# The effects of *Fictibacillus enclensis* and biochar on plant growth

By
Tanisha Ballard

A Thesis Submitted to
Saint Mary's University, Halifax, Nova Scotia
in Partial Fulfillment of the Requirements for
the Degree of Bachelor of Science with Honours in Biology

April, 2022, Halifax, Nova Scotia



| | |
|---|---|
| Approved: | Dr. Clarissa Sit<br>Supervisor |
| Approved: | Dr. Zhongmin Dong<br>Examiner |
| Date: | April 26, 2022 |

The effects of *Fictibacillus enclensis* and biochar on plant growth

By
Tanisha Ballard

## Abstract

Food insecurity is an emerging concern in the world, as global populations are increasing and the yield and quality of crops are declining. Excessive use of agrochemicals designed to improve plant growth is very damaging to the environment, which warrants the need for sustainable alternatives. The exploitation of plant growth-promoting bacteria is one alternative being considered due to the inherent relationship between plants and beneficial bacteria within the soil. The use of biochar provides its own benefits to plants in addition to acting as a carrier for bacterial inoculum.

Here we investigated the effects that a formulation of *Fictibacillus enclensis* and biochar had on the vegetative growth of collard greens, carrots, and barley. Carrots treated with the *F. enclensis* and biochar formulation exhibited little benefit in terms of carrot root weight and length; however, collard greens treated with the *F. enclensis* and biochar formulation displayed improvements in leaf weight. In the barley trial, the formulation of *F. enclensis* and biochar did improve some aspects of the vegetative growth of barley when compared to *F. enclensis* by itself.

However, these results can not be relied upon. The positive controls did not produce any benefits to the vegetative growth of the plants. Therefore, any results that are obtained do not accurately showcase the effects that the *F. enclensis* and biochar formulation has on the vegetative growth of plants; something is wrong in the system being used to grow the plants. The growth of the plants is being restricted by certain limiting resources/factors. Consequently, any interpretation of the results is deemed inaccurate and unreliable.

April 26, 2022

**Acknowledgements**

I would first like to thank Dr. Clarissa Sit for allowing me to be apart of her research group. Her support has helped guide me through this process and the challenges that I faced. I would also like to thank Dr. Zhongmin Dong as well for the guidance that he provided me while I was growing the plants.

Thank you to the past and present members of the Sit research group: Cassie Burns, Nicola Augustin, Michaela Smit, Logan Cox, Lauren Grant, Jenn Kolwich, and Julie Ann Dayrit. They were always there to lend a helping hand and to provide support both in and out of the lab. I would also like to thank the biology and chemistry departments for their assistance as well.

A big thank you goes to my friends and family for all of their encouragement and support. I couldn't ask for a better support system!

# Table of Contents

# 1. Introduction

## 1.0.0. Agriculture – Food Supply and the Economy

Agriculture provides many countries with a means to support their economy while also providing people with essential foods and by-products (Townsend, 2015). Agricultural commodities such as cereals, fruits and vegetables, meat, and dairy products supply people with the necessary nutrition and energy needed to sustain life (Organisation for Economic Co-operation and Development, 2008; Townsend, 2015). The production of these commodities varies across countries and regions requiring reliance on international trade to import and export goods (OECD, 2008; Townsend, 2015). This trade contributes to a country's economy through the profits gained from their exported goods, and it also provides a source of income for individuals that are employed in the agricultural industry (OECD, 2008; Townsend, 2015). For example, Canada generates $139.3 billion from the agricultural industry while also generating 1 in 9 jobs throughout the country (Government of Canada, 2021). However, this is all under threat as the world's agricultural sector is anticipated to struggle to provide goods to a larger global population while simultaneously trying to combat the effects of climate change on crop production (Food and Agriculture Organization, 2009; Lesk et al., 2016; Prosekov & Ivanova, 2018).

## 1.0.1. Food Insecurity Concerns

Looking at the future of agriculture, there is growing concern regarding its ability to contend with an increased demand for food, and the prospect of food insecurity across the globe (FAO, 2009; Prosekov & Ivanova, 2018). A rising global population and the increased frequency of severe weather occurrences related to climate change (i.e., floods, droughts, etc.) have largely

contributed to this concern (FAO, 2009; Lesk et al., 2016). According to The Food and Agriculture Organization of the United Nations (FAO), the world's population is expected to increase by 34% from 2009 to 2050, which will not be sustained by the current availability of food (FAO, 2009). Furthermore, the agricultural products currently produced are under immense strain from the effects that increasing instances of severe weather occurrences have on their production (Lesk et al., 2016). These extreme weather events are attributed to climate change and are only anticipated to worsen as their likelihood of occurrence is predicted to increase in the future (Battisti & Naylor, 2009; Intergovernmental Panel on Climate Change, 2012). Consequently, when combining the effects of climate change on crop production with a larger global population to feed, there emerges concern over how to address this inevitable food insecurity crisis (Spiertz, 2010). Current strategies to address this crisis include supporting the research into, and development of, sustainable agriculture (Canadian International Development Agency, 2009). A major component of this is the reduction of harmful agrochemical use (CIDA, 2009).

### 1.0.2. Agrochemical Use

Agrochemicals are chemically synthesized products, such as fertilizers and pesticides, used in agriculture to increase crop production (Bhandari, 2014). Chemical fertilizers primarily contain nitrogen, phosphorous, and potassium; important nutrients needed for a plant to grow (Stewart et al., 2005). Chemical pesticides function to deter or kill pests and pathogens that would otherwise damage plants, thereby increasing crop yield (Bhandari, 2014). Therefore, both fertilizer and pesticide use in agriculture serves to increase the yield of crops (Bhandari, 2014).

Excessive application of agrochemicals has contributed to various environmental issues (Davidson, 2009; Erisman et al., 2008; Good & Beatty, 2011). To ensure optimum increases in crop production, agrochemicals need to be applied at the right time and with the correct dose (Bhandari, 2014). In spite of this knowledge, agrochemicals, and specifical fertilizers, have not been applied to agricultural fields correctly (Good & Beatty, 2011; Sun et al., 2012). Its application has been excessive, and this is based on the belief that the application of more fertilizer will equate to greater yields of crops (Good & Beatty, 2011; Sun et al., 2012). While greater crop yields were observed, this overapplication has undoubtedly had long-standing impacts on the environment (Davidson, 2009; Erisman et al., 2008; Good & Beatty, 2011). This is because the estimated efficiency of these applied chemical fertilizers is low (Baligar & Bennett, 1986). For example, in tropical conditions, the efficiency of applied chemicals fertilizers is averaged to be around 50% or less (Baligar & Bennett, 1986). In other words, plant uptake of the nutrients in chemical fertilizers is around 50% or less in this environment (Baligar & Bennett, 1986). When an excess of chemical fertilizer is applied, thereby surpassing what the crops requires, the resulting excess is lost through runoff, release into the atmosphere, or it is retained by soil microbes (Baligar & Bennett, 1986). This loss inevitably has negative impacts on the environment (Davidson, 2009; Erisman et al., 2008; Good & Beatty, 2011).

### 1.0.3. Agrochemical Application Complications

The presence and accumulation of algae in aquatic ecosystems has been attributed to the excessive application of chemical fertilizer on agricultural fields (Rabalais et al., 2009; United States Environmental Protection Agency, 2007; Withers et al., 2014). The excessive growth of algae results in response to an increased amount of nutrients that have leached from fields

supplied with excess fertilizer (US EPA, 2007). The process in which an aquatic ecosystem becomes supplied with excess nutrients is known as eutrophication (Withers et al., 2014). The algae produces either harmful toxins or causes oxygen depletion of the water (Shumway, 1990). This occurs when the algae die and undergo decomposition by bacteria (Prepas & Charette, 2003). Decomposition requires oxygen, which inevitably results in oxygen depletion of the water when there is a surge in algal growth (Müller et al., 2012; Prepas & Charette, 2003; Rabalais et al., 2009; US EPA, 2007). This severely impacts many marine organisms who are now faced with hypoxia, which is the decrease in normal oxygen concentrations to a level that is unsustainable for most marine life (US EPA, 2007). The toxins produced from toxic algae are taken up by filter feeders, such as shellfish, where they can accumulate and ultimately lead to shellfish poisoning when consumed by humans (Shumway, 1990). In addition to this public health risk, toxic algae cause harm to the shellfish themselves as mass mortalities have been attributed to this algal accumulation (Shumway, 1990). In the Alaskan clam industry, 5 million pounds of shellfish products were produced in 1917; however, today the industry is nonexistent because of the mass mortality of shellfish (Shumway, 1990). This affects the economic prosperity of such industries and the individuals employed by them (Shumway, 1990). Therefore, eutrophication has emerged as an economic threat, an environmental threat, and a public health risk requiring immediate action to halt its primary causative agent: the overapplication of chemical fertilizer (Withers et al., 2014).

Another threat that the overapplication of chemical fertilizer poses to the environment is the increase in nitrous oxide ($N_2O$) emissions into the atmosphere (Davidson, 2009; Erisman et al., 2008; Good & Beatty, 2011). $N_2O$ is primarily produced by microbes within the soil undergoing nitrification and denitrification processes in response to nitrogen applied in chemical

fertilizers (Davidson, 2009; Good & Beatty, 2011). Nitrification is the oxidation of ammonium ($NH_4^+$) or ammonia ($NH_3$), two forms of nitrogen present within the soil, to nitrate ($NO_3^-$) (Wrage et al., 2001). This reaction produces several intermediate species that are decomposed to form $N_2O$ (Wrage et al., 2001). Denitrification is the reduction of $NO_3$ to nitrogen ($N_2$) where $N_2O$ is an intermediate species set free during the reaction (Wrage et al., 2001). Along with carbon dioxide ($CO_2$), methane ($CH_4$), and chlorofluorocarbon-12 (CFC), $N_2O$ is categorized as one of the major anthropogenic greenhouse gases contributing to climate change (Davidson, 2009). However, $N_2O$ appears to be a much stronger greenhouse gas (Lashof & Ahuja, 1990). In comparison to $CO_2$, the calculated global warming potential for $N_2O$ is 180 times that of the global warming potential for $CO_2$ (Lashof & Ahuja, 1990). Other sources attribute an even higher global warming potential of $N_2O$, with some estimates stating that its global warming potential is 296 times that of $CO_2$ (US EPA, 2010). Nevertheless, both numbers demonstrate how powerful $N_2O$ is as a greenhouse gas (Lashof & Ahuja, 1990; US EPA, 2010). The continued use of chemical fertilizers will undoubtedly stimulate a greater release of $N_2O$ into the atmosphere, which will inevitably contribute to higher rates of global warming (Lashof & Ahuja, 1990). This in turn poses a great environmental threat if the use of chemical fertilizers is continued, and so alternatives need to be implemented to enhance crops in a more sustainable way (Spiertz, 2010).

## 1.1. Sustainable Agriculture

Sustainable agriculture aims to address problems like eutrophication and $N_2O$ emissions by ensuring that crops are produced in a sustainable way (Reganold, et al., 1990). The basis of sustainability aims to provide for the present while ensuring that there will be a means to provide for the future; something that cannot be upheld if agrochemicals are continuously used (Spiertz,

2010). Ideally, the crops produced from sustainable agriculture should be comparable in quality and quantity to crops supplied with agrochemicals, but it does so by a more natural means of enhancing the plant growth of these crops (Reganold, et al., 1990). Suggestions for sustainable agriculture include the use of plant growth promoting bacteria, biological pest and disease control, as well as nutrient/organic matter cycling (Spiertz, 2010).

### 1.0.0. Plant Growth-Promoting Bacteria

Plant growth-promoting bacteria are a means of sustaining agriculture due to the beneficial relationships that they form with plants (Pathan et al., 2020; Spiertz, 2010). The soil surrounding a plant contains various microorganisms including bacteria, fungi, and algae that can influence its growth (Glick, 1995; Glick, 2012). Bacteria are the most common microorganism in the soil with higher concentrations located around the rhizosphere (Glick, 2012). The rhizosphere is the region of soil immediately surrounding the plant roots that can be affected by secretions of the root hairs (Berendsen et al., 2012). Bacteria present within this rhizosphere can either negatively, neutrally, or positively affect the plant. (Glick, 1995; Glick, 2012). Those that positively affect the plant do so by promoting plant growth, and are therefore termed plant growth-promoting bacteria (Glick, 1995; Glick, 2012). The plant's health and success often depend on these plant-bacteria interactions as they allow the plant to experience greater nutrient acquisition, tolerance to stress, and resistance to pests and diseases (Glick, 1995; Glick, 2012; Santoyo et al., 2016). Plant growth-promoting bacteria can be exploited in agriculture as a means of sustainably promoting plant growth due to their inherently symbiotic nature with plants (Glick, 1995; Glick, 2012).

The bacteria that can positively influence plant growth do so in a variety of ways (Glick, 1995; Glick, 2012). They can promote plant growth either directly or indirectly (Glick, 1995; Glick, 2012; Santoyo et al., 2016). Indirect promotion of plant growth by plant growth-promoting bacteria occurs when the bacteria act as a biocontrol agent that inhibits the effects of plant pathogens and diseases (Santoyo et al., 2016). In contrast, bacteria that promote plant growth directly do so by adjusting the levels of hormones produced by the plant and by assisting in resource acquisition of essential nutrients, both of which regulate the growth and development of a plant (Glick, 1995; Glick, 2012; Santoyo et al., 2016).

Plant hormones and nutrients have different stimulatory effects on plants (Glick, 2012). Plant growth-promoting bacteria are involved in the acquisition of these nutrients and hormones, which allow for the successful growth of plants (Glick, 2012). For example, nitrogen can be provided to plants through either the production of $NH_3$ or through the nitrogen-fixing capabilities of plant growth-promoting bacteria (Bhattacharyya et al., 2020; Glick, 2012). Nitrogen is involved in the creation of amino acids, proteins, and other components needed for the growth of cells and plant organs, which includes the components that constitute the yield of the crop (Lawlor et al., 2001). This is why nitrogen plays a critical role in determining the yield of a crop (Spiertz, 2010). In regards to the modulation of plant hormones, plant growth-promoting bacteria can influence the levels of auxins, gibberellin, cytokinin, and ethylene, which have various effects on plant growth and development (Santoyo et al., 2016). For example, the secretion of indole acetic acid (IAA), an auxin, by plant growth-promoting bacteria affects the root development of plants (Glick, 2012). Successful utilization of these direct, and indirect mechanisms, of plant growth promotion would address the food insecurity crisis while also maintaining agricultural sustainability (Spiertz, 2010).

### 1.1.1. Experimental Evidence Supporting the Effects of Plant Growth-Promoting Bacteria

There is substantial evidence supporting the use of plant-growth-promoting bacteria due to their significant effects on crop yields (Cui et al., 2019; Helaly et al., 2020; Viljoen et al., 2019). In collard greens that were supplied with specific plant growth-promoting bacteria (*Serratia marcescens*; *Pseudomonas poae*; *Plantibacter flavus*; and *Bacillus amyloliquefaciens subsp. plantarum*), there were significant increases in plant height, leaf size, number of leaves, and crop yield when compared to the control treatment (Helaly et al., 2020). Another study noted improvements in plant height, grain yield, and straw yield of wheat when treated with *Bacillus megaterium*, *Arthrobacter chlorophenolicus*, and *Enterobacter sp.* (Kumar et al., 2014). These results are attributed to each bacteria's own production of certain plant hormones and their ability to provide important nutrients to the plant (i.e., IAA production, $NH_3$ production, and nitrogen-fixing capabilities) (Kumar et al., 2014).

There is also evidence supporting the use of plant growth-promoting bacteria as biocontrol agents (Cui et al., 2019; Viljoen et al., 2019). In testing the biocontrol properties of *Bacillus amyloliquefaciens B9601-Y2* against southern corn leaf blight caused by *Bipolaris maydis,* the disease index decreased from 59.58% in the control treatment to 18.30 % in the bacterial treatment group (Cui et al., 2019). This likely contributed to the increased crop yields that resulted: 1.01 kg/plant in the control treatment to 1.64 kg/plant in the bacterial treatment (Cui et al., 2019). Another experiment looked at the biocontrol potential of five plant growth-promoting bacteria - *Bacillus firmus*, *Bacillus aryabhattai*, *Paenibacillus barcinonensis*, *Paenibacillus alvei,* and *Bacillus cereus* - against the nematode, *Meloidogyne incognita*, in

carrots (Viljoen et al., 2019). The bacterial strains reduced nematode-induced gall numbers by 81.5% - 86.0%, thereby contributing to a likely increase in crop yield (Viljoen et al., 2019). These studies give an indication of the effectiveness of plant growth-promoting bacteria to increase crop yields, thereby providing an alternative to agrochemical use to address future food insecurity.

## 1.2. Biochar

In addition to plant growth-promoting bacteria, biochar has also been found to influence plant growth (Verheijen, et al., 2010; Caroline et al., 2016). Biochar is defined as a product of pyrolysis, which involves the heating of organic matter at high temperatures without oxygen being present (Verheijen et al., 2010). The raw materials that produce biochar vary, and they can include crop residues, forest waste, and manure (Rawat et al., 2019). Its application to soil has been found to increase microbial activity, which is beneficial to the plant, in response to an increase in the availability of nutrients (Caroline et al., 2016; Verheijen, et al., 2010;). This is because of the biochar's involvement in maintaining a high soil organic matter, which reserves nutrients and also water (Lehmann & Kleber; Verheijen, et al., 2010). Soil organic matter is composed primarily of plant materials that contain plant nutrients; therefore, this organic matter serves as a storage unit for plant nutrients (Lickacz & Penny, n.d.). Consequently, plant growth will be benefitted from the increased availability of nutrients that are stored within this soil organic matter (Caroline et al., 2016; Lehmann & Kleber; Verheijen, et al., 2010).

Biochar also serves as a carrier for plant growth-promoting bacteria (Vecstaudza et al., 2017). The delivery of plant growth-promoting bacteria to a plant can benefit from a carrier in terms of its successful inoculation of the soil (Vejan et al., 2019). The function of the carrier is to

protect and nourish the bacterium to ensure its survivability before and after its application to the soil (Hale et al., 2014; Vecstaudza et al., 2017). Using qualitative PCR (qPCR) and selective plate count assays, Hale et al. were able to confirm that bacterial survival was improved when using biochar as a carrier for the bacterium as opposed to directly inoculating the soil with the bacterium (Hale et al., 2014). Other studies have noted an increase in the vegetative growth of plants inoculated with both bacteria and biochar, compared to bacteria and biochar alone (Vecstaudza et al., 2017). This represents a synergistic relationship between the biochar and bacterium that provides greater benefits than having the plant growth-promoting bacteria applied by itself (Vecstaudza et al., 2017).

## 1.3. Use of Plant Growth-Promoting Bacteria in Agriculture

Currently, there still exist limitations to using plant growth-promoting bacteria (Bashan et al., 2013; Egamberdiyeva, 2007; Rilling et al., 2019). Often times, laboratory results fare better than field trials due to the bacteria's susceptibility to harsher and more realistic environmental conditions (Bashan et al., 2013; Rilling et al., 2019). Studies have investigated the plant growth-promoting properties of bacteria applied to various types of soil and have noticed differences in their effectiveness at promoting plant growth (Egamberdiyeva, 2007). Within one study the selected bacterial strains performed better (i.e., promoted greater plant growth and nutrient uptake) in nutrient deficient calcisol soil rather than in loamy sand soil (Egamberdiyeva, 2007). Plant growth-promoting properties of the bacteria can also differ depending on what crop it is being used on and what environmental conditions it may be exposed to (Rilling et al., 2019). These findings have resulted in a lack of success in implementing the commercialization of plant growth-promoting formulations (Bashan et al., 2013; Rilling et al., 2019). Therefore, the

continued discovery and testing of plant growth-promoting bacteria in different formulations is warranted to increase the likelihood of their successful inoculation and effect on plants (De Souza et al., 2015). In addition, a better understanding of the factors affecting the bacteria's colonization and activity within the soil of a plant is also needed to combat their limitations (Rilling et al., 2019).

## 1.4. *Fictibacillus enclensis*

The recent discovery of *Fictibacillus enclensis* as a potential plant growth-promoting bacteria, with a biochar formulation, provides a new avenue to sustainably promote plant growth (Blatt-Janmaat et al., 2020). *F. enclensis* was discovered and identified using 16S rRNA sequencing, in a contaminated batch of biochar (Blatt-Janmaat et al., 2020). Bacteria within the *Fictibacillus* genus have previously been categorized as plant endophytes (Yan et al., 2018). Endophytes are bacteria or fungi that populate the internal structures of plants without causing any harm to the plant itself (Reinhold-Hurek & Hurek, 2011). In fact, endophytes promote plant growth by increasing the availability of necessary nutrients for the plant, and by producing compounds that inhibit the effects of plant pathogens (Rosenblueth & Martínez-Romero, 2006). There is a possibility that *F. enclensis* is an endophyte, because it belongs to the *Fictibacillus* genus; therefore, there is potential for it to be a plant growth-promoting bacterium (Blatt-Janmaat et al., 2020).

Blatt-Janmaat et al. tested the effects of *F. enclensis* with biochar on the vegetative growth of barley plants (Blatt-Janmaat et al., 2020). This study revealed that *F. enclensis* exhibited the highest fruit:shoot ratio (fruit weight divided by shoot weight) compared to the control treatment and to the other bacterial treatment groups (Blatt-Janmaat et al., 2020). The

authors attributed this to a shift in resource allocation from the shoots to the fruits (Blatt-Janmaat et al., 2020). Past attempts at altering the resource allocation of crops have focused on modifying shoot architecture, such as removing the lateral branches of a plant, in an effort to produce crops with larger fruits (Bennett et al., 2012). Therefore, resource allocation is an avenue of interest among researchers involved in the development of sustainable agriculture, and the resource allocation strategies of some plant growth-promoting bacteria have been tested as well (Dai et al., 2016).

Additional plant trials were conducted on the effects of *F. enclensis* with biochar and an investigation into the plant growth-promoting properties of *F. enclensis* was also initiated (Burns, 2021). Compared to the control, benefits were noted in the strawberry leaves, collard leaves, and carrot weight when they were treated with *F. enclensis* and biochar (Burns, 2021). Leaf damage on the strawberry leaves treated with *F. enclensis* and biochar was also significantly less than the leaf damage of the control treatment group (Burns, 2021) This was attributed to the bacteria's potential as a biocontrol agent (Burns, 2021). Similar to the study conducted by Blatt-Janmaat et al., a high head:shoot ratio in broccoli and a high leaf:shoot ratio in collard greens were also noted in the plants treated with *F. enclensis* and biochar (Burns, 2021; Blatt-Janmaat et al., 2020). Various qualitative tests on the plant growth-promoting properties of *F. enclensis* revealed that it could fix nitrogen and produce IAA (Burns, 2021).

## 1.5. Research Goals and Questions

In an effort to sustain the agricultural sector, by reducing the use of agrochemicals, the goal of this research is to gain more information on the potential plant growth-promoting bacteria, *Fictibacillus enclensis*, as well as how it influences plant growth when supplemented

with biochar. Both plant growth-promoting bacteria and biochar represent sustainable means of improving crop yields, therefore providing a potential solution to the food insecurity crisis. Questions remain regarding the bacteria's effect on different plant species and how it may influence their growth.

The objective of this study is to test the effects that *F. enclensis* and biochar had on the vegetative growth of various plants. The present study conducted more trials looking at the effects that *F. enclensis* and biochar have on collard greens, carrots, and barley. Additionally, this study is looking to uncover whether or not the bacteria is still present within the soil after inoculation with one and multiple applications. This is to better understand the bacteria's activity within the soil, and to get a better understanding on whether or not plants would require more than one application of this formulation to have significant effects on plant growth.

# 2. Materials and Methods

## 2.1. Collard Greens and Carrot Trials

### 2.1.0. Treatments and Set-Up

The collard and carrot trials began with the planting of their seeds to grow into the seedling stage. Top Bunch collard seeds, and Canada carrot seeds, were purchased from West Coast Seeds located in Vancouver, British Columbia, Canada. The collard and carrot seeds were planted in 2 separate 6 x 6 seed starting trays (each cell was 4 cm by 4 cm), filled with autoclaved Golfgreen potting soil purchased from Canadian Tire. The soil was autoclaved using a Getinge Vacuum Steam Sterilizer (Model 533Ls) that was set to run for 30 minutes at 121°C. In each cell, 2 seeds were planted in their respective seed starting tray. Both trays were watered when the soil became dry, and were left to grow. The collards grew for 20 days and the carrots

grew for 30 days. They were grown at room temperature and under natural sunlight. No additional supplements were applied to the plants during this time.

In preparation for the transplanting process, 2 L plastic nursery pots (n = 40) were washed with soap and water, bleached, rinsed with water, and sprayed with 70% ethanol to limit contamination. The 40 nursery pots encompassed 8 pots per treatment with a total of 5 treatment groups. The treatment groups included a control (negative control), nutrient broth and biochar (negative control), *Pseudomonas rhodesiae* (a known plant growth-promoting bacteria) and biochar (positive control), *Fictibacillus enclensis* and biochar, and fertilizer (positive control) as outlined in Table 1. The negative controls should not yield any benefits to the vegetative growth of plants. The positive controls should yield benefits to the vegetative growth of plants. Lack of beneficial results in the positive control could signify that there is something wrong in the system being used to grow the plants (i.e., the conditions of the greenhouse). The nutrient broth was prepared as follows: 5g tryptone, 5g sodium chloride, 3g yeast extract in 1000 mL of water. Biochar was obtained from Canadian Agrichar, a company located in Maple Ridge, British Columbia, Canada. The fertilizer was prepared using 20-20-20 Miracle-Gro® fertilizer, and by following the instructions provided on the box.

**Table 1.** Treatment groups and their components used for both the collard and carrots trials.

| Treatment Group | Components (per 1 pot) |
|---|---|
| Control | 2000 mL topsoil |
| Nutrient Broth and Biochar | 2000 mL topsoil, 100 mL of 1:1 nutrient broth and biochar |
| *P. rhodesiae* and Biochar | 2000 mL topsoil, 100 mL of 1:1 nutrient broth and biochar inoculated with *P. rhodesiae* bacteria |
| *F. enclensis* and Biochar | 2000 mL topsoil, 100 mL of 1:1 nutrient broth and biochar inoculated with *F. enclensis* bacteria |
| Fertilizer | 2000 mL topsoil, 100 mL of 20-20-20 Miracle-Gro® fertilizer |

### 2.1.1. Inoculation of Biochar for the Collard Green and Carrot Trials

The method of biochar inoculation followed a modified version of the procedure set forth by Xiong et al. (Xiong et al., 2017). A colony of *F. enclensis* from a petri dish was transferred to a 50 mL centrifuge tube filled with 10 mL of nutrient broth using a 1 μL inoculation loop. The tube was placed in an Amerex Instruments Gyromax 737 incubator at 30°C for 72 hours. The tube was not shaken; it was placed on the top shelf where it remained stationary. Bacterial growth was verified by visual inspection of the broth, and it was confirmed when the broth exhibited a cloudy appearance. Approximately 2.4 L of both biochar and nutrient broth were

autoclaved for each trial using the Getinge Vacuum Steam Sterilizer (Model 533Ls) for 30 minutes at 121°C. A 1000 mL plastic beaker was used to measure the biochar. To prepare the necessary treatment groups, a 1:1 ratio of biochar to nutrient broth was created by combining 800 mL of nutrient broth and 800 mL of biochar. This was performed for the nutrient broth and biochar, *F. enclensis* and biochar, and *P. rhodesiae* and biochar treatment groups. For the bacterial treatment groups, once the nutrient broth and biochar were added, the mixture was inoculated with the appropriate bacteria. Optical density for bacterial growth was not measured for the collard green and carrot trials. Measuring the optical density would give an idea of the quantity of bacteria added to the soil. Keeping the optical density consistent across each plant trial would ensure that the quantity of bacteria added to the soil is standardized. However, the collard green and carrot trials were already initiated before this could be implemented. After inoculation, each mixture was placed in an Amerex Instruments Gyromax 737 shaking incubator at 100 rpm, at a temperature of 30 °C, for up to 24 hours. This allowed for mixing of all of the ingredients.

### 2.1.2. Collard Greens

To carry out each treatment group, a 1000 mL plastic beaker was used to approximate the 2000 mL of unsterilized Nova Scotia topsoil that each pot received. For the nutrient broth and biochar, *P. rhodesiae* and biochar, and *F. enclensis* and biochar treatment groups, 1000 mL of topsoil was added to each pot and mixed with 50 mL of the 1:1 ratio of the nutrient broth and biochar mixtures described in Table 1. Mixing of these components required the use of a laboratory spoon/spatula. This was repeated for the remaining 1000 mL of topsoil and 50 mL of the 1:1 nutrient broth/biochar mixture to ensure that all of the necessary components were

equally distributed. For the fertilizer treatment group, instructions attached to the 20-20-20 Miracle-Gro® fertilizer (mix 2.5 mL of contents with 4 L of water) were followed to prepare 800 mL of fertilizer that would be applied to the soil of the 8 pots in that treatment group (100 mL of fertilizer/pot).

Collard green seedlings (described above – pg. 18) were transplanted into each pot (1 seedling/pot). All of the pots were watered and subsequently transported to the greenhouse at the southwest corner of the Saint Mary's University (SMU) campus in Halifax, Nova Scotia, Canada. The placement of the pots within the greenhouse was random. The collard greens were left to grow for an additional 2 months in the greenhouse where they were watered 1-2 times per week. During this time, the temperature inside the greenhouse was recorded whenever the collard greens were watered. Additional applications of the fertilizer treatment group were given every 2 weeks as recommended by the 20-20-20 Miracle-Gro® fertilizer box. The remaining treatment groups received only 1 application of their respective components.

Measurements of the collards were performed after the 2-month growth period. They were harvested by loosening the topsoil within the pot and subsequently pulling the collard green out by its stem. The leaves were removed from the collard green plants at the point where the base of the leaf meets the end of the petiole. Pictures were taken of each leaf from each individual collard green plant the following day and uploaded to ImageJ for further analysis. Any damage on the collard green leaves was measured using ImageJ as well. The collard greens were then left to dry in the lab at SMU for 6 weeks to ensure adequate drying. Following the 6 weeks, the weight of the leaves on each individual plant were measured in grams using a Sartorius analytical lab balance. This was repeated for the weight of each individual shoot as well. Shoot

length was measured in centimeters with a ruler. The leaf:shoot ratio was calculated by dividing the leaf weight of the collard greens by its shoot weight.

### 2.1.3. Carrots

The procedures used to start the carrot trial followed the same procedures employed for the collard green trial with the exception of the soil that was used. Due to time constraints, unsterilized Fafard garden soil was purchased and used in this trial. After carrot seedlings (described above – pg. 18) were transplanted into the 2 L plastic nursery pots, they were also transported to the greenhouse at SMU where they received the same treatment as the collards. However, the carrots were allowed to grow for an additional 3 months, instead of the additional 2 months that the collards were grown for, due to their slower growth. Harvesting of the carrots was accomplished by pulling on the stem of each carrot and removing it from the topsoil. Immediately upon harvest, the carrot taproots were removed from any soil debris by manual brushing of the taproot surface. Each carrot taproot was then measured in grams using an Ohaus Navigator portable scale. The length of each individual carrot taproot was measured in centimeters using a ruler.

## 2.2. Barley Trial

### 2.2.0. Treatments and Set-Up

The barley trial contained only 4 treatment groups with 8 plants in each. The treatment groups are as follows: water and biochar (negative control), *F. enclensis*, *F. enclensis* and biochar, and fertilizer (positive control) as outlined in Table 2. Water and biochar were used in this trial instead of nutrient broth and biochar due to concerns regarding excess nitrogen

application, which causes the plants to fall over (lodge). In this trial, 18-ounce red solo cups were used as pots that were placed on a light bench within the laboratory. The cups were washed, bleached, rinsed, and sprayed with ethanol in the same manner that was employed in the collard and carrot trials. The light bench allowed the plants to receive a controlled amount of light. Therefore, the amount of time the plants spent under the light source could be adjusted using this light bench, and the distance between the lights and the plants could also be adjusted. For the barley, the light bench was programmed to emit light for 14 hours and was turned off for the remaining 10 hours of a 24-hour time period. This length was determined to ensure that light was not a limiting factor in the barley's growth. The distance between the lights and the barley was adjusted throughout the experiment to coincide with the height of the plants.

**Table 2.** Treatment groups with their appropriate components for the barley trial.

| Treatment Group | Components (per 1 cup) |
|---|---|
| Water and Biochar | 500 mL of top/garden soil, 25 mL of 1:1 sterilized water and biochar |
| *F. enclensis* | 500 mL of top/garden soil, 25 mL of sterilized water inoculated with *F. enclensis* bacteria |
| *F. enclensis* and Biochar | 500 mL of top/garden soil, 25 mL of 1:1 sterilized water and biochar inoculated with *F. enclensis* bacteria |
| Fertilizer | 500 mL of top/garden soil, 25 mL of 2-fold diluted 20-20-20 Miracle-Gro® fertilizer |

### 2.2.1. Inoculation of Biochar and Sterile Water

In order to prepare the bacterial (*F. enclensis*) treatment groups, 50 µL of *F. enclensis* from a frozen stock was placed in 2, 50 mL centrifuge tubes filled with 10 mL of nutrient broth and placed in the Amerex Instruments Gyromax 737 incubator at 30 °C for 48 hours. The tubes were not placed in the shaking component of the incubator but on the top shelf where they remained stationary. Frozen stocks were used in this trial to keep the methods consistent with another group member quantifying the bacteria at various optical densities. The frozen stocks were prepared by swabbing a colony of *F. enclensis* that had been growing on a petri dish for 24 hours into a 1.5 mL cryovial containing a 0.7 ml nutrient broth/0.3 mL glycerol solution. The frozen stocks were kept in a Z-SCI Twincore Technology ULT -80°C freezer (Model DF8517P). After the 48 hour-growth period was completed for the *F. enclensis* in the incubator, the cell density of each tube was measured by calculating the $OD_{600}$ using a BIOCHROM Novaspec Plus Visible Spectrophotometer. The $OD_{600}$ was standardized at approximately 0.070 for both tubes by performing dilutions.

To prepare for the planting process, 1.6 L of Garden Club top/garden soil, 600 mL of deionized water, and 200 mL of biochar were autoclaved for sterilization using the Getinge Vacuum Steam Sterilizer (Model 533Ls) for 30 minutes at 121°C. The method to prepare the 1:1 ratio of sterilized water and biochar followed the same procedure that was outlined with the nutrient broth and biochar for the collard and carrot trials, but with only 200 mL of each of the sterilized water and biochar being mixed together. This was repeated twice for the water and biochar treatment group, and the *F. enclensis* and biochar treatment group. One was inoculated with a tube of the standardized *F. enclensis* and mixed in the same manner as it was in the collard green and carrot trials. The other tube of standardized *F. enclensis* inoculated 200 mL of sterilized water.

### 2.2.2. Barley

The planting process for the barley trial followed the same procedure as the collard and carrot trials except with differing amounts of each of the components. A 500 mL beaker was used to transfer the autoclaved soil into the cups. A 50 mL beaker was used to transfer all of the other components that make up each treatment group as outlined in Table 2 (i.e., the sterilized water/biochar mixture, the sterilized water inoculated with *F. enclensis*, etc.). For the water and biochar, *F. enclensis* and biochar, and *F. enclensis* treatment groups, the components of each were mixed in the same manner that the collard and carrot treatment groups were mixed. The only difference being the increments; in this trial 250 mL increments of soil were mixed with approximately 12.5 mL increments of the other components listed in Table 2. This was done for the water and biochar, *F. enclensis* and biochar, and *F. enclensis* treatment groups. The fertilizer was prepared and applied to the soil in the same manner as the collard and carrot trials, except that it was a 2-fold dilution. Once all of the soil was placed in each cup, with the appropriate components added, the planting process could begin. In each pot, 3 Bere barley seeds, purchased from Salt Spring Seeds located in Salt Spring Island, British Columbia, Canada, were sown approximately 1 cm into the soil. The cups were placed randomly on the light bench in the laboratory, at room temperature, where they were allowed to grow for 5 weeks. The 3 seeds initially planted were later thinned to 1 per pot after 1 week of growth.

Within the 5 weeks that the barley plants were growing, components of each treatment group were applied every 2 weeks. The components are outlined in Table 3. The fertilizer

treatment group was diluted to a 3-fold dilution because of concerns related to too much nitrogen application, which causes the plants to fall over (lodge). For the treatment groups that contained *F. enclensis*, the bacteria were grown in the same manner as described above while keeping a consistent $OD_{600}$ of 0.070.

**Table 3.** Components of each treatment group that was added every 2 weeks to the barley plants.

| Treatment Group | Components (per 1 cup) |
|---|---|
| Water and Biochar | 25 mL of sterilized water |
| *F. enclensis* | 25 mL of sterilized water inoculated with *F. enclensis* bacteria |
| *F. enclensis* and Biochar | 25 mL of sterilized water inoculated with *F. enclensis* bacteria |
| Fertilizer | 25 mL of 3-fold diluted 20-20-20 Miracle-Gro® fertilizer |

The harvesting process for the barley was performed after 5 weeks. Both the root and its associated shoot were removed together before they were separated at the point where both the root and shoot begin. Once they were separated the roots were washed with water to remove any soil present. They were immediately weighed after this using a Sartorius analytical lab balance to obtain their fresh weights. Both the barley shoots and roots were allowed to dry for 1 week and then weighed again to obtain their dry weights. The shoot:root ratio was calculated by dividing the shoot by its respective root.

## 2.3. Statistical Analysis of the Plant Trials

To analyze the significance of each measurement taken from all of the plant trials, a one-way ANOVA test was performed. For this test, the $H_0$ was that the means for each treatment group were equal, and the $H_A$ was that the means for each treatment group were not equal. A Tukey Honestly Significance Difference test was employed to further investigate any individually significant results obtained from the one-way ANOVA test. The $H_0$ was that the 2 treatment group means were equal, and the $H_A$ was that 2 treatment group means were not equal. In all of these tests, the alpha value for significance was set at 0.05. All analyses were conducted using RStudio.

## 2.4. Testing the Soil for the Presence of *Fictibacillus enclensis*

The soil at the end of each plant trial was tested for the presence of *F. enclensis* in the plants treated with the *F. enclensis* and biochar formulation. Soil collection from each plant trial was taken after the trial was complete and the plants had been harvested. Labeled 50 mL polypropylene tubes were taken to the greenhouse site, and the laboratory site, at Saint Mary's University. For each plant trial, a different scoopula was used to collect approximately 40 g of soil from multiple *F. enclensis* and biochar treatment group pots. This was done to ensure that the soil was representative of all of the pots within the *F. enclensis* and biochar treatment group and not just one pot.

The bacteria within the soil samples were plated by dilution plating. To prepare the diluted solutions, six 15 mL polypropylene centrifuge tubes were filled with 9 mL of sterilized water and labeled appropriately from $10^{-1}$ to $10^{-6}$. Approximately 1 g of collected soil, measured using a Sartorius analytical lab balance, was added to the tube labeled $10^{-1}$. That tube was shaken and placed in a balanced PrO-Research Centurion Scientific Benchtop centrifuge at 1000 x g for

5 minutes. After centrifugation, 1 mL of the supernatant in that $10^{-1}$ tube was pipetted into the $10^{-2}$ tube. The $10^{-2}$ tube was gently vortexed and 1 mL of that tube was then pipetted into the $10^{-3}$ tube. This same process was repeated for each tube until the tube labeled $10^{-6}$ was reached. Once this was complete, 1 mL of each tube was pipetted on a 100 mm x 15 mm petri dish, filled with approximately 15-20 mL of nutrient agar (5g peptone, 3g yeast extract, 5g sodium chloride, 15g agar), and spread using a 1 µL inoculation loop. Petri dishes were placed in a drawer at room temperature and allowed to grow. This process was repeated for the soil of each plant trial. Timing also allowed for the centrifugation of the soil in the collard greens trial at 3400 x g for 5 minutes that yielded different bacterial colonies when the prepared tubes ($10^{-1}$ to $10^{-6}$) were plated on petri dishes.

Once bacterial colonies formed, they were inspected and isolated based on their morphological characteristics. Only the bacteria that looked morphologically similar to *F. enclensis* were isolated because that was the bacteria of interest. The isolation of bacteria was performed by swabbing a colony with a 1 µL inoculation loop and streaking it onto another petri dish. Once all of the bacterial colonies of interest were isolated, they underwent PCR amplification in preparation for DNA sequencing.

### 2.4.0. PCR Preparation

Samples of the bacterial colonies were prepared and underwent PCR to amplify their DNA. This would ensure that adequate DNA was present to later undergo DNA sequencing for the identification of each bacterium. To prepare for PCR amplification, 500 µL of 70% ethanol (EtOH) and 500 µL of glass beads were added into a 1.5 mL centrifuge tube. A 1 µL inoculation loop full of bacteria was added to the tube and vortexed on high for 2 minutes. Vortexing the

solution allowed for the glass beads to break up the cells in order to access their DNA. This tube served as the stock solution. Master mix (Promega GoTaq® Green Master Mix (Contains Taq DNA polymerase, dNTPs, MgCl2 and reaction buffers)), primers for bacterial samples (IDT 16S rRNA Forward (AGA GTT TGA TCC TGG CTC AG), IDT 16S rRNA REVERSE (ACG GCT ACC TTG TTA CGA CTT)), and nuclease-free water vials were thawed at room temperature and vortexed. In a PCR strip tube, 19 µL of nuclease-free water, 25uL of master mix, 2uL of the forward primer, 2uL of the reverse primer, and 2uL of the stock solution was added using a micropipette. A negative control was prepared by adding the same components to another PCR strip tube without the addition of the stock solution containing the DNA. This was needed to test the reagents for contamination. This procedure was followed for every microbe that was isolated and deemed as morphologically similar to *F. enclensis*.

### 2.4.1. PCR Thermocycling

PCR tubes were spun on the Eppendorf Centrifuge 5430R at 1000 x g for 1 minute. The BIO-RAD C1000 Touch$^{TM}$ thermocycler was set to run as described in Table 1.

**Table 4.** Thermocycling conditions for bacterial samples deemed similar in morphology to *Fictibacillus enclensis*.

| Phase | Temperature (°C) | Time (minutes) | Number of Cycles |
|---|---|---|---|
| Initial Denaturation | 95 | 2 | 1 |
| Annealing | 52 | 0.5 | 33 |
| Extension | 73 | 2 | |
| Denaturation | 95 | 0.5 | |
| Final Extension | 73 | 5 | 1 |

Once the run was complete, the samples underwent gel electrophoresis to assess the quality and quantity of DNA.

### 2.4.2. Gel Electrophoresis

Gel electrophoresis was performed to separate DNA strands of differing lengths as a way to assess DNA quality and quantity. A 1x TAE buffer was prepared by diluting a 50x TAE stock solution buffer (242g Tris Base, 57.1 mL glacial acetic acid, 100 mL 0.5 M EDTA (pH 8.0)). This was accomplished by adding 20 mL of the 50x TAE stock solution buffer to 980 mL of deionized water into a 1 L glass bottle. To prepare the agarose gel, 100 mL of that 1x TAE buffer was placed in a 500 mL glass bottle and 1 g of agarose was added to create a 1% agarose gel. The agarose was measured using a Ohaus Navigator portable scale. The resulting 1% agarose gel was heated in the microwave and swirled every 30 seconds. Ethidium bromide (3 µL) was added once the gel had cooled slightly. The gel apparatus was assembled and the cooled agarose gel was added thereafter. The chamber was then filled with 1x TAE buffer so that it just rested atop the gel. In the first well, 3 µL of a 1 kilobase (kb) DNA ladder was added with a micropipette. Each DNA sample, and a negative control, was added (3 µL) to the appropriate well and their placement in each well was documented. Once all the samples were loaded, the gel apparatus was run at 100V for approximately 1 hour. Once this was completed, the resulting gel was visualized using the BIO-RAD Gel Doc$^{TM}$ XRT Imaging System. This was done to determine if the quality and quantity of DNA would be sufficient for analysis. For samples that were of good quality, and sufficient quantity, they were sent to Genome Quebec for DNA sequencing.

# 3. Results

To assess the effect that *Fictibacillus enclensis* had on the vegetative growth of collard greens, carrots, and barley, plants exposed to different treatment groups were grown in either a greenhouse or on a light bench. The collard green and carrot trials included 5 treatment groups that were grown in a greenhouse. These treatments groups consisted of a control, nutrient broth and biochar, *Pseudomonas rhodesiae* and biochar, *F. enclensis* and biochar, and fertilizer (Table 1). The barley trial included 4 treatment groups that were grown on a light bench within the laboratory. These 4 treatment groups included a control, *F. enclensis*, *F. enclensis* and biochar, and fertilizer (Table 2).

## 3.1. Collard Greens

The collard greens were grown in the greenhouse at SMU for 2 months to assess their vegetative growth across the different treatment groups. Figure 1 depicts the number of leaves on the collard greens for each treatment group, which consisted of 8 collard greens per group for a total of 40 collard greens within the whole trial. There were no statistically significant differences between treatment groups when a one-way ANOVA test was conducted.

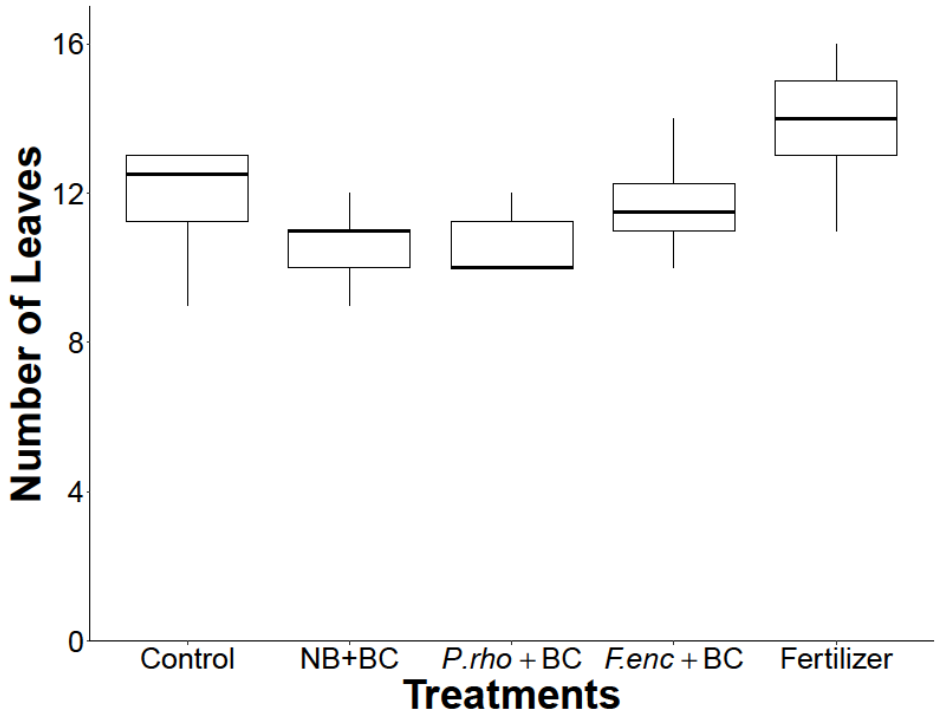


**Figure 1.** Number of collard green leaves. Collard greens were grown for 2 months in the greenhouse at Saint Mary's University. Upon harvesting, the collard green leaves were removed at the point where the base of the leaf meets the end of each petiole and subsequently counted. Each treatment group had a sample size of 8. *F.enc* + BC = *Fictibacillus enclensis* and biochar, NB+BC = nutrient broth and biochar, *P.rho* + BC = *Pseudomonas rhodesiae* and biochar. A one-way ANOVA test yielded no significant differences between the treatment groups.

The collard greens shoot length is shown in Figure 2. A one-way ANOVA test yielded significant results that a Tukey Honestly Significant Difference test revealed to be between the *F. enclensis* and biochar and the fertilizer treatment groups ($p = 0.031$). Therefore, the collard greens treated with chemical fertilizer produced significantly longer shoots than the collard greens treated with the formulation of *F. enclensis* and biochar. The median shoot length of the collard greens treated with chemical fertilizer was 44 cm, whereas the median shoot length of the collard greens treated with the *F. enclensis* and biochar formulation was 31cm.

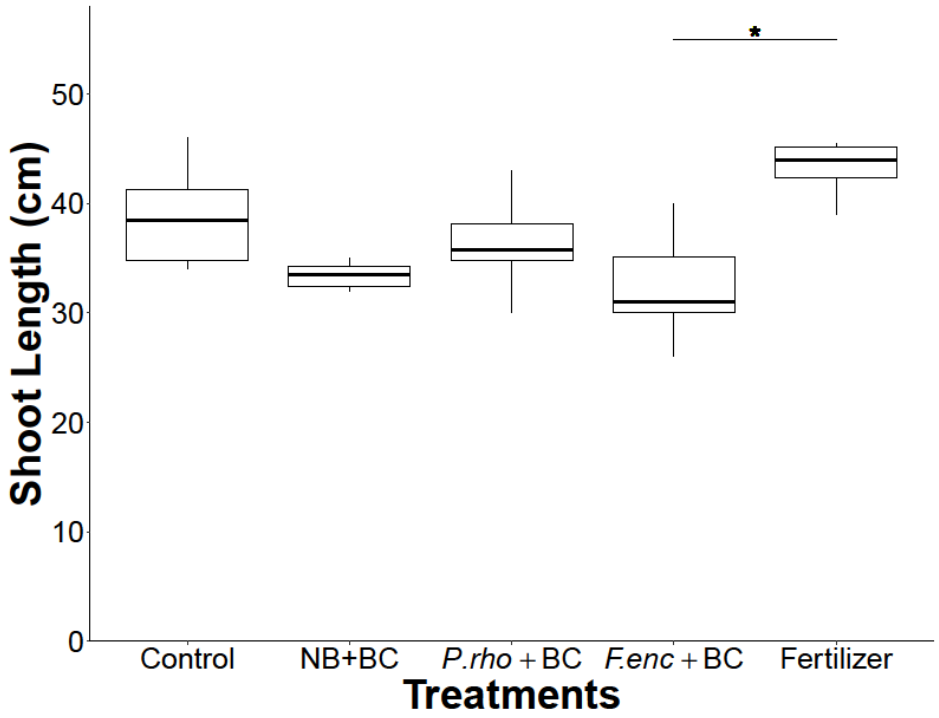


**Figure 2.** Shoot length of collard greens (cm) from the top of the primary root to the shoot apex. Collard greens were grown for 2 months in the greenhouse at Saint Mary's University. After harvesting, they were left to dry before the petioles were removed and the shoots were measured. Each treatment group had a sample size of 8. *F.enc* + BC = *Fictibacillus enclensis* and biochar, NB+BC = nutrient broth and biochar, *P.rho* + BC = *Pseudomonas rhodesiae* and biochar. A one-way ANOVA test yielded significant differences between the treatment groups that was further analysed by a Tukey Honestly Significant Difference test. * Indicates $0.01 \leq p \leq 0.05$.

Figure 3 shows the shoot weight of the collard greens. The collard greens treated with chemical fertilizer produced the heaviest shoots (median value = 0.8754) compared to the other treatment groups. A one-way ANOVA test yielded significant results that was revealed to be between the fertilizer and the nutrient broth and biochar treatment groups ($p = 0.0015$), and the chemical fertilizer and the *P. rhodesiae* and biochar formulation ($p = 0.0068$) through a Tukey Honestly Significant Difference test. The collard greens treated with chemical fertilizer produced significantly heavier shoots than the collard greens treated with nutrient broth and biochar and the *P. rhodesiae* and biochar formulation. The median shoot weight of the chemical fertilizer

treatment group was 0.8754 g compared to the nutrient broth and biochar treatment group (0.3519 g) and the *P. rhodesiae* and biochar formulation (0.4372 g).

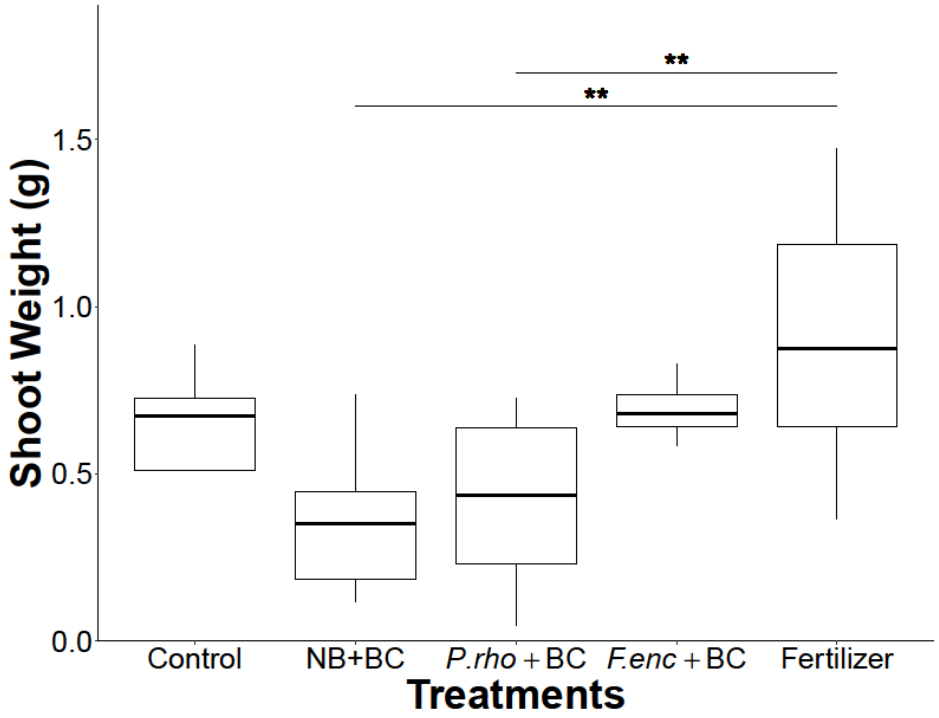


**Figure 3.** Dry shoot weight (g) of collard greens grown for 2 months in the greenhouse at Saint Mary's University. After drying the collard greens, the petioles were removed and the shoots were measured. Each treatment group had a sample size of 8. *F.enc* + BC = *Fictibacillus enclensis* and biochar, NB+BC = nutrient broth and biochar, *P.rho* + BC = *Pseudomonas rhodesiae* and biochar. A one-way ANOVA test yielded significant differences between the treatment groups that was further analysed by a Tukey Honestly Significant Difference test. ** Indicates $0.001 \leq p \leq 0.01$.

The total leaf weight of the collard greens is depicted in Figure 4. A one-way ANOVA test yielded a significant result that was later analyzed by a Tukey Honestly Significant Difference test to reveal where the individual significant differences lay. The collard greens treated with the *P. rhodesiae* and biochar formulation were significantly lighter than the collard greens treated with the *F. enclensis* and biochar formulation ($p = 0.0013$) and the collard greens treated with chemical fertilizer ($p = 0.0024$). The collard greens treated with nutrient broth and

biochar were also significantly lighter than the collards greens treated with the *F. enclensis* and biochar formulation (p = 0.0013) and the collard greens treated with chemical fertilizer (p = 0.0024). This was also true for the control treatment compared to both the collard greens treated with the *F. enclensis* and biochar formulation (p = 0.013) and the collard greens treated with chemical fertilizer (p = 0.022). The collard greens treated with the *F. enclensis* and biochar formulation produced similar leaf weights to the collard greens treated with chemical fertilizer as their median values were 0.995 grams and 0.988 grams respectively.

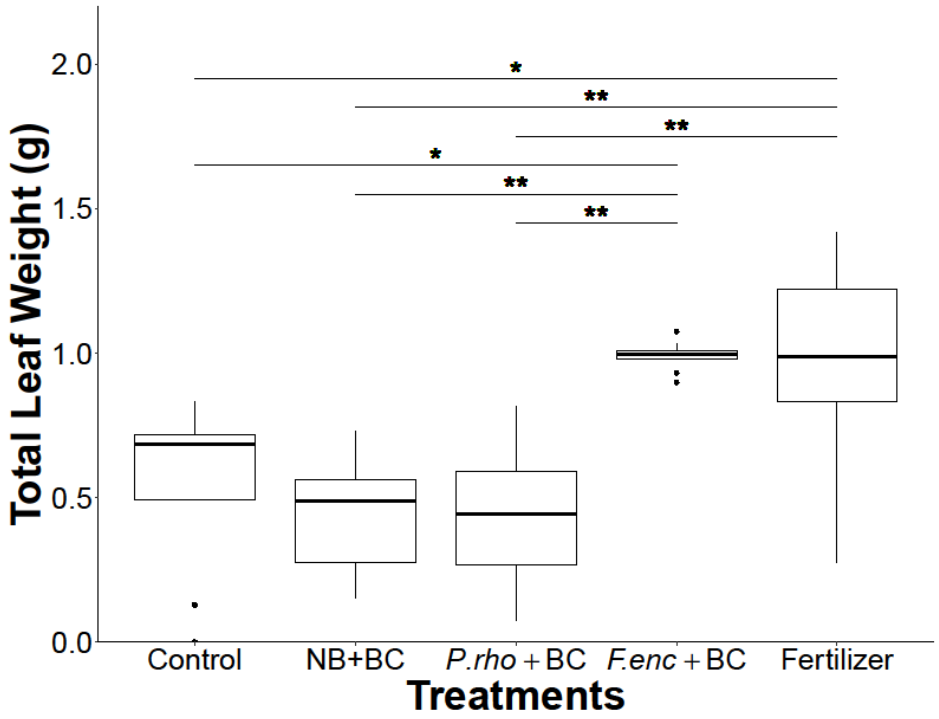


**Figure 4.** Total dry leaf weight of collard greens (g) that were grown for 2 months in the greenhouse at Saint Mary's University. The collard green leaves were removed and left to dry prior to weighing. Each treatment group had a sample size of 8. *F.enc* + BC = *Fictibacillus enclensis* and biochar, NB+BC = nutrient broth and biochar, *P.rho* + BC = *Pseudomonas rhodesiae* and biochar. A one-way ANOVA test yielded significant differences between the treatment groups that was further analysed by a Tukey Honestly Significant Difference test. * Indicates $0.01 \leq p \leq 0.05$, ** Indicates $0.001 \leq p \leq 0.01$.

In Figure 5, the average total leaf area of the collard greens is shown. A one-way ANOVA test yielded no statistically significant results.

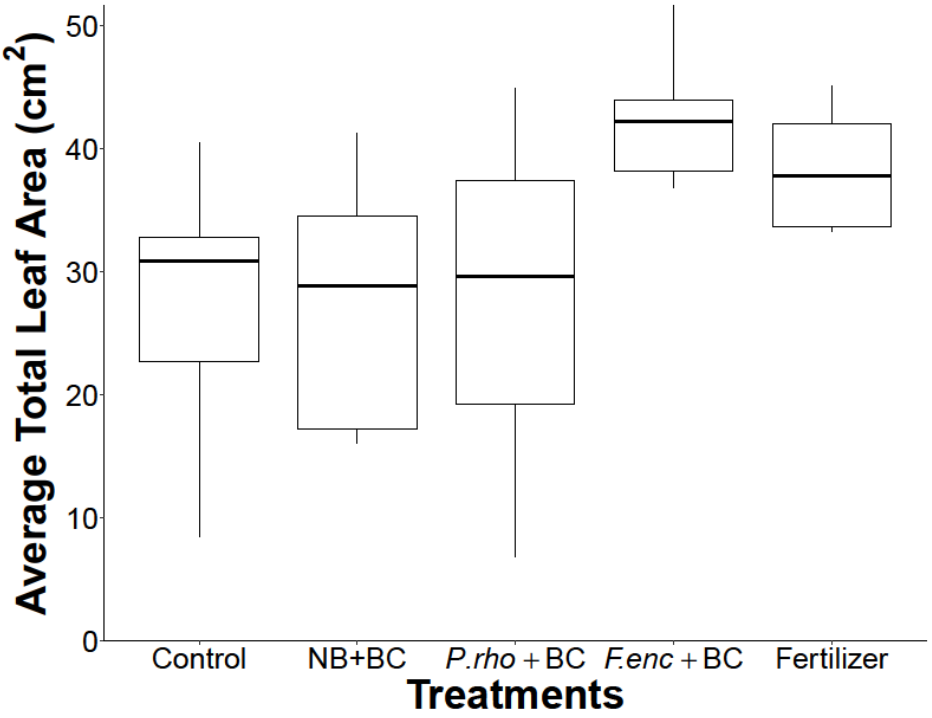


**Figure 5.** Average total leaf area (cm2) of collard greens that were grown for 2 months in the greenhouse at Saint Mary's University. Upon harvesting, the collard green leaves were removed and pictures were taken. Each treatment group had a sample size of 8. *F.enc* + BC = *Fictibacillus enclensis* and biochar, NB+BC = nutrient broth and biochar, *P.rho* + BC = *Pseudomonas rhodesiae* and biochar. A one-way ANOVA test yielded no significant differences between the treatment groups.

The leaf:shoot ratio of the collard greens is shown in Figure 6. This was calculated by dividing the total dry leaf weight by its respective dry shoot weight. A one-way ANOVA test, followed by a Tukey Honestly Significant Difference test, yielded significant results between the *F. enclensis* and biochar formulation and both the control and chemical fertilizer treatment group, and between the nutrient broth and biochar treatment group and the control treatment group. The collard greens treated with the *F. enclensis* and biochar formulation had a significantly higher leaf:shoot ratio than the control treatment ($p = 0.00042$) and the collard

greens treated with chemical fertilizer (p = 0.036). The collard greens treated with nutrient broth and biochar also had a significantly higher leaf:shoot ratio than the control treatment (p = 0.0044).

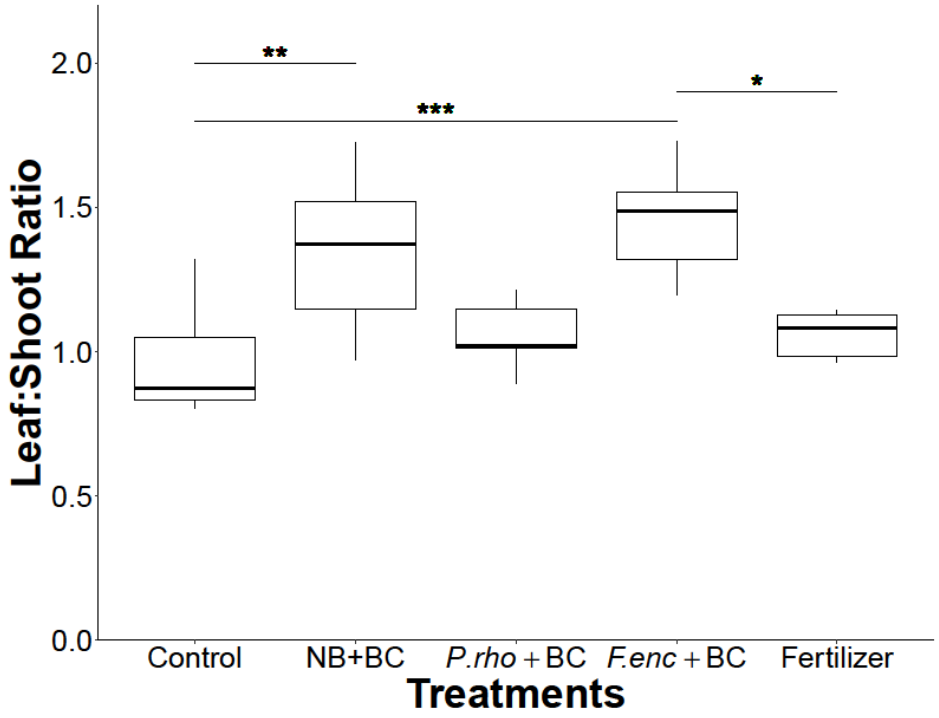


**Figure 6.** Leaf:shoot ratio of collard greens calculated by dividing the total dry leaf weights by their respective dry shoot weights. Collard greens were grown for 2 months in the greenhouse at Saint Mary's University. Each treatment group had a sample size of 8. *F.enc* + BC = *Fictibacillus enclensis* and biochar, NB+BC = nutrient broth and biochar, *P.rho* + BC = *Pseudomonas rhodesiae* and biochar. A one-way ANOVA test yielded significant differences between the treatment groups that was further analysed by a Tukey Honestly Significant Difference test. * Indicates $0.01 \leq p \leq 0.05$, ** Indicates $0.001 \leq p \leq 0.01$, *** Indicates $0 \leq p \leq 0.001$.

Examples of the damaged leaves in each treatment group are shown in Figure 7 to Figure 11. This damage was recognized to be any loss of leaf tissue. The damage depicted on the leaves of the collard greens treated with the *F. enclensis* and biochar formulation (Figure 8) was the extent of any damage present in that treatment group. The collard greens treated with chemical fertilizer suffered the worst in terms of the leaf damage present; the leaves that were affected

were larger and had a significant amount of leaf tissue lost (Figure 11). Additionally, the number of affected leaves were more plentiful in the collard greens treated with chemical fertilizer than any other treatment groups (not shown).

**Figure 7.** The damage on the leaves of the control collard greens. Collard greens were grown for 2 months in the greenhouse at Saint Mary's University.

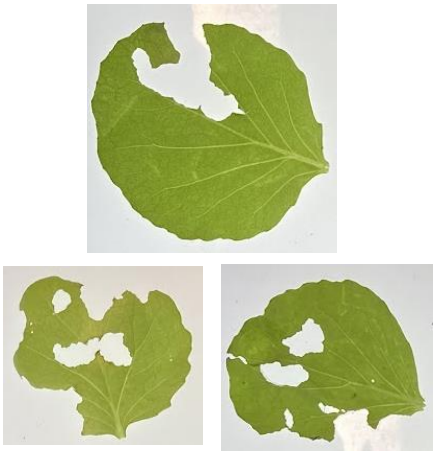

**Figure 8.** The damage on the leaves of the collard greens treated with nutrient broth and biochar. Collard greens were grown for 2 months in the greenhouse at Saint Mary's University.

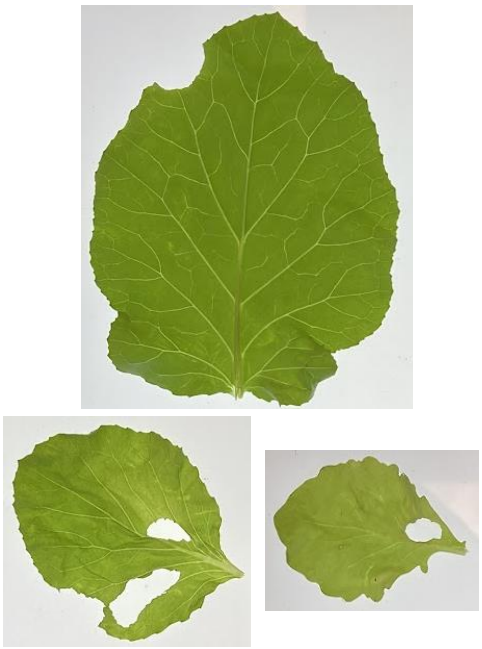

**Figure 9.** The damage on the leaves of the collard greens treated with the *P. rhodesiae* and biochar formulation. Collard greens were grown for 2 months in the greenhouse at Saint Mary's University.

**Figure 10.** The damage on the leaves of the collard greens treated with the *F. enclensis* and biochar formulation. Collard greens were grown for 2 months in the greenhouse at Saint Mary's University.

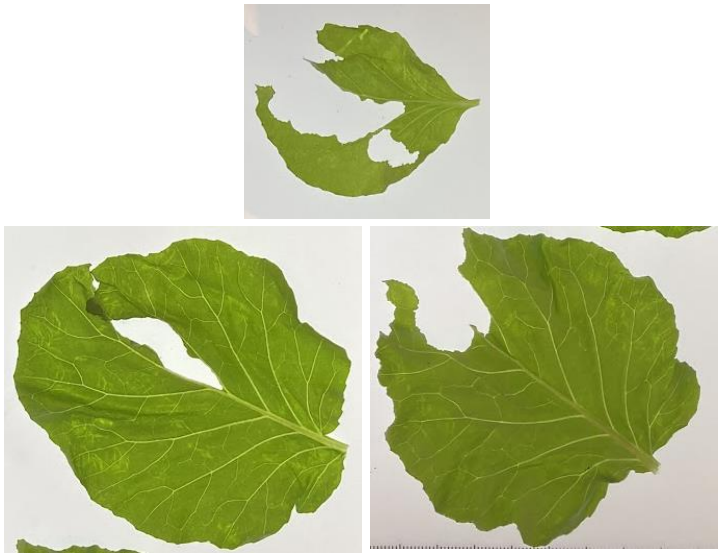

**Figure 11.** The damage on the leaves of the collard greens treated with chemical fertilizer. Collard greens were grown for 2 months in the greenhouse at Saint Mary's University.

Figure 12 shows the average leaf damage across all of the collard green leaves of each treatment group. A one-way ANOVA test yielded significant results that was later analyzed using a Tukey Honestly Significant Difference test. The collard greens treated with chemical fertilizer had significantly more leaf damage when compared with the control ($p = 0.011$), the *F. enclensis* and biochar formulation ($p = 0.001$), and the *P. rhodesiae* and biochar formulation (0.012).

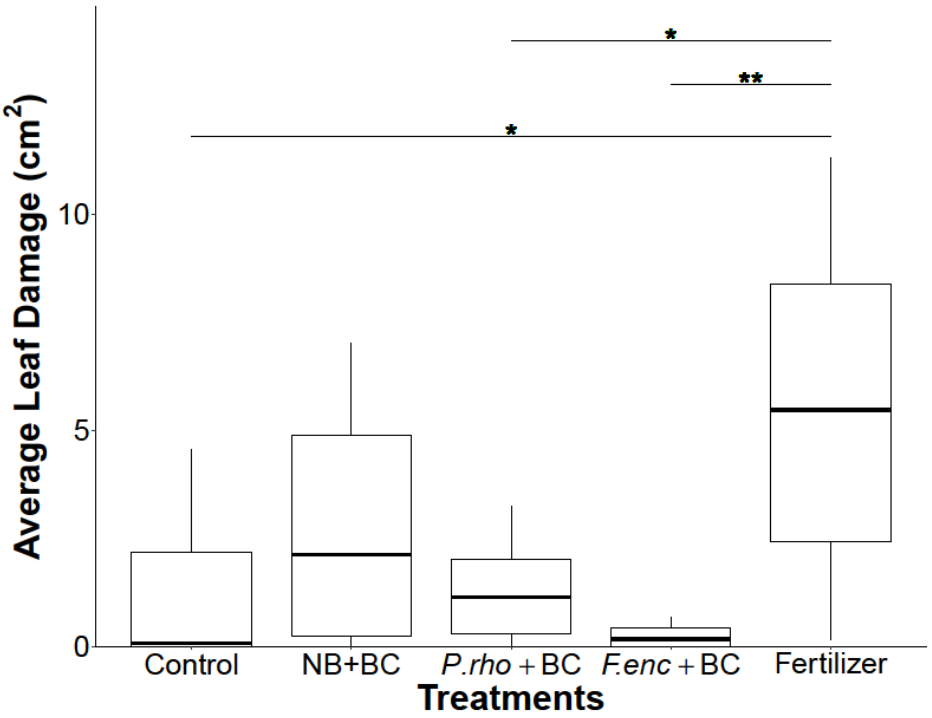


**Figure 12.** Average leaf damage ($cm^2$) on the collard green leaves. Collard greens were grown for 2 months in the greenhouse at Saint Mary's University. Upon harvesting, the collard green leaves were removed at the point where the base of the leaf meets the end of each petiole. Pictures of the leaves were taken and any loss of leaf tissue was measured. *F.enc* + BC = *Fictibacillus enclensis* and biochar, NB+BC = nutrient broth and biochar, *P.rho* + BC = *Pseudomonas rhodesiae* and biochar. A one-way ANOVA test yielded significant differences between the treatment groups that was further analysed by a Tukey Honestly Significant Difference test. * Indicates $0.01 \leq p \leq 0.05$, ** Indicates $0.001 \leq p \leq 0.01$.

## 3.2. Carrots

Carrots were also grown in the greenhouse at Saint Mary's University for 3 months to assess their vegetative growth when supplemented with different treatment groups. The sample size was the same as the collard green trial at 8 plants per treatment group with 5 treatment groups. A one-way ANOVA test yielded no significant differences across the different treatment groups in terms of the carrot taproot length (Figure 13A). Similarly, no statistically significant results were observed when comparing the fresh carrot taproot weights across the different treatment groups (Figure 13B).

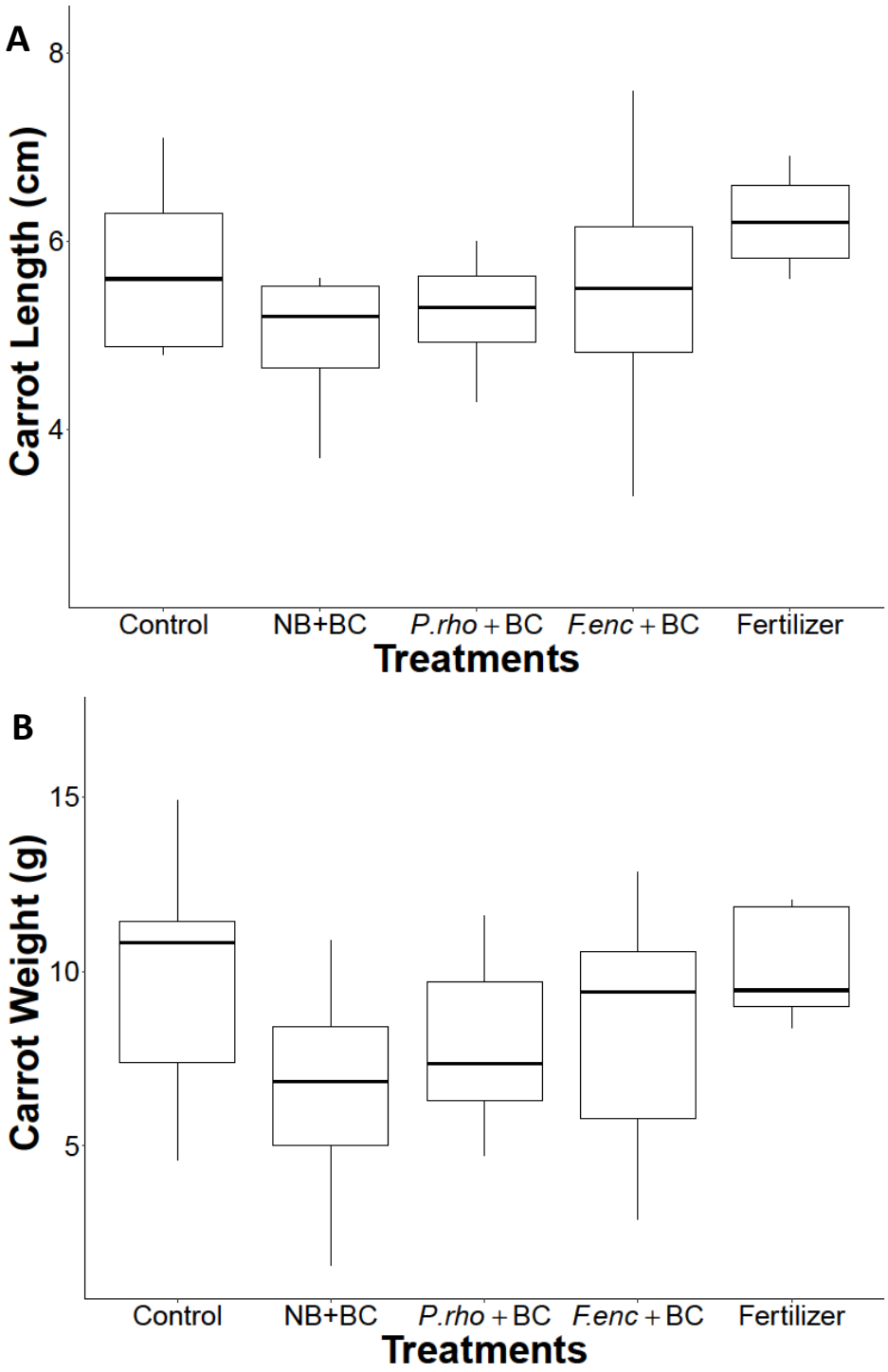


**Figure 13.** Length (cm) (**A**) and fresh weight (g) (**B**) of Canada carrot taproots grown for 3 months in the greenhouse at Saint Mary's University. Immediately upon harvest, the weight of each carrot taproot was measured and their length was recorded. Each treatment group had a sample size of 8. *F.enc* + BC = *Fictibacillus enclensis* and biochar, NB+BC = nutrient broth and biochar, *P.rho* + BC = *Pseudomonas rhodesiae* and biochar. A one-way ANOVA test yielded no significant differences between the treatment groups.

## 3.3. Barley

The barley was grown on a light bench within the laboratory to assess their vegetative growth after 5 weeks. This trial had only 4 treatment groups with 8 barley plants in each for a total of 32 barley plants. The components of each of the treatment groups were applied to the barley plants every 2 weeks. Figure 14 shows the fresh weight of both the barley roots and shoots. A one-way ANOVA test yielded no significant results for either of the fresh root or shoot weights.

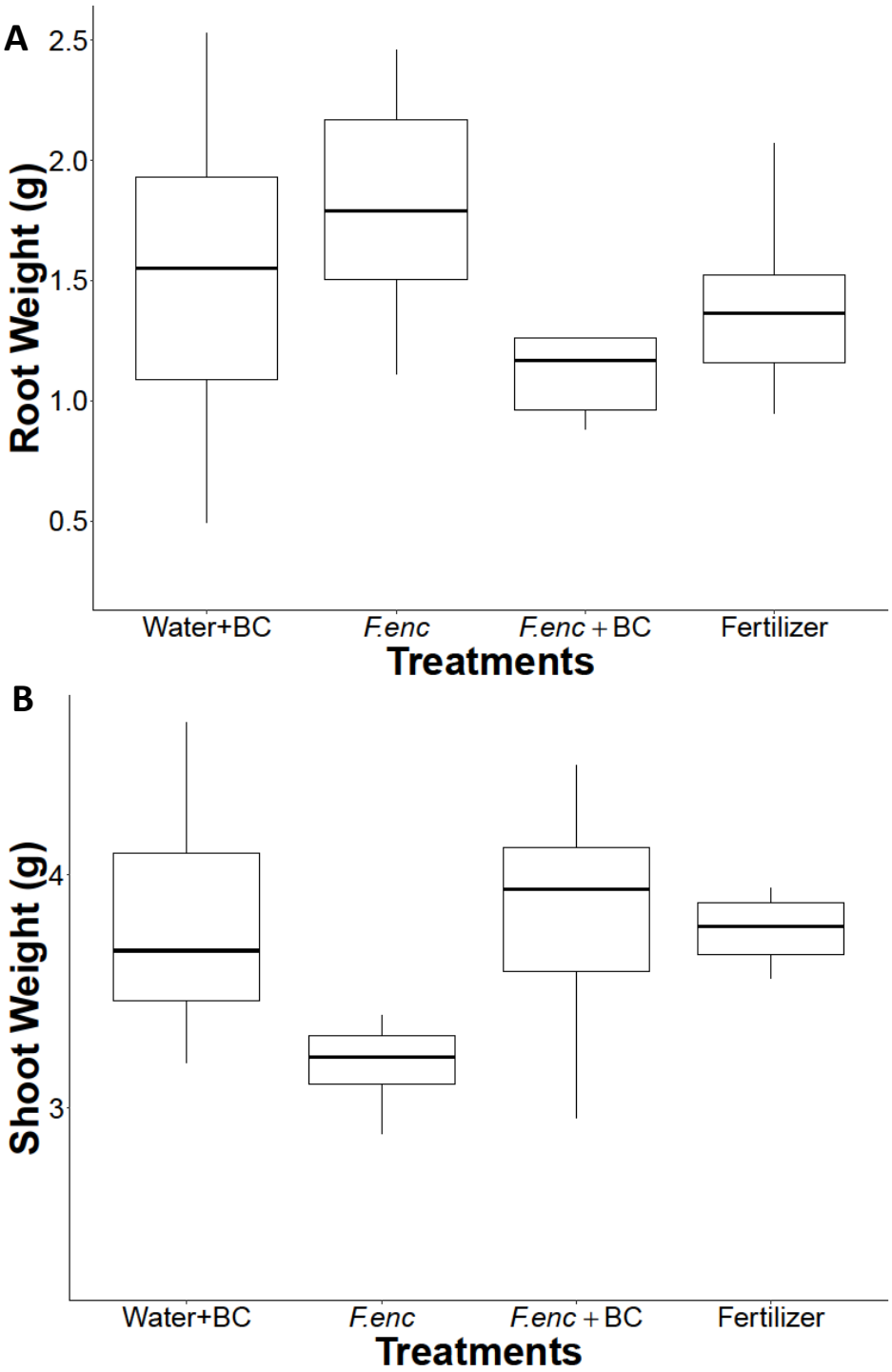


**Figure 14.** Fresh root weight (g) (**A**) and fresh shoot weight (g) (**B**) of barley that were grown for 5 weeks under a light bench within the laboratory. The harvesting process involved the removal of each root and its associated shoot together before they were separated at the point where both the root and shoot begin, and both were weighed thereafter. Each treatment group had a sample size of 8. *F.enc = Fictibacillus enclensis*, BC = biochar. A one-way ANOVA test yielded no significant differences between the treatment groups.

The dry roots and shoots of the barley plants are shown in Figure 15. The dry weight of the barley roots yielded no statistically significant results when a one-way ANOVA test was employed. However, statistical significance was observed in the dry shoot weights from one-way ANOVA testing. The barley plants treated with the *F. enclensis* and biochar formulation were significantly heavier than the barley plants treated with just *F. enclensis* ($p = 0.0042$) based on the results of a Tukey Honestly Significant Difference test.

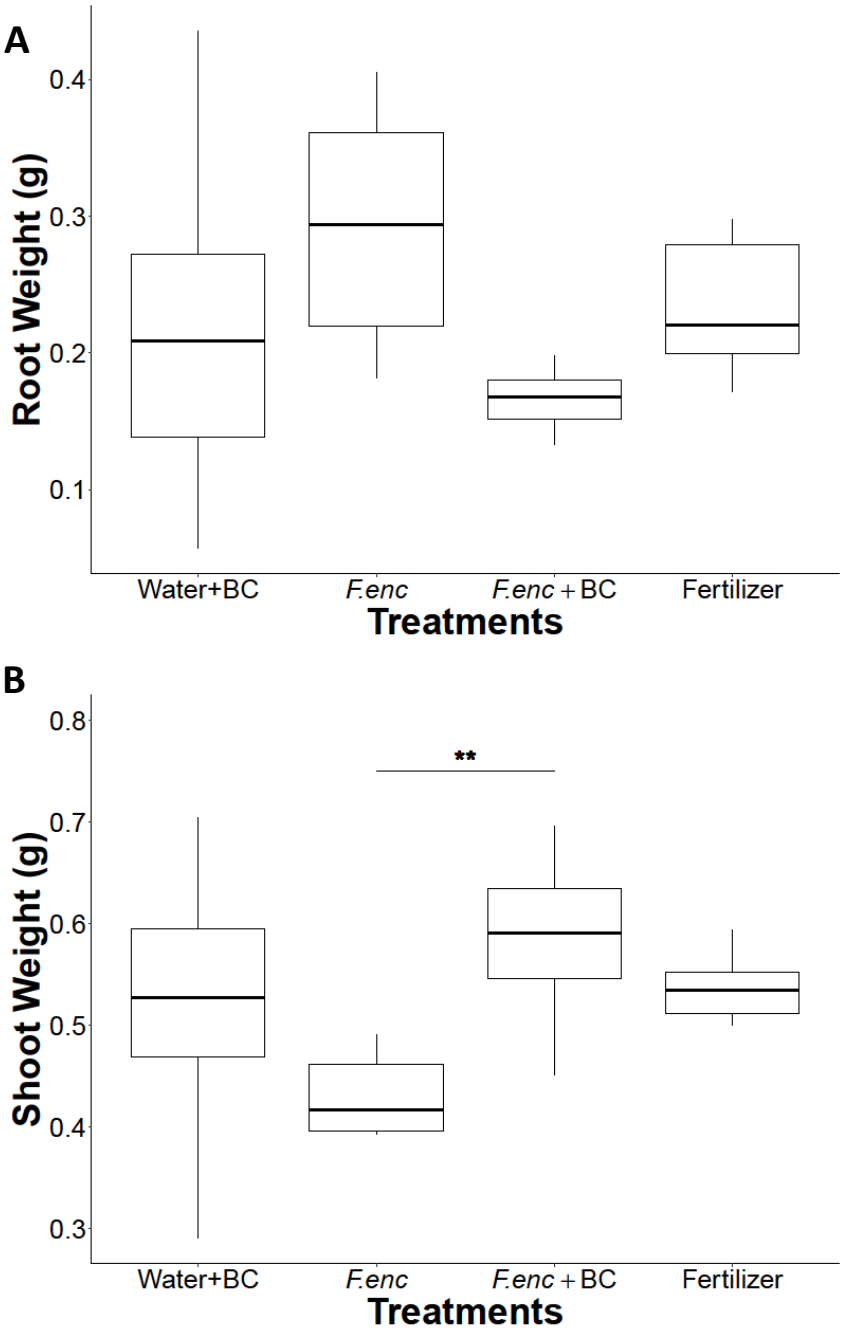


**Figure 15.** Dry root weight (g) (**A**) and of dry shoot weight (g) (**B**) of barley grown for 5 weeks under a light bench within the laboratory. The harvesting process involved the removal of each root and its associated shoot together before they were separated at the point where both the root and shoot begin. Both the root and shoot were then dried. Each treatment group had a sample size of 8. *F.enc = Fictibacillus enclensis*, BC = biochar. A one-way ANOVA test yielded significant differences between the treatment groups that was further analysed by a Tukey Honestly Significant Difference test. ** indicates $0.001 \leq p \leq 0.01$.

Figures 16 and 17 show the fresh and dry shoot:root ratios respectively. The shoot:root ratio was calculated by dividing the shoot weight by its respective root weight. Upon analysis with a Tukey Honestly Significant Difference test, both the fresh and dry shoot:root ratios yielded significant results between the barely treated with *F. enclensis* and the barley treated with the *F. enclensis* and biochar formulation ($p = 0.031$ and $p = 0.00019$ respectively). The barley plants treated with the *F. enclensis* and biochar formulation had a significantly higher shoot:root ratio than the barley plants treated with *F. enclensis* for both the fresh and dry weights. Additionally, the barley plants treated with water and biochar had a significantly higher dry shoot:root ratio than the barley plants treated with *F. enclensis* ($p = 0.0055$), and the barley plants treated with the *F. enclensis* and biochar formulation had a significantly higher shoot:root ratio than the barley plants treated with chemical fertilizer ($p = 0.019$).

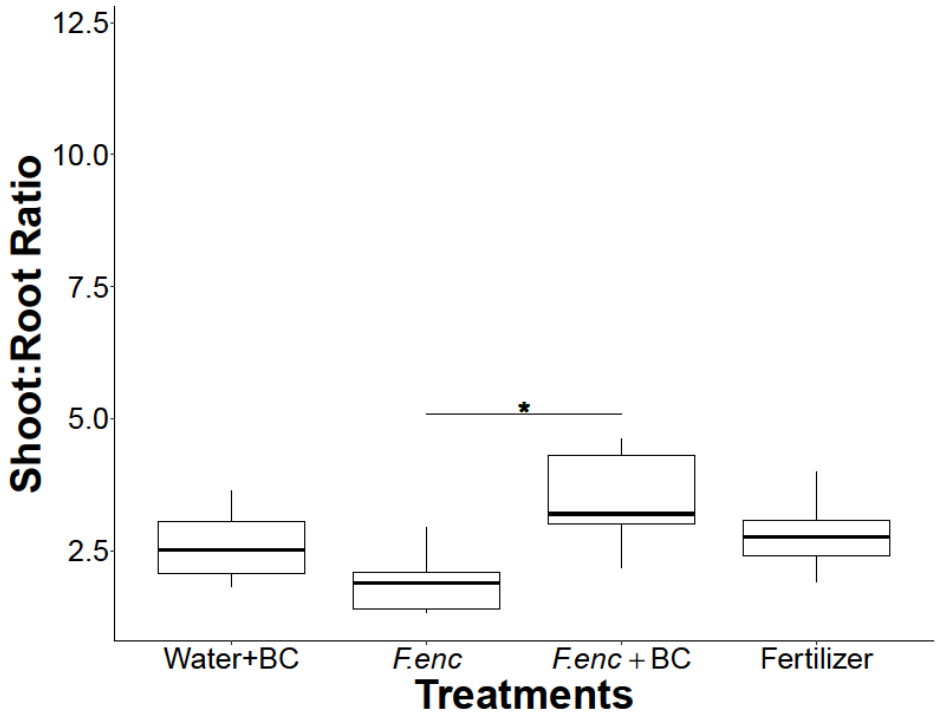


**Figure 16.** Fresh shoot:root ratio of barley calculated by dividing the fresh shoot weight by the fresh weight of its respective root. Barley plants were grown for 5 weeks under a light bench within the laboratory that emitted light for 14 hours out of a 24-hour period. The harvesting process involved the removal of each root and its associated shoot together before they were separated at the point where both the root and shoot begin and the fresh measurements were taken. Each treatment group had a sample size of 8. *F.enc = Fictibacillus enclensis*, BC = biochar. A one-way ANOVA test yielded significant differences between the treatment groups that was further analysed by a Tukey Honestly Significant Difference test. * Indicates $0.01 \leq p \leq 0.05$.

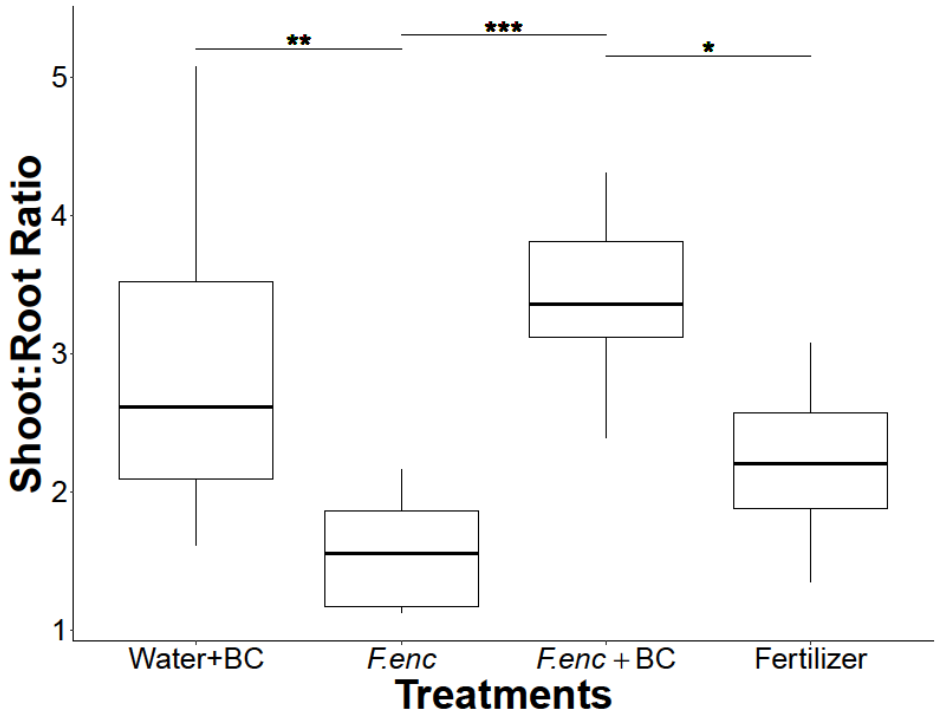


**Figure 17.** Dry shoot:root ratio of barley calculated by dividing the dry shoot weight by the dry weight of its respective root. Barley plants were grown for 5 weeks under a light bench within the laboratory that emitted light for 14 hours out of a 24-hour period. The harvesting process involved the removal of each root and its associated shoot together before they were separated at the point where both the root and shoot begin. They were left to dry before measurements were taken. Each treatment group had a sample size of 8. *F.enc = Fictibacillus enclensis*, BC = Biochar. A one-way ANOVA test yielded significant differences between the treatment groups that was further analysed by a Tukey Honestly Significant Difference test. * Indicates $0.01 \leq p \leq 0.05$, ** Indicates $0.001 \leq p \leq 0.01$, *** Indicates $0 \leq p \leq 0.001$.

## 3.4. Testing the Soil for the Presence of *Fictibacillus enclensis*

After harvesting the plants, soil samples were collected and tested for the presence of *F. enclensis*. Serial dilution and subsequent plating were performed to grow any bacteria that were present within the soil. Any bacterial colonies that appeared morphologically similar to *F. enclensis* were isolated to undergo further testing, which consisted of gel electrophoresis and DNA sequencing. Figure 18 shows *F. enclensis*, both freshly grown (A) and after 2 weeks of

growth (B) as its color slightly changes. Figure 19 to Figure 24 were the bacterial species that underwent further testing.

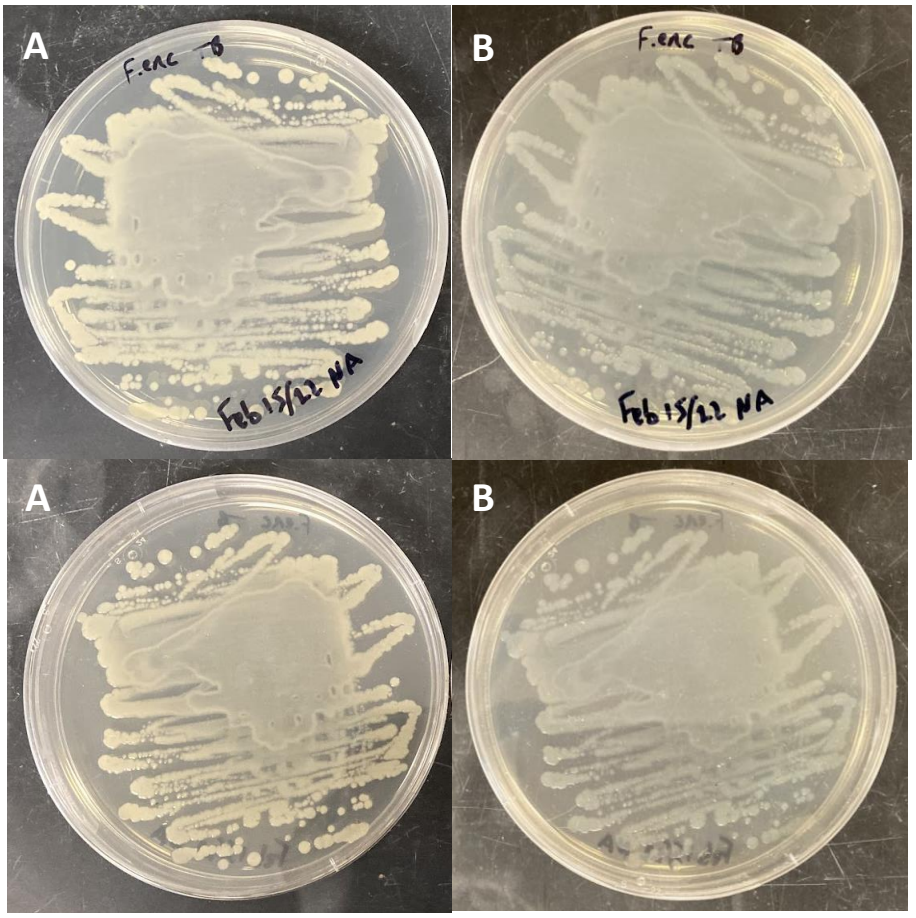


**Figure 18.** *F. enclensis* freshly grown on nutrient agar (A) and after 2 weeks of growth (B). Nutrient agar (5g peptone, 3g yeast extract, 5g sodium chloride, 15g agar).

**Figure 19.** Unknown bacteria grown on nutrient agar (5g peptone, 3g yeast extract, 5g sodium chloride, 15g agar) from the soil of the collard green trial that was treated with the *F. enclensis* and biochar formulation. Soil and sterilized water were centrifuged at 1000 x g for 5 minutes and diluted. This bacterium originated from the 3-fold dilution ($10^{-3}$) of the stock vial ($10^{-1}$).

**Figure 20.** Unknown bacteria grown on nutrient agar (5g peptone, 3g yeast extract, 5g sodium chloride, 15g agar) from the soil of the carrot trial that was treated with the *F. enclensis* and biochar formulation. Soil and sterilized water were centrifuged at 1000 x g for 5 minutes and diluted. This bacterium originated from the 3-fold dilution ($10^{-3}$) of the stock vial ($10^{-1}$).

**Figure 21.** Unknown bacteria grown on nutrient agar (5g peptone, 3g yeast extract, 5g sodium chloride, 15g agar) from the soil of the collard green trial that was treated with the *F. enclensis* and biochar formulation. Soil and sterilized water were centrifuged at 3400 x g for 5 minutes and diluted. This bacterium originated from the 3-fold dilution ($10^{-3}$) of the stock vial ($10^{-1}$).

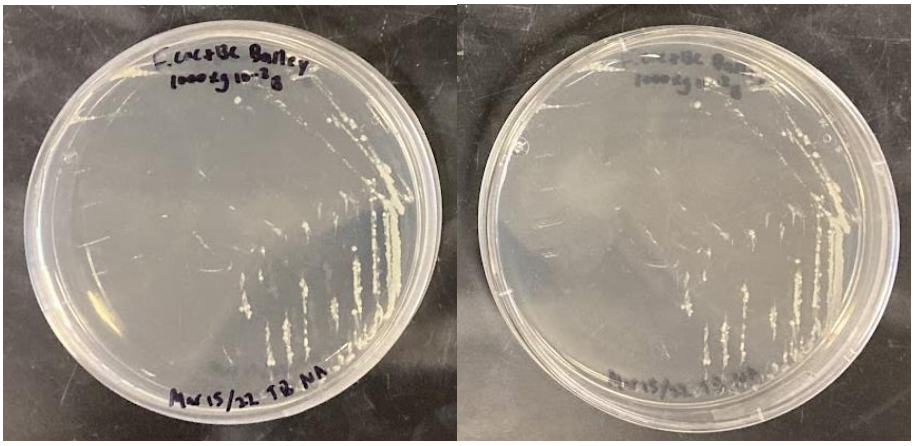

**Figure 22.** Unknown bacteria grown on nutrient agar (5g peptone, 3g yeast extract, 5g sodium chloride, 15g agar) from the soil of the barley trial that was treated with the *F. enclensis* and biochar formulation. Soil and sterilized water were centrifuged at 1000 x g for 5 minutes and diluted. This bacterium originated from the 3-fold dilution ($10^{-3}$) of the stock vial ($10^{-1}$).

**Figure 23.** Unknown bacteria grown on nutrient agar (5g peptone, 3g yeast extract, 5g sodium chloride, 15g agar) from the soil of the barley trial that was treated with the *F. enclensis* and biochar formulation. Soil and sterilized water were centrifuged at 1000 x g for 5 minutes and diluted. This bacterium originated from the 4-fold dilution ($10^{-4}$) of the stock vial ($10^{-1}$).

**Figure 24.** Unknown bacteria grown on nutrient agar (5g peptone, 3g yeast extract, 5g sodium chloride, 15g agar) from the soil of the collard green trial that was treated with the *F. enclensis* and biochar formulation. Soil and sterilized water were centrifuged at 3400 x g for 5 minutes and diluted. This bacterium originated from the 4-fold dilution ($10^{-4}$) of the stock vial ($10^{-1}$).

### 3.4.0. Gel Electrophoresis

The 6 bacterial isolates (Figure 19 to Figure 24) were tested on the quality and quantity of their DNA. This was accomplished through gel electrophoresis. Figure 25 shows the final product of this process. The second lane, containing the negative control, verified that the PCR reagents were not contaminated based on the lack of a band. Looking at the 1 kb DNA ladder in the further most left lane as a reference, all of the DNA samples in the remaining lanes appeared to be around 1500 base pairs. This coincides with the length of the DNA region that the primer amplified (16 rRNA). Based on the brightness of the bands in lanes 3-8, it can be concluded that each DNA sample is of sufficiently high quantity. Slight degradation can be observed in the DNA samples, based on the slight smear present in each lane. However, each sample was deemed to be suitable to send to Genome Quebec for DNA sequencing.

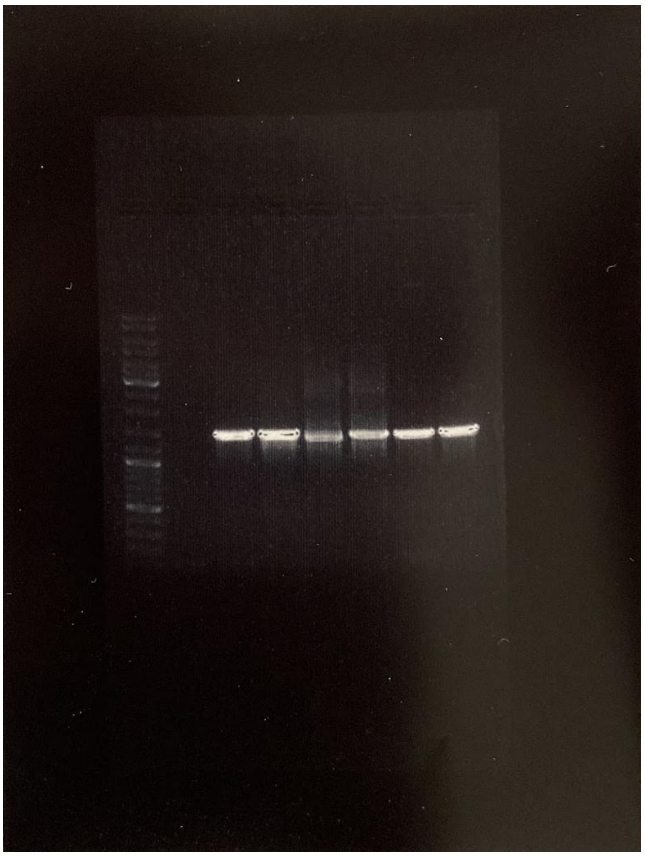

**Figure 25.** End-product of gel electrophoresis. Each well had 3 µL of the respective product added. From left to right the product added to each lane are as follows: 1 kb DNA ladder, negative control, unknown bacteria being tested (lanes 3-8).

### 3.4.1. DNA Sequencing

Most of the chromatograms that were received from Genome Quebec could not be analyzed. The presence of multiple overlapping peaks made the analysis of these chromatograms unreliable. However, an unknown bacterium from the 4-fold dilution of soil from the barley plants treated with the *F. enclensis* and biochar formulation did yield a partially good sequence. The remaining sequence of this bacterium yielded a chromatogram similar to the others, which consisted of multiple overlapping peaks. Using a BLAST search, this partial sample was identified as belonging to either an *Arthrobacter* species or a *Paenarthrobacter* species.

| Description | Scientific Name | Max Score | Total Score | Query Cover | E value | Per. Ident | Acc. Len | Accession |
|---|---|---|---|---|---|---|---|---|
| Arthrobacter sp. strain H406 16S ribosomal RNA gene, partial sequence | Arthrobacter sp. | 1088 | 1088 | 100% | 0.0 | 100.00% | 1452 | MH669311.1 |
| Paenarthrobacter nitroguajacolicus strain PP3 16S ribosomal RNA gene, partial sequence | Paenarthrobacter nitroguajacoli... | 1088 | 1088 | 100% | 0.0 | 100.00% | 1373 | MN519462.1 |
| Arthrobacter sp. CSQXZ2N4.7.4 gene for 16S ribosomal RNA, partial sequence | Arthrobacter sp. | 1088 | 1088 | 100% | 0.0 | 100.00% | 1426 | LC484822.1 |
| Arthrobacter sp. CSQXZN4.3.10 gene for 16S ribosomal RNA, partial sequence | Arthrobacter sp. | 1088 | 1088 | 100% | 0.0 | 100.00% | 1431 | LC484811.1 |
| Paenarthrobacter sp. CSQXZR6.3.8 gene for 16S ribosomal RNA, partial sequence | Paenarthrobacter sp. | 1088 | 1088 | 100% | 0.0 | 100.00% | 1431 | LC484761.1 |
| Paenarthrobacter sp. strain 18JY15-11 16S ribosomal RNA gene, partial sequence | Paenarthrobacter sp. | 1088 | 1088 | 100% | 0.0 | 100.00% | 1441 | MK101054.1 |
| Paenarthrobacter nitroguajacolicus strain PG1 16S ribosomal RNA gene, partial sequence | Paenarthrobacter nitroguajacoli... | 1088 | 1088 | 100% | 0.0 | 100.00% | 1000 | MG458422.1 |
| Paenarthrobacter nitroguajacolicus F-40 gene for 16S ribosomal RNA, partial sequence | Paenarthrobacter nitroguajacoli... | 1088 | 1088 | 100% | 0.0 | 100.00% | 816 | LC430068.1 |
| Paenarthrobacter nitroguajacolicus strain 18JY15-11 16S ribosomal RNA gene, partial seque... | Paenarthrobacter nitroguajacoli... | 1088 | 1088 | 100% | 0.0 | 100.00% | 1454 | MH497646.1 |
| Paenarthrobacter nitroguajacolicus strain YHBT8 16S ribosomal RNA gene, partial sequence | Paenarthrobacter nitroguajacoli... | 1088 | 1088 | 100% | 0.0 | 100.00% | 1374 | MG571714.1 |
| Arthrobacter sp. strain 4N4A 16S ribosomal RNA gene, partial sequence | Arthrobacter sp. | 1088 | 1088 | 100% | 0.0 | 100.00% | 1377 | KX066724.1 |
| Arthrobacter sp. strain 3N5 16S ribosomal RNA gene, partial sequence | Arthrobacter sp. | 1088 | 1088 | 100% | 0.0 | 100.00% | 950 | KX066723.1 |
| Arthrobacter sp. B015-56 16S ribosomal RNA gene, partial sequence | Arthrobacter sp. B015-56 | 1088 | 1088 | 100% | 0.0 | 100.00% | 1388 | KJ190970.1 |
| Arthrobacter sp. HBUM178449 16S ribosomal RNA gene, partial sequence | Arthrobacter sp. HBUM178449 | 1088 | 1088 | 100% | 0.0 | 100.00% | 948 | KR906426.1 |
| Arthrobacter sp. HBUM178470 16S ribosomal RNA gene, partial sequence | Arthrobacter sp. HBUM178470 | 1088 | 1088 | 100% | 0.0 | 100.00% | 902 | KR906425.1 |
| Arthrobacter aurescens strain LK9 16S ribosomal RNA gene, partial sequence | Paenarthrobacter aurescens | 1088 | 1088 | 100% | 0.0 | 100.00% | 730 | KT715775.1 |
| Arthrobacter aurescens strain LK7 16S ribosomal RNA gene, partial sequence | Paenarthrobacter aurescens | 1088 | 1088 | 100% | 0.0 | 100.00% | 720 | KT715774.1 |
| Paenarthrobacter nitroguajacolicus strain ES3 16S ribosomal RNA gene, partial sequence | Paenarthrobacter nitroguajacoli... | 1088 | 1088 | 100% | 0.0 | 100.00% | 1468 | OL851734.1 |
| Arthrobacter nitroguajacolicus partial 16S rRNA gene, isolate SBA 66 | Paenarthrobacter nitroguajacoli... | 1088 | 1088 | 100% | 0.0 | 100.00% | 1111 | HG941837.1 |

**Figure 26.** The result of a BLAST (Basic Local Alignment Search Tool) search of an unknown bacterium (4-fold dilution of soil from the barley plants treated with the *F. enclensis* and biochar formulation) using the National Library of Medicine: National Center for Biotechnology Information site. This result was obtained from BLAST searching the partial sequence (from base 40 to base 642) of the chromatogram obtained from Genome Quebec.

# 4. Discussion

Testing the effects that an *F. enclensis* and biochar formulation had on the vegetative growth of collard greens, carrots, and barley produced results that can not be deemed reliable. The fertilizer and the *P rhodesiae* and biochar treatment groups (positive controls) in each trial did not significantly improve the vegetative growth of the plants as they should. This could signify that the conditions in the greenhouse, or on the light bench, were not sufficient to grow the plants. The plants were not healthy; they did not grow to their full potential. For example, the carrots were all significantly smaller (~ 6 cm in length, ~ 10 g in fresh weight) than what would be considered normal upon visual inspection. This is likely due to a limited resource/factor that was restricting the growth of the plants. For the greenhouse trials, the limiting factor could be

sunlight because nearby trees limited the amount of sunlight that was reaching the greenhouse. For the lab bench trials, the limiting factor could be the amount of room or drainage in the pots used, because they were only grown in red solo cups as opposed to bigger pots with drainage holes. In saying this, the interpretation of the results that is described below can not be trusted because the plants were not grown appropriately.

The collard greens appeared to benefit from the application of the *F. enclensis* and biochar formulation, as noted in their significantly heavier total leaf weights when compared to the control, nutrient broth and biochar, and the *P. rhodesiae* and biochar treatment groups (Figure 4). However, in terms of shoot weight and the number of leaves produced, there were no significant improvements to the collard greens treated with the formulation of *F. enclensis* and biochar (Figures 1 and 3). The collard greens treated with the *F. enclensis* and biochar formulation actually produced significantly shorter shoots when compared to the collard greens treated with chemical fertilizer (Figure 2). Other studies noted improvements in both shoot and leaf growth when inoculated with a plant growth-promoting bacterium (Baset Mia et al., 2010; Gholami et al., 2009). It is unclear why we observed shorter shoots with larger leaves without knowing more about the exact mechanism of plant growth promotion utilized by *F. enclensis*. Previous work suggests that *F. enclensis* produces IAA; however, the exact concentration of IAA produced is not known (Burns, 2021). High concentrations of IAA were observed to reduce the height of maize while increasing its yield (Li et al., 2018). This same effect could be occurring with the collard greens inoculated with *F. enclensis* if the bacterium produces a high concentration of IAA. However, the amount of IAA would need to be quantified in order to support or contradict this theory.

It was also noted that the collard greens treated with the *F. enclensis* and biochar formulation exhibited a significantly high leaf:shoot ratio (leaf weight divided by shoot weight) when compared with the control (Figure 6). The leaf:shoot ratio looks at the weights of both the leaves and the shoots to compare which component is of the greatest biomass. A high leaf:shoot ratio is indicative that the leaves have a greater biomass when compared to the shoot of the plant. This is attributed to a greater allocation of nutrients to the leaves (Kozlowski & Pallardy, 2002; Pereira et al., 2020). Previously, it was thought that *F. enclensis* may be encouraging the plant to allocate nutrients to the leaves, thereby increasing the potential for a greater yield when compared with the control (Burns, 2021). However, a high leaf:shoot ratio was also observed in the collard greens treated with nutrient broth and biochar. Therefore, this result does not support the theory that *F. enclensis* by itself encourages the plant to allocate its resources to the leaves; the nutrient broth and biochar may have more of a role in creating this high ratio. There would need to be data on the application of *F. enclensis* by itself, without the nutrient broth and biochar, to see if *F. enclensis* influences the plant to direct its resources to the leaves.

The collard greens did not appear to benefit in terms of leaf damage from the application of the *F. enclensis* and biochar formulation when compared with the control (Figure 12). It is hard to discern what may have caused the loss of leaf tissue; that would need further investigation. However, a previous study found that strawberry leaves exhibited less leaf damage when treated with the *F. enclensis* and biochar formulation (Burns, 2021). The author attributed the damage on the leaves to pests, and hypothesized that the limited leaf damage was due to the bacteria's ability to act as a biocontrol agent (Burns, 2021). Analysis of strawberry leaves, using the LC-DAD, that were subjected to treatment with *F. enclensis* and biochar, *P. rhodesiae* and biochar, nutrient broth and biochar, biochar, and a control revealed that a resulting peak may be a

potential proanthocyanidin (Burns, 2021). Proanthocyanidins are a group of secondary metabolites produced by plants that are involved in the plant's protection from pests, large herbivores, and pathogens (Dixon et al., 2005; He et al., 2008). All treatment groups had the presence of this peak but the concentration of proanthocyanidin was not determined for each treatment group (Burns, 2021). A greater production of this compound in the *F. enclensis* treated group could explain the limited amount of leaf damage on the strawberry leaves. Positive associations have been detected between the protection of strawberry plant from *Botrytis cinerea* and the concentration of proanthocyanidins (Amil-Ruiz et al., 2011). However, the production of proanthocyanidins is not verified; more work would be needed to confirm this. In saying this, the ability of *F. enclensis* to act as a biocontrol agent is not yet validated; this trial does not support this theory while the study conducted by Burns does (Burns, 2021). Specific plant trials that introduced plant pathogens and or pests, and measured their effect across the different treatment groups, would need to be conducted, to adequately test this. This could be an avenue of research in the future.

The carrots did not exhibit any improvements in their vegetative growth when they were inoculated with the formulation of *F. enclensis* and biochar. The differences observed between the collard greens, which did appear to benefit from the application of *F. enclensis* and biochar, and the carrots could be due to the way in which different plant species respond to the bacteria (Esitken et al., 2003; Orhan et al., 2006). For example, the *Bacillus* strain of OSU-142 was found to improve the growth and yield of apricot, but had negative effects on the growth and yield of raspberry (Esitken et al., 2003; Orhan et al., 2006). However, the differences between the growth of the collard greens and carrots could also be due to the differences in soil used (Egamberdiyeva, 2007). Due to time constraints and a shortage of Nova Scotia topsoil, a bag of

potting soil had to be purchased. The Nova Scotia topsoil used in the collard trial appeared to be more nutrient deficient, whereas the potting soil used in the carrot trials appeared to be more nutrient rich. A previous study looking at the performance of plant growth-promoting bacteria in different soil types observed that 3 different strains of bacteria had a better stimulatory effect on maize in nutrient deficient calcisol soil than in nutrient rich loamy soil (Egamberdiyeva, 2007). Because no statistically significant results were observed across any of the treatment groups, even the fertilizer treatment group, it may be that the soil was already rich in nutrients. Therefore, any added benefits that a chemical fertilizer or a plant growth-promoting bacteria would have provided might be negligible, resulting in non-significant results in terms of the plants growth.

There were no significant effects on the vegetative growth of barley plants treated with *F. enclensis* when compared with the control. The barley treated with chemical fertilizer didn't produce any significant results in terms of their vegetative growth either. Again, this could be due to the soil used in this trial; it was similar to the soil-type used in the carrot trial. In spite of this, however, the barley plants treated with the formulation of *F. enclensis* and biochar did significantly improve their dry shoot weight compared to the barley treated with only *F. enclensis* (Figure 15B). This can be due to a variety of reasons: the biochar performed well as a carrier to successfully inoculate the soil; or if the soil type has made a difference in the effectiveness of the bacteria, it could show that the biochar can work in a variety of soil types while the bacteria cannot. In addition, because we did observe a significantly high fresh and dry shoot:root ratio on barley treated with *F. enclensis* and biochar when compared to just *F. enclensis* (Figure 16 and 17), this does support the idea that the biochar has more of an effect on these ratios rather than the bacteria encouraging the crop to direct nutrients to certain plant

organs. This is further showcased in Figure 17, which shows that the barley plants treated with water and biochar had a significantly higher dry shoot:root ratio when compared to the barley treated with *F. enclensis*.

Testing the soil for the presence of *F. enclensis* did not yield any substantial results. Most of the chromatograms were of poor quality due to the presence of overlapping peaks. This may have resulted due to contamination; multiple peaks at a position in the DNA sequence could correspond to the presence of multiple bacteria. The reading of these sequences is unreliable because it is impossible to determine what peak belongs to each bacterium. The partial sequence of 1 chromatogram could be used, and a BLAST search revealed that it was not *F. enclensis*. However, without the results for the other bacteria, it is hard to propose an explanation for this.

For future plant trials, the addition of a *F. enclensis* treatment group without any biochar is warranted to obtain more data on the previous theory regarding the nutrient allocation capabilities of *F. enclensis*. No work thus far, besides this barley trial, has been conducted on the vegetative growth of various plants treated with *F. enclensis* by itself. From the data that we do have, its hard to differentiate the effects that *F. enclensis* causes to the plant and the effects that the biochar formulation causes to the plant.

Additionally, it is imperative that more research goes into the mechanism of plant growth promotion utilized by *F. enclensis*. Qualitative testing revealed that *F. enclensis* may be capable of fixing nitrogen, and producing IAA (Burns, 2021). However, more information is needed on each of these to decipher the bacteria's mode of action on specific aspects of plant growth. For example, because IAA is involved in root growth, incremental tests should be conducted on the bacteria's ability to alter the root growth of a plant on a small scale (i.e., in a petri dish, 5-week plant trial) and then on a larger scale (i.e., field trial) (Glick, 2012). The concentrations of these

compounds produced by *F. enclensis* would also be of interest to know because high concentrations of IAA, for example, have resulted in deleterious effects on plants (Li et al., 2018).

More tests should also be conducted on the ability of *F. enclensis* to produce other plant growth regulators in addition to its production of IAA. This is especially important as previous research challenges the nitrogen-fixing capabilities of *F. enclensis* (Solano et al., 2008). Bacteria capable of fixing nitrogen make use of most of the nitrogen for themselves, therefore leaving little to none for the plant to use (Solano et al., 2008). It is thought that the benefits that nitrogen-fixing bacteria provide to plants is more so due to the creation of plant growth regulators, such as gibberellin and abscisic acid (Solano et al., 2008). Therefore, it is likely that this notion holds true for *F. enclensis*, which warrants the need for an investigation into the bacteria's ability to produce these additional compounds.

Even with all of this additional testing, these trials would need to be repeated under more suitable growing conditions to produce reliable results. The plants grown in these trials were not healthy because certain limiting factors restricted their growth. These limited factors would need to be remedied before any results can be trusted.

If this proves successful, testing whether or not *F. enclensis* can remain effective at promoting plant growth in the field would be needed as well. Plant trials on a light bench or in the greenhouse are very controlled in terms of light, soil, environmental conditions, and water for example. Field trials are very different from this because many variables exist that cannot be controlled: the environmental conditions at the time, the bacteria's interaction with local bacteria, and its ability to contend with other bacteria for nutrients while it adjusts to new surroundings (Herrmann & Lesueur, 2013).

That is why further investigation into these factors is crucial to observe the limitations of *F. enclensis* in the field and ultimately its effectiveness with the biochar. More knowledge on *F. enclensis* and additional plant trials are needed to understand its mechanism of plant growth promotion. Hopefully in gaining this knowledge, we will have the necessary information to include or exclude the use of this *F. enclensis* and biochar formulation as an alternative to agrochemicals, and a solution to future food insecurity.

# References


Amil-Ruiz, F., Blanco-Portales, R., Muñoz-Blanco, J., & Caballero J. L. (2011). The strawberry plant defense mechanism: A molecular review. *Plant and Cell Physiology, 52*(11), 1873–1903.

Baligar, V. C., & Bennett, O. L. (1986). NPK-fertilizer efficiency - a situation analysis for the tropics. *Fertilizer Research, 10,* 147-164.

Baset Mia, M. A., Shamsuddin, Z. H., Wahab, Z., & Marziah, M. (2010). Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth and nitrogen incorporation of tissue-cultured Musa plantlets under nitrogen-free hydroponics condition. *Australian Journal of Crop Science, 4*(2), 85-90.

Bashan, Y., de-Bashan, L. E., Prabhu, S. R., & Hernandez, J. (2013). Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). *Plant Soil, 378,* 1-33.

Battisti, D. S. & Naylor, R. L. (2009). Historical warnings of future food insecurity with unprecedented seasonal heat. *Science, 323,* 240–244.

Bennett, E., Roberts, J. A., & Wagstaff C. (2012). Manipulating resource allocation in plants. *Journal of Experimental Botany, 63*(9), 3391-3400.

Berendsen, R. L., Pieterse, C. M., & Bakker, P. A. (2012). The rhizosphere microbiome and plant health. *Trends in Plant Science, 17*(8), 478-486.

Bhandari, G. (2014). An overview of agrochemicals and their effects on environment in Nepal. *Applied Ecology and Environmental Sciences, 2*(2), 66-73.

Bhattacharjee, R. B., Singh, A., & Mukhopadhyay, S. N. (2008). Use of nitrogen-fixing bacteria as biofertiliser for non-legumes: prospects and challenges. *Applied Microbiology and Biotechnology, 80,* 199-209.

Bhattacharyya, C., Banerjee, S., Acharya, U., Mitra, A., Mallick, I., Haldar, A., Haldar, S., Ghosh, A., & Ghosh, A. (2020). Evaluation of plant growth promotion properties and induction of antioxidative defense mechanism by tea rhizobacteria of Darjeeling, India. *Scientific Reports, 10,* 1-19.

Blatt-Janmaat, K. L., MacQuarrie, S. L., & Sit, C. S. (2020). Does size matter? An investigation into the impact of coarse and fine ground inoculated biochar on *Hordeum vulgare* (barley) growth and yield. *Rhizosphere, 13,* 1-8.

Burns, C. (2021). Sustainable agriculture: Plant growth promoting bacteria and biochar as biofertilizer and biocontrol [Unpublished master's thesis]. Saint Mary's University.

Canadian International Development Agency. (2009). *Increasing food security.*

Caroline, A., Debode, J., Vandecasteele, B., D'Hose, T., Cremelie, P., Haegeman, A., Ruttink, T., Dawyndt, P., & Maes, M. (2016). Biological, physicochemical and plant health responses in lettuce and strawberry in soil or peat amended with biochar. *Applied Soil Ecology, 107,* 1-12.

Cui, W., He, P., Munir, S., He, P., Li, X., Li, Y., Wu, J., Wu, Y., Yang, L., He, P., He, Y. (2019). Efficacy of plant growth promoting bacteria *Bacillus amyloliquefaciens B9601-Y2* for biocontrol of southern corn leaf blight. *Biological Control, 139,* 1-11.

Dai, Z., Fu, W., Wan, L., Cai1, H., Wang, N., Qi1, S., & Du, D. (2016). Different growth promoting effects of endophytic bacteria on invasive and native clonal plants. *Frontiers in Plant Science, 7(*706), 1-9.

Davidson, E. A. (2009). The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. *Nature Geoscience, 2*, 659-662.

De Souza, R., Ambrosini, A., & Passaglia, L. M. P. (2015). Plant growth-promoting bacteria as inoculants in agricultural soils. *Genetics and Molecular Biology, 38*(4), 401-419.

Dixon, R. A., Xie, D. & Sharma, S. B. (2005). Proanthocyanidins – a final frontier in flavonoid research? *New Phytologist, 165*, 9-28.

Egamberdiyeva, D. (2007). The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. *Applied Soil Ecology, 36*, 184-189.

Erisman, J. W., Sutton, M. A., Galloway, J., Klimont, Z., & Winiwarter, W. (2008). How a century of ammonia synthesis changed the world. *Nature Geoscience, 1*, 636–639.

Esitken, A., Karlidag, H., Ercisli, S., Turan, M., & Sahin F. (2003). The effect of spraying a growth promoting bacterium on the yield, growth and nutrient element composition of leaves of apricot (*Prunus armeniaca* L. cv. Hacihaliloglu). *Australian Journal of Agricultural Research, 54*, 377-380.

Food and Agriculture Organization. 2009. *How to feed the world in 2050*.

Gholami, A., Shahsavani, S., & Nezarat, S. (2009). The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. *International Journal of Agricultural and Biosystems Engineering, 3*(1), 9-14.

Glick, B. R. (1995). The enhancement of plant growth by free living bacteria. *Canadian Journal of Microbiology, 41*(2), 109-117.

Glick, B. R. (2012). Plant growth-promoting bacteria: Mechanisms and applications. *Scientifica,* 1-15.

Good, A. G., & Beatty, P. H. (2011). Fertilizing nature: A tragedy of excess in the commons. *PLOS Biology, 9*(8), 1-9.

Government of Canada. (2021, November 5). *Overview of the Canadian agri-food system.*

Hale, L., Luth, M., Kenney, R., Crowley, D. (2014). Evaluation of pinewood biochar as a carrier of bacterial strain *Enterobacter cloacae* UW5 for soil inoculation. *Applied Soil Ecology, 84*, 192-199.

He, F., Pan, Q., Shi, Y., & Duan, C. (2008). Biosynthesis and genetic regulation of proanthocyanidins in plants. *Molecules, 13*(10), 2674–2703.

Helaly, A. A., Hassan, S. M., Craker, L. E., Mady, E. (2020). Effects of growth-promoting bacteria on growth, yield and nutritional value of collard plants. *Annals of Agricultural Sciences, 65*(1), 77-82.

Herrmann, L & Lesueur, D. (2013). Challenges of formulation and quality of biofertilizers for successful inoculation. *Applied Microbiology and Biotechnology*, *97*, 8859–8873.

Intergovernmental Panel on Climate Change. (2012). *Managing the risks of extreme events and disasters to advance climate change adaptation: Special report of the intergovernmental panel on climate change.*

Kozlowski, T. T., & Pallardy, S. G. (2002). Acclimation and adaptive responses of woody plants to environmental stresses. *The Botanical Review, 68,* 270-334.

Kumar, A., Maurya, B. R., Raghuwanshi, R. (2014). Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (*Triticum aestivum L.*). *Biocatalysis and Agricultural Biotechnology, 3*(4), 121-128.

Lashof, D. A. & Ahuja, D. R. (1990). Relative contributions of greenhouse gas emissions to global warming. *Nature, 344*, 529-531.

Lawlor, D. W., Lemaire, G., & Gastal, F. (2001). Nitrogen, plant growth and crop yield. In P. J. Lea, & J. Morot-Gaudry (Eds.), *Plant nitrogen* (pp. 343-367). Springer.

Lehmann, J., & Kleber, M. (2015). The contentious nature of soil organic matter. *Nature, 528,* 60-68.

Lesk, C., Rowhani, P., & Ramankutty, N. (2016). Influence of extreme weather disasters on global crop production. *Nature, 529,* 84-87.

Li, Z., Zhang, X., Zhao, Y., Li, Y., Zhang, G., Peng, Z & Zhang, J. (2018). Enhancing auxin accumulation in maize root tips improves root growth and dwarfs plant height. *Plant Biotechnology Journal, 16*, 86–99.

Lickacz, J., & Penny, D. *Soil organic matter.* Alberta Agriculture Food and Rural Development.

Müller, B., Bryant, L. D., Matzinger, A., Wüest, A. (2012). Hypolimnetic oxygen depletion in eutrophic lakes. *Environmental Science and Technology, 46,* 9964-9971.

Organisation for Economic Co-operation and Development. (2008). *Environmental performance of agriculture in OECD countries since 1990.*

Orhan, E., Esitken, A., Ercisli, S., Turan, M., & Sahin, F. (2006). Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. *Scientia Horticulturae, 111*, 38-43.

Pathan, S. I., Ceccherini, M. T., Sunseri, F., & Lupini, A. (2020). Rhizosphere as hotspot for plant-soil-microbe interaction. In: Datta, R., Meena, R., Pathan, S., & Ceccherini M. (Eds.), Carbon and nitrogen cycling in soil (pp.17-43). Springer.

Pereira, S. I. A., Abreu, D., Moreira, H., Vega, A., Castro, P. M. L. (2020). Plant growth-promoting rhizobacteria (PGPR) improve the growth and nutrient use efficiency in maize (*Zea mays L.*) under water deficit conditions. *Heliyon, 6*(10), 1-9.

Prepas, E. E., & Charette, T. (2003). Worldwide eutrophication of water bodies: Causes, concerns, controls. In K. K. Turekian & H. D. Holland (Eds.), *Treatise on Geochemistry* (pp. 311-331). Elsevier.

Prosekov, A. Y. & Ivanova, S. A. (2018). Food security: The challenge of the present. *Geoforum, 91*, 73-77.

Rabalais, N. N., Turner, R. E., Díaz, R. J., & Justić D. (2009). Global change and eutrophication of coastal waters. *ICES Journal of Marine Science, 66*(7), 1528-1537.

Rawat, J., Saxena, J., & Sanwal, P. (2019). Biochar: A sustainable approach for improving plant growth and soil properties. In V., Abrol, & P., Sharma (Eds.), *Biochar - An imperative amendment for soil and the environment.* IntechOpen.

Reganold, J. P., Papendick, R. I., & Parr, J. F. (1990). Sustainable agriculture. *Scientific American, 262*(6), 112-121.

Reinhold-Hurek, B., & Hurek, T. (2011). Living inside plants: bacterial endophytes. *Current Opinion on Plant Biology, 14*(4), 435-443.

Rilling, J. I., Acuña, J. J., Nannipieri, P., Cassan, F., Maruyama, F., & Jorquera, M. A. (2019). Current opinion and perspectives on the methods for tracking and monitoring plant growth–promoting bacteria. *Soil Biology and Biochemistry, 130*, 205-219.

Rosenblueth, M., & Martínez-Romero, E. (2006). Bacterial endophytes and their interactions with hosts. *Molecular Plant Microbe Interactions, 19*(8), 827-837.

Santoyo, G., Moreno-Hagelsieb, G., Orozco-Mosqueda, M. C., & Glick, B. R. (2016). Plant growth-promoting bacterial endophytes. *Microbiological Research, 183*, 92-99.

Shumway, S. E. (1990). A review of the effects of algal blooms on shellfish and aquaculture. *Journal of the World Aquaculture Society, 21*(2), 65-104.

Solano, B. R., Maicas, J. B., & Mañero, F. J. G. (2008). Physiological and molecular mechanisms of plant growth promoting rhizobacteria (PGPR). In I. Ahmad, J. Pichtel, S. Hayat (Eds.), *Plant-bacteria interactions. Strategies and techniques to promote plant growth* (pp. 41-54). Wiley.

Spiertz, J. H. J. (2010). Nitrogen, sustainable agriculture and food security: A review. *Agronomy for Sustainable Development, 30*(1), 43-55.

Stewart, W. M., Dibb, D. W., Johnston, A. E., & Smyth, T. J. (2005). The contribution of commercial fertilizer nutrients to food production. *Agronomy Journal, 97*(1), 1-6.

Sun, B., Zhang, L., Yang, L., Zhang, F., Norse, D. & Zhu. Z. (2012). Agricultural non-point source pollution in China: Causes and mitigation measures. *Ambio, 41*, 370-379.

Townsend, R. F. (2015). *Ending poverty and hunger by 2030: An agenda for the global food system.* World Bank Group.

United States Environmental Protection Agency. (2007). *Hypoxia in the Northern Gulf of Mexico: An update by the EPA Science Advisory Board*.

United States Environmental Protection Agency. (2010). *Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990–2008.*

Vecstaudza, D., Senkovs, M., Nikolajeva, V., Kasparinskis, R., Muter, O. (2017). Wooden biochar as a carrier for endophytic isolates. *Rhizosphere, 3,* 126-127.

Vejan, P., Khadiran, T., Abdullah, R., Ismail, S., & Dadrasnia, A. (2019). Encapsulation of plant growth promoting Rhizobacteria-prospects and potential in agricultural sector: a review. *Journal of Plant Nutrition, 42*(19), 2600-2623.

Verheijen, F., Jeffery, S., Bastos, A. C., van der Velde, M., & Diafas, I. (2010). *Biochar application to soils: A critical scientific review of effects on soil properties, processes and functions*. Joint Research Centre - Institute for Environment and Sustainability.

Viljoen, J. J. F., Labuschagne, N., Fourie, H., & Sikora, R. A. (2019). Biological control of the root-knot nematode *Meloidogyne incognita* on tomatoes and carrots by plant growth promoting rhizobacteria. *Tropical Plant Pathology, 44,* 284-291.

Withers, P. J. A., Neal, C., Jarvie, H. P., & Doody, D. G. (2014). Agriculture and eutrophication: Where do we go from here? *Sustainability, 6,* 5853-5875.

Wrage, N., Velthof, G. L., van Beusichem, M. L., & Oenema, O. (2001). Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biology and Biochemistry, 33,* 1723-1732.

Xiong, B., Zhang, Y., Hou, Y., Arp, H. P. H., Reid, B. J., & Cai, C. (2017). Enhanced biodegradation of PAHs in historically contaminated soil by *M. gilvum* inoculated biochar. *Chemosphere, 182,* 316-324.