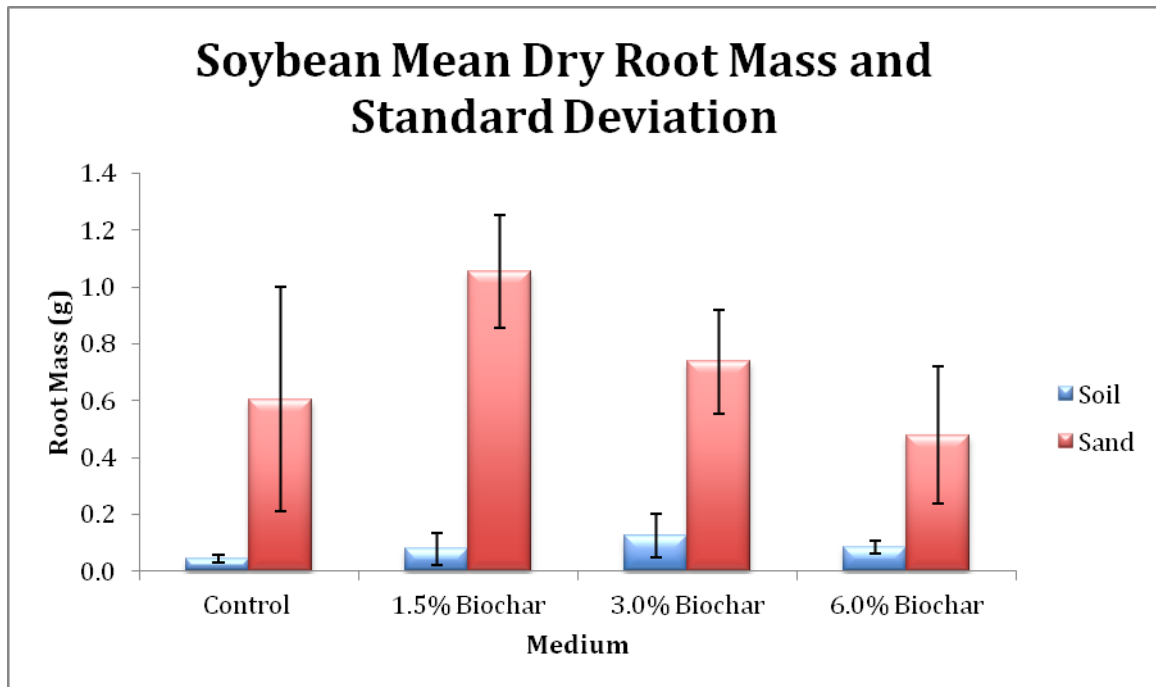
The samples were transported to the Biorenewables Research Lab at Iowa State University for analysis. Lab coats, safety glasses and latex gloves were worn at all times. A review of lab safety protocols was conducted prior to beginning work in the lab. Sixty-four metal boats were labeled and weighed on a balance (scale) to

determine their weight. The root samples were rinsed in a beaker full of distilled water, and squirted with a squeeze bottle to try to get off any remaining excess soil or sand. The roots were then shook gently to get off any excess water and were cut with a pair of scissors. The corn roots were cut off 3mm above the brace roots, while the soybean roots were cut off 3mm above the lateral roots and under the hypocotyl on the stem (Figures 5.2, 8 and 8.1). These roots were placed on the boats with their identification number on the boat tab. The roots on the boats were weighed for the wet weight (full root mass). This weight was recorded to ensure that the dry root mass of the roots would be correct. (i.e. the wet root mass should be higher than the dry root mass). As soon as the wet root mass was taken, the roots were put into a preheated drying oven at 105°C and were left overnight (14 hours). The roots were taken out of the oven and put into a desiccator to ensure that they did not collect moisture while they cooled off. Once the plant roots cooled, they were measured on the balance for the final dry root mass. This weight was subtracted from the boat's mass to get the dry root mass.

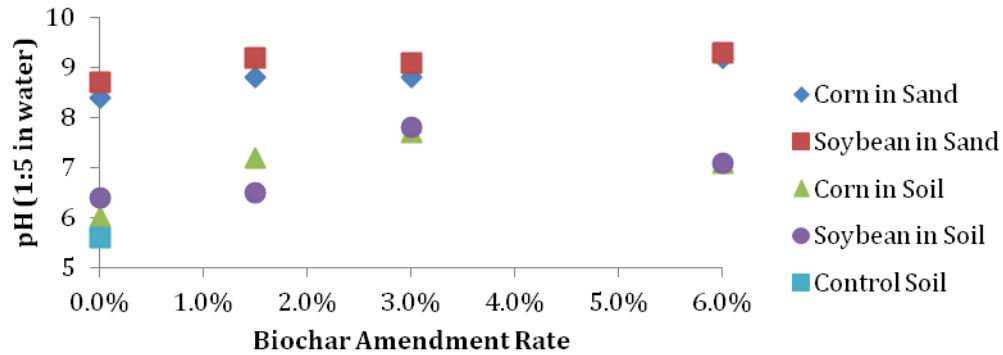
Three grams of the seventeen soil and sand samples were put into pre-weighed vials with a silver spatula. Fifteen milliliters of water was added to the 3 grams of soil or sand since the instruments that measured the pH and electric conductivity needed a water-based solution. Screw-on lids were put on top of the vials. The vials were put onto a reciprocating machine for half an hour to mix the water and soil or sand mixture together. They were then allowed to settle for three hours. When the soil and sand samples were done settling, they were measured with the pH meter and electrical conductivity meter for pH and soil and sand conductivity. The data numbers were recorded and analyzed. Lab glassware and instruments were cleaned and put away.



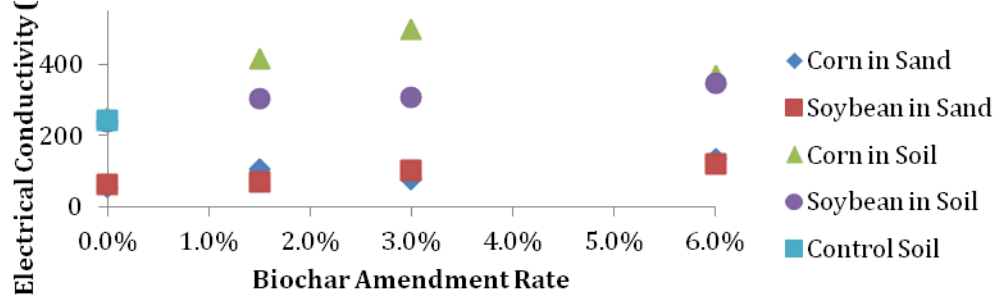
## Data

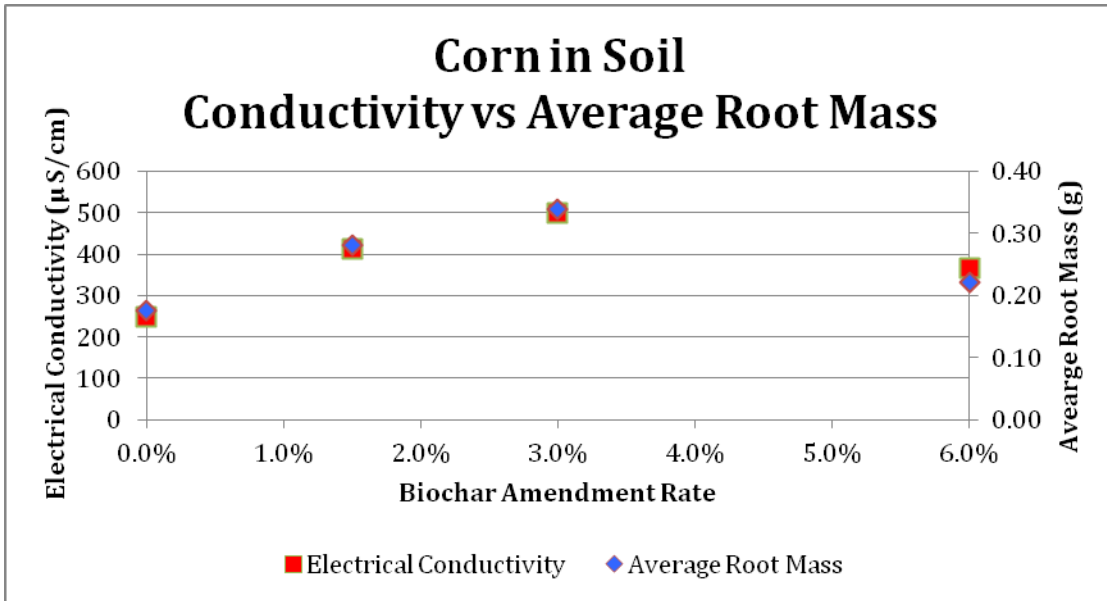
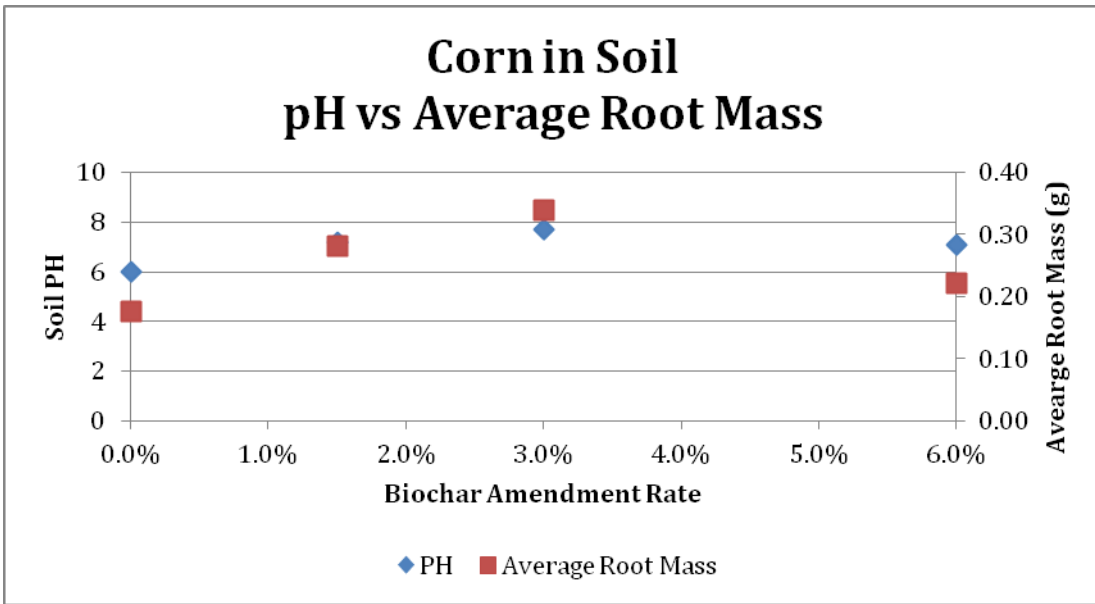


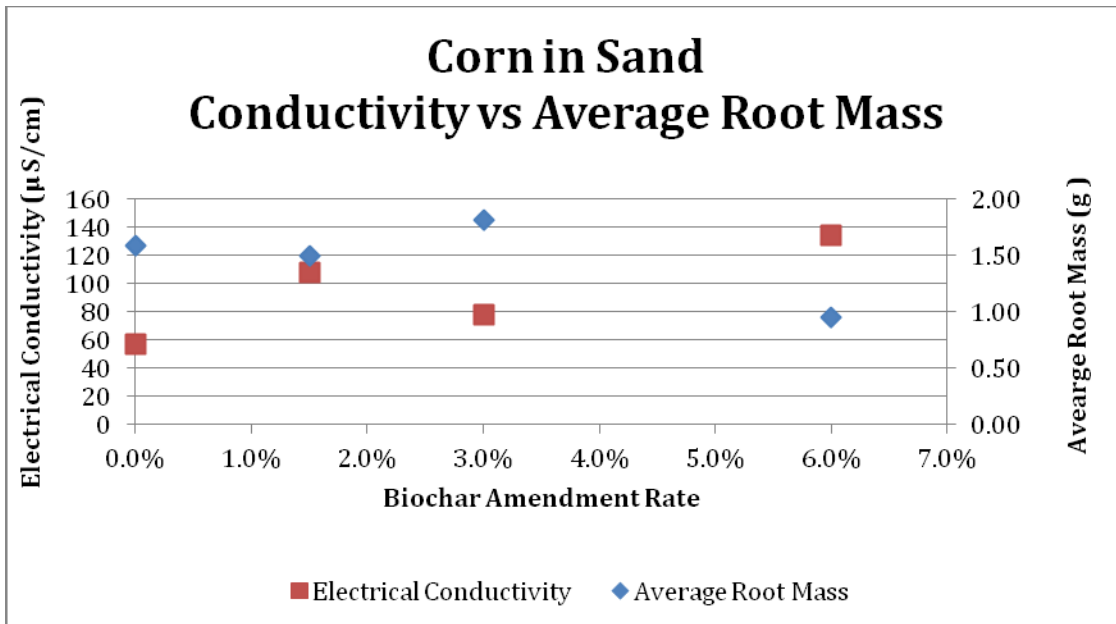
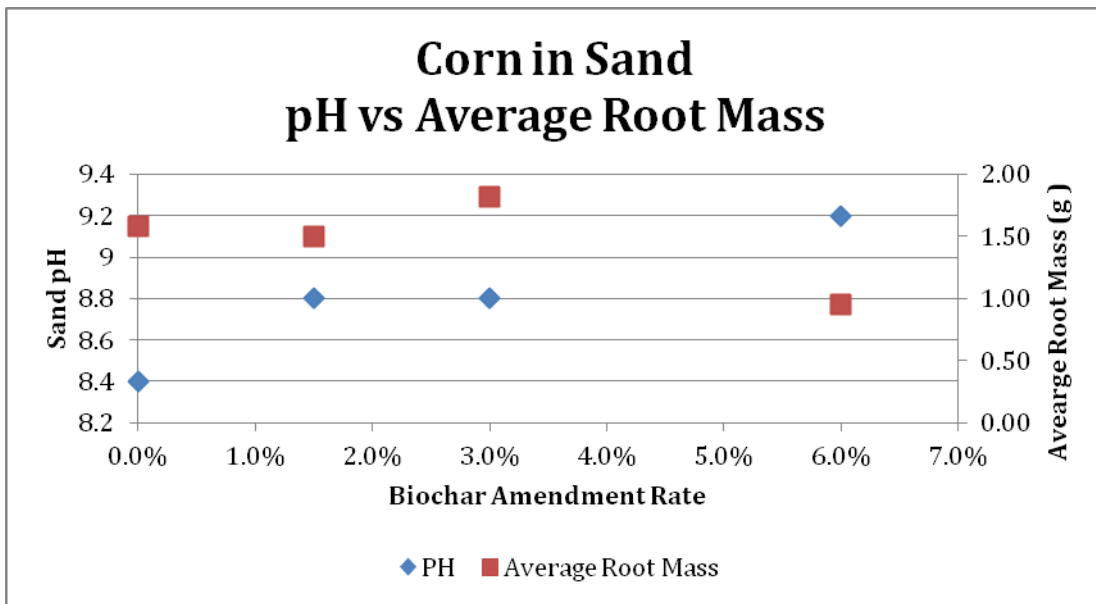
## Overall pH of Sand and Soil Samples with Biochar Amendment

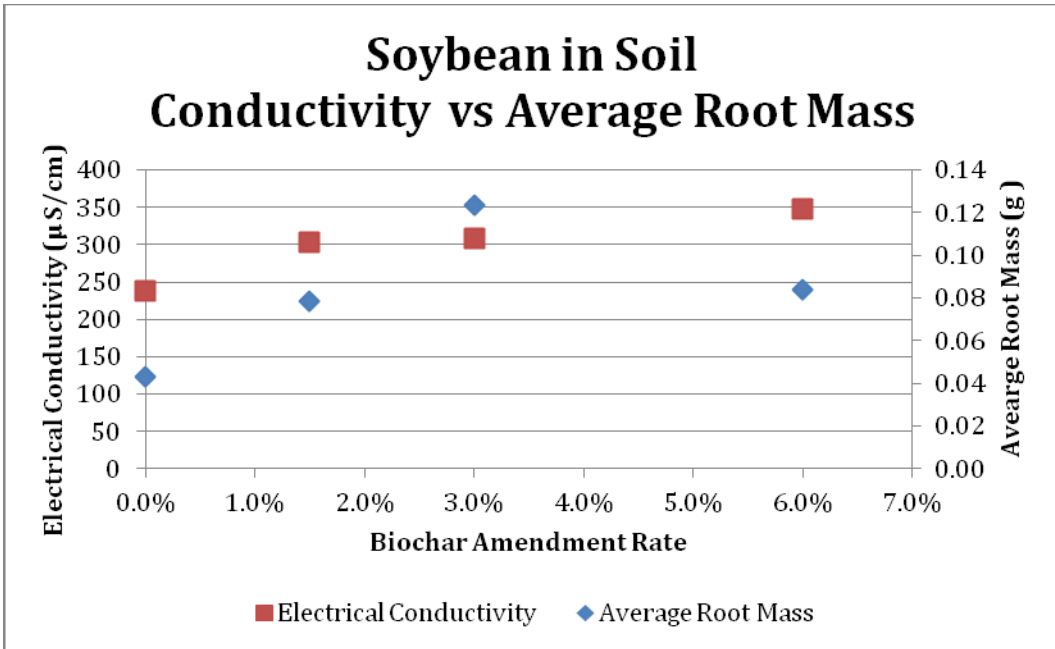
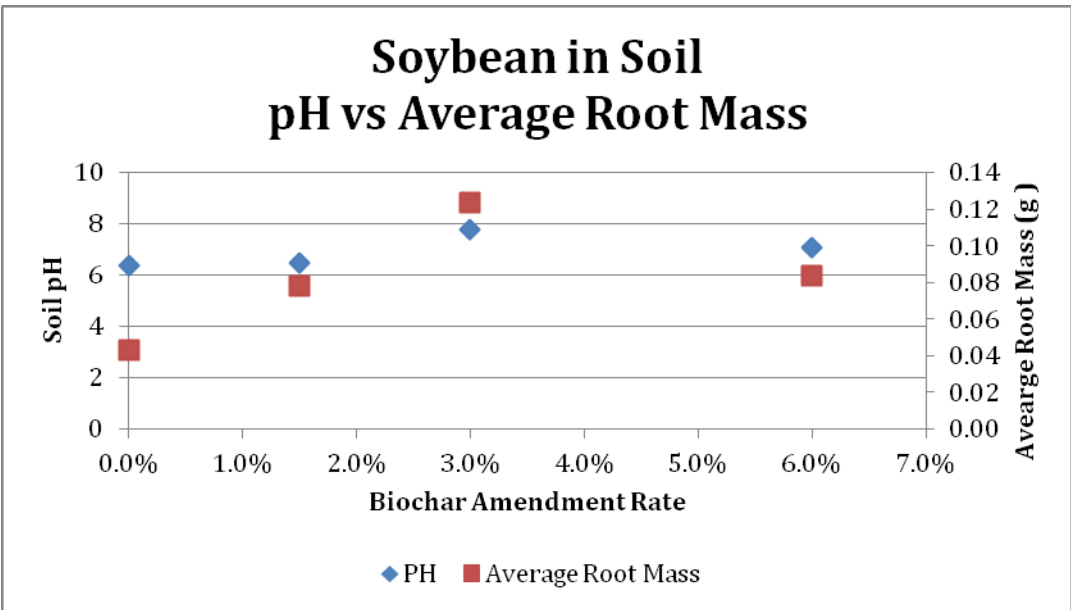


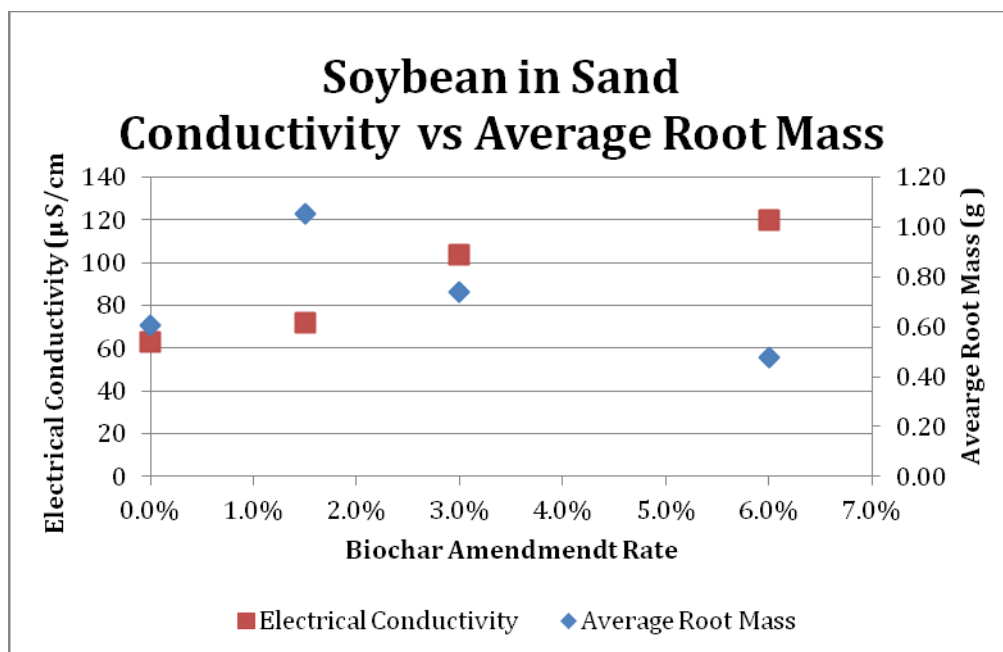
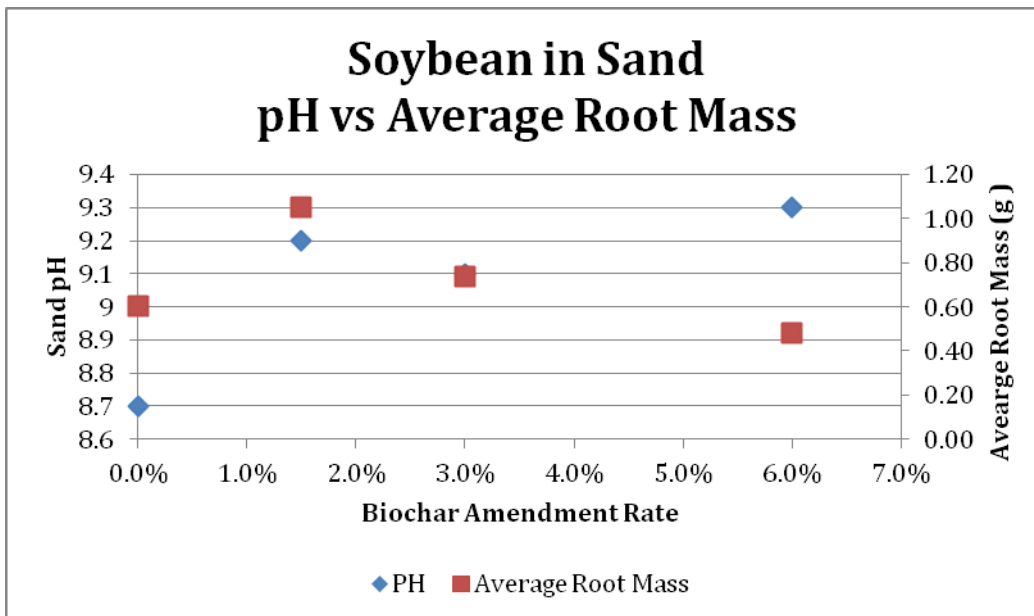
## Overall Electrical Conductivity of Sand and Soil Samples with Biochar Amendment











## Results

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Dry root mass of **soybeans** in **sand** increased in 1.5% biochar as compared to control and decreased with an increase in biochar to 3.0%. Root mass decreased to below control level with an increase in biochar to 6.0%. The pH of soybeans in sand increases to 9.2 at maximum root growth at 1.5% biochar. As the percentage of biochar increases, pH also slightly increases and root mass decreases. The EC of soybeans in sand increases with an increase in percent biochar.

The dry root mass of **soybeans** in **MN Soil** increased to a maximum weight with 3.0% biochar and then decreased as biochar increased to 6.0%, however all the biochar results were still greater than the control. The pH of soybeans in MN Soil increases until it reaches a critical point of 7.8 at 3.0% biochar and maximum root growth. The EC of soybeans in MN soil increases with an increase in percent biochar.

The dry root mass of **soybeans** was at its highest in 1.5% biochar in sand and lowest in MN Soil control with all of the sand trials being higher than all of the MN Soils for all other biochar percentages.

The dry root mass of **corn** in **sand** increased up to 3.0% biochar then decreased to levels below control as the biochar levels increased to 6.0%. The pH of corn in sand gradually increases as the percentage of biochar increases, having a pH of 8.8 at maximum root mass at 3.0% biochar. Root growth then decreases as pH becomes more basic at 6.0% biochar. The EC of corn in sand increased to 107.6 $\mu$ S/cm at 1.5% biochar then decreased to 78.4 $\mu$ S/cm at 3.0% biochar then increases to 134.7 $\mu$ S/cm at 6% biochar.

Dry root mass of **corn** in **MN Soil** increased to 3.0% biochar and decreased as the biochar percentage went up, but stayed higher than the control weight. The pH of corn in MN Soil increased from 6 to 7.7 at 3.0% biochar and maximum root growth. The EC of corn in MN soil increases along with root mass until a critical point of 3.0% biochar, then decreases in parallel with root mass as biochar percentage increases.

The greatest root mass for **corn** was the 3.0% biochar in sand, with the lowest being the control in MN Soil. The sand trials had larger dry root mass measurements than the MN Soil trials for corn.

The highest dry root mass of all of the trials was the 3.0% biochar in the sand and corn. The lowest was the control MN Soil soybean, with all of the corn trials weighing higher than all of the soybean trials.

## Discussion

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Of the soybean trials, the 1.5% biochar in sand had the best outcome. The physical characteristics of the sand, compared to MN soil, likely allowed the roots to grow easier. In addition, the 1.5% biochar must have retained the proper balance of nutrients, microbes and water as compared to the control, 3.0% and 6.0% biochar.

The pH affects the root growth of plants in the soil and sand by enabling certain nutrients to be soluble when in water and taken up by the plant. A slightly acidic soil of 6.0-7.0 pH is optimal. Usually the pH of a soil changes over time from leakage of certain ions. Addition of biochar affects the balance of ions making soil more basic.

Soybeans can handle a pH of 5.8-7.0. In this case, pH in the 1.5% biochar soybeans in sand trial was 9.1 pH, which is higher than the preferred levels. It's possible that the beneficial effects of biochar outweighed the negative effects of the increased pH. As biochar levels increased to 3.0% and 6.0%, root biomass decreased and pH increased. At this point, the alkalinity of the soil may have overcome the positive effect of biochar. The increased concentration of biochar also could have resulted in the over absorption by biochar of too many important elements the plant needed in order to grow.

Of the corn trials, 3.0% biochar in sand had the greatest biomass. Its pH level was 8.8 and soil conductivity 78.4 $\mu$ S/cm. The pH levels at which corn can grow well are 6.0-6.5. The control pH of corn in sand was 8.4, with a conductivity of 57.5 $\mu$ S/cm. Electrical conductivity (EC) is a measurement of how many ions are dissolved into a water solution. It would make sense that the soil conductivity went up because biochar contains certain salts that will dissolve in the water to form ions. The instrument used to measure conductivity only shows how the concentration of ions changes, but does not specify if those ions are harmful, neutral, or helpful. Consequently, the higher conductivity of some of the ions could have been more harmful to the plants than helpful. The 3.0% biochar in sand for corn must have contained the optimal amount of ions as compared to the 1.5% biochar and 6.0% biochar. pH levels were the highest in the 6.0% biochar in sand for corn and as a result some important nutrients may not have been soluble for the roots to take up resulting in lower root biomass. EC was also the greatest in the 6.0% biochar sample of the sand and corn trials, and the root biomass the lowest as a possible result of the increased pH and EC.

The 3.0% biochar in MN soil for corn had the highest dry root mass out of all of the MN soil trials and a pH of 7.7 and EC of 499. As in earlier discussions regarding pH and EC, a pH of 7.7 is the best pH for corn in soil and the higher EC in the sample indicates a larger amount of ions in the soil, which appear helpful since the results reflect improved root growth. However, as the sand still had the greater



root mass but not better pH or EC, this may be attributed to its physical characteristics.

In general, my data did not support my hypothesis because the plants grown in the sand had a better outcome than those grown in MN soil. It seems that the physical characteristics of the soil such as hardness, compaction, and water retention may have been more important than any increase in nutrients the soil might have had over the sand. Biochar is known to help improve soil and sand quality, however this process takes time. For example, over time biochar is supposed to help break down the soil and make an easier path for the roots to grow through which can allow better uptake of water and nutrients. The roots may have grown more in sand than MN soil because the sand offered less resistance to root growth than soil even though the soil may have had more nutrients and microbes living in it. There also might have been a difference in the water absorption of the sand compared to the soil, therefore allowing better drainage for plants in the sand. For example, the soil could have held more water therefore the roots did not need to grow farther down into the soil because they already had all the water at the top of the soil. The sand however, had more of the water drain to the bottom of the pot so the roots would have to grow down more in order to get to the water. If the soil had been finer, the roots may have grown easier, allowing the nutrients and microbes in the soil to be absorbed better, changing the outcome of this experiment. Of note, it appears that the MN soil contained more ions and had a more favorable pH than sand (248.9 vs. 57.5 $\mu$ S/cm; 6 vs. 8.4 for corn) supporting my above discussion that the difference in root mass between sand and soil can largely be attributed to physical characteristics of the soil and sand and not nutrient and microbial content.

A longer growing period could have resulted in greater differences noted in pH and EC as the plants utilized all the resources in the surrounding soil and sand to grow, thus showing how biochar may have been able to provide additional nutrients over time. A larger number of samples could have decreased the standard deviation of the samples and given more weight to the data. Using finer soil or mixing the MN soil to decrease the compaction and hardness may also change future trials results. The fact that the grow lights were raised to accommodate the taller plants could have biased the light received by the smaller plants. Interestingly, a number of the taller plants had lower root biomass, possibly because they used all of their energy to get the sunlight from the grow lights. Uneven distribution of the biochar chips within the soil may have also resulted in some of the plant seeds sprouting later than the majority of the others. This could have been a result of the biochar being near the seed and absorbing some of its stored nutrients and water it needed to start sprouting. As well, it could have taken some time for plant roots to get to some of these pieces of beneficial biochar in the soil. To solve this problem, powdered biochar could be used to permeate the soil more evenly.

## **Conclusion**

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My experiment appears to support the hypothesis that the addition of 3.0 % biochar by weight to corn and soybeans results in an optimal increase in root biomass. The biochar provided nutrients and ions, retained water, housed microorganisms, and controlled pH in such a way as to provide an environment supportive of root growth. Positive root growth declined however as levels of biochar reached 6.0%. It is possible that at these levels the amount of biochar resulted in a net negative effect on root growth due to increased pH, increased EC (of negative ions) and over absorption of beneficial nutrients and ions by biochar.

Future experiments could investigate the effect of powdered biochar on the growth of untreated seeds for a longer period of time of about 40 days. Biochar could be compared to fertilizer addition to evaluate whether biochar amendment helps the seed grow as well as fertilizer, thus giving farmers an ecofriendly option instead of fertilizer. Since biochar improves soil conditions immensely over time, earlier application of biochar to the soil could be done prior to planting to see if a greater improvement is observed.

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<u>ID#</u>	<u>Group</u>	<u>Plant Type</u>	<u>Base Soil</u>	<u>Group Name</u>	<u>Empty(g)</u>	<u>Full(g)</u>	<u>Dry(g)</u>	<u>Dry Rootmass(g)</u>	<u>Full Rootmass(g)</u>
1	1	Corn	soil	Control MN soil Corn	1.0024	1.2	1.1539	0.1515	0.1976
2	1	Corn	soil	Control MN soil Corn	1.0246	1.3229	1.2092	0.1846	0.2983
3	1	Corn	soil	Control MN soil Corn	1.0165	1.2885	1.2301	0.2136	0.272
4	1	Corn	soil	Control MN soil Corn	1.0183	1.2302	1.1761	0.1578	0.2119
5	2	Soya	soil	Control MN soil Soya	1.0097	1.0384	1.0372	0.0275	0.0287
6	2	Soya	soil	Control MN soil Soya	1.0077	1.052	1.0511	0.0434	0.0443
7	2	Soya	soil	Control MN soil Soya	1.0232	1.0727	1.0652	0.042	0.0495
8	2	Soya	soil	Control MN soil Soya	1.013	1.0757	1.0729	0.0599	0.0627
9	3	Corn	Sand	Control Sand Corn	1.0112	5.3037	3.671	2.6598	4.2925
10	3	Corn	Sand	Control Sand Corn	1.0233	4.9934	3.5998	2.5765	3.9701
11	3	Corn	Sand	Control Sand Corn	1.0264	1.8941	1.5392	0.5128	0.8677
12	3	Corn	Sand	Control Sand Corn	1.0018	2.0525	1.5923	0.5905	1.0507
13	4	Soya	Sand	Control Sand Soya	0.9649	2.1406	1.3193	0.3544	1.1757
14	4	Soya	Sand	Control Sand Soya	1.0076	1.9382	1.3278	0.3202	0.9306
15	4	Soya	Sand	Control Sand Soya	1.0046	2.5489	1.5744	0.5698	1.5443
16	4	Soya	Sand	Control Sand Soya	1.0223	3.0662	2.1968	1.1745	2.0439
17	5	Corn	soil	1.5% Biochar MN soil Corn	1.0068	1.9584	1.2883	0.2815	0.9516
18	5	Corn	soil	1.5% Biochar MN soil Corn	1.0091	2.137	1.312	0.3029	1.1279
19	5	Corn	soil	1.5% Biochar MN soil Corn	1.0178	2.1281	1.3313	0.3135	1.1103
20	5	Corn	soil	1.5% Biochar MN soil Corn	0.9948	1.771	1.2249	0.2301	0.7762
21	6	Soya	soil	1.5% Biochar MN soil Soya	1.0246	1.7578	1.1555	0.1309	0.7332
22	6	Soya	soil	1.5% Biochar MN soil Soya	0.9954	1.5763	1.0991	0.1037	0.5809
23	6	Soya	soil	1.5% Biochar MN soil Soya	1.0052	1.3416	1.0839	0.0787	0.3364
24	6	Soya	soil	1.5% Biochar MN soil Soya	0	0	0	0	0
25	7	Corn	Sand	1.5% Biochar Sand Corn	1.015	4.935	3.1964	2.1814	3.92
26	7	Corn	Sand	1.5% Biochar Sand Corn	0.9995	4.5961	3.0704	2.0709	3.5966
27	7	Corn	Sand	1.5% Biochar Sand Corn	0.9928	4.2073	2.7472	1.7544	3.2145
28	7	Corn	Sand	1.5% Biochar Sand Corn	0	0	0	0	0
29	8	Soya	Sand	1.5% Biochar Sand Soya	1.0316	3.7839	2.3766	1.345	2.7523
30	8	Soya	Sand	1.5% Biochar Sand Soya	1.0005	3.5871	1.9632	0.9627	2.5866
31	8	Soya	Sand	1.5% Biochar Sand Soya	1.0047	3.043	1.902	0.8973	2.0383

32	8	Soya	Sand	1.5% Biochar Sand Soya	0.9944	3.2566	2.0087	1.0143	2.2622
33	9	Corn	soil	3.0% Biochar MN soil Corn	0.9754	2.4161	1.3427	0.3673	1.4407
34	9	Corn	soil	3.0% Biochar MN soil Corn	0.9843	1.9633	1.3483	0.364	0.979
35	9	Corn	soil	3.0% Biochar MN soil Corn	0.9969	2.3537	1.4346	0.4377	1.3568
36	9	Corn	soil	3.0% Biochar MN soil Corn	1.0054	1.3738	1.1959	0.1905	0.3684
37	10	Soya	soil	3.0% Biochar MN soil Soya	1.0145	1.844	1.1756	0.1611	0.8295
38	10	Soya	soil	3.0% Biochar MN soil Soya	0.9997	1.1083	1.017	0.0173	0.1086
39	10	Soya	soil	3.0% Biochar MN soil Soya	1.0297	1.6004	1.2254	0.1957	0.5707
40	10	Soya	soil	3.0% Biochar MN soil Soya	0.9725	1.146	1.0921	0.1196	0.1735
41	11	Corn	Sand	3.0% Biochar Sand Corn	0.9799	3.3625	2.6211	1.6412	2.3826
42	11	Corn	Sand	3.0% Biochar Sand Corn	1.022	3.7457	2.9411	1.9191	2.7237
43	11	Corn	Sand	3.0% Biochar Sand Corn	1.0121	5.1883	3.6461	2.634	4.1762
44	11	Corn	Sand	3.0% Biochar Sand Corn	1.0541	2.8622	2.1448	1.0907	1.8081
45	12	Soya	Sand	3.0% Biochar Sand Soya	0.9893	3.193	1.8908	0.9015	2.2037
46	12	Soya	Sand	3.0% Biochar Sand Soya	1.0377	2.5921	1.6798	0.6421	1.5544
47	12	Soya	Sand	3.0% Biochar Sand Soya	0.991	3.1032	1.8726	0.8816	2.1122
48	12	Soya	Sand	3.0% Biochar Sand Soya	1.0101	2.5392	1.539	0.5289	1.5291
49	13	Corn	soil	6.0% Biochar MN soil Corn	1.0133	2.0721	1.2599	0.2466	1.0588
50	13	Corn	soil	6.0% Biochar MN soil Corn	0.9937	1.6213	1.2103	0.2166	0.6276
51	13	Corn	soil	6.0% Biochar MN soil Corn	1.0005	1.7365	1.2742	0.2737	0.736
52	13	Corn	soil	6.0% Biochar MN soil Corn	0.9895	1.2866	1.1389	0.1494	0.2971
53	14	Soya	soil	6.0% Biochar MN soil Soya	1.0039	1.801	1.1115	0.1076	0.7971
54	14	Soya	soil	6.0% Biochar MN soil Soya	0.9963	1.3457	1.0805	0.0842	0.3494
55	14	Soya	soil	6.0% Biochar MN soil Soya	25.5922	25.9712	25.6817	0.0895	0.379
56	14	Soya	soil	6.0% Biochar MN soil Soya	25.095	25.3161	25.1485	0.0535	0.2211
57	15	Corn	Sand	6.0% Biochar Sand Corn	26.0846	26.5253	26.3088	0.2242	0.4407
58	15	Corn	Sand	6.0% Biochar Sand Corn	25.4467	26.0556	25.8326	0.3859	0.6089
59	15	Corn	Sand	6.0% Biochar Sand Corn	31.2783	34.9604	33.6322	2.3539	3.6821
60	15	Corn	Sand	6.0% Biochar Sand Corn	28.9221	30.182	29.7484	0.8263	1.2599
61	16	Soya	Sand	6.0% Biochar Sand Soya	33.35	34.5914	33.6267	0.2767	1.2414
62	16	Soya	Sand	6.0% Biochar Sand Soya	33.5122	35.2718	34.1588	0.6466	1.7596
63	16	Soya	Sand	6.0% Biochar Sand Soya	25.629	26.5489	25.898	0.269	0.9199
64	16	Soya	Sand	6.0% Biochar Sand Soya	30.9452	32.6153	31.6683	0.7231	1.6701

## Group Legend

- Control MN soil
- 1 Corn
- Control MN soil
- 2 Soya
- 3 Control Sand Corn
- 4 Control Sand Soya
- 5 1.5% Biochar MN soil Corn
- 6 1.5% Biochar MN soil Soya
- 7 1.5% Biochar Sand Corn
- 8 1.5% Biochar Sand Soya
- 9 3.0% Biochar MN soil Corn
- 10 3.0% Biochar MN soil Soya
- 11 3.0% Biochar Sand Corn
- 12 3.0% Biochar Sand Soya
- 13 6.0% Biochar MN soil Corn
- 14 6.0% Biochar MN soil Soya
- 15 6.0% Biochar Sand Corn
- 16 6.0% Biochar Sand Soya