Evaluation of Plant Growth Promotion Effects of Coal-Processed Soil Conditioner: Phase 1

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Introduction

The global population has quadrupled in the last century from 1.8B in 1915 to 7.8B in 2020, and it is expected to be over 9.7B by 2050. The world faces the challenge of producing sufficient crops to meet growing food demand for future generations. Use of chemical fertilizers played a key role in the significant increase of crop production; however, soil health has deteriorated severely due to excessive use of chemical fertilizers and other farming practices. Sustainable crop production to meet continued population growth while maintaining soil health is an urgent societal need.

Organic fertilizers are becoming more important in sustainable agriculture thanks to their multiple advantages over chemical fertilizers. In addition to providing essential nutrients for plant growth, organic fertilizers increase soil organic matter, improve soil structure and water holding capacity, reduce soil crusting problems and erosion, maintain and improve the soil microbiome diversity. Advanced Environmental Technologies (AET, Fort Collins, CO) has developed and patented a novel organic fertilizer/soil conditioner, trademarked as “Ginate”, that is based on low grade coal. AET has described three features that distinguish Ginate from other organic fertilizers: (1) organic matter of different molecular sizes at up to 80%; (2) a diversity of indigenous microbes; and (3) a suite of trace mineral elements with no antibiotic residues. As described in this report, Colorado State University has conducted an independent validation of the Ginate product.

The goals of this project were to evaluate the effects of coal-derived soil amendments developed by AET on the growth of lettuce and pepper plants and on the chemical and biological soil properties. To perform these evaluations, the amendments were mixed with a typical agricultural soil. The growth of lettuce and pepper was monitored, and soil samples before and after treatment and plant growth were collected and analyzed. Microbiome compositional analysis of the soil was determined before and after each test.

Lettuce study

Study design

To determine the effects on plant growth of lettuce (*Lactuca sativa*), Ginate was mixed in base soil at three dosages: 4, 8, and 15 g/kg soil. The same amount of commercial organic fertilizer (GRO-WELL Proven Organics 4-lb Natural All-Purpose Food) and equivalent amount of raw coal (lignite) were applied in different treatments. Lettuce seedlings at the 2-true-leaves stage were transplanted into the treatment pots. All lettuce plants were grown at the same conditions during the test period in the greenhouse at CSU. There were 6 plants for each treatment and 1 plant per pot. Plants were harvested at the end of 5 weeks.

Plant growth

As shown in Figure 1, the lettuce plants at 2 weeks were larger in the treatment of 0.4% Ginate in comparison to the reference (soil without amendments) and to the other two treatments including the standard organic fertilizer treatment. When plants were harvested at 5 weeks, the plant fresh weight of the Ginate treatment was approximately twice that of the reference, while the raw coal treatment had similar fresh weight to the reference and fresh weight from the organic fertilizer treatment was about 50% more than the reference (Figure 2).

The test was repeated once in the greenhouse. The second test had similar results as in the first one. As shown in Figure 3, the lettuce plants were larger in the treatments of Ginate at 0.4% and 1.5% than the reference and other treatments, including the organic fertilizer treatment when plants were harvested at 5 weeks. Furthermore, the plant height, fresh weight, and dry weight of the Ginate treatment were all significantly higher than organic fertilizer treatment at different dosages, although the organic fertilizer treatments were higher than the reference which had no addition of fertilizers (Figure 4). Moreover, the application of Ginate (BGF1 and BGF2) at 1.5% showed higher effects on plant growth than those at 0.4%, respectively.

These results indicate that the application of raw coal material does not help plant growth. However, Ginate, produced from coal, had positive effects on promoting the growth of lettuce, and the enhancement is higher than that of the commercial organic fertilizer.

Figure 1. The lettuce plants in the pots of different treatments at 2 weeks after transferring. A: reference-without addition of any treatment. B: 0.4% Ginate; C: 0.4% organic fertilizer; D: 0.4% raw coal.

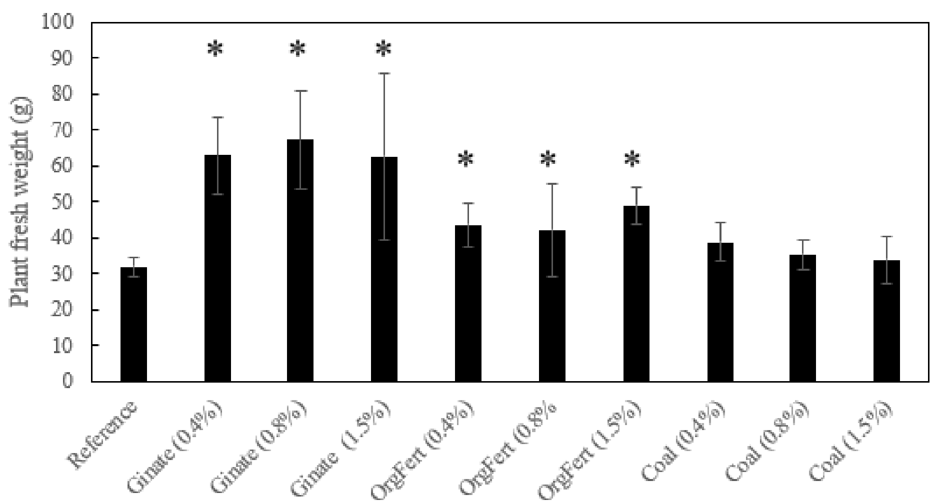

Figure 2. The plant fresh weight of lettuce with different treatments when harvested at 5 weeks after transferring. The values are the average of 6 plants of each treatment. The error bar represents the standard deviation of the 6 biological replicates. The asterisk means the statistical significance (*p<0.5*) in comparison to the reference treatment.

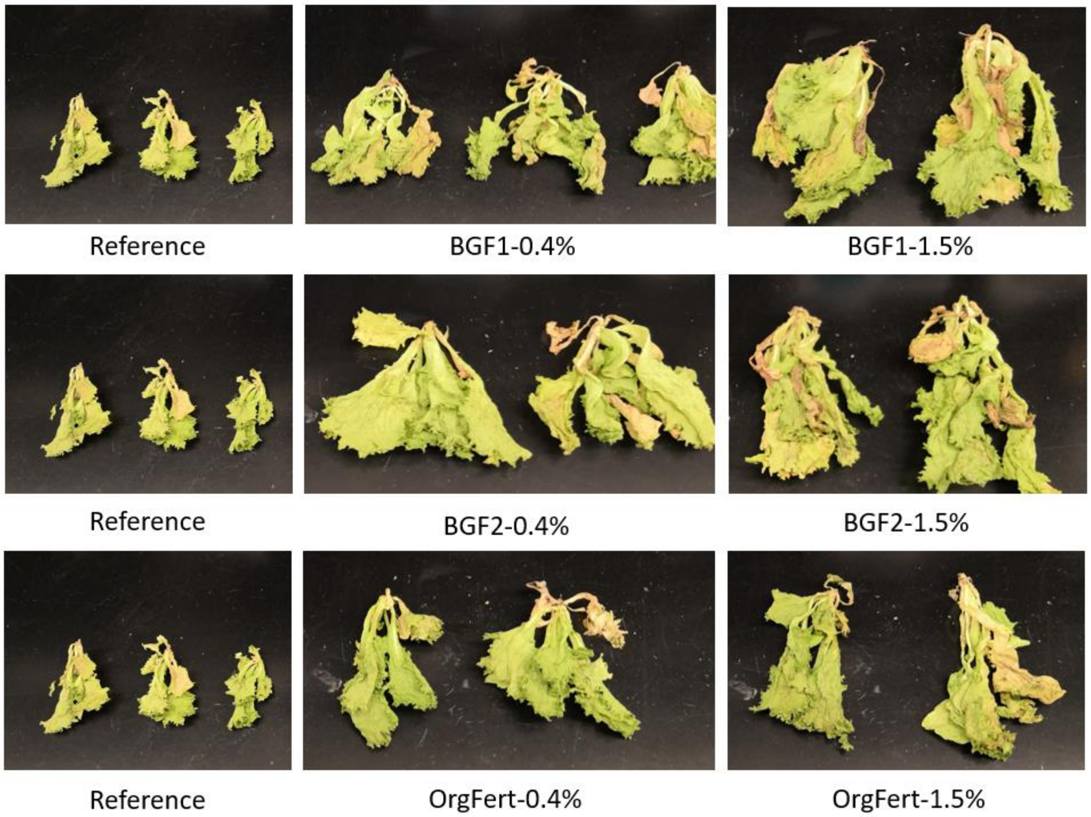

Figure 3. The dried lettuce plants of different treatments after harvested at 5 weeks.

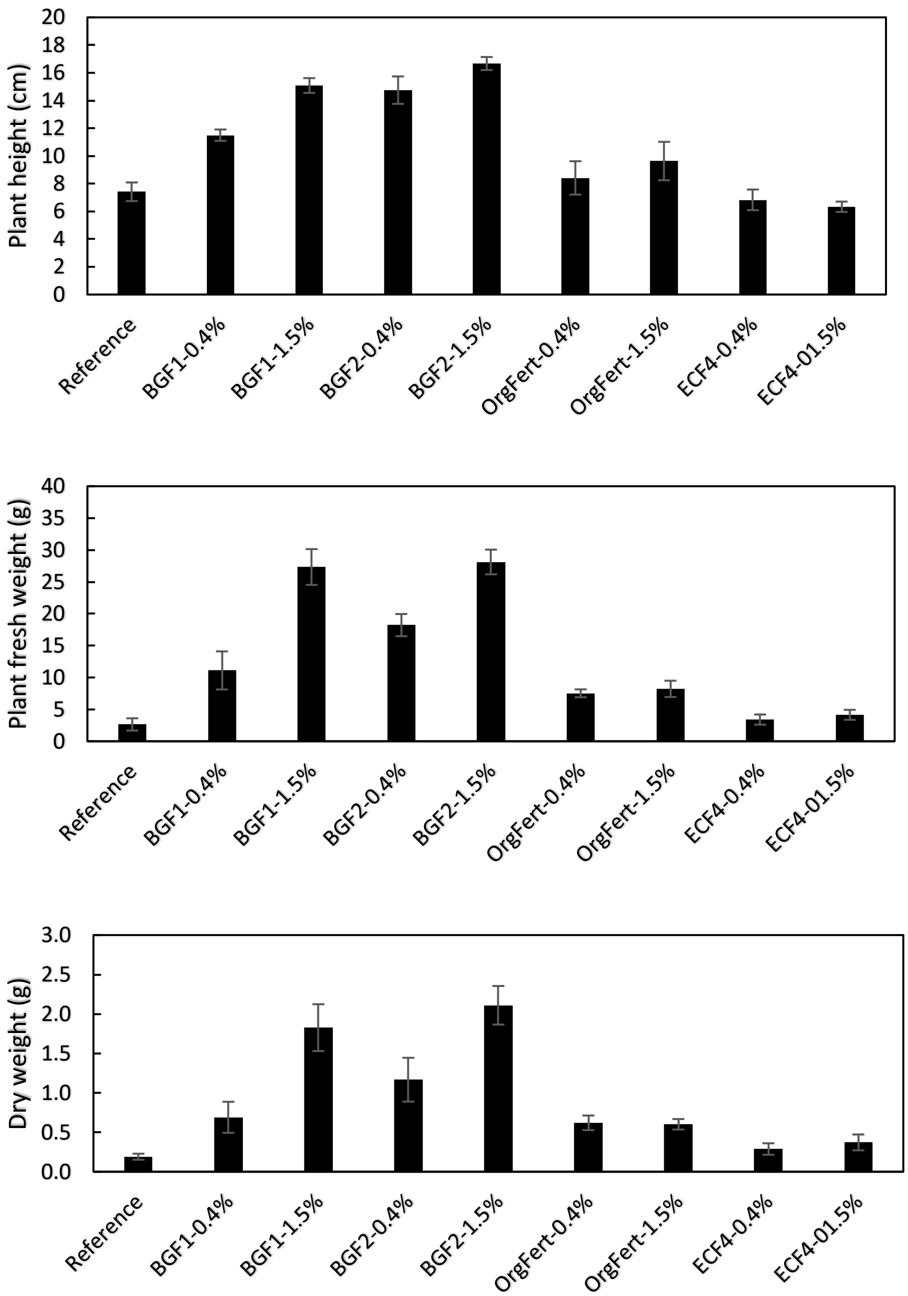

Figure 4. The plant height, fresh weight, and dry weight of lettuce plants when harvested at 5 weeks. The values are the average of 6 plants of each treatment. The error bar represents the standard deviation of the 6 biological replicates.

Soil chemical analysis

Soil samples were collected from all treatment pots to determine the influence of the treatments on key soil parameters. As shown in Table 1, the soil pH was in the range of 5.7 to 7.1 in different treatments. The pH in all the treatments was around 7 when lettuce plants were harvested. The addition of BGFs, organic fertilizer, and raw coal, especially at 1.5%, improved the soil organic matter content of the soils. After lettuce plants were harvested, the organic matter content in all the treatments was still higher than that of the reference. The NO3-N content in all the treatments were similar except organic fertilizer treatments (both 0.4% and 1.5%). There was no significant different in all treatments after harvest. The addition of BGFs, and raw coal in the soils did not significantly increase the content of phosphate and potassium while the organic fertilizer treatments showed higher phosphate and potassium in comparison to the reference. However, both phosphate and potassium contents in all the treatment were at similar level to the reference after lettuce plants were harvested. Furthermore, although the additions of raw coal resulted in higher Cu content and organic fertilizer resulted in higher Fe, Cu and Mn content, the content of Zn, Fe, Cu, and Mn was not significantly different in all these different treatments after lettuce plants were harvested.

Table 1. Soil properties before and after lettuce plants. Values represent the mean of three biological replicates and standard deviation.

	pH	EC (mmhos/cm)	OM (%)	NO3-N (ppm)	P (ppm)	K (ppm)	Zn (ppm)	Fe (ppm)	(
Before									
Reference	7.1+0.1	1.1+0.0	1.6+0.2	102.4+1.5	9.6+0.5	287.0+10.5	2.7+0.3	3.5+0.4	0
BGF2-0.4	7.0+0.0	1.3+0.1	1.8+0.1	118.1+2.7	9.9+0.3	295.4+5.6	2.8+0.2	3.2+0.0	0
BGF2-1.5	7.0+0.0	1.3+0.1	2.4+0.7	115.8+2.3	14.5+0.7	310.9+8.3	2.9+0.1	4.8+0.2	1
OrgFert1-0.4	6.0+0.1	2.8+0.2	2.1+0.0	251.4+8.3	19.1+1.3	661.2+6.7	2.7+0.2	5.2+0.1	1
OrgFert1-1.5	5.7+0.0	4.6+0.4	2.7+0.3	370.9+14.8	26.1+1.5	1320.7+24.4	2.7+0.1	5.2+0.1	24
Coal1-0.4	6.9+0.0	1.0+0.1	1.9+0.3	115.5+9.6	8.0+0.1	259.4+4.8	2.2+0.0	2.7+0.1	0
Coal1-1.5	6.4+0.0	1.1+0.0	2.8+0.1	115.2+2.3	6.9+0.3	269.3+14.4	2.2+0.1	3.6+0.3	0
Harvest									
Reference	7.6+0.2	1.0+0.5	1.8+0.5	68.8+8.2	16.5+3.9	261.1+25.6	2.1+0.0	6.9+0.5	7
BGF2-0.4	7.4+0.4	2.0+0.2	2.4+0.4	63.3+5.7	18.5+2.9	261.2+30.3	2.0+0.1	6.3+0.9	8
BGF2-1.5	6.9+0.1	0.9+0.5	1.8+0.6	71.5+5.0	16.8+2.0	303.2+12.9	1.9+0.1	5.0+1.1	7
OrgFert1-0.4	7.4+0.6	0.8+0.4	2.5+0.3	63.7+5.6	12.8+3.3	254.9+26.6	2.0+0.1	5.9+0.8	8
OrgFert1-1.5	6.8+0.4	2.3+0.4	2.9+0.2	55.7+11.6	19.2+4.2	275.9+15.7	2.1+0.1	5.3+1.6	8
Coal1-0.4	6.8+0.2	2.4+0.4	2.1+0.5	49.4+8.2	15.4+7.2	286.5+14.4	2.0+0.1	5.8+1.2	7
Coal1-1.5	7.0+0.4	1.7+0.9	1.7+0.4	56.5+10.1	20.8+3.8	295.9+15.8	1.9+0.1	5.4+0.3	8

Microbiome Analysis

In order to collect the rhizosphere soil samples, the roots of the lettuce plants were vigorously shaken by hand for 10 minutes until all roots non-adhering soil particles were completely removed. Then, the root was submerged in 50 mL PBS buffer and vortexed vigorously for 5 minutes to remove the adhering soils. The slurry was centrifuged at 4000g and 4 °C for 10 minutes, then discarded the supernatant and kept the soil pellet in -80 °C until DNA isolation.

The rhizosphere soil total DNA was extracted from 0.5 g of rhizosphere soil sample collected as above-mentioned using a *Quick*-DNA Fecal/Soil Microbe Miniprep Kit (ZYMO Research, D6010) according to the manufacturer's instruction. The DNA quality was checked on 1% agarose gel and was quantified using a spectrophotometer (Thermo Scientific NanoDrop 2000c, Vernon Hills, IL). All isolated DNA samples had an absorbance ratio (A260/A280) between 1.8 and 2.0.

Sample pool sequencing libraries were constructed by amplification of the V4 region of the 16S rRNA gene using primers 515F (5´-GTGCCAGCMGCCGCGGTAA-3´) and 806R (5´-GGACTACHVGGGTWTCTAAT-3´). The internal transcribed spacer (ITS) regions of fungal gene were amplified using primers ITS3 (5´-GCATCGATGAAGAACGCAGC-3´) and ITS4 (5´-TCCTCCGCTTATTGATATGC-3´). Briefly, amplicon libraries containing Illumina adaptors and barcodes were generated for each sample. Then the PCR products were cleaned using AmPure beads, quantified with PicoGreen, and pooled in equimolar ratios prior to sequencing using a 2 × 250 Miseq flow cell (Illumina, San Diego, CA) at Genomics Core of University of Colorado Anschutz Medical Campus.

The raw 16S rRNA and ITS gene sequencing reads were demultiplexed, quality-filtered by Trimmomatic (v0.36) and merged by FLASH (v1.20) with the following criteria: (i) an average quality score of <20 over a 50 bp sliding window, and the reads shorter than 120 bp were discarded, reads containing ambiguous characters were also discarded; (ii) only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. The maximum mismatch ratio of overlap region is 0.1. The barcodes and primers have been removed from the raw sequences to obtain clean sequences. The Operational taxonomic units (OTUs) have been obtained using Qiime (V1.8.0) using the clean sequences. The OTUs with 97% similarity cutoff were clustered using UPARSE version 7.1, and chimeric sequences were identified and removed. The taxonomy of each OTU representative sequence was analyzed by RDP Classifier version 2.2 against the 16S rRNA database (Release 128/132/138 http://www.arb-silva.de) and ITS database (Unite Release 8.2 http://unite.ut.ee/index.php).

Bacterial community diversity

Bacterial community profiling of soils of reference and three treatments (Ginate, organic fertilizer, coal) at 2 dosages (0.4% and 1.5%) and the rhizosphere soil samples of lettuces from all these different treatments have been investigated to study the effects of Ginate on the structure of bacterial communities. In total, 2.2M high-quality sequences have been obtained from all 42 samples (14 treatments with 3 biological replicates of each). The average high-quality sequences of all 42 samples is about 52.6K in the range of 17.5K to 98.4K. A total of 11,057 bacterial OTUs were identified at 97% sequence similarity cutoff and 2734 OTUs on average across all samples. There were 8,868 OTUs from the soils mixed with different treatments and 9,171 OTUs from the rhizosphere soils of lettuce plants. In total, 6981 out of the 11,057 OTUs could be found in both soils of different treatments before planting and the rhizosphere soils of lettuce at harvest.

As shown in Figure 5, the rarefaction curves of all samples did not plateau, indicating that additional sequencing would have continued to reveal additional bacterial diversity. Nonetheless, the sequencing covered most of the bacterial diversity within every sample. The average coverage of bacterial community was 0.92 to 0.94 respectively across all samples, suggesting that over 92% species were detected in these samples (Table 2).

The Shannon index, an estimate of alpha diversity in each sample, ranged from 9.13 to 9.81 with an average of 9.58. The richness (Chao1) ranged from 3652 to 4512 with an average of 4030.

The Bray-Curtis distances between samples were visualized using principal coordinates analysis (Figure 6). The soil samples before planting were separated from the lettuce rhizosphere soil samples. Furthermore, the rhizosphere soil samples from different treatment formed distinct clusters although the distance between high and low dosages of the same treatment did not separated well, except organic fertilizer. These clustering patterns suggest the additions of Ginate, organic fertilizer, and raw coal affected the lettuce rhizosphere bacterial communities differently.

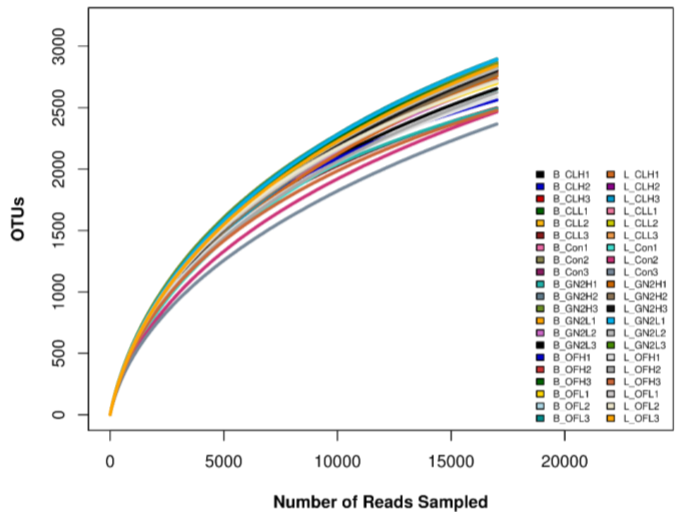

Figure 5. Rarefaction curves of the samples. B_: soil mix before plant; L_: Lettuce rhizosphere; Con: control; CL: coal; GN2: Ginate; OF: Organic fertilizer; H: high dosage (1.5%); L: Low dosage (0.4%).

Table 2. The richness and diversity of bacterial communities. Values in table are the average of 3 replicates. B_: soil mix before plant; L_: Lettuce rhizosphere; Con: control; CL: coal; GN2: Ginate; OF: Organic fertilizer; H: high dosage (1.5%); L: Low dosage (0.4%).

Sample ID	Chao1	Goods_coverage	Observed_species	Shannon	Simpson
B_CLH	4121.7	0.93	2756.8	9.59	1.00
B_CLL	3803.4	0.94	2706.4	9.72	1.00
B_Con	4408.6	0.92	2837.7	9.56	0.99
B_GN2H	3652.8	0.94	2687.2	9.81	1.00
B_GN2L	3890.7	0.93	2736.8	9.69	1.00
B_OFH	3712.7	0.94	2689.5	9.71	1.00
B_OFL	3845.1	0.94	2771.1	9.79	1.00
L_CLH	4512.8	0.92	2807.6	9.25	0.99
L_CLL	4378.1	0.92	2842.8	9.44	0.99
L_Con	3912.2	0.93	2486.2	9.13	0.99
L_GN2H	4191.4	0.93	2779.1	9.47	0.99
L_GN2L	4225.6	0.93	2852.8	9.76	1.00
L_OFH	3691.4	0.94	2563.5	9.50	1.00
L_OFL	4070.0	0.93	2758.8	9.69	1.00

Bacterial community structures

At the phylum level, the most abundant top 5 are Actinobacteria, Proteobacteria, Actinobacteria, Chloroflexi, and Crenarchaeota which occupy roughly 80% of the whole bacterial community in all samples (Figure 7). In comparison to the reference, the Ginate treatments both at low and high dosages decreased the relative abundances of Actinobacteria and Proteobacteria and increased Actinobacteria and Chloroflexi significantly.

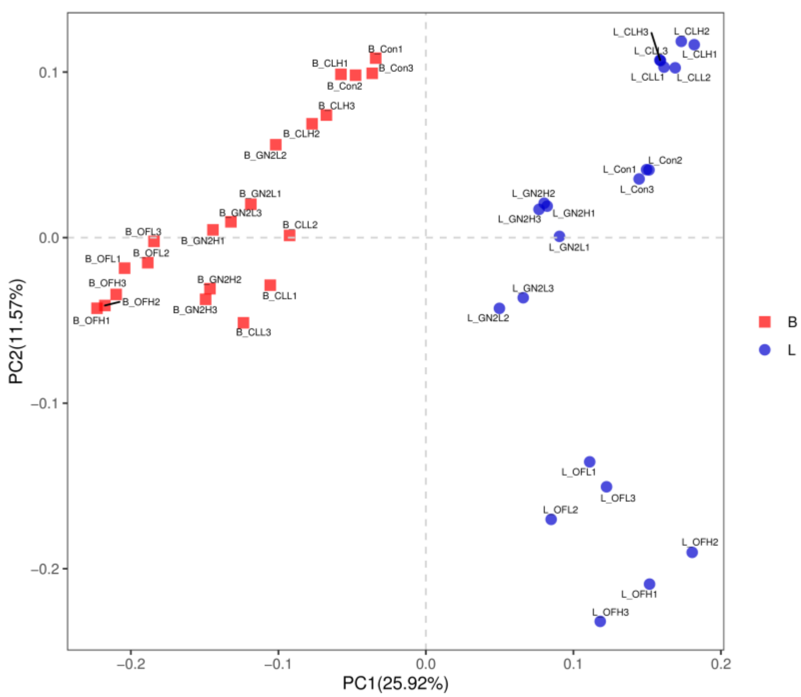

Figure 6. Principal coordinates analysis for visualization of pairwise community similarities (Bray-Curtis index).

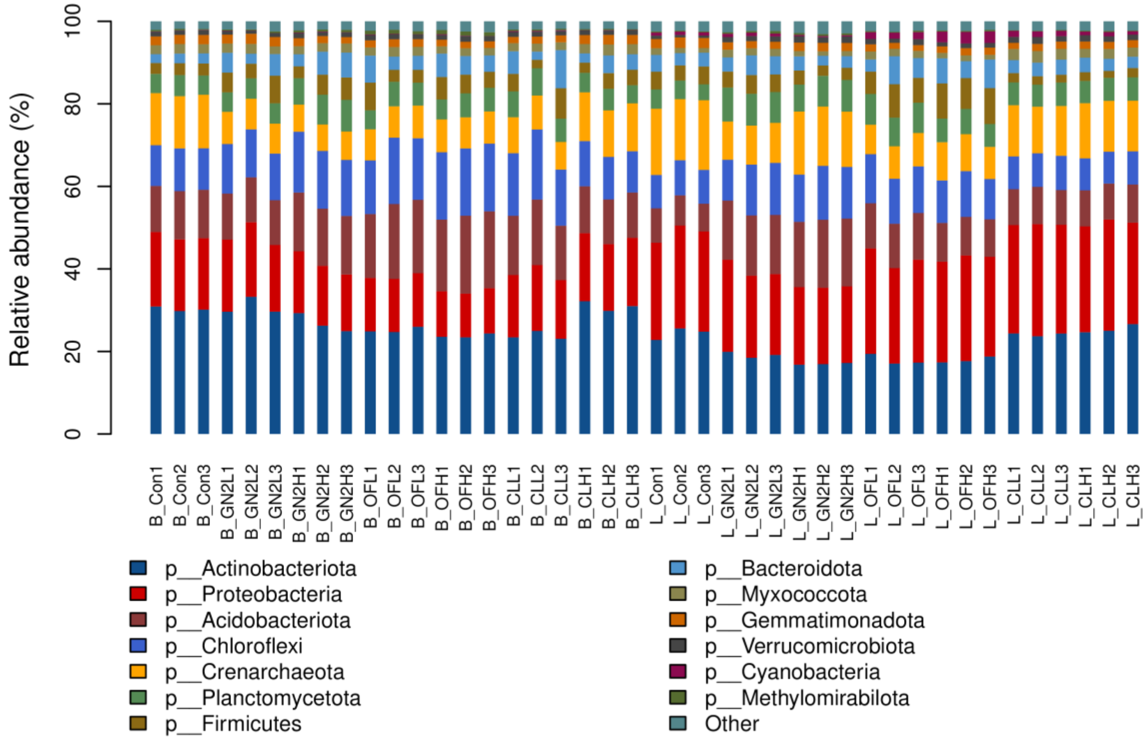

Figure 7. Histogram showing the relative abundance of different groups of bacteria at Phlym level. B_: soil mix before plant; L_: Lettuce rhizosphere; Con: control; CL: coal; GN2: Ginate; OF: Organic fertilizer; H: high dosage (1.5%); L: Low dosage (0.4%).

Fungal community diversity

Like the bacterial community profiling of soils, the fungal communities of the reference and three treatments (Ginate, Organic fertilizer, Coal) at two dosages (0.4% and 1.5%) soils and the rhizosphere soil samples of lettuces from all these different treatments have been investigated to study the effects of Ginate on the structure of fungal communities.

In total, 2.19M high-quality sequences were obtained from all 42 samples (14 treatments with 3 biological replicates). The average high-quality sequences of all 42 samples was about 52K in the range of 19K to 115K. A total of 2,185 fungal OTUs were identified at 97% sequence similarity cutoff and 536 OTUs on average across all samples. There were 1,772 OTUs from the soils from different treatments and 1,629 OTUs from the rhizosphere soils of lettuce plants. Out of the 2,185

OTUs, 1,216 OTUs could be found in both soils of different treatments before planting and the rhizosphere soils of lettuce.

As shown in Figure 8, the rarefaction curves of treatments did not plateau, indicating that additional sequencing would have continued to reveal additional fungal diversity. However, the sequencing covered most of the fungal diversity within every sample. The average coverage of bacterial community was 0.98 to 0.99 respectively across all samples, suggesting that over 98% species were detected in samples (Table 3).

The Shannon index, providing an estimation of alpha diversity in each sample, ranged from 4.85 to 6.67 with an average of 6.22. The richness (Chao1) ranged 559–781 with an average of 688.

The Bray-Curtis distances between samples have been visualized using a principal coordinates analysis (Figure 9). The soil samples from different treatments have been separated from the lettuce rhizosphere soil samples. Furthermore, the rhizosphere soil samples from organic fertilizer treatments formed distinct clusters from other treatments. These clustering pattern suggest the additions organic fertilizer had more significant effects on the lettuce rhizosphere fungal communities than the addition of Ginate and raw coal.

Table 3. The richness and diversity of fungal communities. Values in table are the average of 3 replicates. B_: soil mix before plant; L_: Lettuce rhizosphere; Con: control; CL: coal; GN2: Ginate; OF: Organic fertilizer; H: high dosage (1.5%); L: Low dosage (0.4%).

Sample ID	Chao1	Goods_coverage	Observed_species	Shannon	Simpson
B_CLH	781.5	0.98	605.6	6.61	0.970
B_CLL	688.1	0.98	549.6	6.42	0.963
B_Con	726.2	0.98	561.2	6.39	0.967
B_GN2H	709.1	0.99	554.9	6.36	0.963
B_GN2L	694.7	0.99	537.9	6.13	0.953
B_OFH	738.3	0.99	590.6	6.67	0.963
B_OFL	636.5	0.98	523.2	6.43	0.960
L_CLH	744.8	0.98	552.6	6.39	0.967
L_CLL	673.6	0.99	528.9	6.31	0.960
L_Con	688.3	0.98	545.3	6.19	0.953
L_GN2H	721.9	0.98	560.9	6.52	0.970
L_GN2L	695.9	0.99	552.2	6.54	0.970
L_OFH	559.2	0.99	415.3	4.85	0.883
L_OFL	572.9	0.99	433.3	5.34	0.920

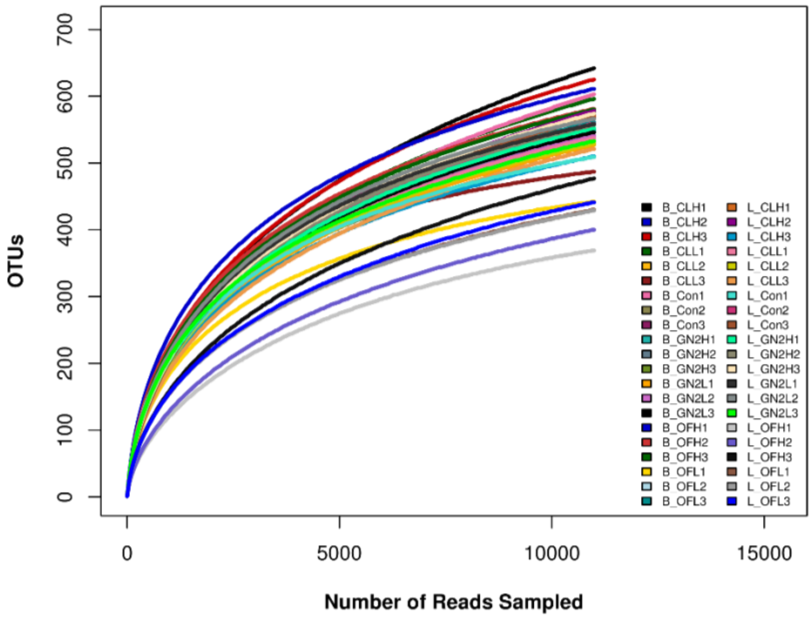

Figure 8. Rarefaction curves of the samples. B_: soil mix before plant; L_: Lettuce rhizosphere; Con: control; CL: coal; GN2: Ginate; OF: Organic fertilizer; H: high dosage (1.5%); L: Low dosage (0.4%).

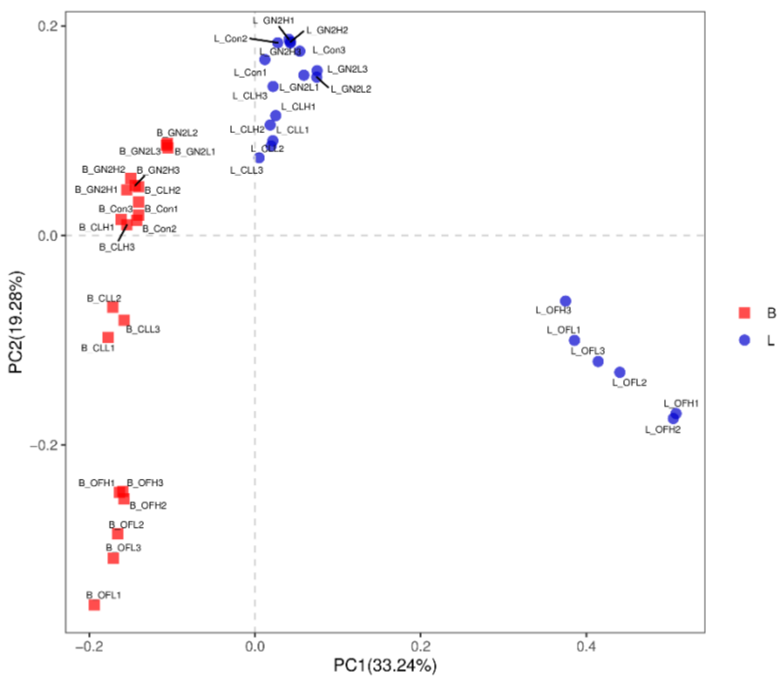

Figure 9. Principal coordinates analysis for visualization of pairwise community similarities (Bray-Curtis index).

The structure of fungal communities

At the phylum level, Ascomycota (about 80%) and Basidiomycota (about 13%) occupy about 93% of the whole fungal community in all the soil samples before planting lettuce (Figure 10). Different treatments resulted in different fungal community structure in the rhizosphere soils of lettuce plants. In comparison to the reference, the Ginate treatments both at low and high dosages decreased the relative abundances of Basidiomycota and increased Ascomycota significantly. The organic fertilizer at both low and high dosages increased the abundance of Mucoromycota much more than other treatments.

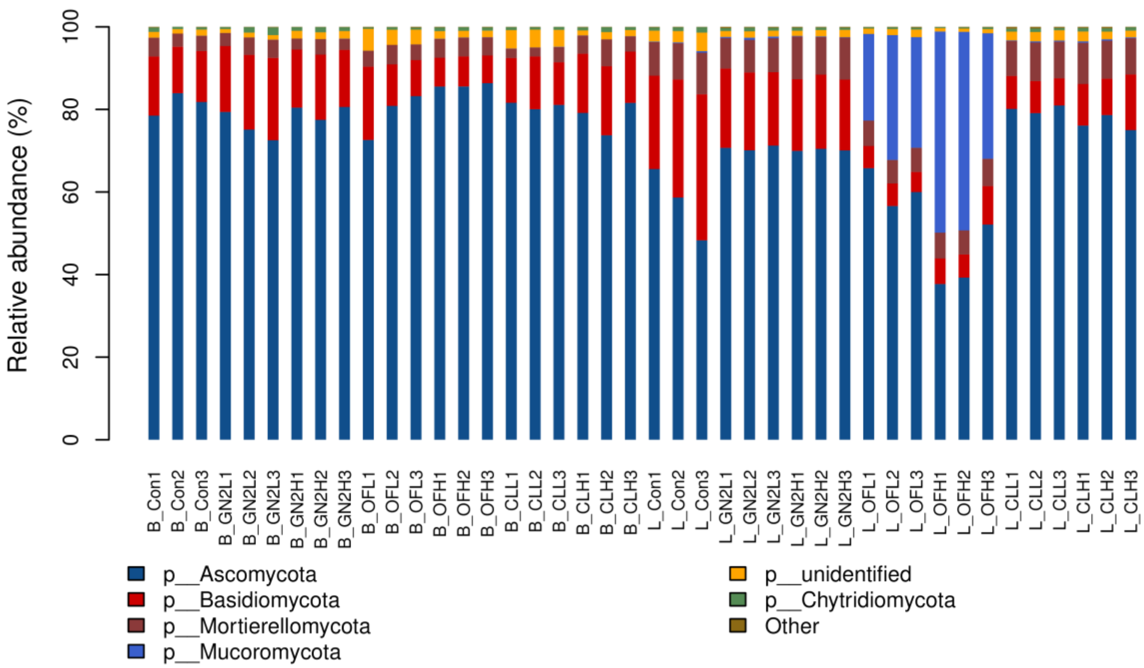

Figure 10. Histogram showing the relative abundance of different groups of fungi at Phlym level. B_: soil mix before plant; L_: Lettuce rhizosphere; Con: control; CL: coal; GN2: Ginate; OF: Organic fertilizer; H: high dosage (1.5%); L: Low dosage (0.4%).

Pepper Study:

Study design

To determine the effects on plant growth of sweet pepper (*Capsicum annuum*), Ginate (BGF1, BGF2) at three dosages: 4 and 15 g/kg soil was mixed in base soil, respectively, and filled in 1 Gallon pots. Commercial organic fertilizer (GRO-WELL Proven Organics 4-lb Natural All-Purpose Food) and an equivalent amount of raw coal (lignite) were mixed in the same base soil as reference treatments. Pepper seeds were germinated in small pots and the seedlings at 2-true-leaves stage were transplanted into the prepared 1 Gallon pots. All pepper plants were growing at the same conditions during the test period in the greenhouse at Colorado State University. There was one pepper plant per pot and 12 plants for each treatment. Six plants were harvested at the end of 5 weeks to monitor the plant growth including plant height, fresh and dry biomass weight. Another six plants were harvested at the end of 12 weeks to monitor the number and weight of pepper fruits in different treatments.

Plant growth

As shown in Figures 11 and 12, the pepper plants at 5 weeks were larger in the treatment of BGF1 and BGF2 at both dosages in comparison to the reference and other 2 treatments including the organic fertilizer treatment. The pepper plant height of BGF1 and BGF2 treatments was approximately twice than that of the reference, while the plant fresh weight was about 10 times higher. Meanwhile, the root weight and plant dry weight were also significantly higher in the BGF1 and BGF2 treatments than those of the reference. Furthermore, the raw coal treatment had similar fresh weight to the reference and fresh weight from the organic fertilizer treatment was slightly higher than the reference (Figure 5).

The second groups of pepper plants were harvested at 12 weeks after transferring. The height and weight of plant and pepper fruits were monitored. As shown in Figures 11 and 13, like the pepper plants at 5 weeks, the plant height and weight of treatments of BGF1 and BGF2 at both dosages were larger in comparison to those of the reference. The organic fertilizer treatment also showed plant promotion effects on pepper plants at week 12. Both BGF1 and organic fertilizer showed higher plant promotion effects at higher dosage (1.5%) than those of lower dosage (0.4%), respectively. Furthermore, the reference treatment had only 1 pepper fruit per plant, while the treatments of BGF1-1.5 and organic fertilizer-1.5% had about 8 pepper fruits per plant (Figure 14).

In addition to the number of pepper fruits, the average fruit weight and fruit weight per plant were higher in all treatments than in the reference which indicates these treatments not only have more pepper fruits but also larger pepper fruits (Figure 14). Moreover, the BGF1-1.5 had the highest pepper fruit productivity as it had the highest fruit weight per plant.

Figure 11. Pepper plants in the pots of different treatments at 5 and 12 weeks after transferring. Top: pepper plants at 5 weeks. Bottom: pepper plants at 12 weeks.

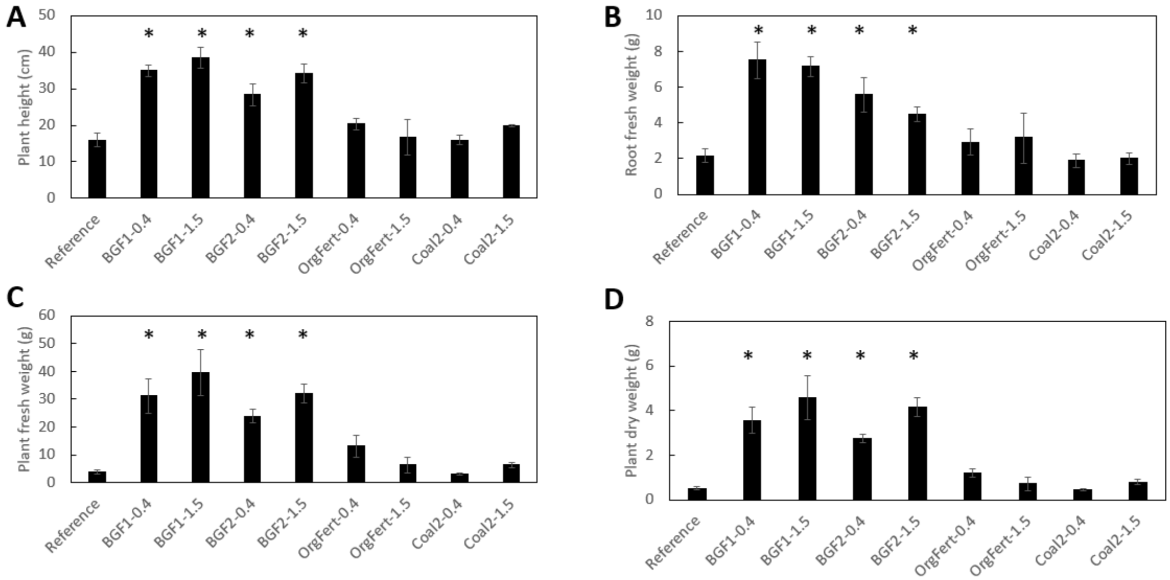

Figure 12. Pepper plants with different treatments when harvested at 5 weeks after transferring. A: plant height; B: root fresh weight; C: plant fresh weight; D: plant dry weight. The values are the average of 6 plants of each treatment. The error bar represents the standard deviation of the 6 biological replicates. The asterisk means the statistical significance (*p<0.5*) in comparison to the reference treatment.

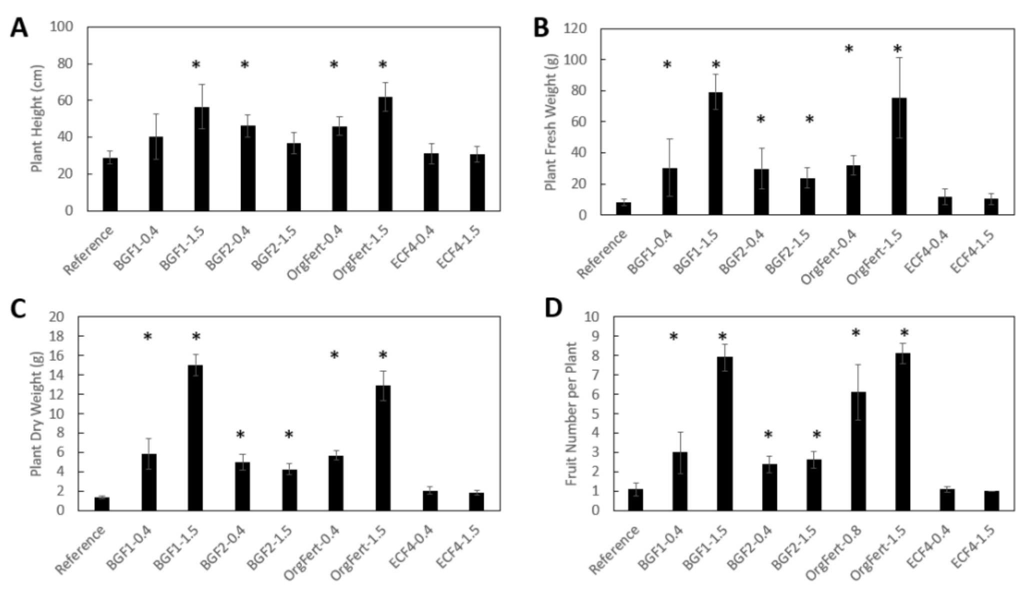

Figure 13. The pepper plants with different treatments when harvested at 12 weeks after transferring. A: plant height; B: plant fresh weight; C: plant dry weight; D: pepper fruit number per plant. The values are the average of 6 plants of each treatment. The error bar represents the standard deviation of the 6 biological replicates. The asterisk means the statistical significance (*p<0.5*) in comparison to the reference treatment.

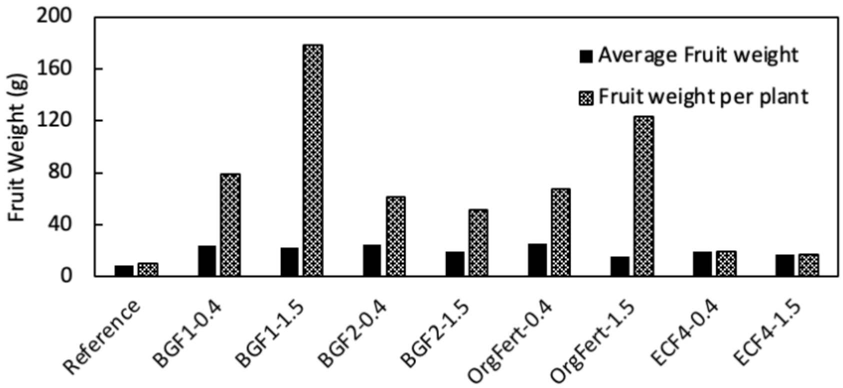

Figure 14. Pepper fruit (average fruit weight and fruit weight per plant) with different treatments when harvested at 12 weeks after transferring.

Soil chemical analysis

Soil samples were collected from all treatments at the start, 5 weeks, and 12 weeks after pepper plants were transferred, and from pots without plants at 12 weeks to determine the influence of the treatments on key soil parameters. As shown in Table 4, the soil pH was in the range of 5.7 to 7.1 in different treatments mixed soils. Organic fertilizer (both 0.4% and 1.5%) decreased the soil pH dramatically when it was blended in the soils while Ginate and raw coal did not. The pH in all the treatments was around 7 at 12 weeks. The addition of BGFs, organic fertilizer, and raw coal, especially at 1.5%, improved the soil organic matter content in the soils. The organic matter content in all the treatments including Ginate, organic fertilizer, and raw coal, still was higher than that of the reference at 5 weeks and 12 weeks. The Ginate treatments (both 0.4% and 1.5%) had higher organic matter content than organic fertilizer and raw coal at 5 weeks and 12 weeks. The content of NPK in all the treatments were like those of the reference except organic fertilizer treatments (both 0.4% and 1.5%) which had higher NPK level. Both Ginate and organic fertilizer had higher NPK level than the reference at 5 weeks and 12 weeks.

The addition of BGFs and organic fertilizer in the soils did not change the soil available water capacity significantly in comparison to the reference treatment. The CEC in the BGFs and organic fertilizer treatments were higher than the reference. At 5 weeks, both BGFs and organic fertilizer treatments showed higher CEC and available water capacity than the reference. However, at 12 weeks, CEC and available water capacity in all the treatments were not significantly changed in comparison to the reference.

Table 4. Soil properties before and after pepper plants. Values represent the mean of three biological replicates and standard deviation.

	pH	EC (mmhos/cm)	OM (%)	NO3-N (ppm)	P (ppm)	K (ppm)	Available Wate Capacity (mc cr 1)
Before							
Reference	7.1+0.1	1.1+0.0	1.6+0.2	102.4+1.5	9.6+0.5	287.0+10.5	0.06+0.01
BGF2-0.4	7.0+0.0	1.3+0.1	1.8+0.1	118.1+2.7	9.9+0.3	295.4+5.6	0.04+0.01
BGF2-1.5	7.0+0.0	1.3+0.1	2.4+0.7	115.8+2.3	14.5+0.7	310.9+8.3	0.04+0.00
OrgFert1-0.4	6.0+0.1	2.8+0.2	2.1+0.0	251.4+8.3	19.1+1.3	661.2+6.7	0.04+0.01
OrgFert1-1.5	5.7+0.0	4.6+0.4	2.7+0.3	370.9+14.8	26.1+1.5	1320.7+24.4	0.08+0.00
Coal1-0.4	6.9+0.0	1.0+0.1	1.9+0.3	115.5+9.6	8.0+0.1	259.4+4.8	0.12+0.01
Coal1-1.5	6.4+0.0	1.1+0.0	2.8+0.1	115.2+2.3	6.9+0.3	269.3+14.4	0.07+0.01
No Plant							
Reference	7.3+0.1	0.5+0.0	1.4+0.1	36.6+2.5	3.8+0.3	154.8+3.5	0.32+0.03
BGF2-0.4	7.0+0.1	0.9+0.0	1.6+0.1	103.0+8.8	9.0+0.8	255.8+4.7	0.28+0.04
BGF2-1.5	7.4+0.0	0.4+0.0	1.8+0.2	23.7+3.4	8.0+0.3	189.7+2.5	0.21+0.01
OrgFert1-0.4	6.0+0.1	1.1+0.0	1.4+0.1	97.5+6.8	21.7+2.5	322.5+4.2	0.25+0.01
OrgFert1-1.5	4.8+0.0	3.8+0.2	1.8+0.1	300.2+82.7	52.2+1.1	817.8+3.4	0.16+0.01
Coal1-0.4	6.9+0.1	0.5+0.0	1.7+0.1	41.5+3.0	3.3+0.2	151.5+3.5	0.05+0.00
Coal1-1.5	6.5+0.1	0.7+0.1	2.1+0.3	56.7+4.9	3.2+0.3	156.4+3.0	0.05+0.00
5-week							
Reference	7.1+0.0	0.6+0.0	1.4+0.0	94.2+0.6	2.7+0.2	143.9+4.5	0.33+0.02
BGF2-0.4	7.1+0.0	0.9+0.0	1.8+0.1	85.3+6.2	5.7+0.1	188.0+2.5	0.92+0.06
BGF2-1.5	6.9+0.0	0.7+0.0	2.0+0.1	74.5+5.6	8.4+0.1	248.8+6.4	0.88+0.05
OrgFert1-0.4	6.1+0.0	1.2+0.0	2.2+0.2	105.9+3.9	6.5+0.0	301.7+17.0	0.64+0.06
OrgFert1-1.5	5.0+0.0	3.1+0.1	1.8+0.2	310.7+25.9	34.7+2.2	746.1+7.1	0.69+0.04
Coal1-0.4	6.8+0.0	0.6+0.0	1.8+0.2	55.5+2.5	3.7+0.1	168.8+3.0	0.76+0.02
Coal1-1.5	6.6+0.0	0.6+0.0	1.7+0.2	37.1+1.3	3.3+0.3	171.4+9.1	0.20+0.00
12-week							
Reference	7.4+0.0	0.4+0.0	1.5+0.2	17.0+0.2	3.5+0.4	157.6+4.8	0.05+0.00
BGF2-0.4	7.4+0.1	0.7+0.1	2.4+0.1	47.4+2.7	8.4+0.3	223.0+2.7	0.05+0.00
BGF2-1.5	7.4+0.0	0.5+0.0	2.6+0.1	26.3+2.6	9.6+0.7	200.1+11.3	0.08+0.00
OrgFert1-0.4	7.1+0.1	0.8+0.2	1.9+0.9	19.6+1.6	14.8+10.2	589.1+404.4	0.07+0.00
OrgFert1-1.5	6.8+0.1	1.6+0.3	2.0+0.1	19.5+0.4	23.3+1.6	1244.0+46.0	0.16+0.00
Coal1-0.4	7.3+0.0	0.4+0.0	1.7+0.3	20.1+0.6	16.9+1.6	234.4+25.4	0.07+0.00
Coal1-1.5	7.1+0.0	0.5+0.0	1.4+0.2	18.5+0.6	5.4+0.3	179.1+2.9	0.07+0.00

The bacterial community diversity

Bacterial community profiling of soils blended with three treatments (Ginate, organic fertilizer, coal) at two doses (0.4% and 1.5%) and reference (without addition) and the rhizosphere soil samples of pepper plants from all these different treatments at 5 weeks and 12 weeks was investigated to study the effects of Ginate on the structure of bacterial communities. In total, 2.86M high-quality sequences have been obtained from all 63 samples (21 treatments with 3 biological replicates). The average high-quality sequences of all 63 samples is about 45.4K in the range of 16.0K to 97.7K. A total of 13,343 bacterial OTUs were identified at 97% sequence similarity and 2375 OTUs on average across all samples. There were 9, 013 OTUs from the soils mixed with different treatments and 9,032 and 9402 OTUs from the rhizosphere soils of pepper plants at 5 weeks and 12 weeks, respectively. Out of the 13,343 OTUs, 5,484 OTUs were shared in the soils of different treatments before planting and the rhizosphere soils of pepper at 5 weeks and 12 weeks, indicating these OTUs may represent the core bacterial groups which have not been changed by different treatments and different time points.

As shown in Figure 15, the rarefaction curves of treatments did not plateau, indicating that additional sequencing would have revealed additional bacterial diversity. However, the sequencing covered most of the bacterial diversity within every sample. The average coverage of bacterial community was 0.89 to 0.97 respectively across all samples, suggesting that approximately 89 to 97% species were detected in these samples (Table 5).

The Shannon index, providing an estimation of alpha diversity in each sample, ranged from 6.59 (Coal 0.4% at 12 weeks) to 9.84 (Ginate 1.5% at 12 weeks). The richness (Chao1) ranged from 1860 (Organic fertilizer 1.5%) to 4513 (Organic fertilizer 0.4%).

The Bray-Curtis distances between samples have been visualized using a principal coordinates analysis (Figure 16). The soil samples have been separated from the pepper rhizosphere soil samples at 5 weeks and 12 weeks. Furthermore, the rhizosphere soil samples at 5 weeks formed a distinct cluster except the high dosage of organic fertilizer at 1.5%. Different treatments of the rhizosphere soil samples at 12 weeks were more spread out and formed distinct clusters, even the same treatment at different dosages were separated, except the raw coal, suggesting the additions of Ginate and organic fertilizer had stronger effects on the pepper rhizosphere bacterial communities at 12 weeks than at 5 weeks.

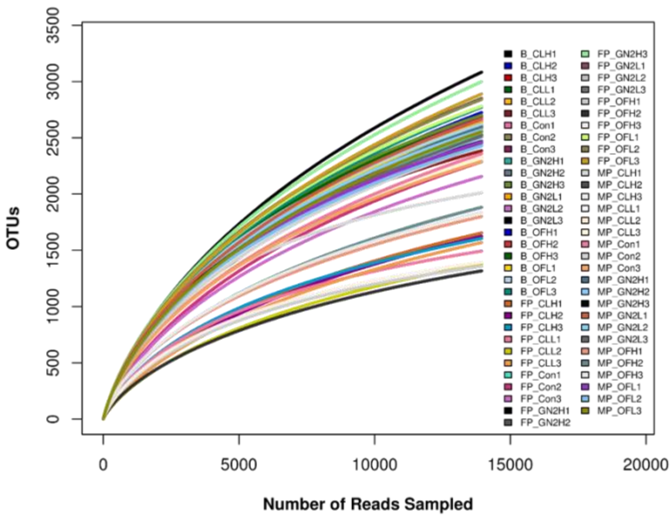

Figure 15. Rarefaction curves of the samples. B_: soil mix before plant; MP_: Pepper rhizosphere at 5 weeks; FP_: Pepper rhizosphere at 12 weeks; Con: control; CL: coal; GN2: Ginate; OF: Organic fertilizer; H: high dosage (1.5%); L: Low dosage (0.4%).

Table 5. The richness and diversity of bacterial communities. Values are the average of triplicates. B_: soil mix before plant; MP_: rhizosphere at 5 weeks; FP_: rhizosphere at 12 weeks; Con: control; CL: coal; GN2: Ginate; OF: organic fertilizer; H: high dose (1.5%); L: Low dose (0.4%).

Sample ID	Chao1	Goods_coverage	Observed_species	Shannon	Simpson
B_Con	4465.4	0.91	2675.8	9.537	0.99
B_CLH	4151.3	0.91	2616.6	9.560	0.99
B_CLL	3806.4	0.92	2570.6	9.693	1.00
B_GN2H	3752.9	0.93	2577.3	9.800	1.00
B_GN2L	3861.5	0.92	2572.9	9.680	1.00
B_OFH	3801.0	0.92	2580.9	9.680	1.00
B_OFL	4015.3	0.92	2658.3	9.777	1.00
MP_Con	3715.8	0.92	2358.3	9.183	0.99
MP_CLH	3289.0	0.93	2316.0	9.480	1.00
MP_CLL	3788.0	0.92	2376.9	9.343	0.99
MP_GN2H	3705.9	0.92	2543.6	9.563	0.99
MP_GN2L	3930.3	0.92	2561.2	9.563	1.00
MP_OFH	2728.6	0.94	1839.6	8.280	0.98
MP_OFL	3740.6	0.93	2481.2	9.607	1.00
FP_Con	3702.3	0.92	2330.0	8.267	0.97
FP_CLH	2580.8	0.94	1627.9	6.873	0.93
FP_CLL	2196.1	0.96	1479.9	6.590	0.92
FP_GN2H	5115.7	0.89	2976.9	9.843	1.00
FP_GN2L	4177.6	0.91	2540.3	8.680	0.98
FP_OFH	1860.0	0.97	1355.9	6.930	0.95

FP_OFL	4513.0	0.90	2836.9	9.707	0.99

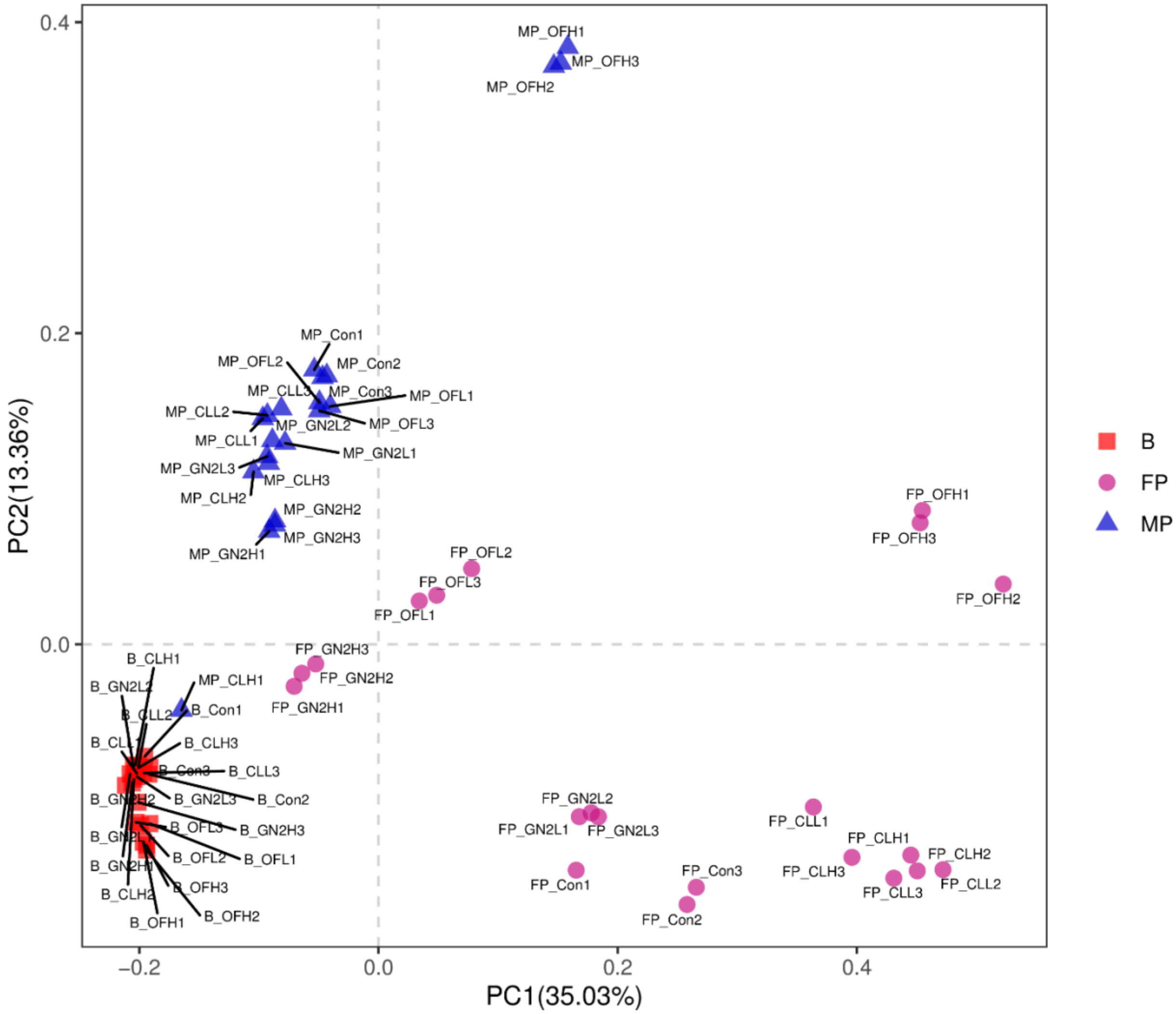

Figure 16. Principal coordinates analysis for visualization of pairwise community similarities (Bray-Curtis index). B_: soil mix before plant; MP_: Pepper rhizosphere at 5 weeks; FP_: Pepper rhizosphere at 12 weeks.

The structure of bacterial communities

At the phylum level, the most abundant top 5 were Actinobacteria, Proteobacteria, Actinobacteria, Chloroflexi, and Crenarchaeota which roughly occupy 82% of the whole bacterial community in the soil samples and the rhizosphere samples at 12 weeks (Figure 17). However, the top 5 most abundant phylum in the rhizosphere samples at 5 weeks were Actinobacteria, Proteobacteria, Chloroflexi, Bacteroidetes, and Crenarchaeota.

In comparison to the reference, the Ginate treatments both at low and high dosages decreased the relative abundances of Actinobacteria and Bacteroidetes and increased Acidobacteria significantly at 5 weeks. The organic fertilizer at 1.5% enriched the abundance of Proteobacteria significantly and reduced the relative abundance of other phyla, including Actinobacteria, Chloroflexi, and Acidobacteria. At 12 weeks, the Ginate at 1.5% decreased the abundance of Proteobacteria but increased Actinobacteria and Chloroflexi, while Ginate at 0.4% not only increased Actinobacteria but also decreased Acidobacteria and Bacteroidetes.

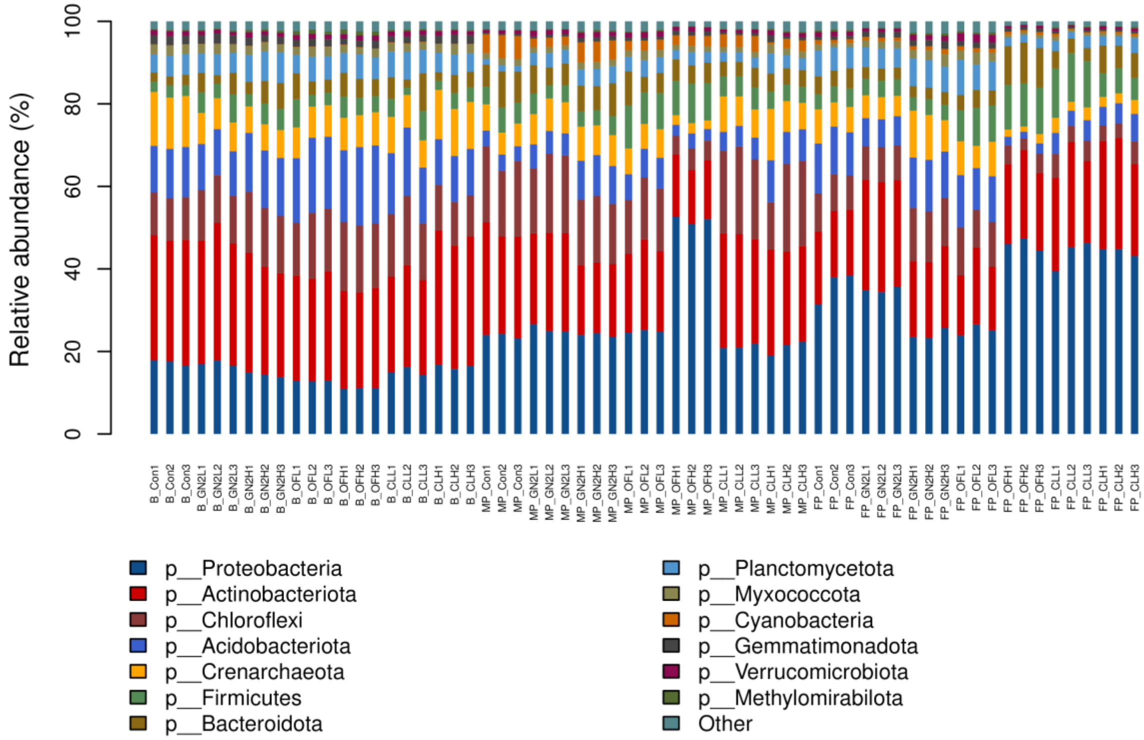

Figure 17. Histogram showing the relative abundance of different groups of bacteria at Phlym level. B_: soil mix before plant; MP_: Pepper rhizosphere at 5 weeks; FP_: Pepper rhizosphere at 12 weeks; Con: control; CL: coal; GN2: Ginate; OF: Organic fertilizer; H: high dosage (1.5%); L: Low dosage (0.4%).

Fungal community

The fungal communities of soils blended with three treatments (Ginate, organic fertilizer, coal) at two dosages (0.4% and 1.5%) and reference (without addition) and the rhizosphere soil samples of pepper plants from all these different treatments at 5 weeks and 12 weeks were investigated. In total, 3.37M high-quality sequences were obtained from all 63 samples (21 treatments with 3 biological replicates). The average high-quality sequences of all 63 samples is about 53.5K in the range of 11.9K to 114.9K. A total of 3,672 fungal OTUs were identified at 97% sequence similarity and 578 OTUs on average across all samples. There were 1,920 OTUs from the soils mixed with different treatments and 2,114 and 2,599 OTUs from the rhizosphere soils of pepper plants at 5 weeks and 12 weeks, respectively. Out of the 3,672 OTUs, 1,003 OTUs were shared in the soils of different treatments before planting and the rhizosphere soils of pepper at 5 weeks and 12 weeks, indicating these OTUs may represent the core fungal groups which have not been changed by different treatments and different time points.

As shown in Figure 18, the rarefaction curves of treatments did not reach a plateau, indicating that additional more sequencing would have continued to reveal additional fungal diversity. However, the sequencing covered most of the fungal diversity within every sample. The average coverage of bacterial community was 0.97 to 0.99 respectively across all samples, suggesting that over 97% species were detected in samples (Table 6).

The Shannon index, providing an estimation of alpha diversity in each sample, ranged from 6.31 to 6.98 in the soil samples. In the rhizosphere soils, Organic fertilizer showed the lowest Shannon index both at 5 weeks and 12 weeks, indicating the organic fertilizer reduced the diversity of the fungal community. The richness (Chao1) ranged from 721 to 841 in the soil samples. In the rhizosphere soils, the organic fertilizer had the lowest richness at 5 weeks and the Ginate had the highest richness at 12 weeks.

The Bray-Curtis distances between samples have been visualized using a principal coordinates analysis (Figure 19). The soil samples have been separated from the pepper rhizosphere soil samples at 5 weeks and 12 weeks. Furthermore, in the rhizosphere soil samples at 5 weeks and 12 weeks, organic fertilizer at both high and low dosages formed a distinct cluster while the other treatments and reference formed another cluster, suggesting the additions of organic fertilizer shaped the pepper rhizosphere fungal community different than other treatments.

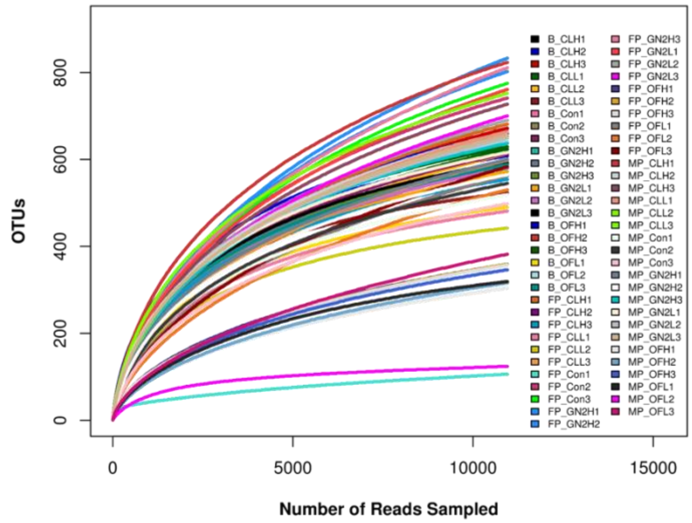

Figure 18. Rarefaction curves of the samples. B_: soil mix before plant; MP_: Pepper rhizosphere at 5 weeks; FP_: Pepper rhizosphere at 12 weeks; Con: control; CL: coal; GN2: Ginate; OF: Organic fertilizer; H: high dosage (1.5%); L: Low dosage (0.4%).

Table 6. The richness and diversity of fungal communities. Values are the average of triplicates. B_: soil mix before plant; MP_: rhizosphere at 5 weeks; FP_: rhizosphere at 12 weeks; Con: control; CL: coal; GN2: Ginate; OF: organic fertilizer; H: high dose (1.5%); L: Low dose (0.4%).

Sample ID	Chao1	Goods_coverage	Observed_species	Shannon	Simpson
B_Con	762.7	0.98	608.3	6.54	0.97
B_CLH	840.9	0.98	650.3	6.74	0.97
B_CLL	771.4	0.98	586.3	6.56	0.97
B_GN2H	720.7	0.98	588.7	6.48	0.96
B_GN2L	729.7	0.98	581.7	6.31	0.96
B_OFH	808.4	0.98	642.3	6.98	0.98
B_OFL	731.4	0.98	573.0	6.68	0.97
MP_Con	677.8	0.98	520.7	5.08	0.87
MP_CLH	992.0	0.97	737.6	6.35	0.94
MP_CLL	967.2	0.98	689.9	6.35	0.95
MP_GN2H	794.8	0.98	600.6	5.27	0.82
MP_GN2L	861.7	0.98	640.0	6.23	0.95
MP_OFH	467.8	0.99	322.0	3.08	0.64
MP_OFL	417.6	0.99	275.0	2.63	0.54
FP_Con	749.9	0.98	540.6	5.97	0.96
FP_CLH	823.1	0.98	632.3	6.52	0.97
FP_CLL	629.1	0.99	507.0	5.59	0.91
FP_GN2H	1238.5	0.97	815.3	6.61	0.96
FP_GN2L	1032.6	0.98	717.0	6.62	0.97
FP_OFH	534.9	0.99	358.3	4.29	0.82

FP_OFL	869.0	0.98	558.6	5.01	0.88

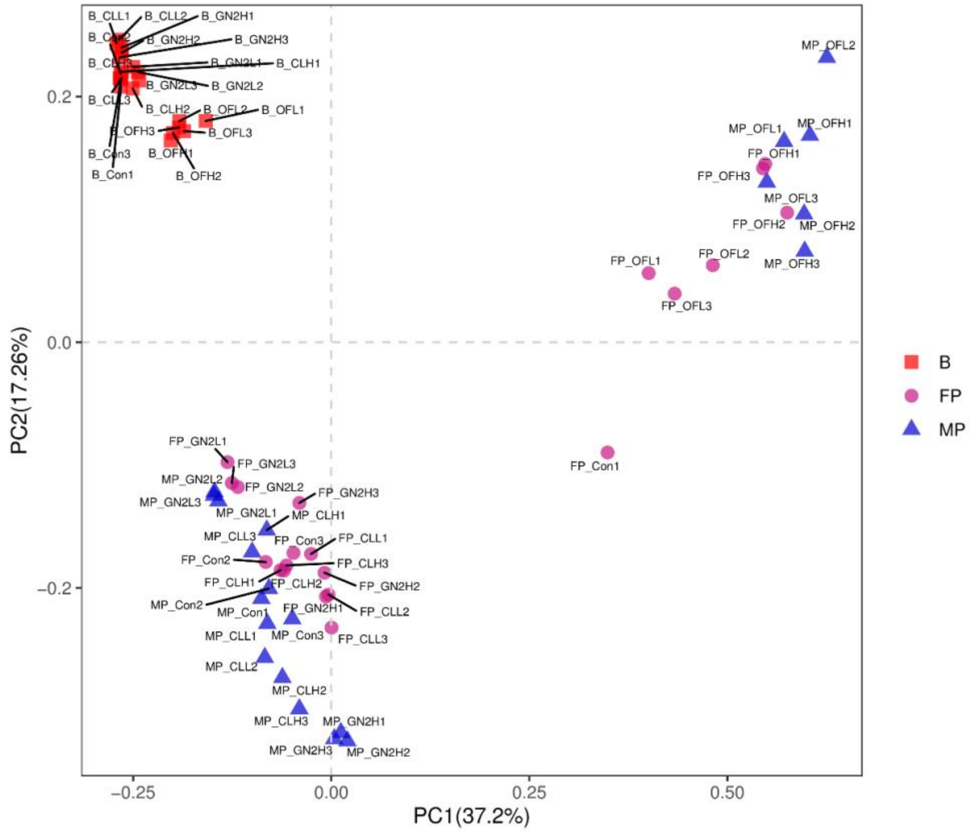

Figure 19. Principal coordinates analysis for visualization of pairwise community similarities (Bray-Curtis index). B_: soil mix before plant; MP_: Pepper rhizosphere at 5 weeks; FP_: Pepper rhizosphere at 12 weeks; Con: control; CL: coal; GN2: Ginate; OF: Organic fertilizer; H: high dosage (1.5%); L: Low dosage (0.4%).

The structure of fungal communities

At the phylum level, Ascomycota (~80%) and Basidiomycota (~13%) occupied about 93% of the whole fungal community in all the soil samples before planting pepper and in rhizosphere soils of pepper plants at 5 weeks and 12 weeks, except organic fertilizer treatments (Figure 20). Different treatments resulted in different fungal community structures in the rhizosphere soils of pepper plants. At 5 weeks, the Ginate treatments both at low and high dosages decreased the relative abundances of Ascomycota and increased Basidiomycota significantly in comparison to the reference. However, the Ginate treatments showed higher Ascomycota and lower Basidiomycota comparing to the reference at 12 weeks. The organic fertilizer at low and high dosages had the relative abundance of Mucoromycota as 76.8% and 69.5% in the rhizosphere soils of pepper plants at 5 weeks, and these numbers dropped to 38.1 and 47.5% at 12 weeks.

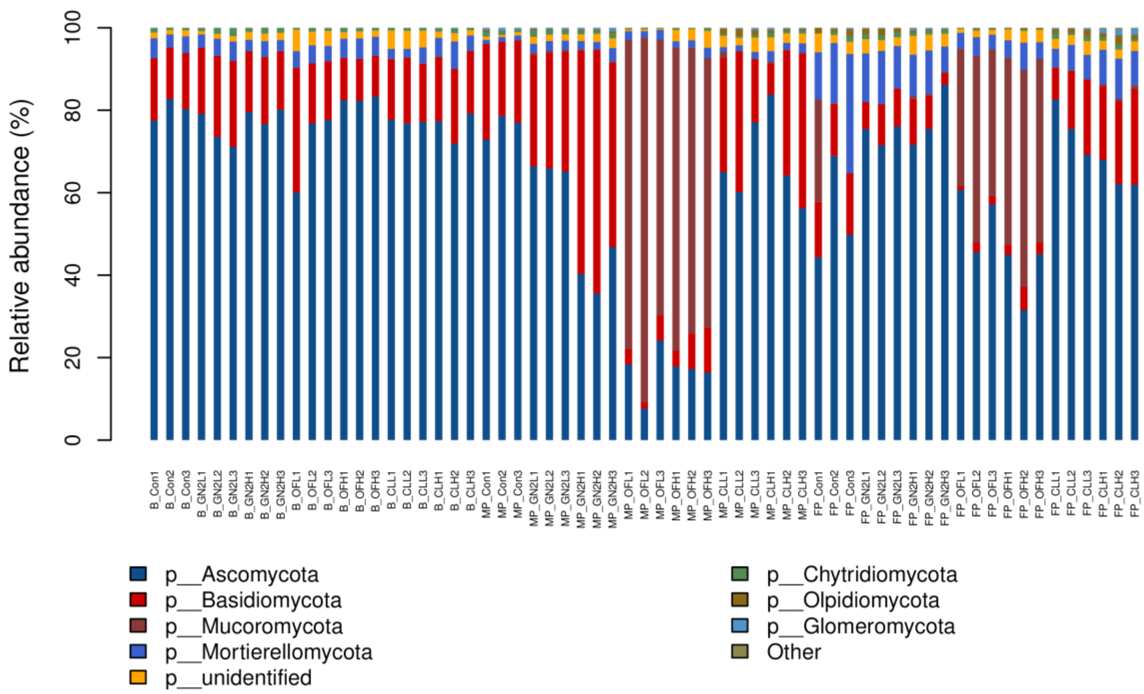

Figure 20. Histogram showing the relative abundance of different groups of fungi at Phlym level. B_: soil mix before plant; MP_: Pepper rhizosphere at 5 weeks; FP_: Pepper rhizosphere at 12 weeks; Con: control; CL: coal; GN2: Ginate; OF: Organic fertilizer; H: high dosage (1.5%); L: Low dosage (0.4%).

Conclusions:

The application of Ginate in soils promoted both lettuce and pepper plant growth and resulted in significant higher plant weight in comparison to the treatment of organic fertilizer. Furthermore, Ginate products (BGF1 and BGF2) improved the pepper fruit productivity as compared with controls and the other treatments tested. In comparison to the commercial organic fertilizer, BGF1 at the same dosage had better effects on pepper plant growth and fruit productivity. Furthermore, Ginate did not add soil nutrients such as N, P, K in soils, indicating that Ginate promotes plant growth by using different compounds rather than the nutrients directly as the commercial organic fertilizer does. Moreover, microbiome analysis showed that the Ginate changed both bacterial and fungal communities in the rhizosphere soils of lettuce. The ability of enrich or attract more beneficial microbes in the rhizosphere may be one of the reasons Ginate promoted plant growth.