IMPACT OF BREWER'S SPENT GRAIN ON SOIL HEALTH AND PRODUCTIVITY

An Undergraduate Research Scholars Thesis

by

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Research activities involving the use of human subjects, vertebrate animals, and/or biohazards must be reviewed and approved by the appropriate Texas A&M University regulatory research committee (i.e., IRB, IACUC, IBC) before the activity can commence. This requirement applies to activities conducted at Texas A&M and to activities conducted at non-Texas A&M facilities or institutions. In both cases, students are responsible for working with the relevant Texas A&M research compliance program to ensure and document that all Texas A&M compliance obligations are met before the study begins.

I, Noriki Miyanaka, certify that all research compliance requirements related to this Undergraduate Research Scholars thesis have been addressed with my Research Faculty Advisor prior to the collection of any data used in this final thesis submission.

This project did not require approval from the Texas A&M University Research Compliance & Biosafety office.

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ABSTRACT

Impact of Brewer’s Spent Grain on Soil Health and Productivity

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Demand for establishing a sustainable food system has increased in past decades. Brewers' Spent Grain (BSG) is main byproduct of beer production process. Global beer industries produce up to 39 million tons of waste annually but usually, but it is usually wasted because it is too perishable to feed animals. BSG contains valuable nutrients for soil including nitrogen (19%-30%). Application of raw BSG to soil for agricultural production has a potential to enhance soil health by supplementing organic materials and stimulating microbial activities. Currently land-application utilizes composted BSG, which is costly and time consuming to produce. The main purpose of this project was to determine if application of fresh BSG enhances soil health and increase yields of plants. In this research, Radish Rover F1 was grown for five weeks in green house, and BSG was applied at three different application rates and two application methods (0, 10 and 40 g applied to the surface or incorporated per 460 g air-dried soil) application) Dry mass of total radish tissue of shoots and roots, nutrient contents (i.e., P, K) in radish tissue, total C:N ratio in soil, and beta glucosidase, N-acetylglutamate (NAG), and phosphatase enzyme activity were assessed to determine effects of BSG on radish growth and soil health improvement. The result showed 40 g incorporated significantly improved dry biomass, P, K contents in plants, total C:N ratio in soil and microbial enzyme activities compared to no BSG application. In conclusion, BSG acted as soil amendment enhancing productivity, microbial activity, and nutrient cycle in soil. The results of this greenhouse evaluation strongly suggest that BSG has potential to enhance soil health and potential crop yields.

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The brewer's spent grain used in this research was produced by Dr. Julie Howe. The total carbon and nitrogen content used for total C:N ratio was provided by Seitz, Paige Graves. The K, and P content analyzed for radish productivity was collected by Isaiah Robertson. The beta glucosidase, N-acetylglutamate (NAG), and phosphatase enzyme activity analysis used for microbial enzyme activity was done with guidance of graduate students under Dr. Payton Smith.

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NOMENCLATURE

BSG	Brewer’s Spent Grain
Sur10	Surface application of 10 g
Sur40	Surface application of 40 g
Inc10	Incorporation application of 10 g
Inc40	Incorporation application of 10 g

1. INTRODUCTION

Demand for establishing a sustainable food system has increased in past decades as a result of increasing scarcity of resources and environmental pollutions during the past century. A sustainable food system delivers food security and nutrition for all in such a way that addresses the economic, social and environmental aspects of production to generate food security and nutrition for future generations (FAO, 2018). Under a sustainable food system, farming system interacts with other industries or institutions to conserve natural elements (e.g., soil, air, water, and minerals) by reducing wastes and pollution in entire community. One example of a sustainable food systems practice is to use by-products of industries for agricultural inputs (i.e., fertilizer). Organic by-products, in particular, have potential to create extra market price on the products in the U.S because consumers are willing to pay additional costs for organic products. Organic sales in the U.S. doubled since 2005, which shows favorable attitudes of U.S consumers towards organically produced goods (USDA, 2021). From this trend, using organic by-products as fertilizer for food production would add market values to both products, and the by-products itself.

One of the organic by-products that can be utilized in food production is spent grain from beer production. Brewers' spent grain (BSG) is the main waste by brewing process, representing ~85% of the total by-products obtained. Average annual global production is estimated to be ~39 million tons (Lynch et al., 2016). During the brewing process, the starchy endosperm is degraded into sugars for the yeast to ferment. The leftover of grain mainly contains fiber (30%- 50%; w/w), which includes cellulose, hemicellulose and lignin, and protein (19%-30%). For these reasons, BSG is a nutrient-rich waste that can be applied as animal feed (Saba et al., 2019). However, this biomass deteriorates rapidly, resulting in logistic difficulties in storage and transportation for animal feed. industries often dispose the material in landfills (de Araujo et al., 2020). Adapting raw BSG to soil for farming instead of feeding livestock has a potential to enhance soil health by supplementing organic materials and support microbial activities. The main objective of this project was to determine if application of BSG can enhance soil health, and influence crop yields.

2. METHODS

Nursery pots were utilized to grow radishes in a greenhouse. In each pot three Rover F1 radish seeds were sowed on 7 Oct, 2020 and harvested on 28 Oct, 2020. Twenty pots (4 × 4 × 10 in) contained finely ground soil (460 g dry weight) collected at Texas A&M University farm. The soil background information was analyzed by the USDA soil survey, pH probe. The soil is classified as Belk clay (Fine, mixed, active, thermic Entic Hapluderts) with 52% clay, 29.3% silt and 18.2% sand in the 0 to 4 inches of topsoil. The pH was 7.78. The BSG was collected and applied within 24 hours after being created and had 69% moisture content. The five treatment were no application, 10 g wet surface application, 40 g wet surface application, 10 g wet incorporation, and 40 g incorporation, with four replications of each treatment (Figure 2-1). Water (100 mL) was irrigated every two days. The same experiment was repeated from 12 Jan, 2021 to 16 Feb, 2021 and extended two weeks (i.e., five weeks instead of three weeks). After harvesting from the first experiment, the impact of BSG was assessed by evaluating the dry biomass of radishes, P and K content in radishes, total C:N ratio in soil, and enzyme activity in soil as parameters of soil health. Due to winter storm in College Station Texas in Feb 2021, the radishes died, and the soil and radish samples were no longer available for data analysis. Therefore, samples only from the first experiment was assessed for BSG impact on soil health and productivity. Pictures of radishes and physical soil structure were taken from the second experiment on 9 Feb, 2021 to visualize the influence of BSG on soil health and plant growth.

Figure 2-1: The experimental setup of 20 pots with no application on the top, 10 g surface applications are on the top left, 40 g surface application on the bottom left, 10 g incorporation on the top right, and 40 g incorporation on the bottom right. Each pot has three seeds sown in about two inches depth.

2.1 Dry Biomass and Nutrient Contents in Radish

Dry biomass and nutrient contents were assessed as an indicator of plant productivity. Harvested radish tissues (i.e., shoots and roots) were air-dried before being gravimetrically weighed. For nutrient contents in radish tissues, Phosphorus and K were selected for analysis because these are two of the macro-nutrients, which plants require the large amount of. Inductively coupled plasma elemental analysis (ICP) was used to measure the P and K contents in radish tissues and converted to mg kg^{-1}, which is equivalent to parts-per-million (ppm). Data were analyzed using t-test to visualize significant difference in each treatment ($p = 0.05$).

2.2 C:N Ratio in Soil

Soil health was assessed with the ratio of total carbon to total nitrogen in soil (C:N ratio). C:N ratio in soil is ratio of carbon content (%) to nitrogen content (%) present in the soil. The C:N ratio was calculated by using a formula below:

$$C{:}N = (\%C) / (\%N) \tag{2.1}$$

The %C refers to percentage of total carbon content in soil on a dry weight basis, and %N indicates percentage of total nitrogen content in soil on a dry weight basis. For example, C:N ratio of 20 means there is twenty mass units of carbon for each mass unit of nitrogen in the soil. The C:N ratio requirement for microorganisms to stay alive is 24:1, and organic amendments have a wide range of C:N based on the original materials. Higher C:N ratio causes a deficit of nitrogen in soil, resulting in competition of nitrogen between microorganisms and plants. On the other hand, a C:N ratio less than 24 provides abundant nitrogen in soil to microbes for digestion of organic matter, resulting in net release of inorganic nitrogen for plant uptake as the nutrient source (Gerald, 2019). Thus, C:N ratio should indicate whether application of BSG to soil can provide nitrogen to plants, as a factor to enhance plant growth. The C:N ratio in each variety was assessed with Vario EL Cube CNS Analyzer (Elementar, Americas Inc. Ronkonkoma, NY 11779), which can measure carbon and nitrogen in samples up to 12,000:1. The C:N data were assessed with t-test ($p = 0.05$) to determine significant difference among treatments.

2.3 Microbial Enzyme Activity in Soil

Enzyme activity in soil was also used for a parameter of soil health. Microbes secrete enzymes when they digest organic materials. Glucosidase enzymes is used for catalyzing hydrolysis of starch to simple sugars for microorganisms. N-Acetylglutamate (NAG) functions as the first intermediate in the arginine bio-synthetic pathway, and phosphatase will be used for obtaining phosphate groups from protein. These enzymes indicate how actively microorganisms are reacting in the soil for organic matter digestion (Shi D et al., 2015). Comparing enzyme activity with each variety evaluates how BSG stimulates nutrient cycling in soil via microbial activity. A microplate reader Synergy H1, BioTek, Winooski, VT) was used to assess enzyme activity in each treatment for the different enzyme activities. Statistical analysis (t-test) was used to show significant difference in each variety for the different enzyme activities associated with soil health ($p = 0.05$).

3. RESULTS

The 1st experiment showed obvious impacts of brewer's spent grain (BSG) on radish growth (Figure 3-1). The incorporated BSG had higher vegetative growth than control and surface application. 40 g-wet application supported higher aboveground growth than 10 g and 0 g application.

Figure 3-1: Picture of radishes in different treatment in 1st experiment. No application on the top, 10 g surface applications are on the top left, 40 g surface application on the bottom left, 10 g incorporation on the top right, and 40 g incorporation on the bottom right.

At the 2nd experiment, visual differences in vegetative growth rate among varieties were the same as the 1st experiment result (Figure 3-2). Overall growth rate looked better in the second experiment because radishes were grown for 21 days in the 1st experiment, compared to 35 days at the 2nd trial. Moreover, presence of BSG on soil surface or underground helped development of visible microfauna, which ws most prevalent with incorporated 40 g BSG application (Inc40) than other treatments.The greatest germination rate was observed in Inc40 treatment.

Figure 3-2: Picture of radishes in different treatment in 2nd experiment after five weeks since planting. No application (cont), 10 g surface applications (Sur10), 40 g surface application (Sur40), 10 g incorporations (Inc10), and 40 g incorporations (Inc40), respectively from the left.

After harvesting, pictures of cross sections of soil from each pot were taken to compare soil structure, root formation, and fungus formation under the soil. The most prominent difference was observed between control and Inc40 treatments (Figure 3-3). Under no BSG application, soil was compacted and few pore spaces were visible. Roots were shallow and water drainage was poor in entire season. On the other hand, BSG created granular soil structure that supported deeper root formation and good water drainage in Inc40. Fungus hyphae were observed surrounding to the radish roots. The initial soil used was dried, sieved and ground prior the experiment, so the initial soil and poor soil structure. The improvement of soil structure and porosity can be attributed to the BSG amendments.

Figure 3-3: Cross sections of control soil (left) and 40g incorporation soil (right) after harvesting.

3.1 Dry Biomass of Radish

Figure 3-4 shows the total dry tissue of radish with each treatment from 1st experiment. Overall, the greatest biomass was harvested from incorporation 40 g BSG application (Inc40) followed by surface 40 g application (sur40). Inc40 and Sur40 had significant difference from control and surface 10g application while the control and 10 g surface application (sur10) were not significantly different.

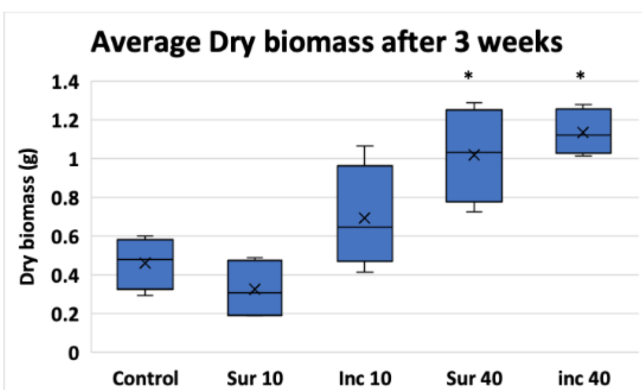
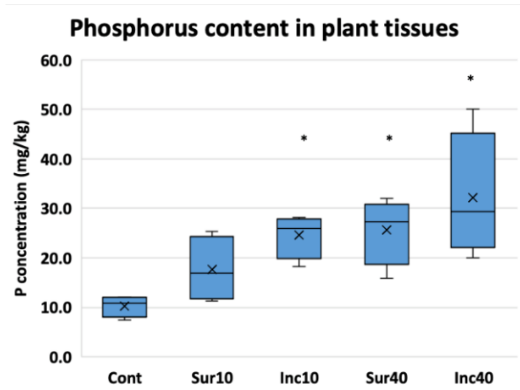


Figure 3-4: Dry biomass after three weeks since planting. Horizontal axis shows each variety group, and Vertical axis shows gravimetric dry biomass (g). The stars indicate significant difference of the group from control group.

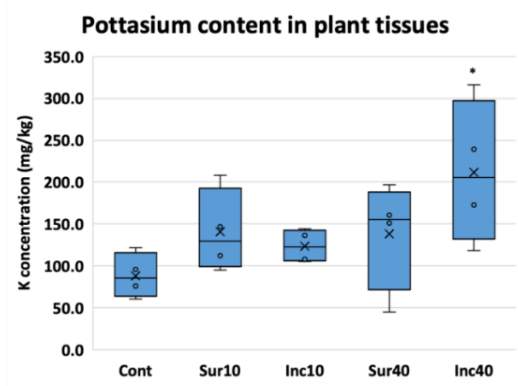
3.2 Phosphorus and Potassium contents in Radish

Phosphorus (P) concentration in radish resulted in differences between Inc 10, Sur 40, and Inc 40 from the control group (Figure 3-5). Radishes with the Inc40 treatment had about 30 mg P/kg, which was three times more P than the control group (10 mg P/kg).



3-5: Phosphorus content in radish tissues. Horizontal axis shows each variety group, and vertical axis shows total phosphorus concentration (mg/kg). The stars indicate significant difference of the group from control group.

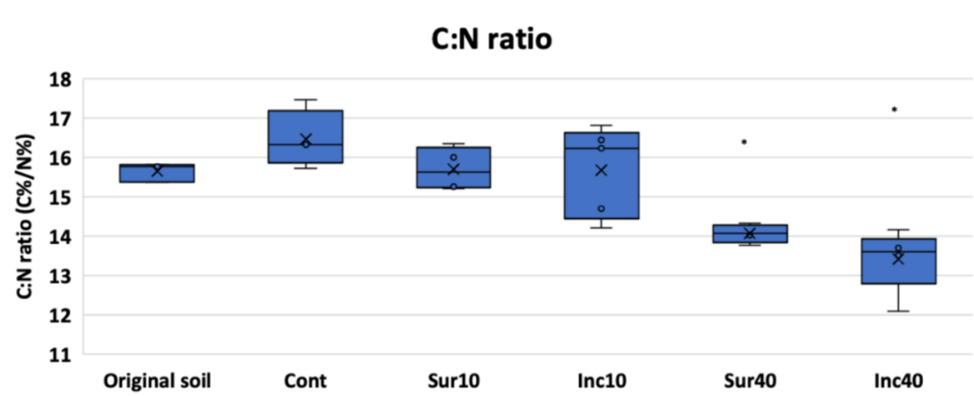
The K concentration in radishes in the Inc40 treatment was significantly different from the control group (Figure 3-6), which was twice that of the control group (200 vs 100 mg K/kg). The K concentration in the Sur10, Inc10, and Sur40 treatments were not significantly different from the control group.



3-6: Potassium content in radish tissues. Horizontal axis shows each variety group, and vertical axis shows total potassium concentration (mg/kg). The stars indicate significant difference of the group from control group.

3.3 C:N Ratio in Soil

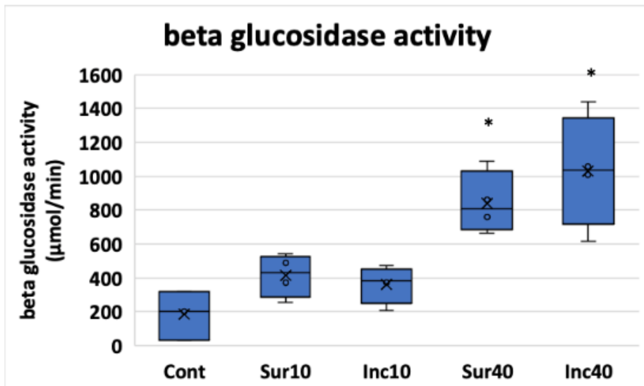
The C:N ratio of Sur40 and Inc40 were significantly less than the control treatment (Figure 3-7). Overall, C:N ratio had decreasing trend as application rate increased, which indicate that total nitrogen content has been increased as more BSG was applied.



3-7: C:N ratio in soil of different varieties. Horizontal axis shows each variety group, and original soil. Vertical axis shows C:N ratio. The stars indicate significant difference of the group from control group.

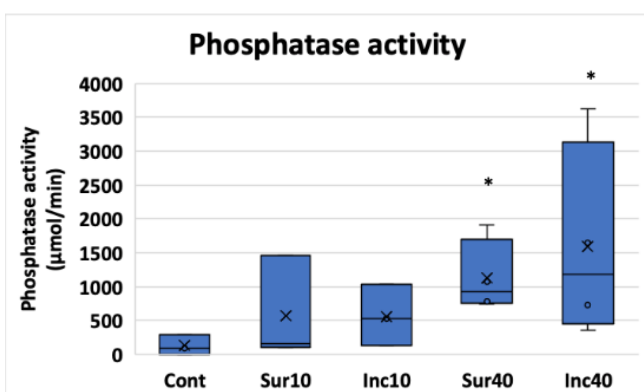
3.4 Microbial Enzyme Activity

Three extracellular enzyme activity were evaluated to indicate the activities of carbon and nitrogen cycling processes. Beta glucosidase activity in Sur40 and Inc40 was significantly greater than all other treatment groups (Figure 3-8). The control, Sur10, and Inc10 application groups did not differ from one another. Average beta glucosidase activity increased by 800 µmol/min from the control to Inc40 treatment.



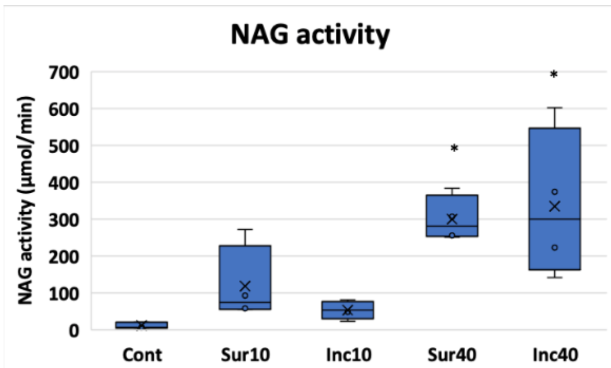
3-8: Beta glucosidase activity in different BSG application. Horizontal axis shows each variety group. Vertical axis shows beta glucosidase activity in umol/min. The stars indicate significant difference of the group from control group.

Phosphatase activity of Sur40 and Inc40 was also greater the control group (Figure 3-9). The control, Sur10, and Inc10 application groups did not different from one another. Average phosphatase activity increased by 1200 µmol/min from the control to the Inc40 treatmemt.



3-9: Phosphatase activity in different application groups. Horizontal axis shows each variety group, and original soil. Vertical axis shows enzyme activity rate in umol/min. The stars indicate significant difference of the group from control group.

N-Acetylglutamate (NAG) enzyme activity was significantly greater in the Sur40 and Inc40 treatments compared to the control group (Figure 3-10). The control, Sur10, and Inc10 treatment groups did not differ from one another. Average phosphatase activity increased by 300 µmol/min from the control to the Inc 40.



3-10: N-Acetylglutamate activity in different application groups. Horizontal axis shows each variety group, and original soil. Vertical axis shows enzyme activity rate in umol/min. The stars indicate significant difference of the group from control group.

4. CONCLUSION

4.1 Discussion

The overall results suggest that brewer's spent grain (BSG) improved biomass of radish, tissue P, K and N, and the extracellular enzyme activities in soil. Although there was no difference between incorporated and surface applied treatments at the same BSG addition rates, incorporated BSG was constantly greater than surface applied. This was readily observed in the cross-sectional images of the soil in the pots where incorporated improved soil structure throughout the pot, while soil structure in the surface applied was concentrated at the surface. Generally incorporated organic matter has better contacts with microbes, creating more chance for bacteria or fungi to decompose organic materials than surface application. Microbial enzymes break down organic materials and release inorganic nitrogen, C:N ratio would become lower, which means more free nitrogen gets available for plants' uptake. Like other organic amendments, BSG showed critical roles to improve nutrient cycles in soil. Based on the amount of BSG application, 40 g of BSG had significant difference than 0 or 10 g application. Because the Sur40 application and Inc 40 application did not have significance difference in the most parameters, the application rate had the key factor in this experiment. More or less, BSG stimulated nutrient cycle in soil and provided nutrients for plants.

4.2 Limitation and Future Work

The germination rate was highly impacted by physical property of soil, which led very poor soil structure. The lack of macro-pores due to poor soil structure kept water from infiltrating and percolating through the soil. Incorporated BSG acted to enhance infiltration creating macropores, which provide a pathway for water to enter the soil. This characteristic can be observed in other organic amendments, enhancing soil structure and infiltration by increasing total soil porosity (Lourdes et al., 2017). However, this experiment did not conduct any analysis for quantitative data to show weather BSG improves infiltration rate and physical soil structure. Evaluating the influence of BSG on infiltration rate, water content, and soil aggregate stability should be added in future work.

The poor drainage of soil water may have contributed to the poor performance of the control and thus differences among. The finely ground soil has poor soil structure and thus few macropores, which allow infiltration and percolation. The 100mL of irrigation water oversaturated the control treatment, which reduced germination. Oversaturation was less in BSG treatments indicating that under these conditions the BSG perform under improved drainage conditions.

This experiment did not identify species of microbes encouraged by BSG. It is important to identify these organisms prior to use of BSG because some fungal or bacterial species can induce serious diseases for plants. We need to figure out in the future if the fungal species formed with BSG is actually safe for plant growth or cause any diseases and abnormality in plant life cycle.

4.3 Conclusion

Brewer’s spent grain (BSG) had influenced soil health and plant growth. The BSG increased radish yield, improved soil structure, provided nutrients, and stimulated nutrient cycling in soil. In the future, it would be good to evaluate in sol with good soil structure to clarify BSG’s ability to enhance plant growth. Also, a field experiment with different crops would further solidify the benefits of BSG in agriculture.

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