Utilization of growth-promoters to improve biomass productivity of *Arundo donax* L. (NileFiber™) as a purpose-grown biofuel feedstock in Nova Scotia.

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A Thesis Submitted to Saint Mary's University, Halifax, Nova Scotia,
in Partial Fulfillment of the Requirements for the
Degree of Master of Science in Applied Science.

August 2016, Halifax, Nova Scotia

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Date:	02 August 2016

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ABSTRACT

Arundo donax L. is a perennial grass species of Mediterranean origin. Its characteristic high growth rate and biomass yield potential have attributed to its viability as a biofuel feedstock in its native climate. The goal of this research was to evaluate the growth potential of a proprietary genotype of *A. donax* (NileFiber™) on low-quality land in Nova Scotia as a purpose-grown biofuel feedstock. Applications of plant growth-promoters (bacterial and fungal species) and plant stimulants (seaweed extract and lipo-chitooligosaccharide) were investigated for their ability to enhance NileFiber™ biomass productivity. Growth-promoters were applied in three experimental trials (two greenhouse, one field) and were compared to untreated plants in both environments. Growth potential was determined by growth measurements taken at harvest and by calculating biomass yield. Experimental evaluation suggests *A. donax* is not a suitable biomass feedstock in Nova Scotia but that growth-promoters can enhance NileFiber™ productivity.

Date: 02 August 2016

ACKNOWLEDGEMENTS

I would like to express my gratitude to many individuals whose support and guidance made this research possible. To my main supervisor, Dr. J. Kevin Vessey, for the research opportunity and for his direction and support throughout my graduate program. Dr. Vessey was able to provide guidance that enabled me to gain independence and confidence in my research capabilities. To my co-supervisor, Dr. Yousef Papadopoulos, I would like to express my sincere appreciation for his unwavering support and confidence in me. To Dr. Balakrishnan Prithiviraj, my external examiner and Drs. Ryan Gibson and Zhongmin Dong, who comprised my supervisory committee, I would like to express thanks for their time and feedback, as their knowledge and expertise propelled the interdisciplinary nature of this thesis.

I would like to express appreciation for Dr. Houman Fei and Matthew Crouse, for their tremendous patience and guidance throughout the extensive experimental procedures of this research. Also, to those who assisted in the lab, greenhouse or the field (Krista Budgell, Carla MacKay, Alex Peters, Mackenzie Rathegeber, Chao Wang, Jingwen Zhao and graduate student helpers from Dalhousie University).

I would also like to acknowledge Dr. Timothy Frasier, without whom I would have never grasped the concept of Bayesian Statisical Analysis and come to appreciate its validity in light of a heavily biased Frequentist world. I would like to thank Dr. Laura Weir for embracing my craziness, and for being an unofficial supervisor in times when I needed guidance from a fellow female academic perspective and when I needed help with RStudio.

I am incredibly thankful to my parents, Jane and Mike, my brother Alex, Jeff, and the rest of my family for their love, support and patience. The number of times I succinctly explained my research for these people enabled me to convey the importance of my research outside of academia.

Lastly, I would like to thank the friends that I met along the way during my Master's degree: Natasha Dazé Querry, Karissa Appleby, Suchinta Arif, and all the other wonderful friends I met at Saint Mary's. Without you, I wouldn't have had the peer support (academic and otherwise) to fulfill the requirements of this program. I feel fortunate to have met such great people throughout my two years!

I would also like to acknowledge BioFuelNet Canada, Saint Mary's University Faculty of Graduate Studies and Research, Nile Fiber Canada Inc., Acadian Seaplants Ltd., and the Government of Canada's Research Support Fund for providing the financial support for this research.

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1.0 INTRODUCTION

The burning of fossil fuels (oil, natural gas and coal (McGlade and Ekins, 2015; Maggio and Cacciola, 2012)) accounts for between 66 and 80% of total global energy consumption (Asif and Muneer, 2007; Berg and Boland, 2014; Hammond and Seth, 2013; IEA, 2015; Youngs and Somerville, 2012). Fossil fuel combustion for energy is a major source of trace atmospheric gases (i.e. carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O)) (Edenhofer et al., 2014; Koçar and Civas, 2013; Mander et al., 2015) and the anthropogenic production of these gases is responsible for Earth's changing climate (Bhullar et al., 2012; Crowley, 2000; Khan et al., 2014; Mander et al., 2015). The non-renewable nature of fossil fuels combined with their prominent share of global energy consumption has initiated concern over reaching peak resource estimates and ultimately depleting resources (Asif and Muneer, 2007; Maggio and Cacciola, 2012). Recent technological advancements in areas such as hydraulic fracturing have resulted in the possibility to exploit previously inaccessible fossil fuel reserves (Fitzgerald, 2013; Meinert et al., 2016; Miller and Sorrell, 2014), however; total global fossil fuel resources are estimated to peak within the next fifty years (Maggio and Cacciola, 2012).

Human energy dependence based on industrialized, modern societies is causing an increase in global energy demand, further augmented by the growth of the global human population (Asif and Muneer, 2007; Blaschek et al., 2010; Demirbas, 2008; Meinert et al., 2016). Potential losses in energy security and deteriorating environmental conditions are only two of the many issues associated with the prevalence of fossil fuel

use on a global scale (Asif and Muneer, 2007; Hughes and Rudolph, 2011). Bioenergy encompasses various forms of renewable energy derived from the conversion of biomass through two routes: using biomass directly as fuel or processing biomass into liquids or gases for fuel (IEA Bioenergy, 2009; Yuan et al., 2008). Bioenergy serves as a potential complement to the current fossil fuel industry resulting in a diversified, sustainable energy industry for the future (Bhullar et al., 2012). Globally, 10% of the primary energy supply is bioenergy (IEA Bioenergy, 2009; Youngs and Somerville, 2012) and the overall investment into the renewable energy sector is steadily growing (Asif and Muneer, 2007).

The growth of global bioenergy depends on the development and implementation of national-scale bioenergy incentives and mandates that will support the development of the industry (Kedron, 2015; Offermann et al., 2011; Yue et al., 2014). As of December 2014, sixty-four countries have biofuel mandates in place ranging from 2 – 27.5 % ethanol content and 2 – 20 % biodiesel content in non-renewable fuels based on regional fuel availability and policy regarding renewable fuels (Lane, 2014). Globalization of the industry through biomass trade is also important for growth (IEA Bioenergy, 2009).

Canada has committed to reducing greenhouse gas emissions 17 % below 2005 levels by the year 2020 (Kedron, 2015). The primary motivation for expanding the renewable energy industry in Canada is to reduce greenhouse gas emissions (the transportation sector is the greatest contributor to national greenhouse gas emissions (Bhullar et al., 2012; Environment Canada, 2013)) and to reduce other negative environmental impacts, such as flooding, drought and acid rain (Asif and Muneer, 2007; Dessureault, 2014; Kedron, 2015; Mabee and Saddler, 2010).

Bioenergy is currently the second most important form of renewable energy in Canada, accounting for 6% of total energy supply while more specifically, biofuels and renewable waste account for 4.4% (Main et al., 2007; NRC, 2015). The growth of this industry can be attributed to the implementation of fuel blending mandates in 2006 (under the Canadian Environmental Protection Act Bill C-33), which requires the addition of renewable content, such as ethanol or biodiesel, to fossil fuels that are produced or imported into Canada, creating market demand for renewable fuels (Bhullar et al., 2012; Bradley, 2010; Environment Canada, 2011). The federal mandate requires at least 5 % renewable content (ethanol) in gasoline and 2 % renewable content (biodiesel) in diesel by volume (Environment Canada, 2011). Similar provincial biofuel mandates were developed before the federal mandate, which was created to simplify and further support interprovincial regulations (Dessureault, 2014).

Despite significant growth since the mandate introduction, current Canadian production of ethanol and biodiesel is below the national blend mandate, therefore requiring the importation of biofuels to meet the mandate (Bhullar et al., 2012; Dessureault, 2014; Dessureault, 2015). Canada must import approximately 20 % of our total ethanol consumption to meet the national blend mandate (Dessureault, 2015), with the United States of America exporting over 50 % of their total ethanol exports to Canada, covering the vast majority of our domestic production shortfall (Beckman, 2015; USDC, 2014).

The demand for renewable fuels to meet the Canadian blend mandate has augmented domestic production (USDC, 2014). This production is mainly from food and

feed crops for first generation (conventional) biofuels (Bhullar et al., 2012). As of 2008, 97 % of ethanol produced in Canada was corn-based, wheat-based or corn-wheat mixture-based (Bhullar et al., 2012). In 2010, 7 million litres of bioethanol was produced in Canada for transport fuel from lignocellulosic material (wheat straw and wood wastes), accounting for approximately 1 % of total national ethanol production (Mabee and Saddler, 2010). There are currently numerous national and provincial incentives in place to support and promote domestic lignocellulosic ethanol production (Mabee and Saddler, 2010; USDC, 2014).

Within Canada, the bioenergy industry in Nova Scotia is still in its infancy, despite growth of the national industry in recent years. The Atlantic Council for Bioenergy Cooperative Limited (ACBC) was founded in 2010 and is the main representative of bioenergy industries in Nova Scotia. ACBC published a public report in 2013 regarding the opportunities for bioenergy in Nova Scotia and the other Atlantic Provinces (Magnus, 2013). In addition to this report and current research of feasibility and sustainability of a bioenergy industry in Nova Scotia, eleven successful bioenergy projects have been established since 2006 (Simmons et al., 2015).

Pertaining to the feasibility of a bioenergy industry in Nova Scotia, the existing infrastructure of the pulp and paper industry can be crucial to bioenergy growth. The pulp and paper industry in Nova Scotia has been very important since its introduction in the 1800s but is declining based on reduced societal dependence on paper products (Kuhlberg, 2015). Despite losses within this integral industry locally, the transition to a bioenergy industry that capitalizes on existing pulp and paper infrastructure could drive a

successful, sustainable industry for the province (Magnus, 2013). Although the opportunity to capitalize on existing forestry biomass previously used for pulp and paper is substantial, diversifying provincial biomass resources (i.e. purpose-grown feedstock and waste materials) will lead to more sustainable resource exploitation.

Arundo donax L., a perennial grass native to Mediterranean climatic conditions (Christou et al., 2001; Mardikis et al., 2001) is one of the primary feedstock crops used for lignocellulosic ethanol production by Beta Renewables Crescentino plant in Vercelli, Italy (Bomgardner, 2013; Palmqvist and Lidén, 2014). There is no record of research regarding *A. donax* as a purpose-grown biofuel feedstock crop in Canada, therefore this project examines the potential of *A. donax* under climatic and edaphic conditions in Nova Scotia, Canada.

The objectives of this research are:

1. To evaluate the growth potential of *A. donax* (NileFiber™) for use as a purpose-grown advanced biofuel feedstock crop in class 3 and 4 soils in Nova Scotia;
2. To investigate the application of growth-promoter microorganisms and stimulants to enhance biomass productivity of *A. donax* (NileFiber™).

To carry out this research, Nile Fiber Atlantic Canada Inc. of Halifax, Nova Scotia, Canada provided rhizomatous tissues from an identifiable genotype of *A. donax* (NileFiber™, US 20140075628 P1 patent pending by TreeFree Biomass Solutions Inc. of Seattle, Washington) for growth potential evaluation in Nova Scotia. Three experiments were conducted within the time frame of this project: two greenhouse trials and one field

trial. The primary purpose of all trials was to investigate the growth of NileFiber™ in unfertilized, marginal soils. By testing the crop under such edaphic conditions in Nova Scotia, we are able to ascertain the production potential of NileFiber™ with low inputs and on land that would not compete with food production – both important factors for the production of biomass feedstock crops. In addition, plant growth-promoting microorganisms and supplements were applied via soil drench to investigate the ability of these promoters to increase biomass yield. The results are discussed in terms of identifying the effect on biomass yield in the absence or presence of growth-promoters.

Exploring the growth of NileFiber™ in Nova Scotia is important in delving into the somewhat daunting task of determining diversified biomass resources without jeopardizing food crop resources or over-exploiting natural forests. Further investigating NileFiber™ growth after growth-promoter inoculation is key to reduce environmental impacts of modern agriculture whilst increasing biomass yield and ultimately increasing biofuel production. The overarching goal of this research is to further the real data necessary to drive the development of a second generation biofuel industry in Nova Scotia.

2.0 LITERATURE REVIEW

2.1 Bioenergy

Biomass for energy is any material of biological origin (Whalen, 2015) that in a solid form, has been used since the discovery of fire as an energy source (Okonko et al., 2009). Biofuel is a term coined for fuel composed from biomass material (Demirbas, 2008) that can be used as an alternative to fossil fuels (Bhullar et al., 2012; Singh, 2013). Fossil fuels are also derived from biomass, but a temporal scale can be used to differentiate between the two forms of biomass: fossil fuel biomass originates from living organisms tens to hundreds of millions of years ago, whereas biofuel biomass originates from living organisms over a significantly shorter scale of one year to tens of years (Whalen, 2015).

Bioenergy is growing in importance in comparison to fossil fuels. Fossil fuels are finite, non-renewable resources (Owen et al., 2010; Speirs et al., 2015) that have replaced primitive biofuels (e.g. wood burned for heat) as human societies have developed a dependence on cheap, readily available fuel (Chapman, 2014; Keeney and DeLuca, 1992). Rapidly growing reliance on fossil fuels has increased societal efficiency by mechanizing numerous daily tasks. Despite the non-renewable nature and the reduced availability of cheap fossil fuel resources, societal consumption has not stalled (Herring, 2006; Murray and King, 2012).

The imminent decline of the global supply of fossil fuels (Owen et al., 2010), coupled with the environmental and social issues associated with fossil fuel consumption,

create ideal conditions for an increased demand for biofuels (Chapman, 2014; Herring, 2006).

2.2 Liquid Biofuels

The earliest commercial use of liquid biofuels dates back to the mid-19th century when whale oil was burned for light in lighthouses and household lamps (Davis et al., 1988; Songstad et al., 2009). The automotive industry also began its long history of biofuel use in the 19th century, as American engineer Samuel Morey and German engineer Nicolaus Otto independently designed modified internal combustion engines that used ethanol as fuel (Mussatto et al., 2010; Soloman et al., 2007). Most notably, Henry Ford designed the first vehicle to run on ethanol: the 1908 Ford Model-T (Graver and Kriss, 2012; Mussatto et al., 2010; Soloman et al., 2007). In this instance, ethanol was sourced from small-scale agricultural operations seeking alternative income during less profitable growing seasons (Soloman et al., 2007).

Ethanol (also known as bioethanol or lignocellulosic ethanol) and biodiesel are the two most common liquid transportation fuels that show global potential of direct product replacement in the current petroleum industry (Demirbas, 2011; Hill et al., 2006). Bioethanol is most commonly derived from biomass that has undergone hydrolysis and fermentation (Demirbas et al., 2008; Yuan et al., 2008) whereas biodiesel is derived from vegetable oils and animal fats (Demirbas, 2008; Ma and Hanna, 1999) that have undergone transesterification (Demirbas, 2008; Vessey, 2015). As secondary liquid biofuels (processed biomass) (Dragone et al., 2010), biodiesel serves as a replacement for

petroleum diesel and bioethanol for petroleum gasoline (Agarwal, 2007; Demirbas, 2008; Demirbas, 2011).

The aforementioned history of the use of biofuels in the automotive industry has enabled blending of biofuels with petroleum fuels for use in unmodified engines without disrupting normal engine function (Demirbas, 2011; Hansen et al., 2005). Ethanol-gasoline blends with ethanol content between 0 – 30 % have been studied in unmodified engines with successful engine performance and decreased emissions (Al-Hasan, 2003; Hsieh et al., 2002; He et al., 2003), enabling a simpler transition to an ethanol-gasoline blended industry. Additionally, flexible fuel vehicles (FFV) have modified engines that are capable of adapting to and using ethanol-gasoline blends with higher ethanol contents (Rosillo-Calle et al., 2007). Fuels with 100 % ethanol content in Brazil and 85 % in North America are used in flexible fuel vehicles (Delgado et al., 2007; Graham et al., 2008). The energy density of ethanol is 33 % less than the energy density of gasoline, contributing to a loss of fuel mileage between 3 and 30 % (increasing with greater ethanol content) compared to pure gasoline (Atsumi et al., 2007; Knoll et al., 2009; Regalbuto, 2009; Swana et al., 2011). The development of a biofuel transportation industry complimentary to the fossil fuel industry has greater traction when infrastructure, such as vehicles and fuel stations with accommodations for renewable fuel already exist.

2.3 Biofuel Characterization

Biofuels are categorized based on biomass characteristics and the biomass to biofuel conversion process (Demirbas, 2011). There are currently four categories of

biofuels: first generation (conventional) and second generation (advanced), both derived from agricultural feedstocks and waste, and third and fourth generation are derived from the use of algae (Demirbas, 2011; Dutta et al., 2014). Considering only first and second generation biofuels, the ability of second generation biofuels to address disadvantages associated with first generation biofuels propels second generation to a more favorable position in the future of the biofuel industry (Dutta et al., 2014; Hamelinck and Faaij, 2006; Naik et al., 2010). Throughout this thesis, only ethanol will be discussed as biodiesel is a completely different form of biofuel, mainly based on feedstock and conversion processing.

2.3.1 First Generation (Conventional) Biofuels

The feedstocks for first generation bioethanol include edible food and feed crops (Bhullar et al., 2012), most notably corn, wheat, soybean and sugarcane (Dutta et al., 2014; Sims et al., 2010).

2.3.1.1 Advantages

The production and conversion of first generation feedstocks are considered commercialized “established technology” (Naik et al., 2010; Serra and Zilberman, 2013; Smith, 2010). The conversion process of carbohydrates (sugars and starches) used for first generation biofuels is also well known as the process used to create alcoholic beverages (Demirbas, 2009; Demirbas, 2011).

2.3.1.2 Drawbacks

2.3.1.2.1 Food versus Fuel Debate

There have been three major commodity booms since World War II (Radetzki, 2006), with the longest lasting boom occurring most recently, concurrent with the "biofuel boom" (Tyner, 2008) in the mid-2000s (Baffes and Haniotia, 2010; Radetzki, 2006; Smith, 2010). The rapid growth of the biofuel industry has created changes in the pre-existing dynamic relationship between energy and agriculture, thus initiating the food versus fuel debate (Myers et al., 2014; Serra and Zilberman, 2013).

The food versus fuel debate, regardless of opposition or agreement, raises two disconcerting issues with the diversion of food feedstock to fuel: food supply (security) and food prices (Smith, 2010; Tokgoz et al., 2007; Tyner, 2008). Food security is defined by the Food & Agriculture Organization of the United Nations (2009) as "when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life". Food insecure individuals do not have access to food, causing undernourishment (FAO, 2009). More than one billion people were classified as undernourished by the FAO in 2009 (Ahmed et al., 2014; FAO, 2009) while the amount of grains in ethanol distilleries was enough to feed 330 million people for one year (Smith, 2010).

Food prices are constantly subjected to volatility, because of the variable nature of agricultural production and consumption (Ahmed, 2014). Agricultural demand can cause food price volatility through unpredictable variability (Ahmed, 2014). At the height of

the “biofuel boom” (Tyner, 2008), the price of corn increased 150 % over the course of two years (Glauber, 2008; Mitchell, 2008; Smith, 2010; Tyner, 2008) while ethanol production grew 8.9 billion litres per year over the same period (Tyner, 2008).

The validity of the food versus fuel debate has been repeatedly scrutinized in varying ways. To oppose biofuel based food insecurity, the land area undertaken to produce biofuel feedstocks amounts to a mere 2 % of global arable land (Baffes and Haniotis, 2010; Sims et al., 2010). To oppose the rising food prices, less than 2 % of the increasing consumer costs for food (Tyner, 2008) and only 36 % of the rise in corn prices can be directly attributed to corn ethanol production (remaining 64 % rise attributed to external economic conditions) (Babcock and Fabiosa, 2011).

The dynamic relationship between energy and agriculture can cause uncertainty when addressing the food versus fuel debate. Ultimately, the validity of arguments opposing and supporting the different sides of the debate are up for interpretation. Myers et al. (2014) concluded that energy and agricultural biofuel feedstock prices move jointly over the short run, but over the long run, agricultural prices are determined by supply and demand patterns that are independent of biofuels. Conversely, Serra and Zilberman (2013) concluded instability and volatility in energy markets have been negatively affecting feedstock markets since the “biofuel boom” (Tyner, 2008). Despite the presence of supporting and opposing evidence, moving biofuel production away from food feedstock is the optimal option for nullifying the debate.

2.3.1.2.2 Land-Use and Land-Use Change

The carbon cycle describes the natural movement of carbon through Earth's spheres: atmosphere, hydrosphere and terrestrial biosphere (combination of lithosphere and biosphere) (Falkowskit et al., 2000; Post et al., 1990; Wigley and Schimel, 2000). Carbon pools in all spheres act as carbon storage, and can either emit (source) or uptake (sink) carbon, creating fluxes between pools (Melillo et al., 2002; Schlesinger and Andrews, 2000). The terrestrial biosphere, composed of soil and plant biomass, is the greatest terrestrial carbon sink (Fargione et al., 2008) and absorbs approximately 30 % of anthropogenic carbon dioxide emissions (Luo et al., 2015; Reichstein et al., 2013). Land-use and land-use change negatively impact the terrestrial carbon sink and the carbon cycle balance by destroying natural ecosystems and eliminating respective ecosystem services (Foley et al., 2011; Wigley and Schimel, 2000).

Foley et al. (2005) define the result of land-use practices as the "acquisition of natural resources for immediate human needs, often at the expense of degrading environmental conditions". Agriculture is the greatest user of Earth's terrestrial land, with approximately 40 % of land used for crop production or pasture (Foley et al., 2005; Foley et al., 2011). Agriculturally cultivated land has expanded since the modernization of food production (Foley et al., 2005) and continues to expand to sustain a growing population (Laurance et al., 2014). Substantial dependence on current arable land causes eventual land degradation through erosion and fertility loss to meet short-term food production at a cost of long-term ecosystem services (Foley et al., 2005). Degradation of arable land leads to agricultural expansion into other land types, typically forest and

grassland, which then undergoes substantial land-use change (Dale, 1994; Don et al., 2012; Laurance et al., 2014; Wigley and Schimel, 2000).

One beneficial aspect of biofuels is the sequestration effect that occurs when atmospheric carbon dioxide is captured through photosynthesis by plants, theoretically balancing the release of carbon dioxide to the environment through fuel consumption (Azar et al., 2013; Searchinger et al., 2008; Smeets et al., 2009). Conventional energy feedstocks (food crops) require multiple inputs to sustain high yields (Don et al., 2012), therefore requiring land expansion once the arable land necessary for crop growth is degraded beyond suitability. Land expansion to grow conventional energy crops prompts land-use change, further inducing land-use change emissions (releasing terrestrial carbon and reducing present and future land sequestration ability) (Lambin and Meyfroidt, 2011; Searchinger et al., 2008; Wigley and Schimel, 2000). This ultimately defeats the carbon sequestration benefits of biofuels (Don et al., 2012; Laurance et al., 2014; Searchinger et al., 2008).

2.3.1.2.3 Anthropogenic Inputs

Conventional energy feedstocks (food crops) require multiple inputs to sustain high yields (Don et al., 2012) and these inputs prove costly economically, socially and environmentally (Groom et al., 2008; Lambin and Meyfroidt, 2011). Additional inputs can be categorized into farm-level inputs and fuel-processing inputs (Hill et al., 2006), of which key inputs will be expanded upon in subsequent sections.

2.3.1.2.3.1 Fertilizer

Fertilizer is a soil amendment used to sustain or elevate crop yield by addressing soil nutrient deficiencies (Xue et al., 2014). In traditional organic agriculture, soil nutrients are replenished through crop rotations including pulse crops and by spreading and incorporating animal manure or crop residues into the soil (Crews and Peoples, 2004; Dwwyor et al., 2005; Smil, 2001; Smil, 1997). Synthetic fertilizer was invented in the early 20th century to address the increasing demand for soil nitrogen in agriculture (Crews and Peoples, 2004; Mulvaney et al., 2009; Smil, 2001; Smil, 1997). The Haber-Bosch process ($N_2 + 3H_2 \rightarrow 2NH_3$) is the industrial process of synthesizing ammonia (NH_3) from a reaction between a fossil fuel (as a source of hydrogen, (H_2)) and nitrogen gas (N_2) under extremely high temperatures and pressures (Kandemir et al., 2013; Smil, 2001; Vojvodic et al., 2014).

Global consumption of fertilizers has grown significantly since the intensification of agriculture during the "Green Revolution" of the 1960s (Foley et al., 2005; Matson et al., 1997). Over the past four decades, consumption has increased approximately 400 % (Alexandratos and Bruinsma, 2012; IFA, 2015). Synthetic fertilizer consumption is based on supplying plant-available primary macronutrients nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O) to the soil, attempting to delimit plant growth based on Liebig's law of the minimum (Smil, 2001).

2.3.1.2.3.1.1 Nitrogen (N)

The Earth's atmosphere contains approximately 80 % dinitrogen gas (N_2), discovered in the late 18th century (Gowariker et al., 2009; Smil, 2001; Sutton and

Bleeker, 2013) and its importance in agriculture was recognized by many individuals, most prominently by French chemist Jean-Babtiste Boussingault early in the 19th century (Smil, 2001; Wisniak, 2007). Nitrogen is important to support vegetative plant growth (Smil, 2001), as it is a vital constituent of plant substances such as amino acids, proteins and chlorophyll (Gowariker et al., 2009).

Agricultural nitrogen use efficiency (NUE) is the amount of above-ground biomass production per unit of plant available nitrogen in the soil (usually measured in grams) (Dawson et al., 2008; Moll et al., 1982). NUE, a function of overall use (Moll et al., 1982) of nitrogen fertilizers is generally low (≤ 50 %) (Crews and Peoples, 2004; Dawson et al., 2008) due to a) competition between soil microbes and plants, b) the mobile nature of nitrogen and c) the many ways in which nitrogen can escape from the ecosystem (Bouwman et al., 2009; Crews and Peoples, 2004; Dawson et al., 2008; Gowariker et al., 2009). The aforementioned factors, combined with plant nutritional demand and further societal demand (from a growing population) for plants and plant products, are the driving force behind the need to increase soil nitrogen through fertilization (Mulvaney et al., 2009).

Nitrogen largely has three forms at standard temperature and pressure: dinitrogen, organic nitrogen and inorganic nitrogen. Dinitrogen (N_2) is the most abundant and most unavailable form of nitrogen because of its non-reactive / highly stable nature (Socolow, 1999). Organic nitrogen, the integral building blocks of larger molecules such as amino acids and proteins (Socolow, 1999). Finally, inorganic nitrogen in the form of nitrogen

ions (Socolow, 1999). Plants uptake ionic inorganic nitrogen as ammonium (NH_4^+) or nitrate (NO_3^-) (Gowariker et al., 2009).

The inefficiency of plant nitrogen utilization (nitrogen accumulation by plants grown in soil with nitrogen fertilizers (Moll et al., 1982)) results in detrimental side-effects. Despite being available for uptake by plants, nitrate (NO_3^-) can be lost through leaching, based on its high solubility, contaminating ground and surface waters and causing soil acidification (Brentrup et al., 2000; Crews and Peoples, 2004; Gowariker et al., 2009). Denitrification of nitrate (NO_3^-) and nitrification of ammonium (NH_4^+) in the soil can create nitrous oxide (N_2O) and nitric oxide (NO), inducing a greenhouse gas flux to the atmosphere (Brentrup et al., 2000; Bouwman, 1996; Crews and Peoples, 2004; Socolow, 1999). Volatilization of ammonia (NH_3) into the atmosphere is another pathway of nitrogen to the environment (Brentrup et al., 2000; Crews and Peoples, 2004; Socolow, 1999).

Synthetic nitrogen fertilizer, accounting for approximately 40 – 68 % of energy use, is the single greatest energetic agricultural input (Crews and Peoples, 2004; Fluck, 2012). Commercial nitrogen fertilizer production accounts for more than half of anthropogenic fixed nitrogen (Socolow, 1999) and its application to agricultural soils is the greatest global anthropogenic source of nitrous oxide (N_2O) emissions (Crews and Peoples, 2004; Matson et al., 1998; Smith et al., 2014). Nitrous oxide has a global warming potential 300 times greater than carbon dioxide (CO_2), has an atmospheric lifetime of 120 years and is a major contributor to ozone destruction (Forster et al., 2007; Victor et al., 2014).

2.3.1.2.3.1.2 Phosphorus (P)

Phosphorus (P) was discovered by German chemist Hennig Brand in 1669 (Brunner, 2010; Weeks, 1932a) and was later recognized for its importance to agriculture by Justus Freiherr von Liebig in the mid 19th century (Brunner, 2010; Liebig et al., 1841; Nagendrappa, 2013). Although availability is scarce in the biosphere, phosphorus is equally as important as nitrogen for plant growth, as it is a key element in nucleic acids (phosphodiester bonds) and is also essential for energy (adenosine diphosphate ↔ adenosine triphosphate, ADP/ATP) (Bouwman et al., 2009; Smil, 2000).

Phosphorus can be categorized into two groups, inorganic and organic (Richardson and Simpson, 2011; Rodríquez and Fraga, 1999; Smil, 2000). With no direct phosphorus flux from the biosphere to the atmosphere, the concentration of atmospheric phosphorus is very small, allowing for 95 % of global phosphorus to be concentrated in the biosphere (Bouwman et al., 2009; Rodríquez and Fraga, 1999; Smil, 2000). Despite its significant proportion in the biosphere, a substantially insignificant concentration of that proportion is in a form available to plants as phosphate anions (HPO_4^{-2} and $H_2PO_4^-$) (Richardson and Simpson, 2011; Richardson et al., 2009; Rodríquez and Fraga, 1999; Smil, 2000).

The limited natural availability of phosphorus in the soil (compared with nitrogen) has lead to increasing synthetic phosphorus fertilizer application (Hart et al., 2004; Smil, 2000). There are fewer pathways for phosphorus loss within the soil phosphorus cycle, potentially resulting in increased probability of exogenously applied phosphorus uptake by plants thus creating a more efficient cycle than the soil nitrogen

cycle (Bouwman et al., 2009). However, there are still losses of phosphorus to report: firstly, the fraction of soluble phosphorus is highly reactive with other soil compounds, and is readily immobilized, rendering it unavailable to plants (Richardson and Simpson, 2011; Smil, 2000). Secondly, soil phosphorus can also be lost through leaching and runoff (Hart et al., 2004; Sharpley et al., 2015). High levels of phosphorus in water systems can induce algal growth, leading to eutrophication in aquatic environments (Sharpley et al., 2015).

Phosphorus-use efficiency (PUE) is the total dry matter production of a crop per unit of phosphorus input (usually measured in kilograms), including naturally available and exogenously applied phosphorus (MacDonald et al., 2011). Interestingly, areas of high P fertilizer application are correlated with low PUE (MacDonald et al., 2011) based on surplus P immobilization (Smil, 2000). Approximately 90 % of global phosphorus applications are agricultural in nature (Brunner, 2010), and crop uptake of that phosphorus fertilizer is merely 20 % (Bouwman et al., 2009).

2.3.1.2.3.1.3 Potassium (K)

The isolation and identification of potassium (K) occurred in 1807 by Sir Humphry Davy (Thomas et al., 2008; Weeks, 1932b). Potassium is the most abundant inorganic nutrient in plants (Spalding et al., 1999), functioning as an ionic osmoticum regulating turgor pressure (Lokhande and Reddy, 2015; Walker et al., 1996) and somewhat as a catalyst for plant development (Gowariker et al., 2009; Parmar and Sindhu, 2013).

Soil potassium can be categorized into three groups: 1) water-soluble / exchangeable, 2) non-exchangeable / fixed and 3) mineral / structural (Moody and Bell, 2006; Parmar and Sindhu, 2013; Zörb et al., 2014). The mineral / structural forms of potassium are the form least available for uptake by plants, followed by non-exchangeable potassium, constituting between 90 – 98 % of soil potassium combined (Parmar and Sindhu, 2013; Römheld and Kirkby, 2010; Zörb et al., 2014). The water-soluble / exchangeable potassium is readily taken up by plants, but constitutes a mere 2 % of soil potassium (Parmar and Sindhu, 2013; Zörb et al., 2014). Plant availability of soil potassium is dependent on physical and chemical properties of the soil (Blake et al., 1999; Zörb et al., 2014).

Despite a reduced uptake by plants compared to exchangeable potassium, non-exchangeable potassium in the soil can provide a long term supply, creating a reserve in the soil through synthetic fertilizer application, as it is more easily available for plant uptake than mineral potassium (Römheld and Kirkby, 2010; Zörb et al., 2014).

Nitrogen, phosphorus and potassium are often mixed and applied together as synthetic fertilizer with varying compositions of each nutrient (N-P-K). These macronutrients are harvested within biomass material, resulting in a loss from the soil (Jungers et al., 2015). The most prominent indirect effect of synthetic fertilizers is the resultant emissions that occur across the span of production to application, as the entire process is heavily energy dependent, thus generating emissions (Mulvaney et al., 2009; Xue et al., 2014).

2.3.1.2.3.2 Pesticides

Pesticides, broadly encompassing insecticides, herbicides and fungicides, are substances used to protect agricultural plants from potential pests (Gowariker et al., 2009; National Research Council U.S. et al., 2000; Canada Department of Justice, 2006). Pests have been a cause of reduced yield since the development of agriculture (between 16,000 and 10,000 years ago) (Popp et al., 2013; Thacker, 2002). Initial responses of early agricultural producers to a pest outbreak were to suffer or to abandon the land and move agricultural production (NRC U.S. et al., 2000; Thacker, 2002). In modern agriculture, pesticides are applied to maintain agricultural yield with minimal suffering and movement.

Natural, plant-derived chemicals, such as nicotine (*Nicotiana tabacum*) (NRC U.S. et al., 2000; Thacker, 2002) and Pyrethrum (*Chrysanthemum cinerariaefolium*) (Casida and Quisad, 1998; Isman, 2006; Thacker, 2002) were popular pest control substances from the mid-16th to the late 19th century while inorganic compounds (such as arsenic compounds) (Casida and Quisad, 1998; Rodrigo et al., 2014; Thacker, 2002) were also introduced in the 16th century and application increased steadily as availability increased (Thacker, 2002; Zadocks and Waibel, 2000).

Synthetic chemical pesticides (organochlorines, organophosphates, carbamates and pyrethroids) were discovered in the mid-20th century, more specifically during and after World War II (Isman, 2006; NRC U.S. et al., 2000; Thacker, 2002; Zadocks and Waibel, 2000). The pest control properties of Dicholorodiphenyltrichloroethane (DDT), one of the most prominent global synthetic chemicals to date, were discovered by Paul

Müller in 1939 (organochlorine) (Thacker, 2002; Zadocks and Waibel, 2000). DDT was used during World War II to prevent insect borne diseases (NRC U.S. et al., 2000; Thacker, 2002) and was used after the war as a crop pesticide (Thacker, 2002; Zadocks and Waibel, 2000).

Globally, pesticide application has experienced steady growth to sustain high crop yields to supply a growing human population (Carvalho, 2006; Popp et al., 2013). There are direct and indirect negative effects associated with pesticide application that are not justified for biofuel feedstock production. Similar to synthetic fertilizers, pesticides can contaminate surrounding non-target ecosystems through soil leaching (Hill et al., 2006), soil runoff into water (Carvalho, 2006; Hill et al., 2006; Pimental and Burgess, 2014) and spray drift, which can lead to pesticide losses up to 70 % (Popp et al., 2013). Indirect effects of pesticide application include negative impacts on non-target species, including humans (through ingestion of contaminated material) and beneficial species, despite target specificity (Carvalho, 2006; Newsom, 1967). Finally, increased pesticide application can lower the threshold of crop pest defense, increasing future reliance on synthetic inputs (Landis et al., 2008).

2.3.1.2.3.3 Energy

The most prominent source of energy on Earth is the Sun, whether the end-use of that solar energy is direct or indirect (Fluck, 2012). Modern, industrialized agriculture is extremely energy intensive, requiring energy supplementary to the sun (commonly in the form of non-renewable fossil fuels) to surpass subsistent agricultural production (Bardi et al., 2013; Fluck, 2012; Horrigan et al., 2002). An agricultural system boundary outlines

the processes associated with the entire life cycle of the agricultural output, and is a major step in an overall Life Cycle Analysis (LCA) to determine energy use and associated emissions (Boland and Unnasch, 2014; Xue et al., 2014). Production and application of anthropogenic inputs (fertilizers and pesticides), fueling agricultural equipment and additional transportation of output are few of many aspects within the system boundary that are fossil fuel dependent (Bardi et al., 2014; Boland and Unnasch, 2014; Fluck, 2012; Xue et al., 2014). Biofuel feedstocks require further processing to create the final fuel product, adding to the processes in the system boundary and the LCA (Boland and Unnasch, 2014).

The energy balance of biofuel systems is a key factor in determining sustainability of the feedstock for biofuel (López-Bellido et al., 2014; Cherubini et al., 2009). LCA focuses on energy balance (energy outputs : energy inputs) and associated environmental impacts (Cherubini et al., 2009; Davis et al., 2009). Energy balance of corn-ethanol has been extensively studied with variable results across studies. In the early expansion of corn-ethanol production, studies showed promising results of reduced greenhouse gas emissions when compared with equivalent non-renewable fuel production (Farrell et al., 2006; Kim and Dale, 2005). More recently, the culmination of substantive non-renewable fossil fuel inputs and land-use change associated with corn-ethanol production has shown less emissions reduction and in some cases, emission increases (Bonin and Lal, 2012; Farrell et al., 2006; Jaradat, 2010; Searchinger et al., 2008).

2.3.2 First Generation (Conventional) Biofuels – Summary

In summary, the drawbacks associated with first generation (conventional) biofuels are:

- **Food versus Fuel**: Despite opposing and supporting research validating the food versus fuel debate, the optimal scenario is to be able to nullify the debate by moving away from food feedstock for biofuels.
- **Land-Use and Land-Use Change**: Greenhouse gas emissions reductions through biofuel production are negligible when 1) arable land is over-exploited, being supplied with extraneous fossil fuel derived inputs and 2) extensive changes in land-use occur to overcome losses in arable land. The resultant soil carbon storage through production of annual crops does not balance the carbon storage of undisturbed land.
- **Anthropogenic Inputs**: Significant dependence on non-renewable energy to fuel the production of first generation biofuel feedstocks based on the need for synthetic fertilizers and pesticides. Additionally, resultant emissions from production and application of synthetic inputs and agricultural equipment generate less sustainable production conditions for renewable fuel.

2.3.3 Second Generation (Advanced) Biofuels

Sources of biomass for second generation biofuels consist mostly of non-food biomass (Demirbas, 2011; Naik et al., 2010). Herbaceous energy crops, such as perennial grasses (*Panicum virgatum* and *Miscanthus* spp.) (Lewandowski et al., 2003) and short rotation coppice (SRC), including *Populus* spp. and *Salix* spp. (Hinchee et al., 2009) all

combine to encompass purpose-grown feedstocks. Additionally, waste materials can also be sourced for biofuel: agricultural waste, including food crop residues (corn stover, wheat straw, etc.), forestry waste (by-products of conventional forestry activities) (Tilman et al., 2009) and municipal solid waste (Demirbas, 2008; Tilman et al., 2009).

The production and conversion of second generation biomass is less established than first generation biofuel technology, in terms of industrial scale production, but the processing allows for less species specificity of biomass. Plant biomass is largely composed of cellulose, hemicellulose and lignin; these complex sugars are bound by non-fermentable lignin (Sarkar et al., 2012; Smith, 2015; Yuan et al., 2008). Cellulose and hemicellulose must be separated from lignin through a pre-treatment process (less established technology, variable between biomass sources) (Naik et al., 2010; Sarkar et al., 2012) before the sugars can proceed through a similar hydrolysis / fermentation process as first generation biofuels (Smith, 2015; Yuan et al., 2008).

2.3.3.1 Addressing the Drawbacks of First Generation Biofuels

Many of the described drawbacks associated with first generation biofuel production have been addressed through the development of second generation biofuels (Naik et al., 2010; Wang et al., 2012).

- **Food versus Fuel**: The capability of diverse biomass to be converted into second generation biofuel reduces the profound reliance on food feedstock (Sims et al., 2010).
- **Land-Use and Land-Use Change**: Lignocellulosic feedstocks show greater potential of substantial growth on lower-quality land (Gelfand et al., 2013; Sims et al., 2010).

Additionally, resultant carbon storage in lignocellulosic feedstocks can increase (compared to annual food crops) based on the perennial nature of the plants (Don et al., 2012; Wang et al., 2012). Greenhouse gas emissions are offset more efficiently through an atmospheric carbon recycling process that sequesters carbon in plant biomass (Cherubini et al., 2009; Demirbas, 2008). The implementation of these feedstocks on degraded soils can initiate a natural soil restoration process thus enhancing biodiversity (Holland et al., 2015; López-Bellido et al., 2014).

- **Anthropogenic Inputs**: Lignocellulosic feedstocks are perennial species (Cherubini et al., 2009; Yuan et al., 2008). These crops have a higher nitrogen use efficiency than their annual counterparts, requiring less synthetic nitrogen fertilization and losing less nitrogen to external sources (Don et al., 2012). Feedstock production is significantly less dependent on non-renewable energy for production as minimal annual management is required for perennial species (Yuan et al., 2008).

2.4 *Arundo donax* L.

Arundo donax L. is a C3 perennial rhizomatous grass (Corno et al., 2014; Pompeiano et al., 2015) of the *Poaceae* (Graminae) family (Corno et al., 2014; Polunin and Huxley, 1966) and is the largest species of the *Arundo* genus (Bell, 1997; Perdue, 1958). *A. donax* thrives in Mediterranean conditions (Christou et al., 2001; Lambert et al., 2014; Mardikis et al., 2001) but originates from Asia (Corno et al., 2014; Polunin and Huxley, 1966). *A. donax* has spread locally through natural invasions, such as dispersal of vegetative propagules through flooding (Ahmad et al., 2008; Lambert et al., 2014; Perdue, 1958) and globally through anthropogenic introductions (Corno et al., 2014).

2.4.1 History

The initial introduction of *A. donax* to North America dates back to the early 1800s, when it was implemented as a form of erosion control in California, USA (Ahmad et al., 2008; Bell, 1997; Mariani et al., 2010). Additionally, *A. donax* has been used for many more purposes, including baskets and roofing material created from leaf weaving (Bell, 1997; Mariani et al., 2010). The aerial stems of *A. donax* have been used for over 5,000 years as reeds in musical instruments (Fiore et al., 2014; Perdue, 1958). Although the musical reed market has sustained greatly over time, *A. donax* has also been exploited for non-wood paper production in Greece (Ververis et al., 2004), as a natural reinforcement in polymer composites (as opposed to synthetic reinforcements) (Fiore et al., 2014; Porras et al., 2016) and as a biofuel crop in Italy (Cavallaro et al., 2011; Palmqvist and Lidén., 2014).

2.4.2 Reproduction

A. donax rarely produces viable seed (Bell, 1997; Bhanwra et al., 1982), therefore reproducing asexually through vegetative plant propagules, including rhizomes, nodes and fragmented stems (Corno et al., 2014; Cosentino et al., 2006; Dragoni et al., 2015; Tauler and Baraza, 2015). Under optimal growing conditions, *A. donax* can attain a high growth rate of 0.3 to 0.7 m per week (Bell, 1997; Perdue, 1958). Consequently, high biomass productivity has been noted in the Mediterranean climates of Italy (37.7 t DM ha^{-1} – 38 t DM ha^{-1}) (Angelini et al., 2009; Mantineo et al., 2009) and Virginia, USA (39 t DM ha^{-1}) (Smith et al., 2015a). Layering is a form of clonal reproduction that occurs when plant shoots make contact with the soil and produce adventitious roots (Grace,

1993). Layering is a less common reproductive strategy associated with *A. donax* (Boland, 2006; Dragoni et al., 2015) but has since been studied to have a greater rate of dispersal than the dispersal by both rhizomes and stem fragments (Boland, 2006; Dragoni et al., 2015).

2.4.3 Invasive Potential

Characteristics of *A. donax* as a promising biomass feedstock are similar to those of an invasive weed (as *A. donax* has been classified in some areas) (Cosentino et al., 2014; Dragoni et al., 2015; Quinn et al., 2015; Williams et al., 2009). *A. donax* is a stress tolerant species (Tauler and Baraza, 2015) that can grow in differing soil types (Alshaal et al., 2014; Corno et al., 2014) that vary in salinity (Nackley and Kim., 2015; Perdue, 1958), moisture content (Lewandowski et al., 2003; Perdue, 1958) and pH (Alshaal et al., 2014). *A. donax* is also drought tolerant (e Silva et al., 2015; Lewandowski et al., 2003), however, due to naturally high growth and occurrence in riparian areas, it is often considered an aquatic plant (Saikia et al., 2015).

The invasive potential of *A. donax* has been recognized in many areas, resulting in the species being classified as one of thirty-two land plants listed as the World's Worst Invasive Species (Boland, 2006). The *A. donax* characteristics mentioned above contribute to this invasive classification. *A. donax* can outcompete native flora (Lambert et al., 2014) and fauna (Herrera and Dudley, 2003) in alien environments thus supporting declining ecosystem diversity and habitat availability (Coffman et al., 2010; Kui et al., 2013; Lambert et al., 2010a). *A. donax* is highly flammable and resilient (Coffman et al., 2010) and its extensive vertical growth is characteristic of a ladder fuel (Brooks et al.,

2004). After a wildfire, *A. donax* has grown up to four times faster than native vegetation from re-growth of the rhizomatous material below-ground (Coffman et al., 2010; Lambert et al., 2010b). These characteristics combined augment the potential intensity of wildfires, especially in warmer climatic areas, and pose increased risks to ecosystem diversity and habitat availability (Brooks et al., 2004; Coffman et al., 2010).

Although identified in some areas as an invasive species, there is evidence that *A. donax* can be cultivated as a purpose-grown feedstock without posing harm. Firstly, genetic diversity is important in determining the invasive nature of a species, as adaptability is crucial to an invader (Khudamrongsawat et al., 2004) and previous studies have shown low genetic diversity throughout many *A. donax* populations (Ahmad et al., 2008; Balogh et al., 2012; Khudamrongsawat et al., 2004; Mariani et al., 2010).

A. donax does not commonly produce viable seeds or pollen (Balogh et al., 2012; Bell, 1997; Bhanwra et al., 1982; Mariani et al., 2010). In *A. donax*, ovule development is somewhat disrupted and there is no further maturation to the gametophyte stage (Balogh et al., 2012; Mariani et al., 2010). Pollen development is also disrupted by an autolytic process in the pollen grains, substantially reducing the number of mature pollen grains (Balogh et al., 2012; Mariani et al., 2010). In addition, the anther of *A. donax* showed dehiscence before the release of pollen grains, in contrast to other fertile *Arundo* species (Mariani et al., 2010). Based solely on vegetative reproduction, there is opportunity for dispersal in areas close to water courses or frequent flooding / erosion events, but there is also evidence to suggest that *A. donax* does not spread vegetatively outside of a cultivated plot (Balogh et al., 2012; Smith et al., 2015b). The reduced genetic

diversity coupled with lack of sexual reproduction markedly reduces the potential for invasion. Finally, the invasion potential of *A. donax* in northern climates is unknown.

2.4.4 Control

Difficulties in controlling unwanted dispersal of *A. donax* are largely due to its characteristic clonal reproduction (Boose and Holt, 1999; Douhovnikoff and Dodd, 2014; Kui et al., 2013). Numerous control methods have been studied over time, as permanent control of *A. donax* is hindered by the many factors that attribute to its tolerance (Bell, 1997).

2.4.4.1 Physical Control

Complete physical removal of *A. donax* stands can be very difficult based upon the extensive and essentially uncontrollable spread of vegetative material below-ground (Ahmad et al., 2008; Bell, 1997; Quinn, 2015). Persistent *A. donax* stem cutting as an alternative to stand removal reduced the size of re-emerging stems after repetitive destruction and removal of stem material (Racelis, 2012). Further, harvested biomass material can be desiccated and chipped to reduce sprouting potential (Boose and Holt, 1999). A combined control protocol of physical removal followed by chemical treatment (cut-stem treatment) has been investigated as an alternative to isolated physical control: a concentrated herbicide is applied directly to the freshly cut stem left in the ground (Bell, 1997). Physical control of *A. donax* is labour and economically intensive and is not sufficient for dispersal control (Bell, 1997; Boose and Holt, 1999; Racelis, 2012; Quinn, 2015).

2.4.4.2 Chemical Control

Numerous studies have investigated various aspects of chemical control in *A. donax*, including mode and timing of application and pre-treatment management. Herbicidal treatments applied to *A. donax* plants near the end of the growing season (pre-dormancy) can be very effective based on co-translocation of nutrition and herbicide below-ground for sustenance during the dormancy period (Arundale et al., 2014; Bell, 1997; Spencer et al., 2011).

Glyphosate is a broad spectrum herbicide that has been investigated thoroughly in conjunction with *A. donax* control (Bell, 1997; Lawson, 1996; Santín-Montanyá et al., 2013; Spencer et al., 2011) based on its extensive history for controlling unwanted plant populations (Bradshaw et al., 1997; Santín-Montanyá et al., 2013). Foliar application of glyphosate on *A. donax* was more effective in decimating plants and in reducing time and monetary costs associated with application than the cut-stem treatment (Lawson et al., 1996). Further, autumn foliar applications of glyphosate on *A. donax* showed significant reductions in new stem growth and living stems compared to other application timings (Spencer et al., 2011). Interestingly, recent research has advanced the former cut-stem treatment method by injecting glyphosate directly into the stem, which can be useful in areas where foliar herbicidal spray is not an option (such as in an area with endangered native species) (Spencer, 2014).

Other herbicidal treatments have been studied for their effects of controlling *A. donax* (Table 2-1), but none had as significant effects as glyphosate. Chemical control,

as a lone control of *A. donax* is more effective than a lone physical control, but combined efforts ensure more persistent and successful population regulation (Bell, 1997).

Table 2-1. Herbicide treatments tested as chemical control agents for *A. donax* by different authours.

Chemical	Application Method to *A. donax*	References
Asulam	Foliar spray	Odero and Gilbert, 2012
Azimsulfuron	Precision spray	Santín-Montanyá et al., 2013
Cyhalofop-butyl	Precision spray	Santín-Montanyá et al., 2013
Imazapyr	Cut-stem injection	Spencer, 2014
Penoxsulam	Precision spray	Santín-Montanyá et al., 2013
Triclopyr	Cut-stem injection	Spencer, 2014
Trifloxysulfuron	Foliar spray	Odero and Gilbert, 2012

2.4.4.3 Biological Control

Biological control is the regulation of a pest species by natural enemies (van den Bosch et al., 2013). Biological control is not intended to completely eliminate the pest species, but to induce enough environmental stress to naturally control its presence (DiTomaso, 2000; van den Bosch et al., 2013). Plant species that reproduce solely by asexual reproduction are less likely to develop resistance to biological control agents due to low genetic diversity (Khudamrongsawat et al., 2004). Many candidates have been evaluated for biological control of *A. donax* because of the lack of other effective control mechanisms and the growing presence of *A. donax* (Table 2-2).

Table 2-2. Biological control candidates investigated for the control of *A. donax* by different authours.

Scientific Name	Common Name	References
Tetramesa romana	*Arundo* wasp	Goolsby and Moran, 2009 Seawright et al., 2009 Cortés et al., 2011
Rhizaspidiotus donacis	*Arundo* scale	Goolsby et al., 2009 Seawright et al., 2009
Cryptonevra sp.	*Arundo* fly	Dudley et al., 2008 Seawright et al., 2009
Lasioptera donacis	*Arundo* leafminer	Goolsby et al., 2009 Seawright et al., 2009

Thus far, the most effective control candidate is the *Arundo* wasp, based upon host specificity to the *Arundo* genus (Goolsby and Moran, 2009; Pilu et al., 2012) and sufficient post-release evidence supporting this mode of control in *A. donax* (Goolsby et al., 2015). The *Arundo* wasp deposits eggs into *A. donax* shoots, and larval development within the shoot causes gall formation, stunting shoot growth (Goolsby et al., 2015; Goolsby and Moran, 2009).

2.4.5 Biofuel Feedstock

A. donax is a promising species for energy and biofuel production (Dragoni et al., 2015; Pilu et al., 2012; Saikia et al., 2015). Historically high growth rates (Bell, 1997; Perdue, 1958) and subsequently high biomass yields (Perdue, 1958; Rüggeberg et al.,

2010) produced with low anthropogenic inputs (Bell, 1997; Tauler and Baraza, 2015) substantiate the potential of *A. donax* as a biofuel feedstock. Although the growth characteristics of *A. donax* are similar to an invasive species, the sterility and vegetative propagation of *A. donax* reduce the invasive potential of this species in cultivated plots away from water courses, which is encouraging from the perspective of biofuel production (Cosentino et al., 2006; Quinn, 2015). Additionally, *A. donax* is one of the main feedstocks currently fueling the first commercial scale lignocellulosic ethanol plant (Crescentino Bio-Refinery, Crescentino, Vercelli, Italy) (Guo et al., 2015; Nogué and Karhumaa, 2015).

2.5 Plant-Growth Promoters

Agriculture has evolved tremendously since the beginning of time, and while most of the evolution is progressive, the majority of this evolution has required direct or indirect use of non-renewable fossil fuels (Bardi et al., 2013; Cuéllar and Webber, 2010). Cultivating crops for biofuels is meant to reduce societal dependence on fossil fuels, making production impractical while supplementing with fossil fuel derived inputs (Xue et al., 2014).

Plant growth-promoters can be loosely defined in the context of this research as rhizospheric microorganisms and substances that are beneficial to plants under certain conditions (Bashan and de-Bashan, 2005; Bhattacharyya and Jha, 2012; Vessey, 2003). Promoters can directly or indirectly promote plant growth through various relationships and mechanisms when in association with a host plant (Glick, 2012; Gray and Smith, 2005; Vessey, 2003). Rhizospheric bacterial promoters colonize the plant rhizosphere,

including soil surrounding plant roots and the surface of plant roots (Gray and Smith, 2005; Vessey, 2003). Endophytic bacterial promoters colonize the plant itself, including extracellular (within intercellular spaces of the plant) and intracellular (within plant cells) (Gray and Smith, 2005; Bhattacharyya and Jha, 2012; Vessey, 2003). Plant growth-promoting fungi, such as mycorrhizae and *Penicillium bilaii* are rhizospheric promoters that augment plant access to soil nutrients (Vessey, 2003) and also provide protection against pathogenic organisms and diseases (Ahemad and Kibret, 2014; Bhattacharyya and Jha, 2012).

2.5.1 Modes of Action

2.5.1.1 Biological Nitrogen Fixation

Biological nitrogen fixation (BNF) is a mechanism of growth-promotion that increases the amount of plant-available nitrogen supply to the plant (Ahmed and Kibret, 2014; Gowariker et al., 2009). Certain prokaryotic organisms reduce atmospheric nitrogen (N_2) by the nitrogenase enzyme complex to produce plant-available ammonia (NH_3) (Ahmed and Kibret, 2014; Gowariker et al., 2009).

There are three types of nitrogen fixing microorganisms: 1) symbiotic, 2) associative and 3) free-living (Ahmed and Kibret, 2014; Vitousek et al., 2002). Symbiotic bacteria form a direct relationship with host plants, such as rhizobia forming nodules on leguminous plant roots (Ahmed and Kibret, 2014; Vessey, 2003). Associative and free-living bacteria are non-symbiotic, forming indirect relationships with host plants (Ahmed and Kibret, 2014). Associative bacteria live on root surfaces or in intercellular

spaces in the root systems of host plants (Santi et al., 2013; Vitousek et al., 2002) whereas free-living bacteria live within the host plant rhizosphere (Glick, 1995).

2.5.1.2 Nutrient Availability

Plant nutrient balance is one of the most important factors for sustaining high yield through proper plant growth and development (Gowariker et al., 2009). This balance is acquired by the plant through uptake of nutrients from the soil (Gowariker et al., 2009). Nutrient deficient soils may have low concentrations of specific nutrients or have insoluble or plant unavailable stores (Richardson et al., 2009). Nutrient availability is the third greatest limiter of plant growth following water and temperature (Lambers et al., 2009).

Phosphorus is a primary plant macronutrient and is acquired by plants in the form of soluble inorganic anions hydrogen phosphate (HPO_4^{2-}) and dihydrogen phosphate ($H_2PO_4^-$) (Richardson et al., 2009; Shen et al., 2011). The fraction of soluble soil phosphorus is highly reactive with other soil compounds, and is readily immobilized, rendering it plant unavailable (Richardson and Simpson, 2011; Smil, 2000). Phosphate-solubilizing microorganisms (PSM) increase mobility of soil phosphorus by increasing solubilisation through organic acid production (Richardson et al., 2009; Shen et al., 2011).

Iron is one of many plant micronutrients and is important in plant respiratory reactions (Gowariker et al., 2009; Vigani et al., 2016). The most common naturally occurring form of iron in the soil is the insoluble highly plant unavailable ferric ion, iron(III) (Fe^{3+}) (Brumbarova et al., 2015; Hayat et al., 2010; Vigani et al., 2013).

Widespread insolubility of iron in soil has enabled plants to respond through various mechanisms of uptake (Brumbarova et al., 2015; Vigani et al., 2013).

Vascular plants (Strategy I) acquire iron through a "reduction-based strategy" (Brumbarova et al., 2015): iron is solubilized, iron(III) is reduced to iron(II) and iron(II) is subsequently taken up by the plant (Brumbarova et al., 2015; Vigani et al., 2013). Non-vascular plants (Strategy II) acquire iron through a "chelation-based strategy" (Brumbarova et al., 2015): plants produce iron-chelating compounds (phytosiderophores) that solubilize iron(III) and the iron(III)-phytosiderophore complex is consumed by the plant (Brumbarova et al., 2015; Vigani et al., 2013). Strategy III was termed by Bienfait (1989) as the uptake of microbial siderophores as a source of iron (Yang and Römheld, 1999). Iron(III) is bound to the siderophore, and the complex is reduced to plant-available iron(II) (Aznar et al., 2014). Siderophores increase concentration of soluble iron in the soil, although this soluble store may not be more available to the plant (Aznar et al., 2014).

Modification of plant roots is an alternative mode of action of growth-promoters to supplement plant nutrition. Mycorrhiza, a symbiotic relationship between fungus and plant roots (Nadeem et al., 2014) most commonly increases root surface area and the number of roots to enhance plant uptake of nutrients (Nadeem et al., 2014; Treseder, 2013), especially phosphorus (Nadeem et al., 2014; Smith and Smith, 2012; Vessey, 2003) and micronutrients with lower mobility, including copper (Cu) and zinc (Zn) (Cornejo et al., 2013; Lehmann and Rillig, 2015; Nadeem et al., 2014).

2.5.1.3 Phytohormones

Phytohormones are directly involved with plant growth (Gowariker et al., 2009; Ma et al., 2011) and are often referred to as plant growth-regulators based on their impact on promotion or suppression of growth (Saharan and Nehra, 2011). Phytohormones are organic compounds that act as signal molecules in low concentrations to stimulate and regulate plant response (Lugtenberg et al., 2013; Saharan and Nehra, 2011). Common phytohormone classification suggests five major groups: auxins, cytokinins (CKs), gibberellins (GAs), ethylene (ET) and abscisic acid (ABA) (Lugtenberg et al., 2013; Saharan and Nehra, 2011) but there are other phytohormone-like compounds that fall outside these major groups. Soil microorganisms can produce and synthesize phytohormones and agricultural producers can take advantage of these mechanisms as natural soil amendments to enhance crop growth (Ma et al., 2011; Farrar et al., 2014).

2.5.1.3.1 Auxins

The Greek derivation of the term auxin ("auxein") and its Greek meaning is "to grow" (Enders and Strader, 2015). Auxins were discovered in 1928 by Frits Warmolt Went, a Dutch biologist, through the use of the *Avena* test (Enders and Strader, 2015; Schneider and Went, 1938). Auxins serve roles in various functions of plant growth, but most significantly through cell elongation and root development and growth (Cassán et al., 2014; Gowariker et al., 2009; Van Overbeek, 1959).

Indole-3-acetic acid (IAA) and 4-indole-3-butryic acid (IBA) are naturally occurring auxins that stimulate root development and growth (Gowariker et al., 2009;

Van Overbeek, 1959) but also act as signal molecules throughout the plant to develop under variable environmental conditions (Sauer et al., 2013).

IAA is present in an extract of seaweed *Ascophyllym* (Craigie, 2011; Sharma et al., 2012) and many growth-promoter species produce IAA that augments root growth to further promote plant growth (Lugtenberg et al., 2013; Van Overbeek, 1959). Plant growth is stimulated through low concentrations of exogenous IAA supplemented by beneficial microorganisms and in turn, the plant secretes metabolites that stimulate microorganism development (Ma et al., 2011; Patten and Glick, 1996). Higher concentrations of exogenous IAA can inhibit growth, hence the investigation into synthetic application of IAA as an auxinic herbicide (Christoffoleti et al., 2015; Park et al., 2015; Patten and Glick, 1996; Xie et al., 1996).

2.5.1.3.2 Cytokinins (CKs)

The first cytokinin, kinetin, was discovered in 1955 by American scientist Carlos O. Miller and Swedish-American plant physiologist Folke K. Skoog in 1955 (Cassán et al., 2014; Kamínek, 2015; Miller et al., 1955). Since this discovery, many other compounds have been identified as cytokinins and can be divided based on structure: 1) natural adenine-type cytokinins (kinetin) and 2) synthetic phenylurea-type cytokinins (Thidiazuron, TDZ) (Bajguz and Piotrowska-Niczyporuk, 2014; Cassán et al., 2014). Cytokinins control cell division and other important processes involved with plant growth and development (Cassán et al., 2014; Gowariker et al., 2009; Kamínek, 2015).

Cytokinins and cytokinin-like compounds are produced by the majority of rhizospheric bacteria and provide an exogenous source to plants (Cassán et al., 2014;

Lugtenberg et al., 2013; Timmusk et al., 1999). In addition to the well-known function of cytokinins, the endogenous supply and microbial production of cytokinins can also initiate and regulate plant response to environmental stress (Ha et al., 2012; Liu et al., 2013).

2.5.1.3.3 Gibberellins (GAs)

Gibberellins were first identified in 1926 by Japanese plant pathologist Eiichi Kurosawa, upon investigating secretions from the pathogenic fungus *Gibberella fujikuroi* (Gowariker et al., 2009; Takahashi, 1997; Tamura, 2012). The acid was subsequently isolated and termed "gibberellin" in 1935 by Japanese agricultural chemist Teikiro Yabuta (Takahashi, 1997; Tamura, 2012). GAs serve many functions to plants, including stem elongation and regulation of many developmental processes such as seed germination and flowering (Cassán et al., 2014; Lugtenberg et al., 2013; Tanimoto, 2012).

There are more than 130 identified gibberellin compounds produced by plants, bacteria and fungi, making GAs the largest phytohormone class (Cassán et al., 2014; Lugtenberg et al., 2013; Tsavkelova et al., 2006a). Many bacterial and fungal species can produce and synthesize exogenous GAs and GA-like compounds, and can stimulate plant growth through various mechanisms including increasing root growth (Cassán et al., 2014; Lugetnberg et al., 2013; Tanimoto, 2012).

2.5.1.3.4 Ethylene

Gaseous ethylene (C_2H_4), through extensive research was identified as a phytohormone in 1934 by R. Gane (Gane, 1934). Ethylene is produced by the plant

through a mechanism associated with the methionine (Yang) cycle from s-adenosylmethionine (SAM) (Buddendorf-Joosten and Woltering, 2012; Saltveit et al., 1997; Yang and Hoffman, 1984) and is involved in many processes of plant growth and development (Glick et al., 2007; Glick, 2014; Lugtenberg et al., 2013). Although ethylene is important in processes of plant growth and development, stress responses induced by high ethylene concentrations include root inhibition and subsequent plant senescence (Lugtenberg et al., 2013; Martínez-Viveros et al., 2010).

1-aminocyclopropane-1-carboxylate (ACC) is the precursor of ethylene (Buddendorf-Joosten and Woltering, 2012; Lugtenberg et al., 2013) and is converted into ammonia (NH_3) and α-ketobutyrate via the ACC deaminase enzyme (Bal et al., 2013; Lugtenberg et al., 2013; Pande et al., 2016). Several growth-promoting organisms contain the ACC deaminase enzyme, and through synergistic interactions with plant or bacteria produced IAA, promote plant growth through the suppression of ethylene production (Glick et al., 2014; Glick et al., 2007; Pande et al., 2016).

2.5.1.4 Phytohormone-like Compounds

The aforementioned phytohormones were described in detail based on evidence of production by microorganisms to stimulate plant growth. There are other important phytohormone-like compounds associated with plant growth regulation that are also produced by microorganisms.

2.5.1.4.1 Brassinosteroids (BRs)

Brassins were first identified in the pollen of *Brassica napus* L. in the early 1970s by John W. Mitchell and his associates at the United States Department of Agriculture

(Clouse, 2015; Mitchell et al., 1970). The first isolated brassinosteroid was named brassinolide in 1979 (Clouse, 2015; Grove et al., 1979; Tsavkelova et al., 2006b) and over 60 similarly-structured compounds have been identified since (Tsavkelova et al., 2006b). Brassinosteroids serve many functions in plant growth, most notably tissue elongation (Mitchell et al., 1970; Grove et al., 1979) and responding to environmental stresses (Lozaon-Durán and Zipfel, 2015; Tsavkelova et al., 2006b). No known bacteria or fungi produce brassinosteroids (Lugtenberg et al., 2013).

2.5.1.4.2 Jasmonates

The effect of jasmonic acid (JA), methyl jasmonate (MeJA) and their derivatives on plant growth was discovered in the 1980s, as higher concentrations of these substances induced growth inhibition and senescence, similar to the effects of ethylene (Srivastava, 2002; Staswick, 1997). In addition to growth inhibition, jasmonates also induce plant defense mechanisms against pathogens (Pozo et al., 2005) and further develop beneficial plant-microbial interactions (Tsavkelova et al., 2006b; Wasternack, 2014).

2.5.1.5 Symbioses

Mutualistic symbiosis is a mechanism of growth-promotion in which two dissimilar but closely associated organisms interact and both organisms benefit from the interaction (Gowariker et al., 2009). Two major classifications of growth-promotion symbioses are 1) rhizobia-legume symbioses and 2) fungal-plant symbioses (Vessey et al., 2003).

2.5.2 PGP in Biofuel Crops

Growth-promoters have been researched since the 1960s, when bacteria were first studied as fertilizer (Mishustin and Naumova, 1962). The earliest exogenous application of growth-promoters was applied to food crops (radish (Kloepper and Schroth, 1978) and potato (Burr et al., 1978)) to evaluate growth post-inoculation.

More recently, exogenous applications of growth-promoters have been studied in non-food crops for biofuel production. A significant increase in plant biomass was exhibited by poplar trees inoculated with *Enterobacter* sp. strain 638 (an endophytic bacterium) compared with non-inoculated control trees (Taghavi et al., 2009). The inoculation of switchgrass (*Panicum virgatum*) with native rhizospheric bacterial strains (Ker et al., 2012) and more specifically, *Burkholderia phytofirmans* strain PsJN (Kim et al., 2012) have also demonstrated growth-promotion compared to non-inoculated plants.

The following growth-promoters will be investigated for potential growth-promotion effects on NileFiber™ in this research.

- ***Penicillium bilaii*** is a phosphate-solubilizing fungus originally isolated from soils in southern Alberta, Canada (Kucey, 1983). *P. bilaii* mobilizes unavailable (insoluble) soil phosphorus and enhances phosphorus cycling to the microbial pool (Shen et al., 2011; Wakelin et al., 2007).
- ***Gluconacetobacter diazotrophicus*** (previously known as *Acetobacter diazotrophicus*) is a nitrogen-fixing endophytic acetic acid bacterium (Muthukumarasamy et al., 2002; Fuentes-Ramírez et al., 2001) originally distributed in areas of large sugarcane production (Cavalcante and Döbereiner,

1988; Thangaraju and Jayakumar, 2002). In addition to nitrogen fixation, *G. diazotrophicus* also stimulates plant growth through the production of IAA (Saravanan et al., 2008), increasing photosynthesis (Rangel de Souza et al., 2016), phytohormone production (de Paula Soares et al., 2015; Urzúa et al., 2013) and increasing availability of soil nutrients (de Oliveira et al., 2016; Urzúa et al., 2013).

- ***Gluconacetobacter azotocaptans*** is a nitrogen-fixing acetic acid bacterium (Fuentes-Ramírez et al., 2001; Saravanan et al., 2008) and was isolated from the rhizospheres of coffee and corn (Saravanan et al., 2008). This bacterium functions very similarly to *G. diazotrophicus*, stimulating plant growth through the production of IAA, increasing availability of soil nutrients and providing anti-fungal defensive mechanisms to the plant (Saravanan et al., 2008).
- ***Azospirillum brasilense*** is a free-living bacterium most widely studied for its growth-promotion effects in cereal crops (Bashan et al., 2004). *A. brasilense* stimulates plant growth through various mechanisms, such as nitrogen fixation (Fibach-Paldi et al., 2012; Lugtenberg et al., 2013), phytohormone production (Fibach-Paldi et al., 2012; Lugtenberg et al., 2013; Rivera et al., 2014) and enhancing root structure for better nutrient and water uptake (Díaz-Zorita and Fernández-Canigia, 2009; Lugtenberg et al., 2013).
- ***Variovorax paradoxus*** is an endophytic rhizobacterium (Chen et al., 2013; Han et al., 2011; Satola et al., 2013) classified as a plant growth-promoter based on its abilities to enhance plant stress tolerance through its diverse metabolism (Han et

al., 2011; Satola et al., 2013) and through the production of ACC deaminase (Chen et al., 2013; Satola et al., 2013).

- ***Ascophyllum nodosum*** is a brown macro algae (seaweed) native to shores of the northern Atlantic Ocean (Wally et al., 2013) that have been used as soil fertilizers (Fan et al., 2011; Rayirath et al., 2009). Seaweed extracts are more commonly used to stimulate agricultural crop growth (Ali et al., 2015; Rayirath et al., 2009; Wally et al., 2013). Many modes of action of plant growth-promotion have been associated with *A. nodosum* applications, but the most common mode is through enhanced supplies of nutrients and phytohormones (Ali et al., 2015; Craigie et al., 2011).
- **Lipo-chitooligosaccharide** (LCO) is a signal molecule (nodulation (Nod) factor) produced by the microsymbiont of legume plants, generically known as rhizobium (Muñoz et al., 2014; Schwinghamer et al., 2016). The primary function of LCO is to induce root nodule growth on leguminous plants for nitrogen fixation (Muñoz et al., 2014; Prithiviraj et al., 2003; Schwinghamer et al., 2016). Other growth-promotion effects have been noted, including biomass accumulation (Prithiviraj et al., 2013; Souleimanov et al., 2002) and increased cell division (Souleimanov et al., 2002).

2.6 Conclusion

The further development of a second generation biofuel industry in Nova Scotia is dependent on sustainable biomass feedstock and biofuel conversion technology. Current development of pulp and paper infrastructure into biofuel conversion technology is a

critical developmental stage in the local industry. Evaluating the growth of NileFiber™ in Nova Scotia is important to diversify provincial biomass resources without jeopardizing food crop resources or over-exploiting abundant natural forests. Evaluating NileFiber™ growth after growth-promoter inoculation is important to reduce environmental impacts of modern agriculture whilst increasing biomass yield and ultimately increasing biofuel production.

3.0 METHODOLOGY

This research consisted of three experiments: a) two greenhouse experiments: Experiment 1 – Greenhouse Soil Drench (2015) and Experiment 3 – Greenhouse Root Soaking (2016) to investigate the effects of growth-promoters on NileFiber™ and b) one field experiment (Experiment 2 – Field Soil Drench (2015)) to evaluate the growth and survival of NileFiber™ in Nova Scotian climatic and edaphic conditions. Greenhouse experiments allow for controlled conditions to disentangle different factors contributing to growth, while field experiments mimic natural conditions to provide estimates of growth in conjunction with uncontrolled environmental influences.

The first greenhouse experiment, completed during the 2015 summer season evaluated plant growth after delayed growth-promoter inoculation through soil drench. The field experiment, concurrently completed during the 2015 summer season investigated the effect of growth-promoters as in the first greenhouse experiment, but under natural Nova Scotian conditions. The second greenhouse experiment, completed during the winter of 2016, evaluates plant growth after immediate growth-promoter inoculation through root soaking.

In this section, the laboratory protocols for a) *in vitro* plant tissue culture propagation and b) growth-promoter treatment preparation are included, as well as protocols for all three experiments.

3.1 Greenhouse Description

The greenhouse was located on the north section of the Green Roof Testing Facility at Saint Mary's University in Halifax, NS, Canada (latitude 44°39'N, 63°35'W)

(Google Earth, 2016a; MacIvor and Lundholm, 2011). Greenhouse conditions were monitored using a HOBO® Relative Humidity/Temperature/Light/External Data Logger (Onset® HOBO® Data Loggers). Supplemental lighting was used to maintain a photoperiod of 16/8 hours (day/night). The minimum photosynthetic photon flux density (PPFD) at plant height was 300 μmol $m^{-2}s^{-1}$ provided by 600 W SON-T Green Power Lamps (Philips). Heating and ventilation systems in the greenhouse were used to attempt to maintain air temperature of 25/18 °C (day/night).

3.2 Field Site Description

The research site, composed of numerous experimental fields, was located 20 metres above mean sea level at the Nappan Research Farm of Agriculture & Agri-Food Canada (AAFC) in Nappan, NS, Canada (latitude 45°46'N, 64°14'W) (Google Earth, 2016b; Webb and Langille, 1995). The site is located in a cool, humid, temperate climate (Webb and Langille, 1995) with an average annual daily temperature of 6.0 °C and an annual precipitation of 1154.8 mm (averages calculated from 1981 – 2010, Environment Canada, 2016). Meteorological conditions were monitored through pre-existing Environment Canada weather monitoring equipment at the AAFC research facility.

Field-based research in this study is based on two experimental plantings of NileFiber™ in different fields within the AAFC facility. The initial planting of NileFiber™ in 2014 was on Field C3-A following a 2013 forage crop. In 2015, the second planting of NileFiber™ was planted on C1 following a 2014 barley crop (Figures 3-1 & 3-2).

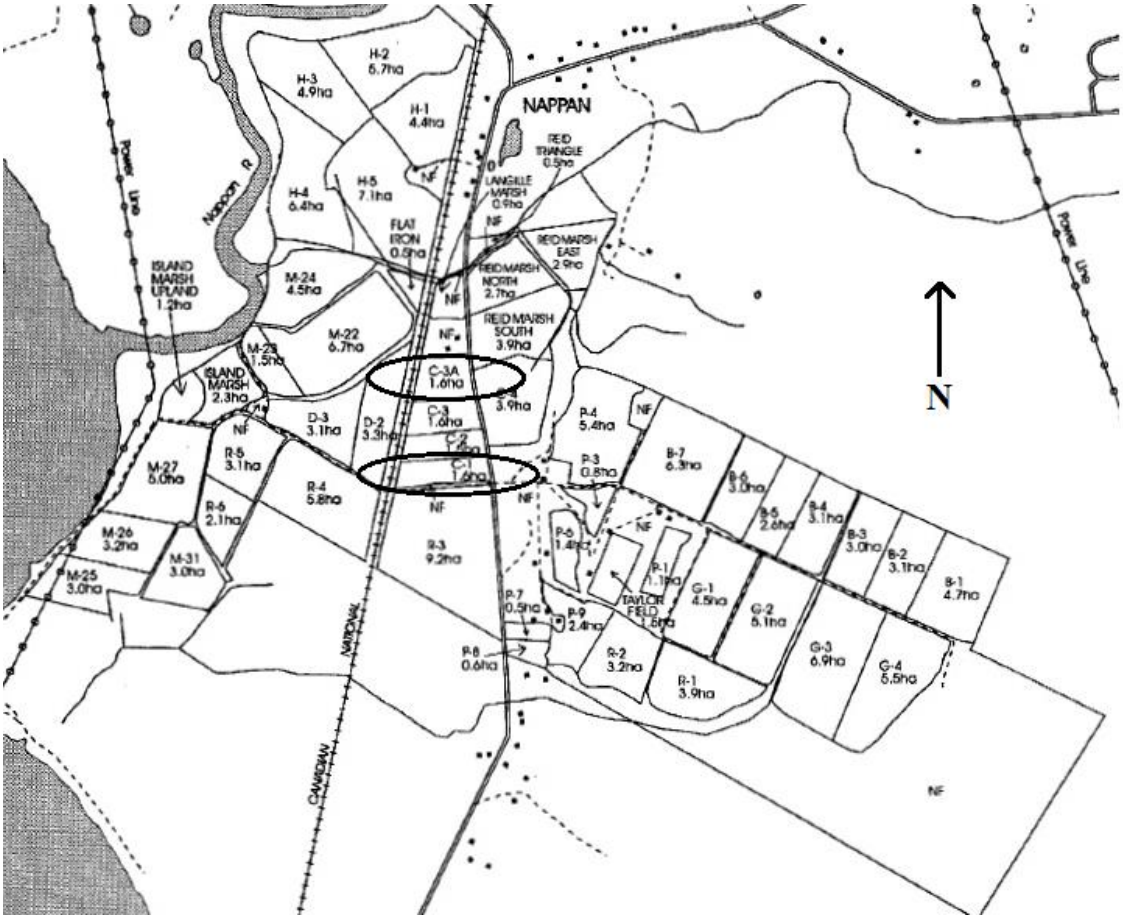

Figure 3-1. Field identification map for the Nappan Research Farm (AAFC), Nappan, NS, Canada; fields C3-A (2014 NileFiber™ planting) and C1 (2015 NileFiber™ planting) encircled (Adapted from Webb and Langille, 1995).

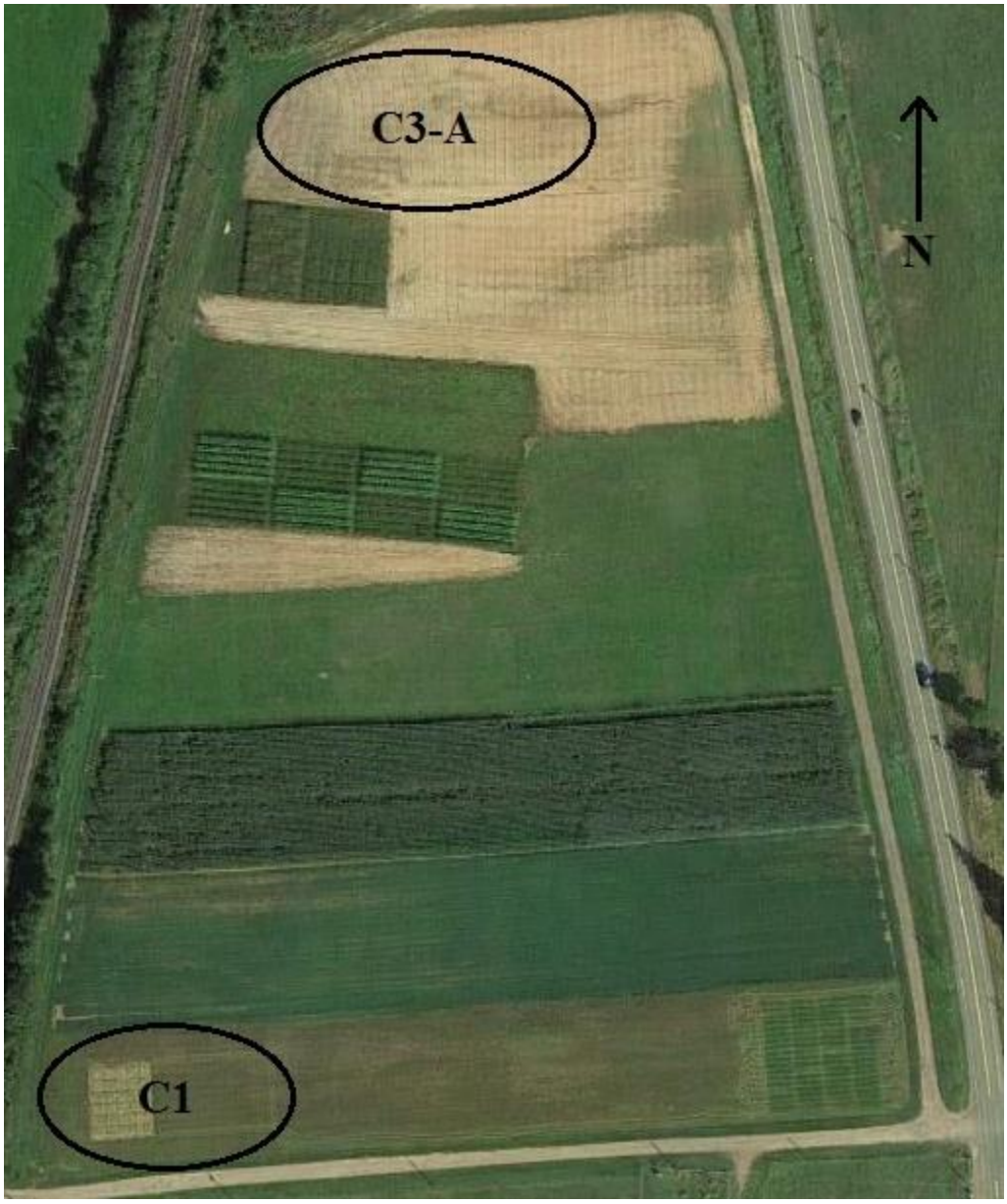

Figure 3-2. Field identification map for the Nappan Research Farm (AAFC), Nappan, NS, Canada; fields C3-A (2014 NileFiber™ planting) and C1 (2015 NileFiber™ planting) encircled (Adapted from Google Earth, 2016b).

3.3 Plant Material

Rhizomatous materials of NileFiber™ were received from Nile Fiber Atlantic Canada Incorporated (Halifax, NS, Canada) and planted on 23 July 2014 on Field C3 (Figure 3-3).

Figure 3-3. NileFiber™ rhizomatous material received in July 2014. Material was cut into smaller pieces (each piece containing at least one bud) prior to planting (Peters, 2014).

Due to low establishment and NileFiber™ emergence during the 2014 growing season, dormant buds were excised from NileFiber™ nodes beneath the soil (Figure 3-4) and used for *in vitro* plant tissue culture propagation.

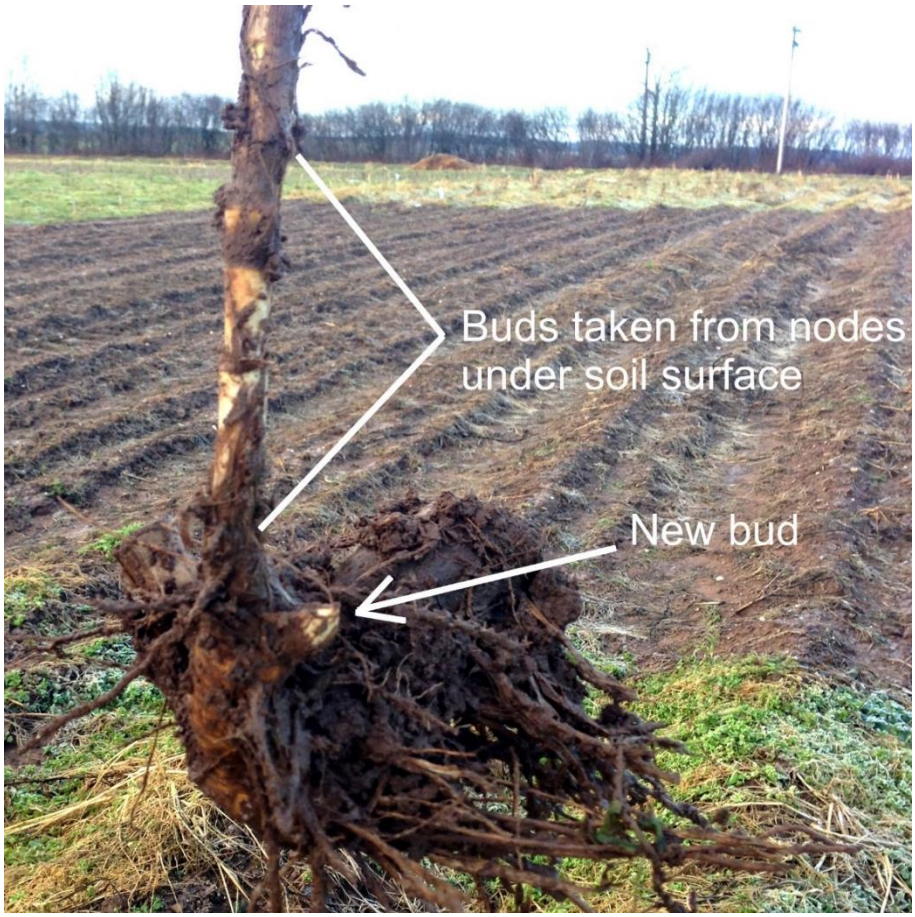

Figure 3-4**.** Dormant buds identified for excision from NileFiber™ node; 15 December 2014 (Fei, 2014).

3.4 Laboratory Protocol I – *In vitro* Plant Tissue Culture Propagation

In vitro plant tissue culture propagation was performed following protocols modified from Cavallaro et al., (2011) and Cavallaro et al., (2014).

3.4.1 Bud Sterilization

Excised NileFiber™ buds were rinsed with cold tap water, soaked in 70 % (v/v) ethanol for one minute, sterilized with 20 % Javex® (1 % available chlorine) for twenty minutes and further rinsed with autoclaved distilled water three times. Sterilized buds were dried using autoclaved filter paper.

3.4.2 Plant Tissue Culture Medium

The basal propagation medium was composed of macro- and micro-nutrients of Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), Morel and Wetmore's vitamins (Morel and Wetmore, 1951), sucrose and Gelrite® (Cavallaro et al., 2014). Stock solutions of all medium constituents were prepared in the laboratory (Appendix A). The propagation procedure consists of three different phases based on the stage of tissue culture growth: a) shoot growth, b) rooting (plantlet generation) and c) shoot proliferation. Varying constituents, including plant hormones, were added to the basal medium depending on the tissue culture growth stage (Appendix A). The pH of all media was adjusted to 5.8 with the addition of 1 N sodium hydroxide (NaOH) (Murashige and Skoog, 1962).

Magenta™ culture boxes (GA-7 Vessels) were filled with 50 mL of media and autoclaved at 121.1 °C (Getinge 533LS-E Steam Sterilizer) for fifteen minutes. Upon removal from the sterilizer, the culture boxes were placed under a fume hood

(Labconco® Purifier Horizontal Clean Bench, Catalog. No. 36100-00) to solidify the media.

3.4.3 Shoot Growth and Medium

Sterilized buds (3.4.1 Bud Sterilization) were placed on solidified shoot growth medium (nine buds per culture box), then securely sealed using Parafilm M® and placed in the plant growth chamber (BioChambers SPC-7-2H). New shoots differentiated adventitiously from the buds (Figure 3-5), and secondary shoots were divided into single shoots and re-cultured on the same type of medium until a sufficient plant population was attained. Shoot growth medium-specific constituents are outlined in Table 3-1.

Figure 3-5. A mass of shoots adventitiously developed from a single bud. These shoots were separated into individual shoots and re-cultured until a sufficient plant population was attained (Peters, 2015a).

Table 3-1. Shoot growth medium-specific constituents and respective concentrations added to basal propagation medium (Adapted from Cavallaro et al., 2011).

Constituent	**Abbreviation**	**Concentration ($mg\ L^{-1}$)**
Benzylaminopurine	BA	1
Indole-3-butyric acid	IBA	1
Thidiazuron	TDZ	0.1[1]
Gibberellic Acid	GA_3	0.05

[1] %

3.4.4 Plantlet Generation (Rooting) and Medium

Single shoots (minimum height 2 cm) were removed from shoot growth medium and rinsed with a solution of autoclaved distilled water and 0.01 % plant preservative mixture (PPM) multiple times to remove residual medium. The shoots were transferred onto solidified rooting medium (sixteen shoots per culture box), securely sealed with Parafilm M® and placed in the growth chamber to generate complete plantlets (Figure 3-6). The basal medium was modified by reducing the volume of MS macro- and micro-nutrients by 50 %. In addition, napthaleneacetic acid (NAA), an exogenous auxin (Aloni, 2004) responsible for root differentiation (Imin et al., 2005) was added at 2 mg L^{-1}.

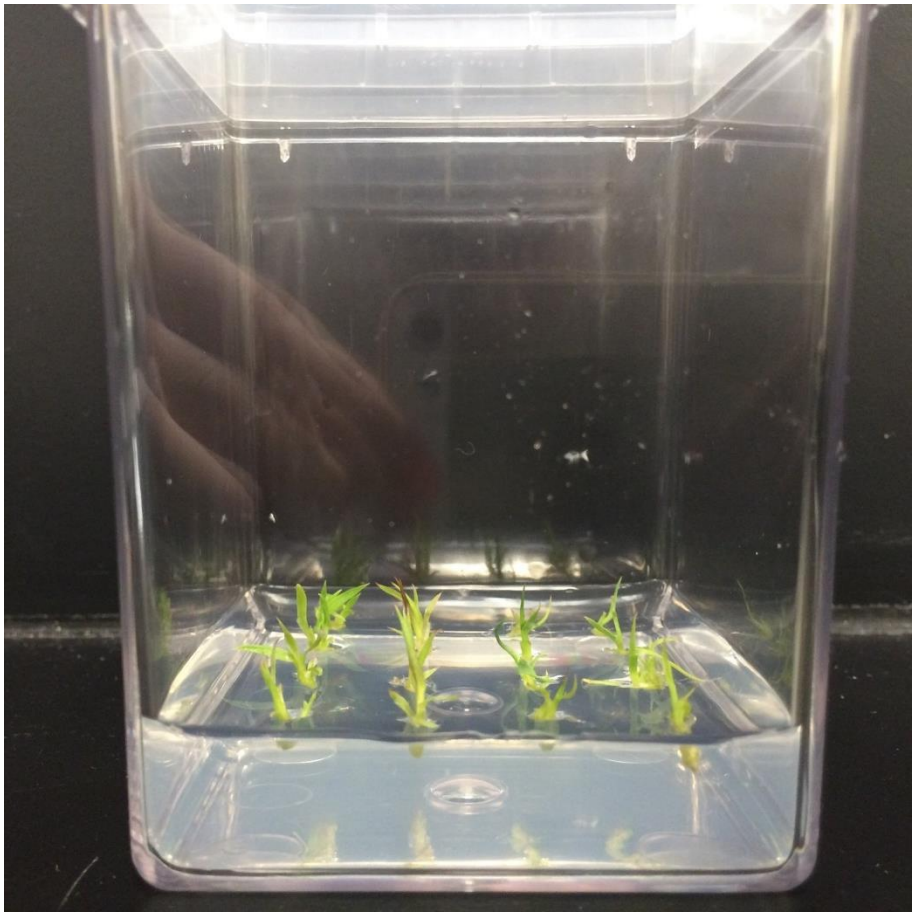

Figure 3-6**.** Individual shoots transferred onto solidified rooting medium (sixteen shoots per culture box) to generate plantlets (Peters, 2015a).

3.4.5 Growth Conditions

In vitro plant tissue culture propagation was performed in the aforementioned BioChambers SPC-7-2H plant growth chamber. Growing conditions for shoot growth and plantlet generation are listed in Table 3-2.

Table 3-2. Growing conditions in BioChambers SPC-7-2H plant growth chamber for shoot growth and plantlet generation stages of *in vitro* plant tissue culture propagation of NileFiber™.

	Temperature (°C)	**Photoperiod (hours)**	**Light Intensity (µmol m^{-2} s^{-1})**
Day	25	16	200
Night	18	8	0

3.4.6 Shoot Proliferation: Medium and Growing Conditions

Upon attaining a sufficient plant population, the remaining shoots were stored in the plant growth chamber for future use. These shoots were transferred onto solidified shoot proliferation medium (nine shoots per culture box), securely sealed with Parafilm M® and placed in the plant growth chamber. Shoot proliferation medium-specific constituents are outlined in Table 3-3. Storage conditions in the plant growth chamber were modified to stall shoot growth, outlined in Table 3-4.

Table 3-3. Shoot proliferation medium-specific constituents and respective concentrations added to basal propagation medium (Adapted from Cavallaro et al., 2011).

Constituent	**Abbreviation**	**Concentration (mg L^{-1})**
Benzylaminopurine	BA	3
Indole-3-butyric acid	IBA	1
Thidiazuron	TDZ	0.1[1]
Gibberellic Acid	GA_3	0.05

[1] %

Table 3-4. Growing conditions in BioChambers SPC-7-2H plant growth chamber for shoot proliferation stage of *in vitro* plant tissue culture propagation of NileFiber™.

	Temperature (°C)	**Photoperiod (hours)**	**Light Intensity (µmol $m^{-2}s^{-1}$)**
Day	12	16	200
Night	10	8	0

3.5 Laboratory Protocol II – Growth-Promoter Treatment Preparation

Various growth-promoter treatments were used throughout this research, including in-lab cultured bacteria and commercially available products (Table 3-5).

Table 3-5. Commercial name (if applicable), type and source of growth-promoters prepared for application to NileFiber™.

Scientific Name	Commercial Name	Type	Source
Ascophyllum nodosum	100% Liquid Seaweed Concentrate	Stimulant	Acadian Seaplants Limited
Azospirillum brasilense N8	-	Bacteria	George Lazarovits' lab, AAFC, London
Gluconacetobacter azotocaptans DS1	-	Bacteria	George Lazarovits' lab, AAFC, London
Gluconacetobacter diazatrophicus PAL5T Lsd B++	-	Bacteria	Lazaro Hemandez, CGEB1, Cuba
Gluconacetobacter diazotrophicus PAL5T	-	Bacteria	Caballero-Mellado's lab, EMBRAPA, Brazil
Gluconacetobacter diazotrophicus SRT4	-	Bacteria	Lazaro Hemandez, CGEB1, Cuba
Lipo-chitooligosaccharide (LCO)	Torque® ST	Stimulant	Novozymes®
Penicillium bilaii	JumpStart®	Fungus	Novozymes®
Variovorax paradoxus JM63	-	Bacteria	Zhongmin Dong's lab, SMU

3.5.1 *Penicillium bilaii*

The recommended application rate of *P. bilaii* in this form (as per commercial label) was followed for treatment applications in these experiments. The active ingredient in the mixture (7.2×10^8 CFU g^{-1}) outlined on the commercial label was used to determine the proper concentration for application.

3.5.2 LGI-P Medium

All but two (*A. brasilense* N8 and *V. paradoxus* JM63) in-lab cultured bacterial strains were cultured using modified liquid LGI-P medium (Cavalcante and Dobereiner, 1988; Pan and Vessey, 2001).

The modified LGI-P medium consisted of (quantities per litre): 0.2 g K_2HPO_4; 0.6 g KH_2PO_4; 0.2 g $MgSO_4 \bullet 7H_2O$; 0.02 g $CaCl_2 \bullet 2H_2O$; 0.002 g $NaMoO_4 \bullet 2H_2O$; 0.01 g $FeCl_3 \bullet 6H_2O$; 5 mL 0.5% bromothymol blue solution in 0.2 M KOH; 0.0001 g Biotin; 0.0002 g Pyridoxal HCl; 100 g sucrose; 1.32 g $(NH_4)_2SO_4$ (Appendix B).

Each bacterial suspension was removed from -80 °C (Thermo Scientific™ Forma™ -86 °C Upright Ultra-Low Temperature Freezer) and thawed. Sterilized Erlenmeyer flasks were filled with modified LGI-P broth and autoclaved at 121.1 °C (Getinge 533LS-E Steam Sterilizer) for fifteen minutes. Upon cooling, 0.5 mL of the suspension was added to the autoclaved broth and placed in an orbital shaker (New Brunswick C24KC Refrigerated Incubator Shaker) at 125 rpm and 30 °C for four days.

3.5.3 LGI-P Cultured Bacteria Quantification

Total bacterial population count was measured directly (plate count method) and indirectly (turbidity measurement method) after four days of incubation. The optical density (OD) value was 0.574, λ 600 nm (Thermo Scientific™ GENESYS 20 Visible Spectrophotometer). Serial dilutions were performed to enumerate the colony forming units (CFU) in solution. Dilutions were plated on LGI-P agar plates (0.1 mL $plate^{-1}$) and counted seven and fourteen days after incubation at 28.1 °C (Thermo Scientific™ Forma Direct Heat CO_2 Incubator). Quantification was completed to ensure an approximate application rate of 10^8 CFU per plantlet.

3.5.4 LGI-P Cultured Bacteria Preparation

For the first greenhouse experiment and the field experiment (2015), bacterial cultures were centrifuged (IEC 21000R Refrigerated Centrifuge) for ten minutes at room temperature and 4500 rpm – broth was discarded and replaced with distilled water and mixed thoroughly with bacterial culture. To create the individual and combination treatments in the first greenhouse experiment, the bacteria-distilled water solution was split in half. For the second greenhouse experiment (2016), 40 mL of the bacterial culture broth was combined with 40 mL of phosphate buffer (34mM, pH = 6.0).

3.5.5 LB Medium

Two bacterial strains (*A. brasilense* N8 and *V. paradoxus* JM63) cultured in-lab were cultured using lysogeny broth (LB) liquid medium (Bertani, 1951).

The LB medium consisted of (quantities per litre): 10 g tryptone; 5 g yeast extract; 10 g NaCl (Appendix B).

Both bacterial suspensions were removed from -80 °C (Thermo Scientific™ Forma™ -86 °C Upright Ultra-Low Temperature Freezer) and thawed. Sterilized Erlenmeyer flasks were filled with LB broth and autoclaved at 121.1 °C (Getinge 533LS-E Steam Sterilizer) for fifteen minutes. Upon cooling, 1 mL of the suspension was added to the autoclaved broth and placed in an orbital shaker (New Brunswick C24KC Refrigerated Incubator Shaker) at 125 rpm and 30 °C for three days.

3.5.6 LB Cultured Bacteria Quantification

Total bacterial population count of *A. brasilense* N8 and *V. paradoxus* JM63 was measured directly (plate count method) and indirectly (turbidity measurement method) after three days of incubation. The OD value was 1.252, λ 600 nm (Thermo Scientific™ GENESYS 20 Visible Spectrophotometer). Serial dilutions were performed to enumerate the colony forming units in the solution. Dilutions were plated on LB agar plates (0.1 mL $plate^{-1}$) and counted seven and fourteen days after incubation at 28.1 °C (Thermo Scientific™ Forma Direct Heat CO_2 Incubator). Quantification was completed to ensure an approximate application rate of 10^8 CFU per plantlet.

3.5.7 LB Cultured Bacteria Preparation

For the first greenhouse experiment and the field experiment (2015), the *A. brasilense* N8 bacterial culture was centrifuged (IEC 21000R Refrigerated Centrifuge) for ten minutes at room temperature and 4500 rpm (*V. paradoxus* not used in these trials). The broth was discarded and replaced with distilled water and mixed thoroughly with the bacterial culture. To create the individual and combination treatments in the first greenhouse experiment, the bacteria-distilled water solution was split in half. For the

second greenhouse experiment (2016), 40 mL of both *A. brasilense* and *V. paradoxus* bacterial culture broth was combined with 40 mL of phosphate buffer (34mM, pH = 6.0).

3.5.8 *Ascophyllum nodosum* (Seaweed Extract)

The recommended application rate of *A. nodosum* in this commercial formulation (100 % Liquid Seaweed Concentrate, Acadian Seaplants Ltd.) was followed for treatment applications in the first greenhouse experimemt and the field experiment (2015). The recommended application rate followed was 1 mL of seaweed extract per plant (J. Norrie [Acadian Seaplants Ltd.], personal communication). Upon further evaluation, a lower application rate (2 mL L^{-1}) applied every three weeks was used in the second greenhouse experiment (2016).

3.5.9 Lipo-chitooligosaccharide (LCO, Torque® ST)

There was no recommended application rate of LCO in this commercial formulation on perennial grasses, therefore the recommended application rate for corn was modified for treatment applications in these experiments. The active ingredient in the solution (1.3×10^{-9} % LCO) and the recommended application rate for corn (0.09 mg per seed) are outlined on the commercial label.

3.6 Experiment 1 – Greenhouse Soil Drench

Nine growth-promoter treatments applied to NileFiber™ through soil drench thirty-five days after the plantlets were transplanted from the growth chamber to the greenhouse. Treatments included: A = *Azospirillum brasilense* N8; AL = *A. brasilense* N8 + LCO; C = Control; G = *Gluconacetobacter diazotrophicus* PAL5T; GL = *G. diazotrophicus* PAL5T + LCO; L = LCO (lipo-chitooligosaccharide); P = *Penicillium bilaii*; PL = *P. bilaii* + LCO; S = Seaweed extract (*Ascophyllum nodosum*).

3.6.1 Plantlet Acclimatization

Upon completion of the plantlet generation stage of tissue culture propagation, the plantlets were transferred from controlled environmental conditions to greenhouse conditions. Plantlets were transferred out of Magenta® culture boxes, rinsed thoroughly with cold tap water to remove residual medium and placed in a cold tap water bath for transport to the greenhouse. Plantlets were transplanted into plastic plant cell packs (one plantlet per cell) and filled with Pro-Mix® HP Mycorrhizae™ (Halifax Seed Co., Halifax, NS, Canada) on 01 May 2015. Cell packs were covered with clear plastic domes to create greater environmental humidity then placed in seed trays without drainage (Figure 3-7). The domes were shifted off (Figure 3-8) and eventually removed to gradually reduce the humidity experienced by plantlets (Figure 3-9). For the final phase of acclimatization, the plantlets were moved outside the greenhouse.

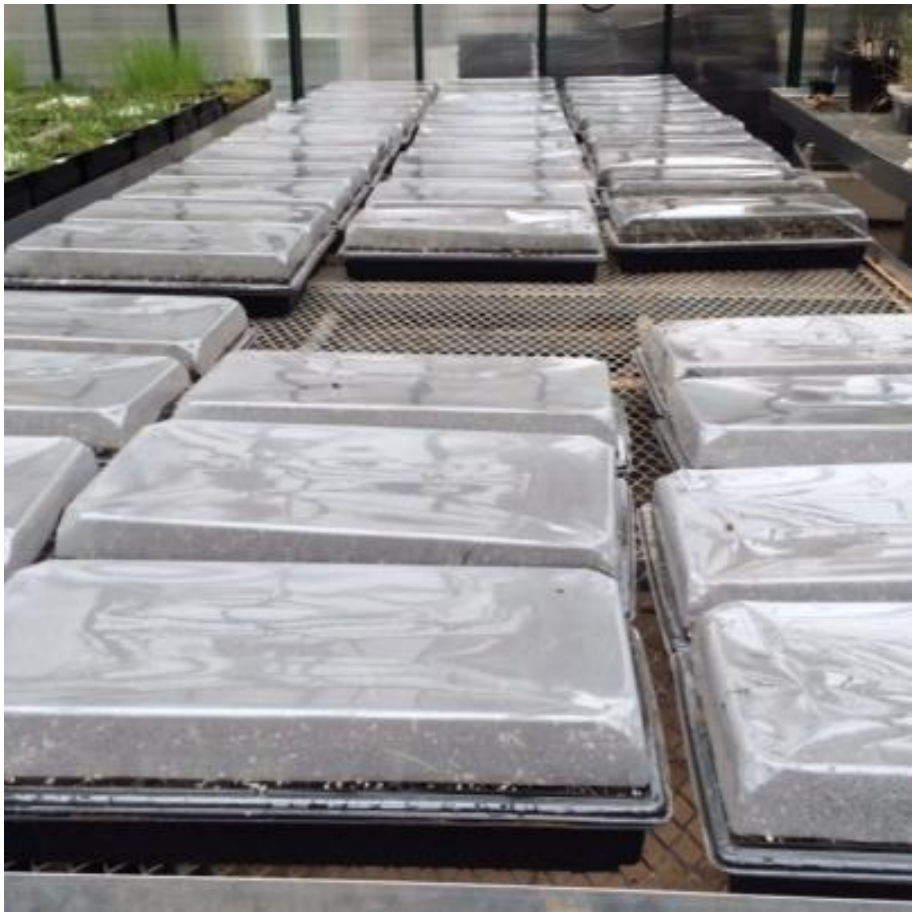

Figure 3-7. NileFiber™ plantlets during initial acclimatization stage in the greenhouse. Clear dome lids create a tight seal with the seed tray, locking moisture in (Peters, 2015a).

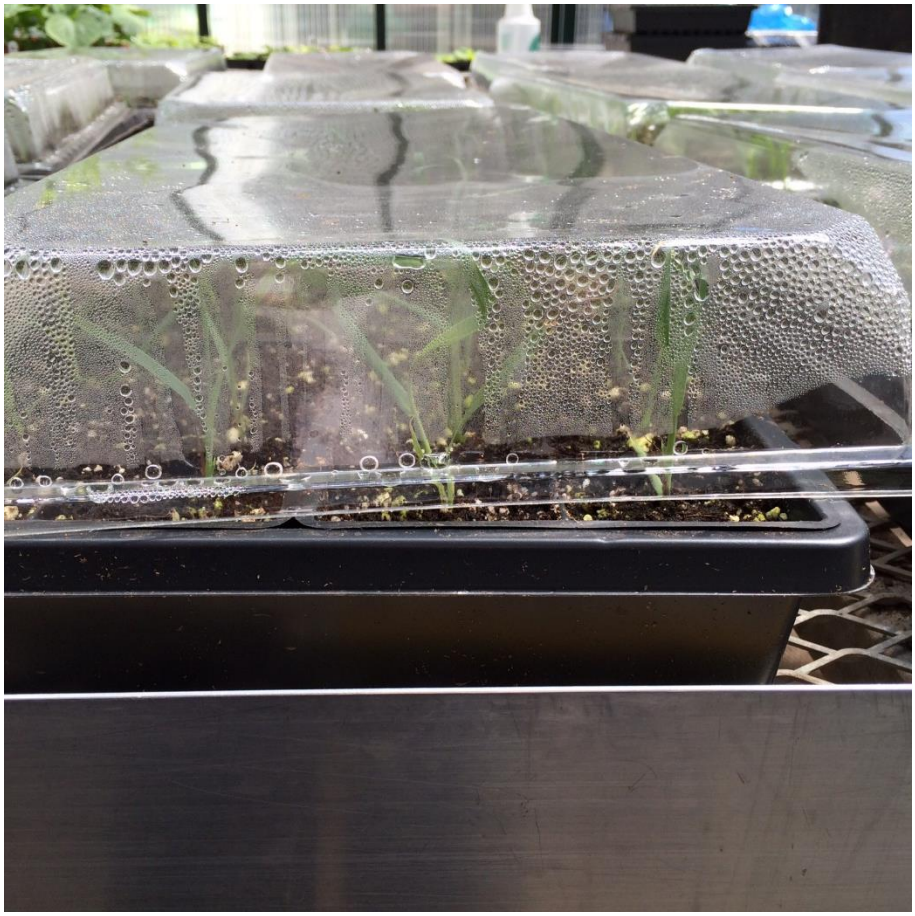

Figure 3-8. NileFiber™ plantlets during later acclimatization stage: the seal between the dome lids and the seed trays was slightly broken to reduce humidity (Peters, 2015a).

Figure 3-9. NileFiber™ plantlets upon removal of dome lids (Peters, 2015a).

3.6.2 Soil Type, Potting and Replicates

Single plantlets (30 – 35 cm height from soil surface) were transplanted into 3 L pots (ITML® Horticultural Products Inc.) filled with 2 kg of PREMIER® Top Soil (Halifax Seed Co., Halifax, NS, Canada) on 28 May 2015 (Figure 3-10). Each plantlet was planted approximately 7 cm into the soil. Replicates (12) within each growth-promoter treatment were originally arranged by treatment, but were completely randomized every two weeks to compensate for variation in temperature and light in the greenhouse.

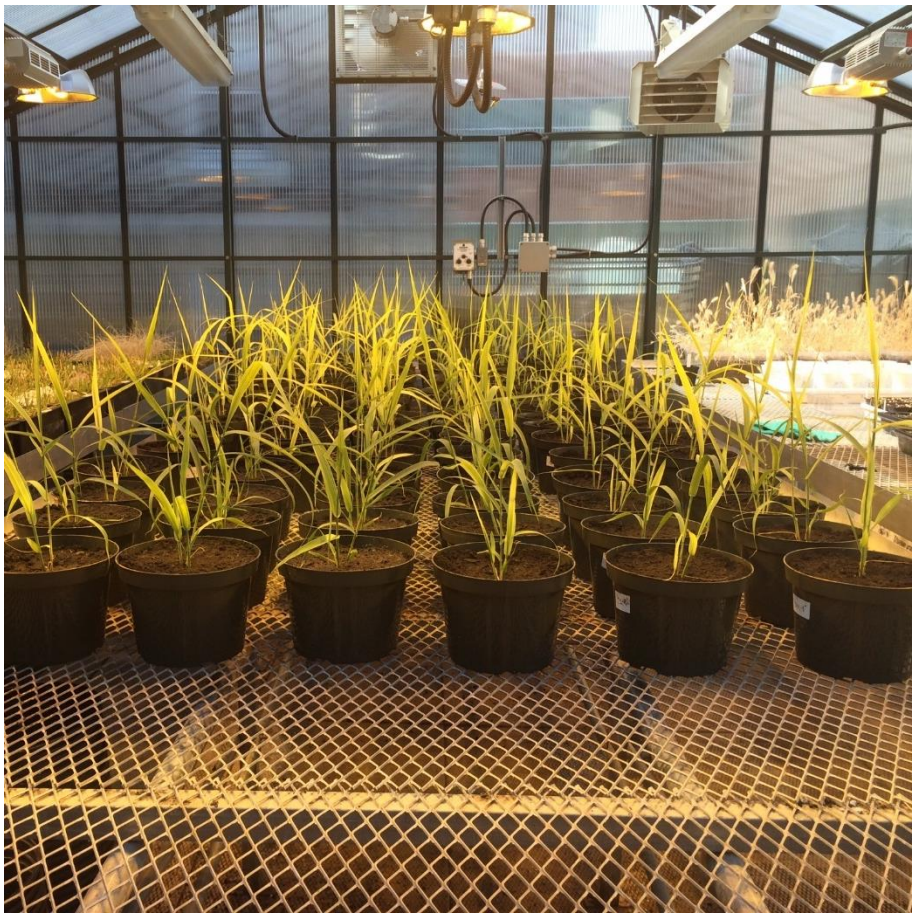

Figure 3-10. NileFiber™ plantlets transplanted into larger pots for first greenhouse experiment (Peters, 2015a).

3.6.3 Treatment and Application

Plantlets were treated once with a synthetic fertilizer treatment on 16 May 2015 to minimally supplement the plantlets until experimental set-up was complete. Approximately 14.3 g of Plant-Prod® 20-20-20 Classic Fertilizer (Halifax Seed Co., Halifax, NS, Canada) was dissolved per 3.8 L water (3.76 g L^{-1}). Each plastic seed tray was filled with 2 L of the fertilizer-water solution, supplying each plant with approximately 40 mg of nitrogen, phosphorus and potassium.

There were nine total growth-promoter treatment applications tested in the greenhouse (including the untreated control) (Table 3-6), as resource availability enabled additional combinatory treatments to be prepared and evaluated. Through bacterial quantification, 1 mL of bacterial culture broth contained approximately 10^8 CFU of bacteria, thus each plant received 5×10^8 CFU of bacteria diluted in 45 mL distilled water through a soil drench application on 05 June 2015.

Table 3-6. Concentration of growth-promoter treatment diluted in soil drench solution (total volume 50 mL) per plant used in first greenhouse trial application on NileFiber™ plantlets (05 June 2015). The same concentrations used in individual treatments were combined to create combination treatments.

Treatment	Concentration (CFU)	Volume per Plant (µL)
P. bilaii	5×10^8	-
G. diazotrophicus PAL5T	5×10^8	-
A. brasilense N8	5×10^8	-
A. nodosum	-	1000
LCO	-	100

3.6.4 Growth Conditions and Maintenance

This greenhouse experiment was conducted during the summer of 2015 (01 May – 15 September). A shade cloth was placed over the greenhouse (Figure 3-11) on 07 July 2015 to regulate the internal greenhouse temperature to better simulate a typical Nova Scotian growing season.

Figure 3-11. Saint Mary's University greenhouse with shade cloth placed on 07 July 2015 (Peters, 2015a).

Plants were watered as needed to keep the soil moist, with watering days logged in the greenhouse maintenance log (Appendix C). Initially, plants were carefully watered using a watering can to ensure the growth-promoter treatments were not sprayed off the soil surface. Each plant received approximately 250 mL of water per pot per day with this approach. Thirteen days post-treatment, a new watering protocol was implemented in which the standard watering can was exchanged for 250 mL plastic ladle dippers (Dynalon®). These dippers ensured soil saturation of the root system as well as the soil

surface, and also ensured greater accuracy of the volume of water received. Each plant received water until soil saturation (water began dripping from the drainage holes on the bottom of the pots). This protocol was followed on subsequent watering days, whether it be 250 mL or 500 mL per pot per day (Appendix C).

3.6.5 Growth Measurements

Growth measurements collected for biomass yield analysis included stem height (soil surface to highest collar region on main stem), number of shoots (height ≥ 5 cm), number of visible buds (height ≤ 5 cm), root dry weight, and above-ground biomass fresh and dry weight. Measurements were taken at harvest time, after approximately four months of growth to mimic a typical Nova Scotia growing season.

3.6.6 Harvest

Plants were harvested 14 – 15 September 2015. Plant height (soil surface to highest collar region on main stem) was measured prior to harvest using a tape measure. The number of shoots and visible buds were also counted before the shoots were cut. Shoots were cut level with the top of the pot and placed in labelled paper bags. Shoot fresh weight was determined using a battery operated digital balance (Pelouze® PE10) immediately after collection. Roots were unearthed from pots, soil was gently shaken off and roots were thoroughly rinsed with water and placed in labelled paper bags (Figure 3-12). All root and above-ground biomass samples were placed in one of two drying ovens (Fisher Scientific™ Isotemp™ 637G Standard Lab Oven or VWR Signature™ Forced Air Safety Oven) and dried at 80 °C for eight days. Upon removal from the ovens, any remaining shoots (below soil surface) were removed from the root systems and dry

weights were recorded for roots and above-ground biomass using an electric digital balance (Denver Instruments PK – 352). Biomass samples were ground using a Wiley Laboratory Mill (Standard Model No. 3, Arthur H. Thomas Co.) (Figure 3-13) and were analyzed for nutrient composition by the Department of Agriculture Analytical Lab in Truro, Nova Scotia.

Figure 3-12. Cleaned root system of NileFiber™ plant C-2 prior to drying (Peters, 2015a).

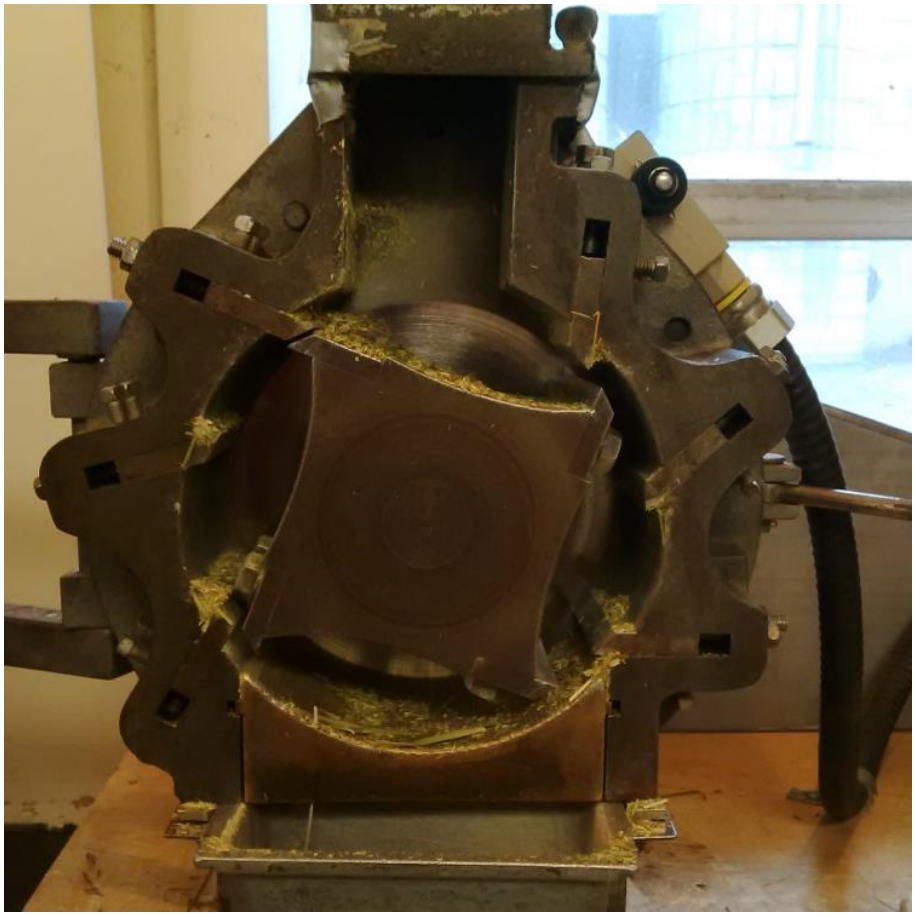

Figure 3-13. Wiley Laboratory Mill (Standard Model No. 3, Arthur H. Thomas Co.) used to grind biomass samples for further analysis (Peters, 2013).

3.7 Experiment 2 – Field Soil Drench

Six growth-promoter treatments applied to NileFiber™ through soil drench seventy days after plantlets were transplanted out of the growth chamber. Treatments included: A = *Azospirillum brasilense* N8; C = Control; D = *Gluconacetobacter azotocaptans* DS1; G = *Gluconacetobacter diazotrophicus* PAL5T; P = *Penicillium bilaii*; S = Seaweed extract (*Ascophyllum nodosum*).

3.7.1 Plantlet Acclimatization

Upon completion of the plantlet generation stage of tissue culture propagation, the plantlets were exposed to less controlled environmental conditions by continuing further growth in the greenhouse. Plantlets were transferred out of the Magenta® culture boxes, rinsed thoroughly with cold tap water to remove residual medium and placed in a cold tap water bath for transport to the greenhouse. Plantlets were transplanted into plastic plant cell packs (one plantlet per cell) and filled with Pro-Mix® HP Mycorrhizae™ (Halifax Seed Co., Halifax, NS, Canada) on 01 May 2015. Cell packs were covered with clear plastic domes to create greater environmental humidity and then placed in seed trays without drainage (Figure 3-7). Domes were shifted off (Figure 3-8) and eventually removed to gradually reduce the humidity the plantlets were experiencing (Figure 3-9). For the final phase of acclimatization, the plantlets were moved outside the greenhouse (Figure 3-14).

Figure 3-14. NileFiber™ plantlets moved outside the greenhouse for additional acclimatization (Peters, 2015a).

3.7.2 Experimental Design

NileFiber™ plantlets not used in the first greenhouse experiment were transported in seed trays to the field site. In total, the plantlets acclimatized for nineteen days outdoors before planting in the field. Single plantlets were transplanted into Field C1 (Figure 3-1) in Nappan, NS, Canada on 15 June 2015. Each plantlet was planted approximately 7 – 10 cm into the soil. The growth-promoter treatments were arranged in a randomized complete block design with five plot replicates per treatment. Plant density

was 6.25 plants m^{-2}, row distance was 50 cm and there was a buffer zone of 1 m between plots (Appendix D).

3.7.3 Treatment and Application

The plantlets were treated twice with a synthetic fertilizer treatment on 16 May 2015 and 29 May 2015. Approximately 14.3 g of Plant-Prod® 20-20-20 Classic Fertilizer (Halifax Seed Co., Halifax, NS, Canada) was dissolved per 3.8 L water (3.76 g L^{-1}). Each plastic seed tray was filled with 2 L of the fertilizer-water solution, supplying each plant with approximately 40 mg of nitrogen, phosphorus and potassium. The fertilizer was added to minimally supplement the plantlets until experimental set-up was complete.

There were six total growth-promoter treatment applications tested in the field experiment (including the untreated control) (Table 3-7), as regulations regarding certain commercial treatments disabled their use in the field. Through bacterial quantification, 1 mL of bacterial culture broth contained approximately 10^8 CFU of bacteria, thus each plant received 5×10^8 CFU of bacteria diluted in 245 mL water through a soil drench application on 10 July 2015.

Table 3-7. Concentration of growth-promoter treatment diluted in diluted in soil drench solution (total volume 250 mL) per plant used in the field trial of growth-promoter application to NileFiber™ plantlets (10 July 2015).

Treatment	**Concentration (CFU)**	**Volume per Plant (mL L^{-1})**
P. bilaii	5×10^8	-
G. diazotrophicus PAL5T	5×10^8	-
A. brasilense N8	5×10^8	-
A. nodosum	-	1
G. azotocaptans DS1	5×10^8	-

3.7.4 Growth Conditions and Maintenance

The field experiment was conducted during the summer of 2015 (15 June – 08 October). Experimental plots were manually weeded using hoes (within plots) and a rototiller (between plots) 11 August 2015 and 25 August 2015 (Figures 3-15 & 3-16). After watering in the greenhouse, the fertilizer treatment applications and the growth-promoter treatment application, plants in the field only received water through rainfall. Soil samples were also taken from the experimental plots on 17 July 2015 from the spots indicated in Appendix D. Samples were taken from 0 – 15 cm and 15 – 30 cm depths.

Figure 3-15. NileFiber™ plants prior to weeding, 10 July 2015 (Fei, 2015).

Figure 3-16. NileFiber™ plants after weeding, 11 August 2015 (Peters, 2015b).

3.7.5 Growth Measurements

Growth measurements collected for biomass yield analysis included stem height (soil surface to highest collar region on main stem), number of shoots (height ≥ 5 cm) and above-ground biomass fresh and dry weight. Measurements were taken at harvest time, after approximately four months of growth to mimic a typical Nova Scotia growing season.

3.7.6 Harvest

Plants were harvested 08 October 2015. Plant height (soil surface to highest collar region on main stem) was measured prior to harvest using a tape measure. The number of shoots were counted before the shoots were cut. Shoots were cut 5 cm above ground level (Figure 3-17) and placed in labelled paper bags. Above-ground biomass fresh weight was determined using a battery operated digital balance (Taylor® 3830-48) immediately after collection. Above-ground biomass samples were placed in a drying oven (Precision Quincy Corporation 40 Series Large Low Temperature Utility Oven) and dried at 80 °C for seven days. Upon removal from the ovens, dry weights were recorded using an electric digital balance (Denver Instruments PK – 202). Biomass samples were ground using a Wiley Laboratory Mill (Standard Model No. 3, Arthur H. Thomas Co.) and were analyzed for nutrient composition by the Department of Agriculture Analytical Lab in Truro, Nova Scotia.

Figure 3-17. NileFiber™ plants during harvest, 08 October 2015 (Peters, 2015b).

3.8 Experiment 3 – Greenhouse Root Soaking

Fifteen growth-promoter treatments applied to NileFiber™ through root soaking immediately after being removed from the growth chamber. Treatments included: AB = *Azospirillum brasilense* N8; AL = *A. brasilense* N8 + LCO; C = Control; D = *Gluconacetobacter azotocaptans* DS1; DL = *G. azotocaptans* DS1 + LCO; G = *Gluconacetobacter diazotrophicus* PAL5T; GL = *Gluconacetobacter diazotrophicus* PAL5T + LCO; PL = *Gluconacetobacter diazotrophicus* PAL5T LsdB++; PLL = *Gluconacetobacter diazotrophicus* PAL5T LsdB++ + LCO; GS = *Gluconacetobacter diazotrophicus* SRT4; GSL = *Gluconacetobacter diazotrophicus* SRT4 + LCO; P = *Penicillium bilaii*; S = Seaweed extract (*Ascophyllum nodosum*); VP = *Variovorax paradoxus* JM63; VPL = *Gluconacetobacter diazotrophicus* SRT4 + LCO.

3.8.1 Plantlet Acclimatization

In comparison to the two previous experiments, the plantlet acclimatization period for the second greenhouse experiment was markedly different. Upon completion of the plantlet generation stage of tissue culture propagation, plantlets were immediatelu subjected to their growth-promoter treatment (through root soaking) then moved to the greenhouse. Potted plants were covered with clear plastic domes to create greater environmental humidity and placed in seed trays without drainage (Figure 3-18). Domes were shifted off and eventually removed to gradually reduce the humidity the plantlets experienced (Figure 3-19).

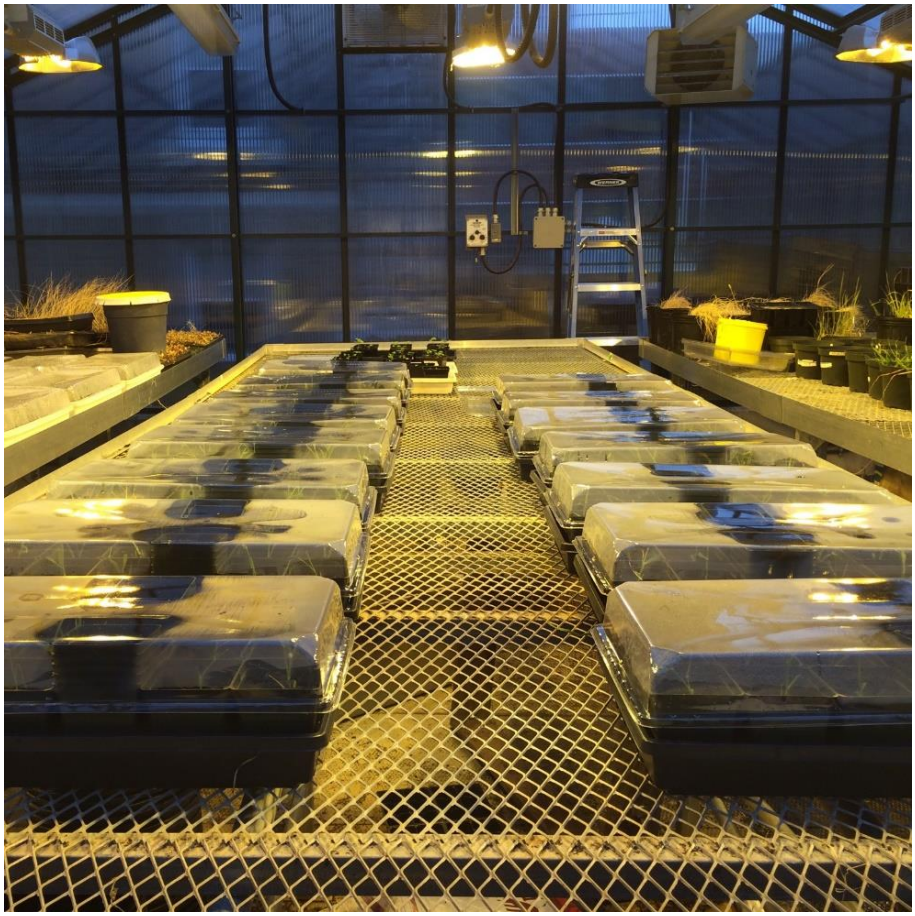

Figure 3-18. NileFiber™ plantlets during acclimatization stage in the greenhouse. Clear dome lids create a tight seal with the seed tray, locking moisture in (Peters, 2016a).

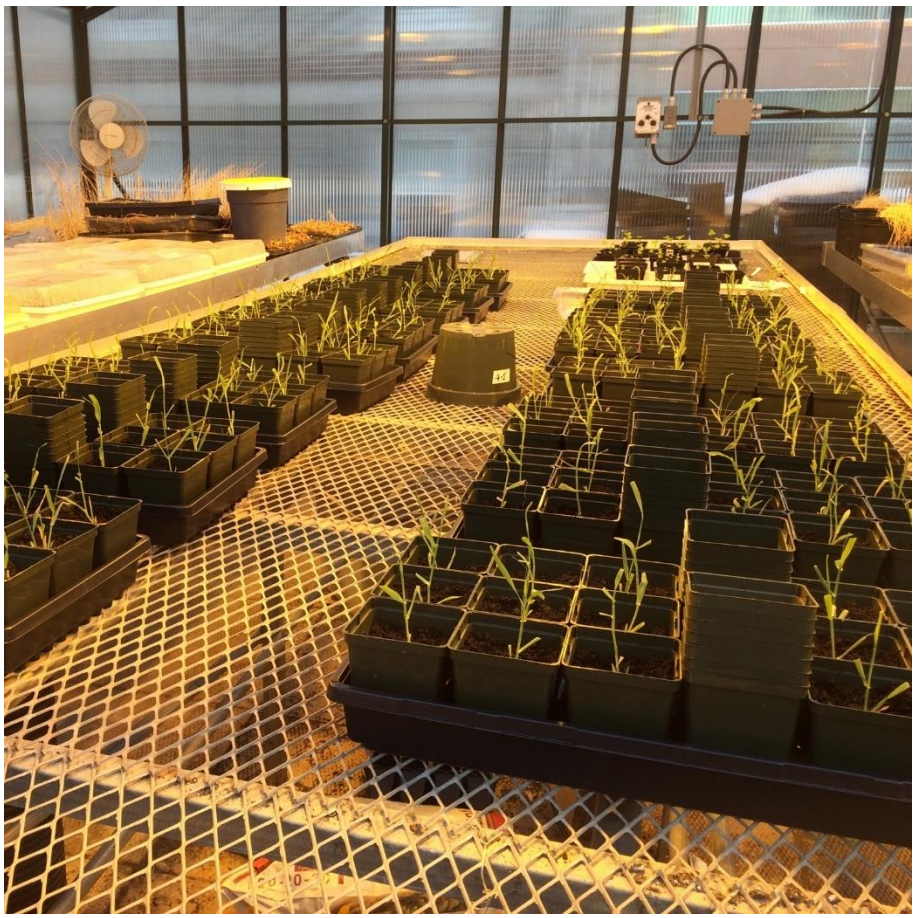

Figure 3-19. NileFiber™ plantlets upon removal of dome lids (Peters, 2016a).

3.8.2 Soil Type, Potting and Replicates

Single plantlets (10 – 15 cm height from soil surface) were transplanted into 0.8 L square pots (3.5” Kordlok Square Pot) and filled with 130 g of sterilized PREMIER® Top Soil (Halifax Seed Co., Halifax, NS, Canada) on 08 February 2016. Soil was sterilized for five hours at 200 °C using an electric soil sterilizer (PRO-GROW Supply Corp., Model SS-15). Each plantlet was planted approximately 7 cm into the soil. Replicates (15) within each growth-promoter treatment were arranged by treatment in seed trays.

3.8.3 Treatment and Application

There were fifteen total growth-promoter treatment applications tested in the greenhouse (including the untreated control) (Table 3-8), as resource availability enabled additional combinatory treatments to be prepared and evaluated. Root systems were suspended in an inoculum broth containing 0.5×10^8 CFU bacteria or fungal spores per mL (1 mL L^{-1} *A. nodosum*) and phosphate buffer (34mM, pH = 6.0) for 30 minutes (Figure 3-20). *P. bilaii* inoculum contained distilled water rather than phosphate buffer, and also contained 0.1 % Tween®20 (Sigma-Aldrich®) to stabilize the emulsion of fungal spores in the inoculum. The broth of the combinatory treatments (addition of LCO) received 1.8 % LCO, the equivalent of 100 μL $plant^{-1}$. *P. bilaii* and *A. nodosum* treated plants received 5 mL $plant^{-1}$ inoculum through soil drench, and remaining plants received 5 mL $plant^{-1}$ phosphate buffer through soil drench.

Table 3-8. Concentration of growth-promoter treatments in inoculum broth used for root soaking in second greenhouse trial application on NileFiber™ plantlets (08 February 2016). The same concentrations used in individual treatments were combined with 1.8 % LCO to create combination treatments.

Treatment	**Concentration in Inoculum Broth (CFU)**	**Concentration in Inoculum Broth (mL L^{-1})**
P. bilaii	10^8	-
G. diazotrophicus PAL5T	10^8	-
A. brasilense N8	10^8	-
A. nodosum	-	1
G. diazotrophicus PAL5T Lsd B++	10^8	-
G. azotocaptans DS1	10^8	-
G. diazotrophicus SRT4	10^8	-
V. paradoxus JM63	10^8	-

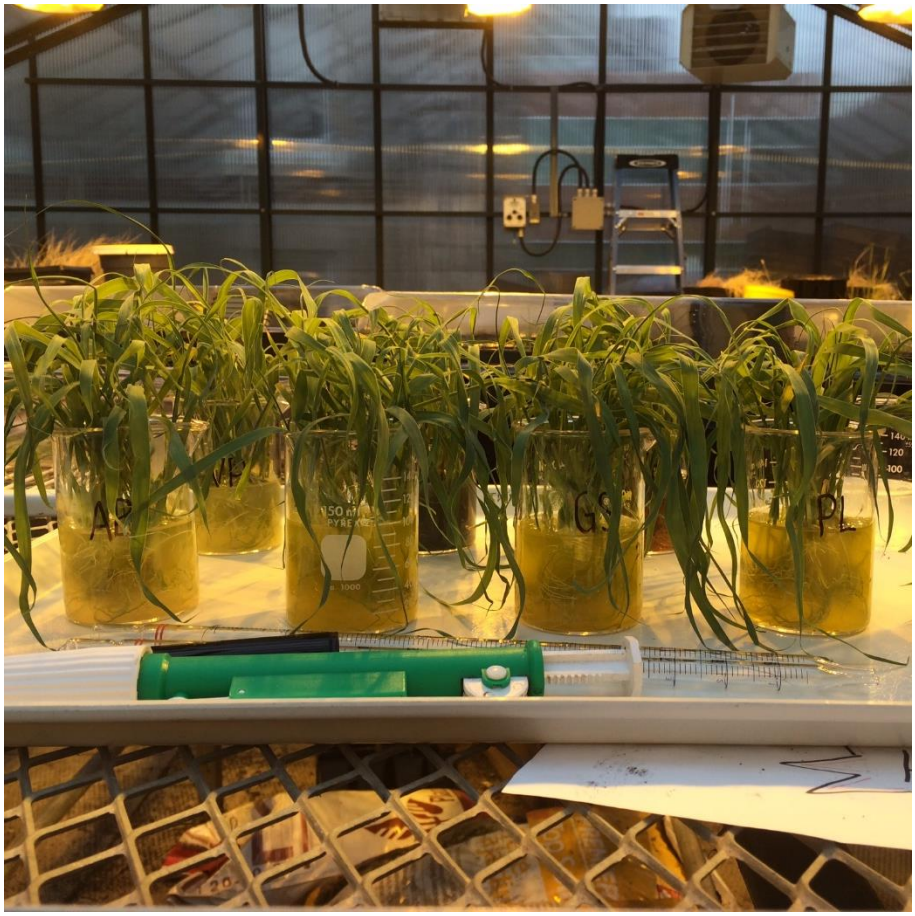

Figure 3-20**.** NileFiber™ plantlets soaking in growth-promoter treatments. Roots were soaked for 30 minutes, then transplanted to sterilized soil (Peters, 2016a).

3.8.4 Growth Conditions and Maintenance

The second greenhouse experiment was conducted during the winter of 2016 (08 February – 14 April). The plants treated with *A. nodosum* were subsequently treated at three week intervals with 2 mL L^{-1} concentration (50 mL $plant^{-1}$) (Appendix C).

Plants were watered as needed to keep the soil moist, with watering days logged in the greenhouse maintenance log (Appendix C). Plants were watered by pouring water into the seed tray using a 4000 mL plastic beaker, each tray received 1000 mL of water.

3.8.5 Growth Measurements

Growth measurements collected for biomass yield analysis included stem height (soil surface to highest leaf tip on main stem), number of shoots (height ≥ 5 cm), root dry weight, and above-ground biomass fresh and dry weight. Measurements were taken at harvest time, approximately two months after treatment.

3.8.6 Harvest

Plants were harvested 11 – 12 April 2016. Plant height (soil surface to highest leaf tip on main stem) was measured prior to harvest using a tape measure. The number of shoots were also counted before the shoots were cut. Plants were unearthed from pots, soil was gently shaken off and roots were thoroughly rinsed with water (Figure 3-21). Above-ground biomass was separated from roots and placed in separate labelled paper bags. Above-ground biomass fresh weight was determined using a battery operated digital balance (Pelouze® PE10) immediately after collection. All root and above-ground biomass samples were placed in one of two drying ovens (Fisher Scientific™ Isotemp™ 637G Standard Lab Oven or VWR Signature™ Forced Air Safety Oven) and dried at 80 ℃ for eight days. Upon removal from the ovens, dry weights were recorded for roots and above-ground biomass using an electric digital balance (Denver Instruments PK – 352).

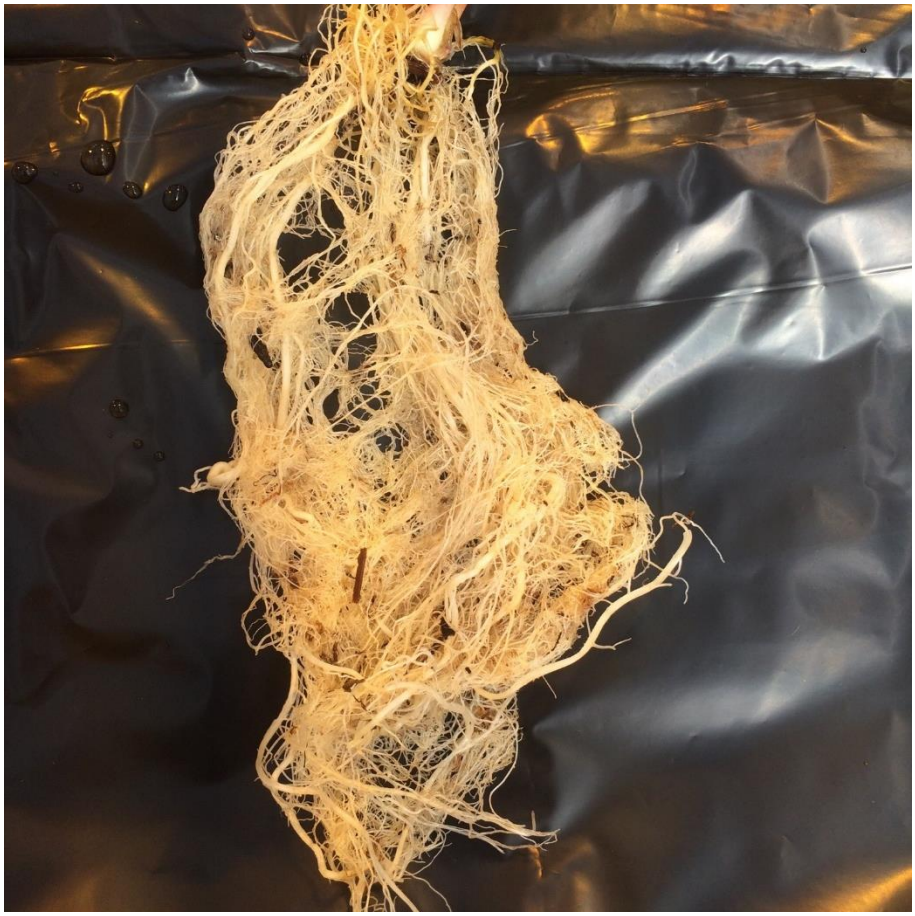

Figure 3-21. Cleaned root system of untreated control NileFiber™ plant prior to drying (Peters, 2016a).

3.9 Statistical Methods

Experimental data was analyzed through two statistical approaches: 1) the frequentist (classical) approach and 2) the Bayesian approach. All analyses were performed using RStudio Version 0.99.484.

3.9.1 Frequentist (Classical) Approach

One-way ANOVA testing was performed on all variables in which data was collected. When the F-statistic was significant, the treatment means of each variable were analyzed using various Post-Hoc analyses, including least significant difference (LSD) tests and pairwise comparisons (RStudio Version 0.99.484).

3.9.2 Bayesian Approach

Experimental data was analyzed using Bayesian statistics in addition to frequentist (classical) statistical analyses. Bayesian statistics were used because these analyses provide a probability of the hypothesis given the observed experimental data that is used to quantify the belief in the hypothesis (Barker, 2015). Frequentist statistical analyses provide evidence against one hypothesis that is used to quantify the frequency of occurrence of the observed data given the hypothesis (Barker, 2015). Ultimately, the nature of Bayesian statistical analyses allows for more informative statistical inferences, and despite potential controversy, it is useful as a supplementary analysis to the less informative frequentist analyses. Formally, Bayes' rule takes the following form:

$$\mathrm{P}(\theta|\mathrm{D}) = \frac{\mathrm{P}(\theta) \times \mathrm{P}(\mathrm{D}|\theta)}{\mathrm{P}(\mathrm{D})} \quad \text{(x.1)}$$

where:

P(θ|D) is the "posterior probability", the probability of parameter values given the data. P(θ) is the "prior probability", the probability assigned to parameters prior to viewing the data. P(D| θ) is the "likelihood", the probability of parameter values given the hypothesis (model) and P(D) is a normalizing constant (Krushcke 2015).

The prior probability (P(θ)) in Bayes' rule can be extremely controversial because in non-repeated experiments, prior information about the data is absent, creating

subjectivity in the prior selection. Weakly-informed priors are loosely based on the data, and do not pose bias upon the outcome of the posterior probabilities.

Historically, the normalizing constant (P(D)) of Bayes' rule has been the main reason for the hiatus from Bayesian inference. Until recently, there was not enough computational power available to solve the integral leading to (P(D)). With significant technological advances, and availability of this technology to the masses, Bayes' rule can be solved for complex problems, using Markov Chain Monte Carlo (MCMC) methodology.

Markov Chain Monte Carlo (MCMC) is a method of generating "a series of random numbers in which the value of each is conditional on the previous number" (McCarthy, 2007). MCMC algorithms are constructed so that each value from the Markov chain represents the relative likelihood of that value in the posterior probability distribution, rendering the calculation of the denominator of Bayes' rule unnecessary (Kruschke, 2015; McCarthy, 2007).

3.9.2.1 Experimental (Observed) Data

The experimental design produced two variables of differing data types. The response (predicted) variable was growth measurement (metric data) and the explanatory (predictor) variable was growth-promoter treatment (nominal data). Given these data types and the desired outcome of the statistical analysis (comparison between nominal categories and comparison between nominal categories across experimental trials), there were two mathematical models used: a) Metric Predicted Variable with One Nominal Predictor Variable, analogous to the traditional one-way analysis of variance (ANOVA)

and b) Metric Predicted Variable with Multiple Nominal Predictor Variables, analogous to the traditional two-way analysis of variance (ANOVA) (Kruschke, 2015; McCarthy, 2007).

3.9.2.2 Metric Predicted Variable with One Nominal Predictor Variable

$$\mu_i = \beta_0 + \sum \beta_{[j]} x_{[j]}$$

(x.2)

In the model, μ_i is the distribution of predicted values. β_0 is the baseline value for the "population", and β_j is the coefficient representing the "effect" of the predictor variables (i.e. the degree to which the predicted values increase or decrease from the baseline in response to the predictor values being in a particular category) (Krushchke, 2015).

3.9.2.2.1 Statistical Model

After defining the mathematical model, the next step in Bayesian data analysis is to outline a descriptive statistical model, defining associated parameters based on mathematical formulae (Krushcke, 2015). Illustrating the statistical model using a hierarchical diagram defining all pertinent parameters is a helpful means of visualizing these models.

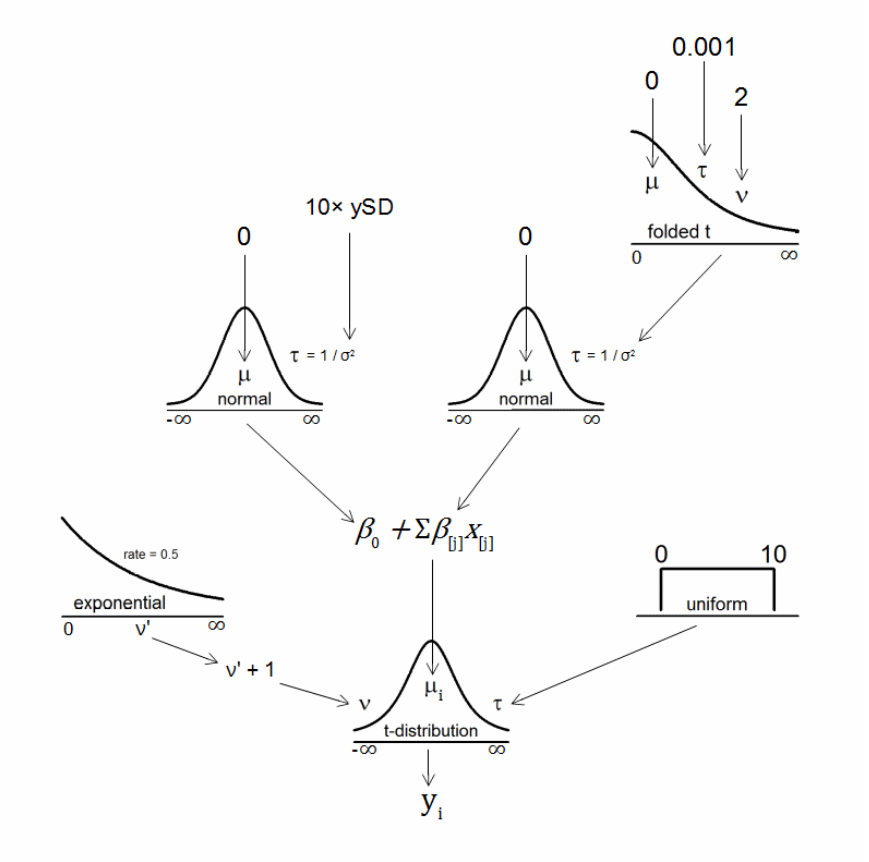

Figure 3-22. Hierarchical diagram illustrating the statistical model for the analyses of a metric predicted variable with one nominal predictor variable (Peters, 2016b).

Starting from the bottom of the hierarchical diagram (Figure 3-22), the growth measurement data (y_i) is from a *t*-distribution around the predicted value (μ_i). Equation (x.1) defines the predicted value, also illustrated in the diagram (Figure 3-22). A *t*-distribution model allows for a robust estimation that better accommodates outliers compared to a normal distribution. The scale parameter (τ) defines the width of the *t*-distribution, and is given a uniform distribution with a range of 0 to 10. Prior to analyses, the data were standardized ($\mu = 0$, $\sigma = 1$), therefore allowing (τ) to range from 0 to 10

should be conservative (the true (τ) value should lay well within this prior distribution). The parameter (ν) defines the degree of normality of the *t*-distribution, where low values give the distribution heavy tails, and as (ν) increases, the tails become lighter, and when (ν) reaches 30, the *t*-distribution is approximately a normal distribution. Here, the prior for (ν) is given an exponential distribution to preferentially allow (ν) to take on very low values, without excluding large values as possibilities. The original range of an exponential distribution is 0 to infinity, however, due to the range of the normality parameter being from 1 to infinity, the prior for the normality parameter is changed (ν + 1).

The baseline parameter, (β_0) is given a normal prior distribution around a mean (μ) of 0, because the data were standardized. The value for precision (τ) uses a standard deviation (σ) 10 times as large as the standard deviation of the observed data (y_i) to capture variation across all categories.

The group deflection parameters (β_j) are also given a normal prior distribution around a mean (μ) of 0, as the sum of all deflection parameters should be 0, and *a priori*, we assume no relationship between predictor and predicted variables. The standard deviation (σ) of the distribution of deflection parameters is given a "folded-*t*" prior distribution for three reasons: 1) the standard deviation (σ) values must be positive (denominator in precision calculation), 2) because the data were standardized, the probability of lower standard deviation values is higher and 3) there is not an infinite density as the distribution approaches 0 (Gelman, 2006). The "folded-*t*" prior

distribution has a mean (μ) of 0 (standardized data), scale parameter (τ) of 0.001 and a normality parameter (ν) of 2 to accommodate outliers.

3.9.2.3 Metric Predicted Variable with Multiple Nominal Predictor Variables

$$\mu_i = \beta_0 + \sum_{j} \beta_{1[j]} x_{1[j]} + \sum_{k} \beta_{2[k]} x_{2[k]} + \sum_{j,k} \beta_{1\times2[j,k]} x_{1\times2[j,k]}$$

(x.3)

In the model, μ_i is the predicted value, β_0 is the baseline value for the "population", β_1 and β_2 are deflections from the baseline of each individual factor and $\beta_{1\times2}$ is the deflection due to the interaction of factors (Kruschke, 2015, p.584).

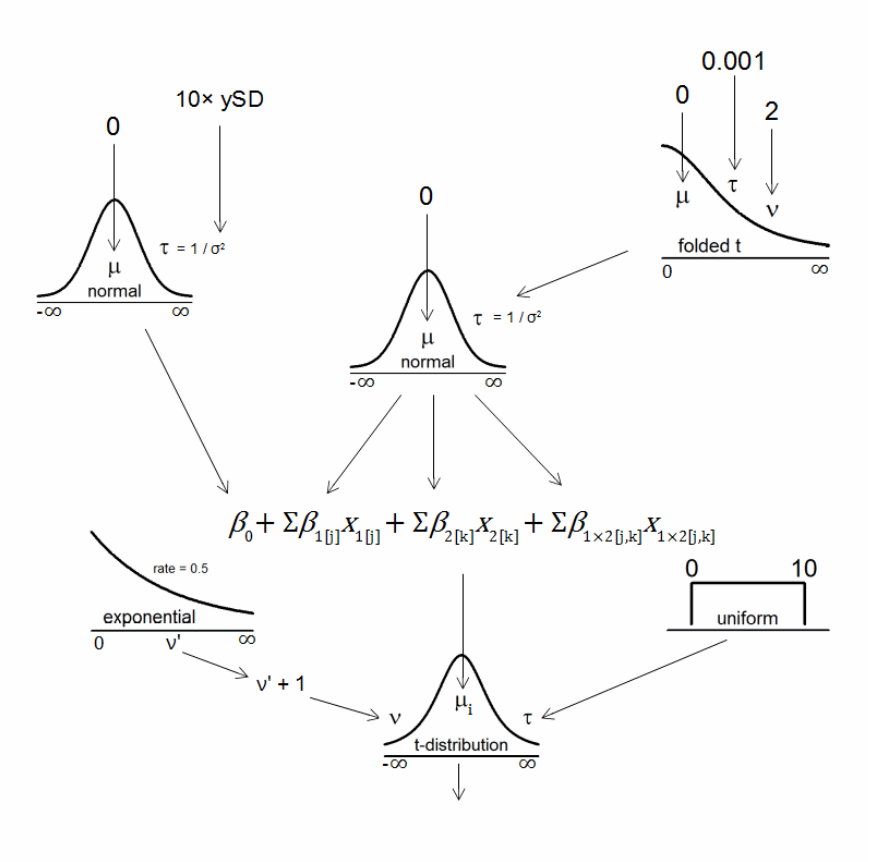

Figure 3-23. Hierarchical diagram illustrating the statistical model for the analyses of a metric predicted variable with multiple nominal predictor variables. β_1, β_2, and $\beta_{1\times2}$ all come from different distributions with the same characteristics. All distributions follow the same characteristics as explained in Figure 3-22 (Peters, 2016c).

3.9.2.3.1 Statistical Model Checking

Once the models were developed, they were each validated through the use of simulations. Briefly, data were generated with similar properties to the experimental data, but in these cases the true values for the parameter values were known. These data were then analyzed with the appropriate model to ensure that the analyses recovered the true values. Once the models were validated, the experimental data were analyzed.

4.0 RESULTS

The objectives of this research were to 1) evaluate the growth potential of *Arundo donax* (NileFiber™) as a purpose-grown feedstock in Nova Scotia and 2) to investigate the effects of growth-promoter treatments. In 2015, a greenhouse experiment and a field experiment were completed using a delayed soil drench inoculation method. In 2016, a second greenhouse experiment was completed with an immediate root soaking inoculation method.

Experimental data was analyzed through two statistical approaches: 1) the frequentist (classical) approach and 2) the Bayesian approach. All analyses were performed using RStudio Version 0.99.484.

4.1 Experiment 1 – Greenhouse Soil Drench

Nine growth-promoter treatments applied to NileFiber™ through soil drench thirty-five days after the plantlets were transplanted from the growth chamber to the greenhouse. Treatments included: A = *Azospirillum brasilense* N8; AL = *A. brasilense* N8 + LCO; C = Control; G = *Gluconacetobacter diazotrophicus* PAL5T; GL = *G. diazotrophicus* PAL5T + LCO; L = LCO (lipo-chitooligosaccharide); P = *Penicillium bilaii*; PL = *P. bilaii* + LCO; S = Seaweed extract (*Ascophyllum nodosum*).

4.1.1 Greenhouse Conditions

The mean hours of daily illumination recorded for Halifax, NS during the 2015 growing season showed insignificant deviation from the daily illumination records for the past twenty years (Figure 4-1). Supplemental lighting was used in the greenhouse to maintain a photoperiod of 16/8 hours (day/night), with minimum photosynthetic photon

flux density (PPFD) at plant height of 300 µmol m^{-2} s^{-1}. The greenhouse was covered with a shading cloth on 07 July 2015 to regulate the internal greenhouse temperature.

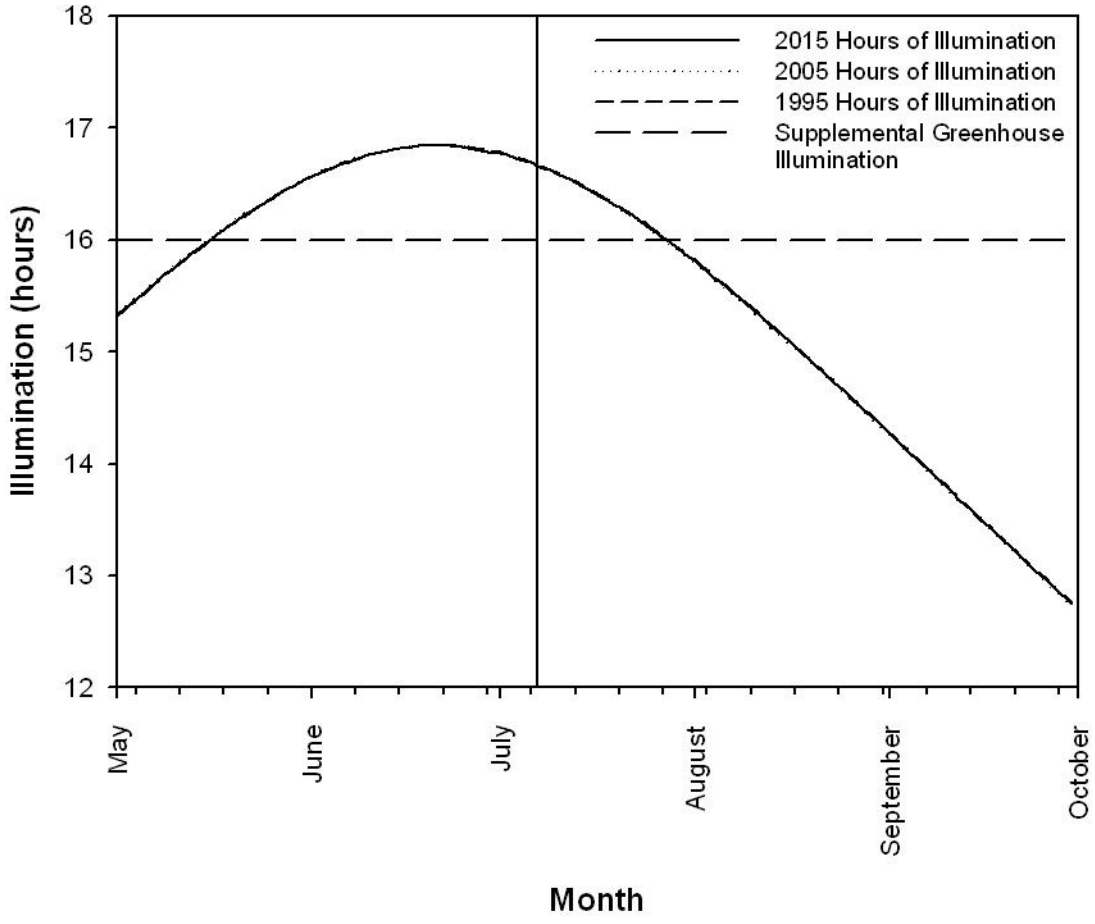

Figure 4-1. Mean hours of daily illumination for Halifax, NS for the duration of three growing seasons (1995, 2005 and 2015) (National Research Council, 2016) and the supplemental greenhouse illumination (16 hours day^{-1}). The vertical line indicates shading cloth application.

The internal greenhouse temperature was set at 25/18 °C (day / night) and controlled by heating, ventilation and the shading cloth (upon application). The internal temperature fluctuated consistently with external temperature (Figure 4-2).

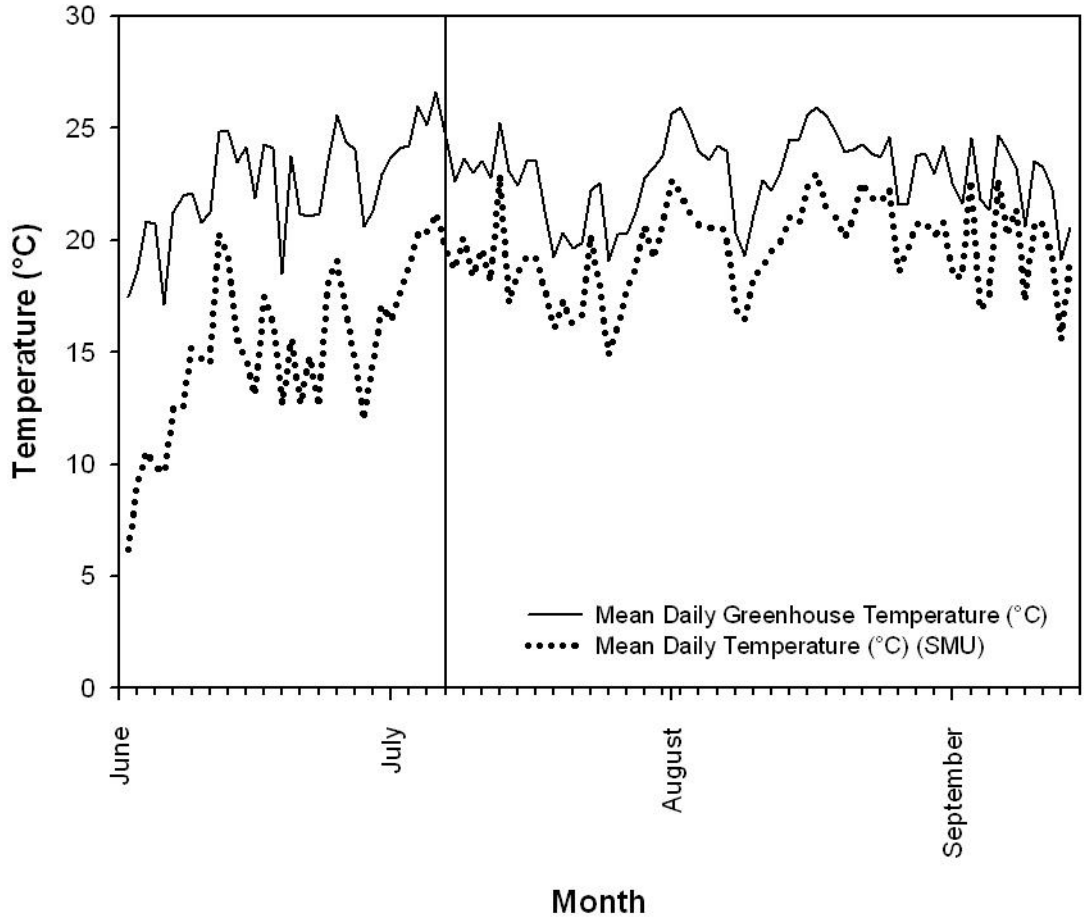

Figure 4-2. Mean daily internal temperature of the greenhouse (HOBO® Relative Humidity / Temperature / Light / External Data Logger) and mean daily external greenhouse temperature (SMU Observatory / Environmental Science **INOVASCO115**, 2016) during the first greenhouse experiment (2015). The vertical line indicates shading cloth application.

4.1.2 Results & Statistical Analysis – Frequentist Approach

4.1.2.1 Plant Height

The height of NileFiber™ was measured from the soil surface to the highest collar region on the main stem. Mean height per plant ranged from 103 cm (AL) to 119 cm (PL) (Table 4-1). Plants treated with the commercial LCO treatments (except PL) had significantly lower mean heights than control plants (Figure 4-7). NileFiber™ plants treated with a single growth-promoter (G, P) were not significantly different than their corresponding combination treatments (GL, PL), whereas treatment (A) was significantly taller than its counterpart (AL). The one-way ANOVA P-value was $P < 0.01$ (Appendix E).

Table 4-1. Mean values of growth parameters measured in the first greenhouse experiment at the time of harvest (14 September 2015) and eight days post-harvest (22 September 2015).

Treatment	Mean Height (cm)	Mean Number of Shoots	Mean Number of Visible Buds	Mean Above-Ground Biomass Fresh Weight (g)	Mean Root Dry Weight (g)	Mean Above-Ground Biomass Dry Weight (g)	Mean Above-Ground Biomass Dry Matter Content (%)
C	119	4	1	46.57	4.11	16.94	36.40
A	116	3	1	44.90	4.00	16.12	35.89
AL	103	4	1	39.49	3.10	16.85	37.56
G	115	4	0	45.32	3.56	16.50	36.50
GL	108	3	0	38.24	3.07	13.90	36.32
L	108	4	0	41.99	3.44	14.99	35.69
P	115	4	0	43.24	3.85	15.12	35.01
PL	119	3	0	43.24	3.60	15.41	35.53
S	116	3	1	41.99	3.75	15.49	36.92

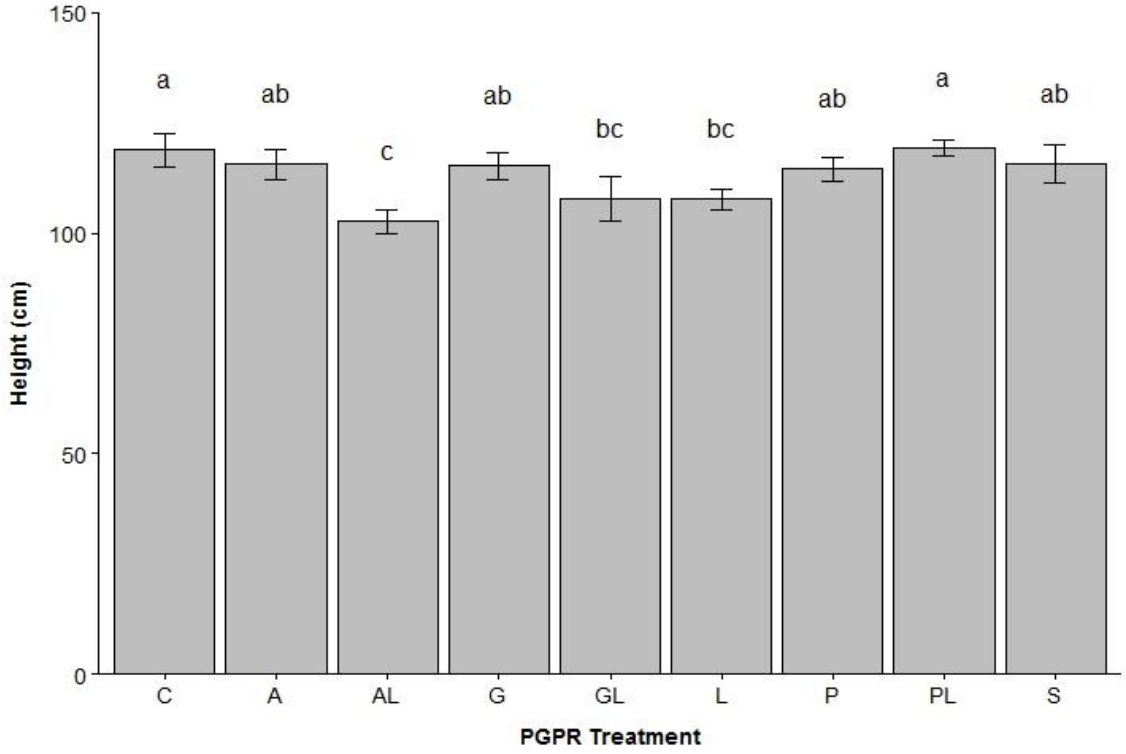

Figure 4-3. Mean NileFiber™ height (cm) per plant measured on 14 September 2015. C = Control; A = *Azospirillum brasilense* N8; AL = *A. brasilense* N8 + LCO; G = *Gluconacetobacter diazotrophicus* PAL5T; GL = *G. diazotrophicus* PAL5T + LCO; L = LCO (lipo-chitooligosaccharide); P = *Penicillium bilaii*; PL = *P. bilaii* + LCO; S = Seaweed extract (*Ascophyllum nodosum*). Different letters indicate significant difference at $P < 0.05$. Bars indicate standard error. (n = 12).

4.1.2.2 Number of Shoots

Shoot number per plant was recorded at the time of harvest, as all shoots greater than 5 cm in height were included. Mean shoot number per plant ranged from 3 (A, GL, PL & S) to 4 (C, AL, G, L & P), showing no statistically significant difference in shoot number between growth-promoter treatments (P = 0.552) (Table 4-1) (Appendix E).

4.1.2.3 Visible Buds

The mean number of visible buds per plant were recorded at the time of harvest, as all shoots less than 5 cm in height were included. Mean number of visible buds per plant ranged from 0 (G, GL, L, P & PL) to 1 (C, A, AL & S), showing no statistically significant difference in mean number of visible buds between growth-promoter treatments (P = 0.609) (Table 4-1) (Appendix E).

4.1.2.4 Above-Ground Biomass Fresh Weight

NileFiber™ above-ground biomass fresh weight was weighed immediately after being harvested. The control treatment had the greatest mean above-ground biomass fresh weight per plant (46.57 g) and the (GL) treatment had the lowest (38.24 g) (Table 4-1). The plants treated with the commercial LCO treatments (except PL) had significantly lower mean fresh weights than the control plants (Figure 4-4). NileFiber™ plants treated with a single growth-promoter (A, G) had shoots that weighed significantly more than their corresponding combination treatment (AL, GL) except in the case of (P) and (PL) (Figure 4-4). The one-way ANOVA P-value was P < 0.01 (Appendix E).

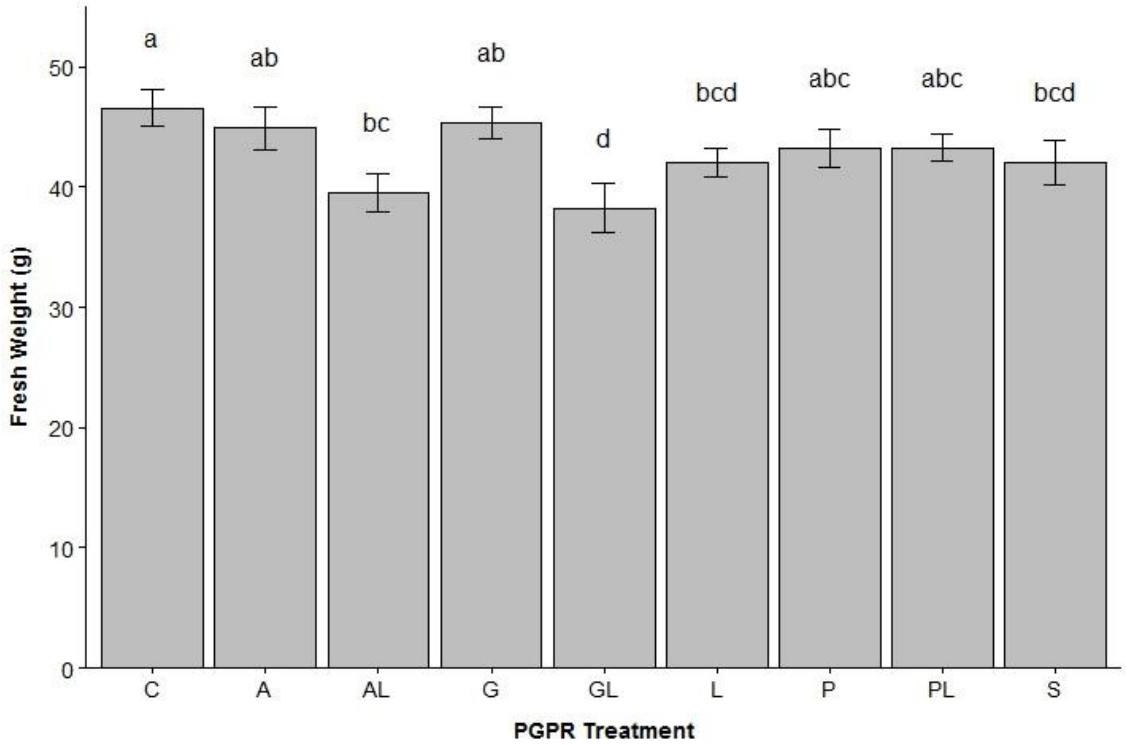

Figure 4-4. Mean NileFiber™ above-ground biomass fresh weight (g) per plant measured on 14 September 2015. C = Control; A = *Azospirillum brasilense* N8; AL = *A. brasilense* N8 + LCO; G = *Gluconacetobacter diazotrophicus* PAL5T; GL = *G. diazotrophicus* PAL5T + LCO; L = LCO (lipo-chitooligosaccharide); P = *Penicillium bilaii*; PL = *P. bilaii* + LCO; S = Seaweed extract (*Ascophyllum nodosum*). Different letters indicate significant difference at $P < 0.05$. Bars indicate standard error. (n = 12).

4.1.2.5 Root Dry Weight

NileFiber™ roots were dried in an oven and weighed eight days post-harvest. Mean root dry weight per plant ranged from 3.07 g (GL) to 4.11 g (C) (Table 4-1). All commercially treated LCO plants weighed significantly less than the control plants (Figure 4-5). NileFiber™ plants treated with a single growth-promoter (A, G) had roots that weighed significantly more than their corresponding combination treatment (AL, GL) except in the case of (P) and (PL) (Figure 4-9). The one-way ANOVA P-value was $P < 0.001$ (Appendix E)

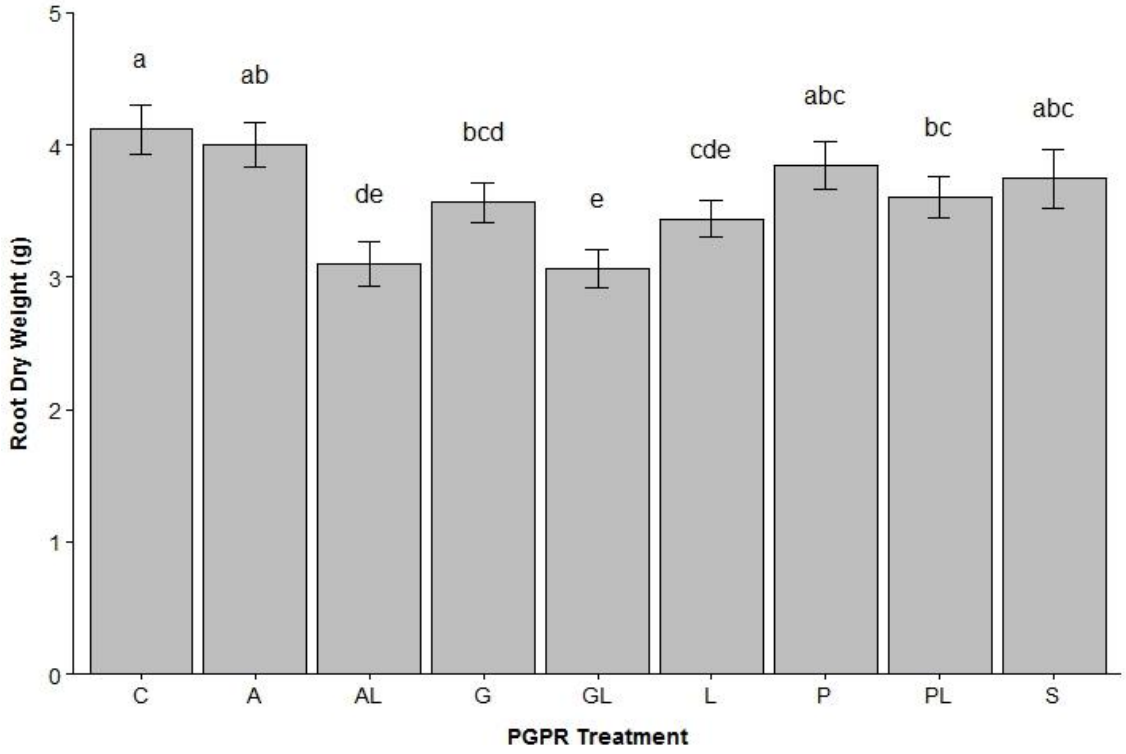

Figure 4-5. Mean NileFiber™ root dry weight (g) per plant measured on 22 September 2015. C = Control; A = *Azospirillum brasilense* N8; AL = *A. brasilense* N8 + LCO; G = *Gluconacetobacter diazotrophicus* PAL5T; GL = *G. diazotrophicus* PAL5T + LCO; L = LCO (lipo-chitooligosaccharide); P = *Penicillium bilaii*; PL = *P. bilaii* + LCO; S = Seaweed extract (*Ascophyllum nodosum*). Different letters indicate significant difference at $P < 0.05$. Bars indicate standard error. (n = 12).

4.1.2.6 Above-Ground Biomass Dry Weight

NileFiber™ above-ground biomass was dried in an oven and weighed eight days post-harvest. Mean above-ground biomass dry weight per plant ranged from 13.90 g (GL) to 16.94 g (C) with no statistical significance to report between treatments (P = 0.6) (Table 4-1) (Appendix E).

4.1.2.7 Above-Ground Biomass Dry Matter Content

Dry matter content is calculated by taking the quotient of sample dry weight over sample wet weight and multiplying by 100 to get the percentage. The mean dry matter content per NileFiber™ plant ranged from 35.01% (P) to 37.56% (AL) with no statistical significance to report between treatments (P = 0.39) (Appendix E). Interestingly, the (AL) treatment had the second lowest mean shoot fresh weight of all the treatments, but had the highest mean dry matter content (Table 4-1).

4.1.3 Results & Statistical Analysis – Bayesian Approach

4.1.3.1 Metric Predicted Variable with One Nominal Predictor Variable

4.1.3.1.1 Plant Height

The height of NileFiber™ was measured from the soil surface to the highest collar region of the main stem at harvest time. The estimate for average plant height was 112.99 cm with a 95 % highest density interval (HDI) from 110.62 – 115.31 cm. From the posterior distribution plots for the group deflection parameters, all of the growth-promoter treatments containing the "L" treatment except "PL" showed a decrease in height, with the "AL" treatment showing the greatest decrease. The control "C" treatment showed the greatest increase in plant height. The posterior distribution for "AL" and "PL" show a credible nonzero difference from the other treatments (given that zero is not within the 95 % HDI). Further, the posterior distribution shows a non-credible difference between the "PL" treatment and the "C" treatment (Appendix E) and a credible nonzero difference between the "AL" treatment and the "C" treatment (Figure 4-6).

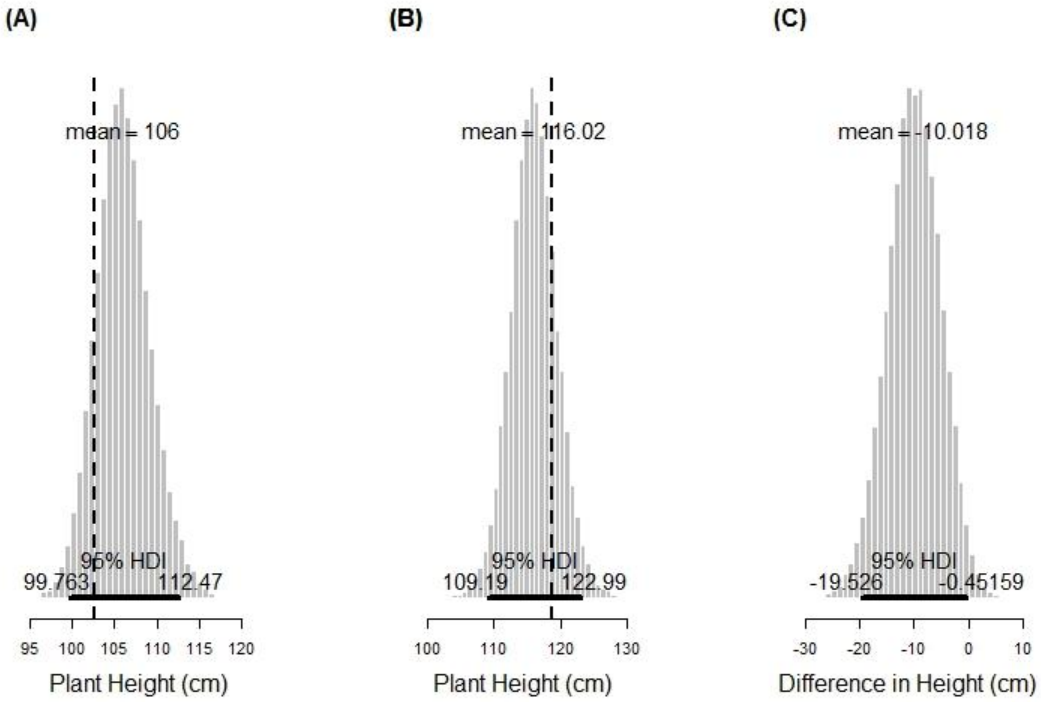

Figure 4-6. Effect of AL treatment on plant height. Included are the posterior distributions for plant heights (cm) under conditions: **(A)** AL treatment (mean = 102.6 cm); **(B)** C treatment (mean = 118.8 cm); and **(C)** the difference between posterior distributions of the AL and C treatments. AL = *A. brasilense* N8 + LCO; C = Control.

4.1.3.1.2 Number of Shoots

The number of shoots (≥ 10 cm) per plant were recorded at harvest time. Unfortunately, the combination of the ordinal nature of these data and the nature of the analysis, this analysis could not be completed using Bayesian statistics.

4.1.3.1.3 Above-Ground Biomass Fresh Weight

NileFiber™ above-ground biomass fresh weight was weighed immediately after being harvested. The estimate for the average above-ground biomass fresh weight per plant was 43.13 g (95 % HDI 41.95 – 44.32 g). None of the treatments showed a credible effect (either an increase or decrease) from this average value. However, some patterns were observed. For example, all of the growth-promoter treatments containing the "L" treatment except "PL" showed a decrease in above-ground biomass fresh weight. The control "C" treatment showed the greatest increase in above-ground biomass fresh weight out of all treatments, while the "GL" treatment showed the greatest decline.

4.1.3.1.4 Root Dry Weight

NileFiber™ roots were oven-dried and weighed eight days post-harvest. The estimate for the average root dry weight per plant was 3.6 g (95% HDI 3.47 – 3.73 g). All of the growth-promoter treatments containing the "L" treatment except "PL" showed a decrease in root dry weight, although the increase in shoot dry weight of the "PL" treatment was minimal. The control "C" treatment showed the greatest increase in root dry weight, while the "GL" treatment showed the greatest decline. The posterior distributions for "AL" and "GL" show a credible nonzero difference from the other treatments (given that zero is not within the 95 % HDI). Further, the posterior

distribution shows credible nonzero differences between “AL” and “C” treatments (Figure 4-7) and “GL” and “C” treatments (Figure 4-8).

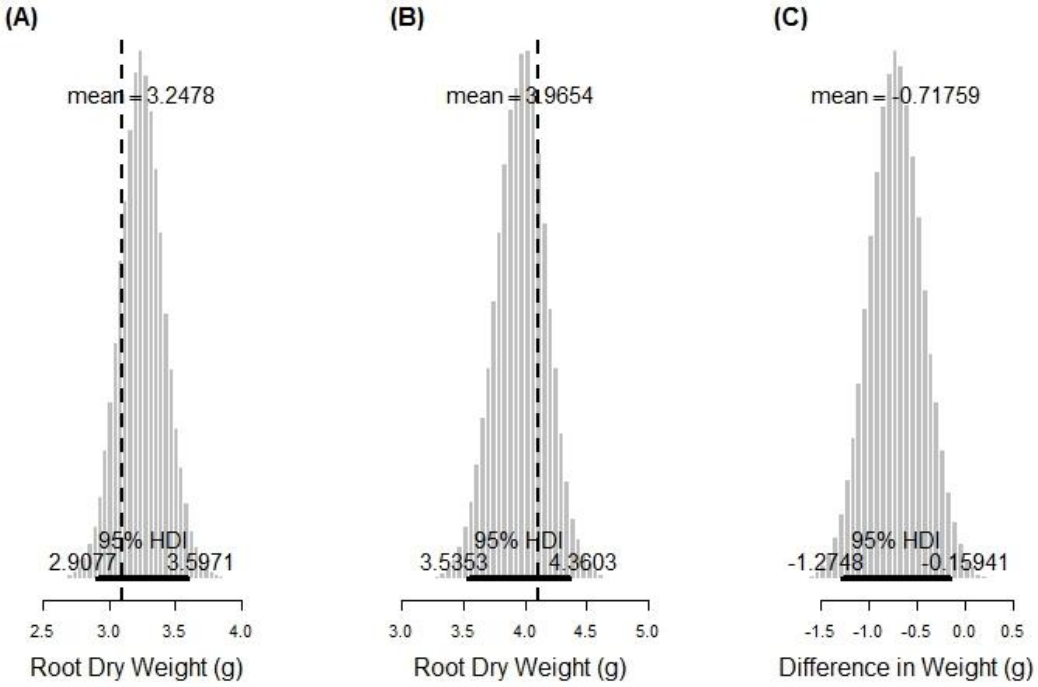

Figure 4-7. Effect of AL treatment on root dry weight (g). Included are the posterior distributions for root dry weight (g) per plant under conditions: **(A)** AL treatment (mean = 3.10 g); **(B)** C treatment (mean = 4.11 g); and **(C)** the difference between posterior distributions of the AL and C treatments. AL = *A. brasilense* N8 + LCO; C = Control.

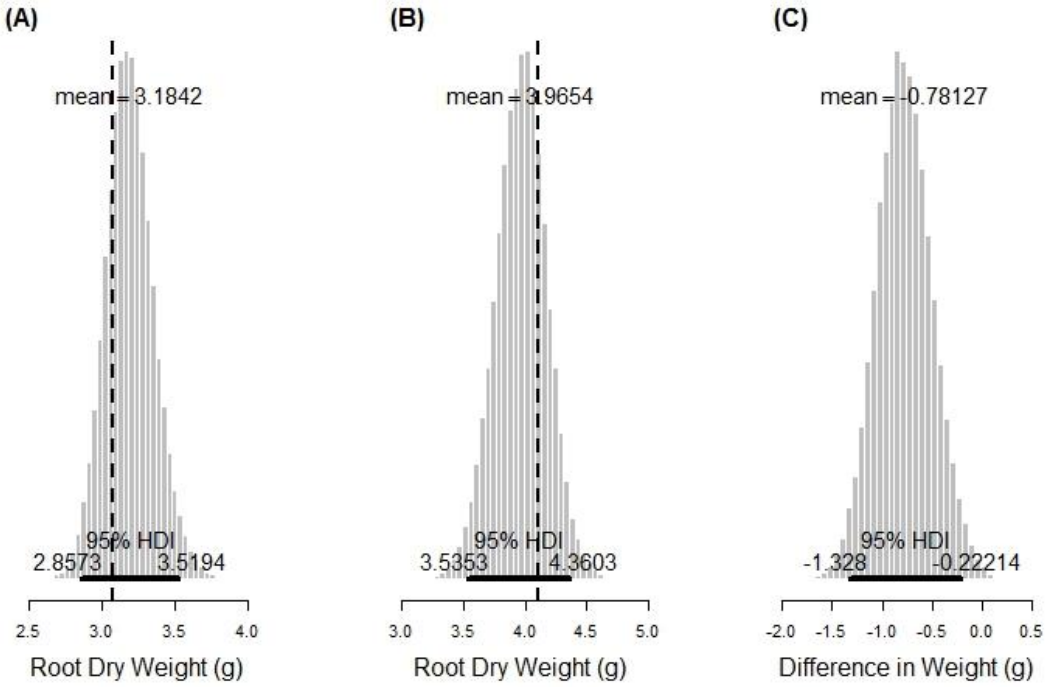

Figure 4-8. Effect of GL treatment on root dry weight (g). Included are the posterior distributions for root dry weight (g) per plant under conditions: **(A)** GL treatment (mean = 3.07 g); **(B)** C treatment (mean = 4.11 g); and **(C)** the difference between posterior distributions of the GL and C treatments. GL = *G. diazotrophicus* PAL5T + LCO; C = Control.

4.1.3.1.5 Above-Ground Biomass Dry Weight

NileFiber™ above-ground biomass was oven-dried and weighed eight days post-harvest. The estimate for the average above-ground biomass dry weight per plant was 15.54 g (95% HDI 15.081 – 16.003 g). All of the growth-promoter treatments containing the "L" treatment showed a decrease in above-ground biomass dry weight. The control "C" treatment showed the greatest increase in above-ground biomass dry weight, while the "GL" treatment showed the greatest decline. The posterior distributions showed no credible nonzero differences between the treatments.

4.1.3.1.6 Above-Ground Biomass Dry Matter Content

Dry matter content (DMC) is the quotient of sample dry weight by sample wet weight multiplied by 100 to make a percentage. The estimate for the average dry matter content was 36.2 % (95 % HDI 35.774 – 36.535 %). The "S" treatment showed the greatest increase in DMC while the "P" treatment showed the greatest decline. The posterior distributions showed no credible nonzero differences between the treatments.

4.2 Experiment 2 – Field Soil Drench

NileFiber™ treated with six growth-promoter treatments through soil drench inoculation seventy days after the plantlets were transplanted out of the growth chamber. Treatments included: A = *Azospirillum brasilense*; C = Control; D = *Gluconacetobacter azotocaptans*; G = *Gluconacetobacter diazotrophicus*; P = *Penicillium bilaii*; S = Seaweed extract (*Ascophyllum nodosum*).

4.2.1 Field Conditions

Mean monthly temperatures during the condensed growing season of 2014 (July - October) were 19.9, 17.6, 13.8 and 10.4 ºC, respectively (Environment Canada 2015a). The total precipitation during this condensed growing season was approximately 369.8 mm; 92.7, 84.0, 65.1 and 128.0 mm from July to October respectively (Environment Canada 2015a). Precipitation was slightly above average, with the exception of below average precipitation in September (Figure 4-9).

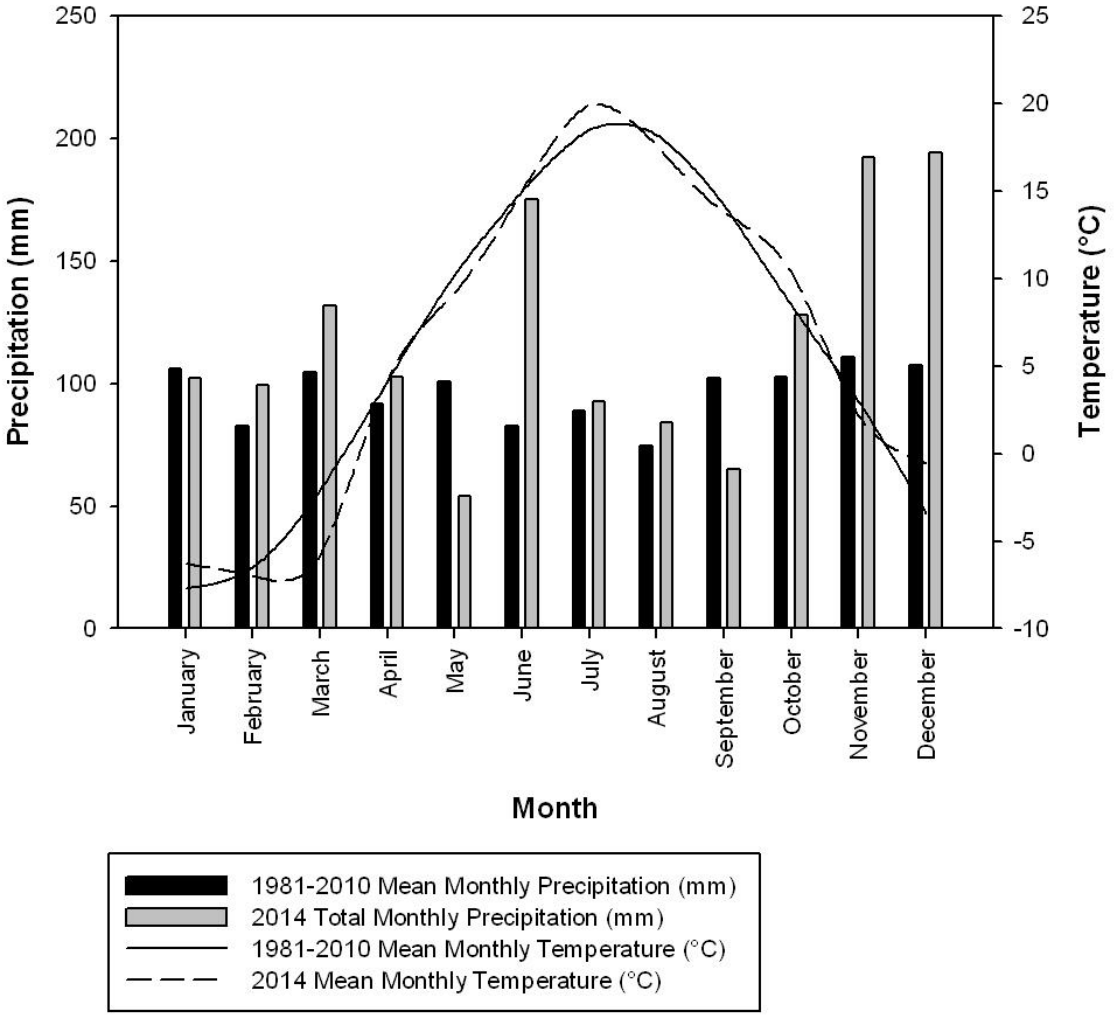

Figure 4-9. Total precipitation (mm) and mean temperature (°C) at Nappan, NS in 2014 compared to mean precipitation (mm) and temperature (°C) from 1981-2010 (Environment Canada 2015b).

Mean monthly temperatures during the growing season of 2015 were 10.8, 13.0, 17.8, 20.6, 16.0 and 7.6 ºC, from May to October respectively (Environment Canada 2015a). The total precipitation during the 2013 growing season was approximately 603.8 mm; 58.6, 202.1, 52.8, 100.5, 85.2 and 104.6 mm from May to October (Environment Canada 2015a). Precipitation was inconsistent in comparison to climate averages. May and September experienced below average precipitation and June, July, August and October experienced above average precipitation (Figure 4-10).

The soil pH was measured at 6.5 in 2013.

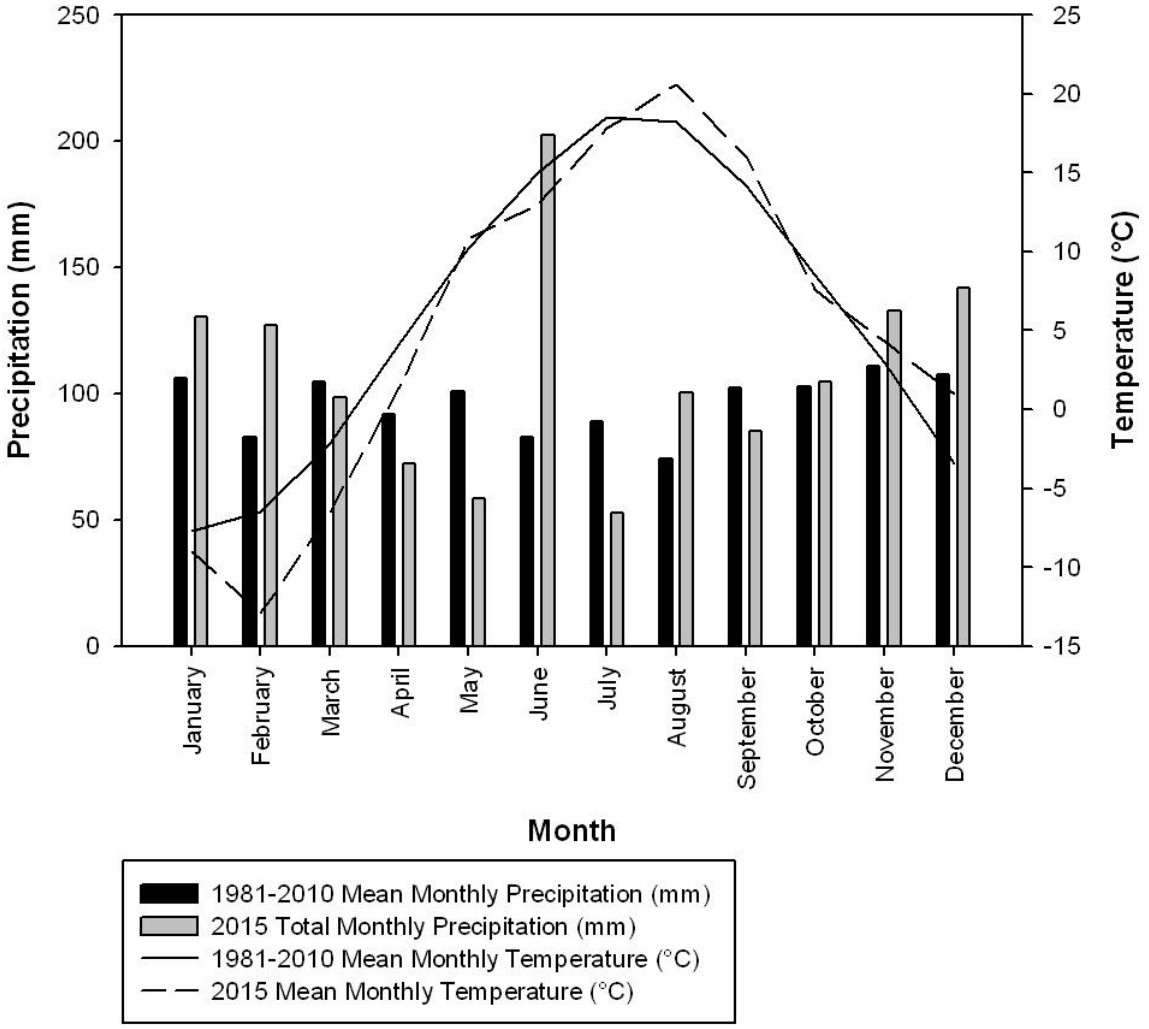

Figure 4-10. Total precipitation (mm) and mean temperature (°C) at Nappan, NS in 2015 compared to mean precipitation (mm) and temperature (°C) from 1981-2010 (Environment Canada 2015b).

4.2.2 Results & Statistical Analysis – Frequentist Approach

4.2.2.1 Plant Height

The height of NileFiber™ was measured from the soil surface to the highest collar region on the main stem at the time of harvest. The mean height per plant ranged from 48 cm (D) to 52 cm (P) with no statistical significance to report between treatments (P = 0.487) (Table 4-2) (Appendix E).

Table 4-2. Mean values of growth parameters measured in the field experiment at the time of harvest (08 October 2015) or seven days post-harvest (15 October 2015).

Treatment	Mean Height (cm)	Mean Number of Shoots	Mean Above-Ground Biomass Fresh Weight (g)	Mean Above-Ground Biomass Dry Weight (g)	Mean Above-Ground Biomass Dry Matter Content (%)
C	50	9	2255.93	711.32	31.56
A	49	7	2280.93	697.19	30.59
D	48	9	2362.93	738.34	31.25
G	49	8	2198.93	671.88	30.64
P	52	8	2378.53	730.06	30.65
S	51	8	2309.53	716.10	30.98

4.2.2.2 Number of Shoots

Shoot number per plant was recorded at the time of harvest, as all shoots greater than 10 cm in height were included. Mean shoot number per plant ranged from 7 (A) to 9 (C) shoots per plant (Table 4-2) (Figure 4-11). The one-way ANOVA P-value was $P < 0.01$ (Appendix E).

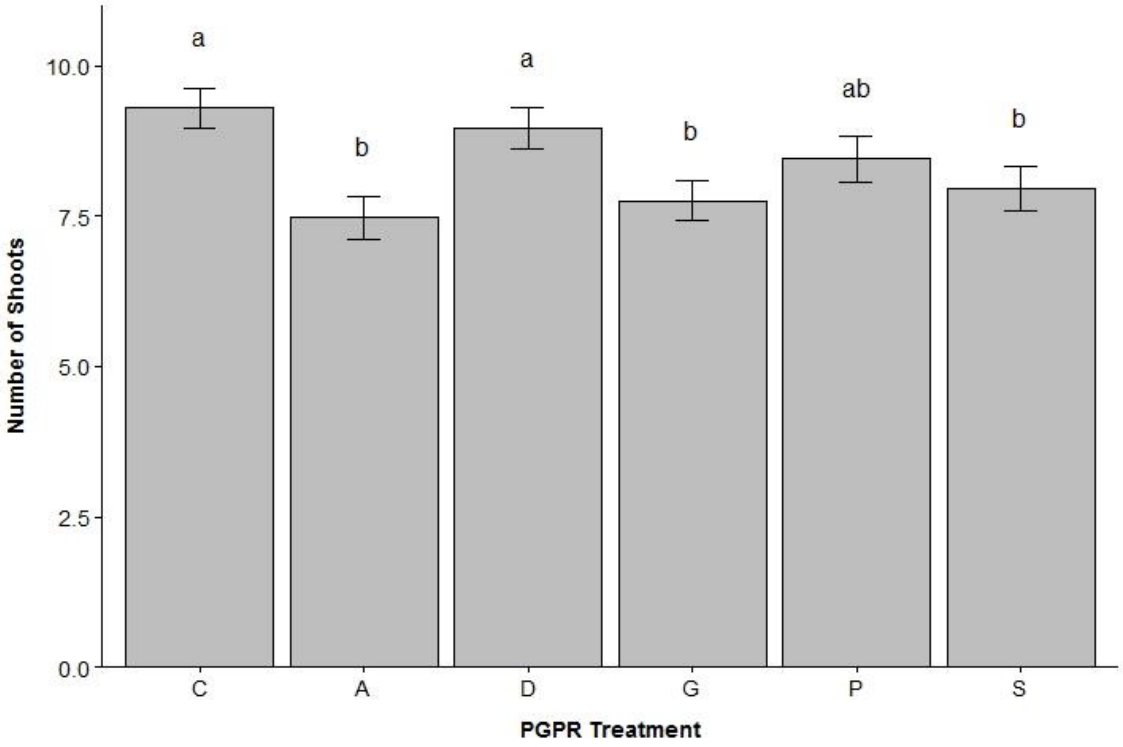

Figure 4-11. Mean NileFiber™ number of shoots per plant measured on 8 October 2015. C = Control; A = *Azospirillum brasilense* N8; D = *Gluconacetobacter azotocaptans* DS1; G = *Gluconacetobacter diazotrophicus* PAL5T; P = *Penicillium bilaii*; S = Seaweed extract (*Ascophyllum nodosum*). Different letters indicate significant difference at $P < 0.05$. Bars indicate standard error. (n = 45).

4.2.2.3 Above-Ground Biomass Fresh Weight

NileFiber™ above-ground biomass fresh weight was weighed immediately after being harvested. Mean above-ground biomass fresh weight per plot ranged from 2198.93 g (G) to 2378.53 (P) with no statistical difference to report between treatments (P = 0.949) (Table 4-2) (Appendix E).

4.2.2.4 Above-Ground Biomass Dry Weight

NileFiber™ above-ground biomass was dried in an oven and weighed seven days post-harvest. Mean above-ground biomass dry weight per plot ranged from 671.88 g (G) to 738.34 g (D) with no statistical significance to report between treatments (P = 0.908) (Table 4-2) (Appendix E).

4.2.2.5 Above-Ground Biomass Dry Matter Content

The mean dry matter content per NileFiber™ plant ranged from 30.59% (A) to 31.56% (C) with no statistical significance to report between treatments (P = 0.182) (Table 4-2) (Appendix E).

4.2.3 Results & Statistical Analysis – Bayesian Approach

4.2.3.1 Metric Predicted Variable with One Nominal Predictor Variable

4.2.3.1.1 Plant Height

The height of NileFiber™ was measured from the soil surface to the highest collar region of the main stem at harvest time. The estimate for average plant height was 49.6 cm (95 % HDI 48.34 – 50.853 cm). From the posterior distribution plots for the group deflection parameters, all growth-promoter treatments except S and P show a decrease in height. The S treatment showed the greatest increase in plant height while the D treatment showed the greatest decline. The posterior distributions showed no credible nonzero differences between the treatments.

4.2.3.1.2 Number of Shoots

The number of shoots (≥ 10 cm) per plant were recorded at harvest time. The estimate for the average shoots per plant was 8.181 (95 % HDI 7.88 – 8.4786). From the posterior distribution plots, the growth-promoter treatments “S”, “A” and “G” showed a decrease in shoots per plant while treatments “C”, “D” and “P” treatments showed an increase in shoots per plant. The posterior distribution for “A” and “C” show a credible nonzero difference from the other treatments (given that zero is not within the 95 % HDI). Further, the posterior distribution shows a non-credible difference between the “A” treatment and the “C” treatment (Figure 4-12) and the “G” treatment and the “C” treatment (Figure 4-13).

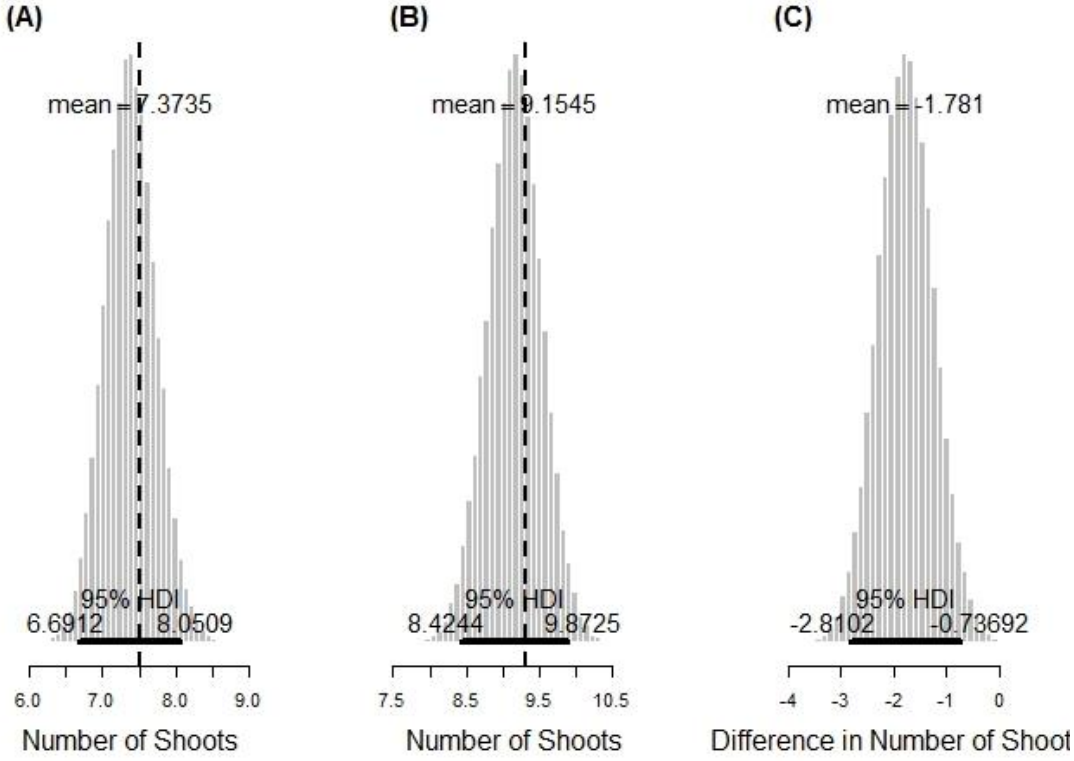

Figure 4-12. Effect of A treatment on number of shoots per plant. Included are the posterior distributions for number of shoots per plant under conditions: **(A)** A treatment (mean = 7.5); **(B)** C treatment (mean = 9.3); and **(C)** the difference between posterior distributions of the A and C treatments. A = *A. brasilense* N8; C = Control.

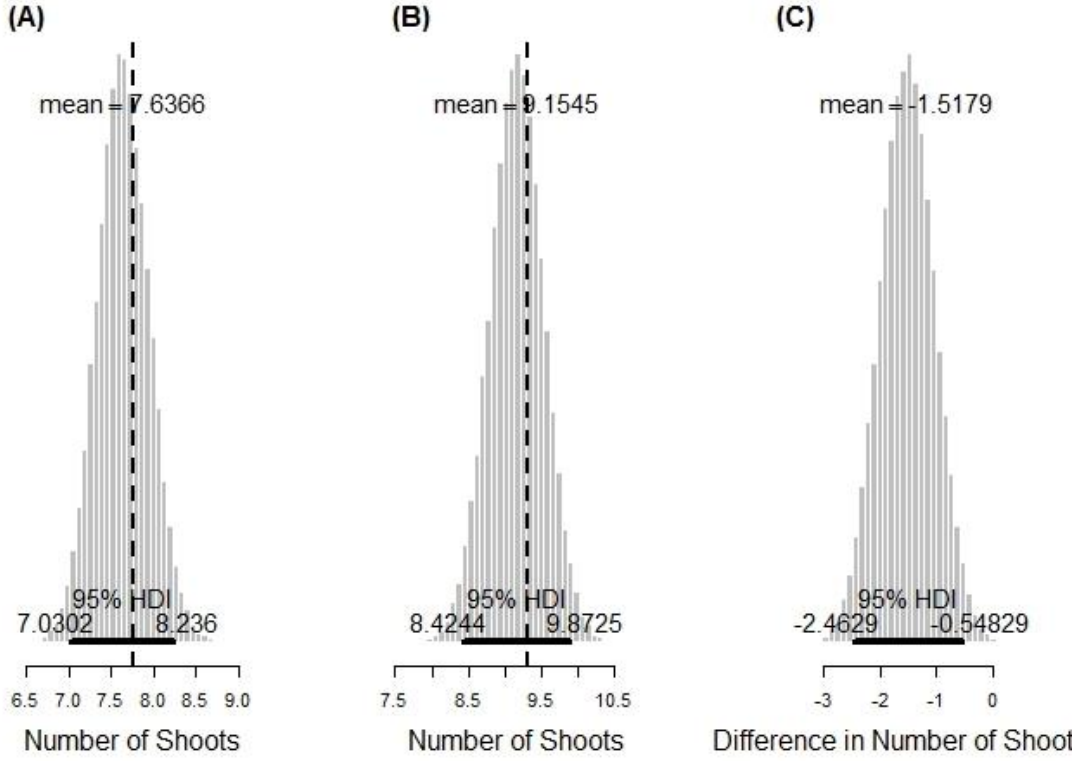

Figure 4-13. Effect of G treatment on number of shoots per plant. Included are the posterior distributions for number of shoots per plant under conditions: **(A)** G treatment (mean = 7.75); **(B)** C treatment (mean = 9.3); and **(C)** the difference between posterior distributions of the G and C treatments. G treatment = *G. diazotrophicus* PAL5T; C = Control.

4.2.3.1.3 Above-Ground Biomass Fresh Weight

NileFiber™ above-ground biomass fresh weight was weighed immediately after being harvested. The estimate for the average above-ground biomass fresh weight per plant was 2296 g (95 % HDI 2168.6 – 2422.6 g). None of the treatments showed a credible effect (either an increase or decrease) from this average value. The control "C" treatment showed the greatest decline in above-ground biomass fresh weight out of all treatments, while the "P" treatment showed the greatest increase. The posterior distributions showed no credible nonzero differences between the treatments.

4.2.3.1.4 Above-Ground Biomass Dry Weight

NileFiber™ above-ground biomass was oven-dried and weighed seven days post-harvest. The estimate for the average above-ground biomass dry weight per plant was 711.36 g (95% HDI 671.03 – 749.99 g). None of the treatments showed a credible effect (either an increase or decrease) from this average value. The "D" treatment showed the greatest increase in above-ground biomass dry weight, while the "G" treatment showed the greatest decline. The posterior distributions showed no credible nonzero differences between the treatments.

4.2.3.1.5 Above-Ground Biomass Dry Matter Content

The estimate for the average dry matter content was 30.875 % (95 % HDI 30.583 – 31.185 %). From the posterior distribution plots, the growth-promoter treatments "S", "D" and "C" showed an increase in DMC while treatments "A", "G" and "P" showed an decrease in DMC. The "C" treatment showed the greatest increase in DMC while the

"A" treatment showed the greatest decline. The posterior distributions showed no credible nonzero differences between the treatments.

4.2.3.2 Metric Predicted Variable with Multiple Nominal Predictor Variables

4.2.3.2.1 Plant Height

The height of NileFiber™ was measured from the soil surface to the highest collar region of the main stem at harvest time. The estimate for average plant height across experiments was 82.782 cm (95 % HDI 80.941 – 84.615 cm). From the posterior distribution plots of the differences between experiments, the greatest difference in plant height between experiments was the "C" treatment, and the least difference was the "P" treatment. The posterior distributions showed credible nonzero differences between plant height in the greenhouse and the field in all treatments (Figure 4-14).

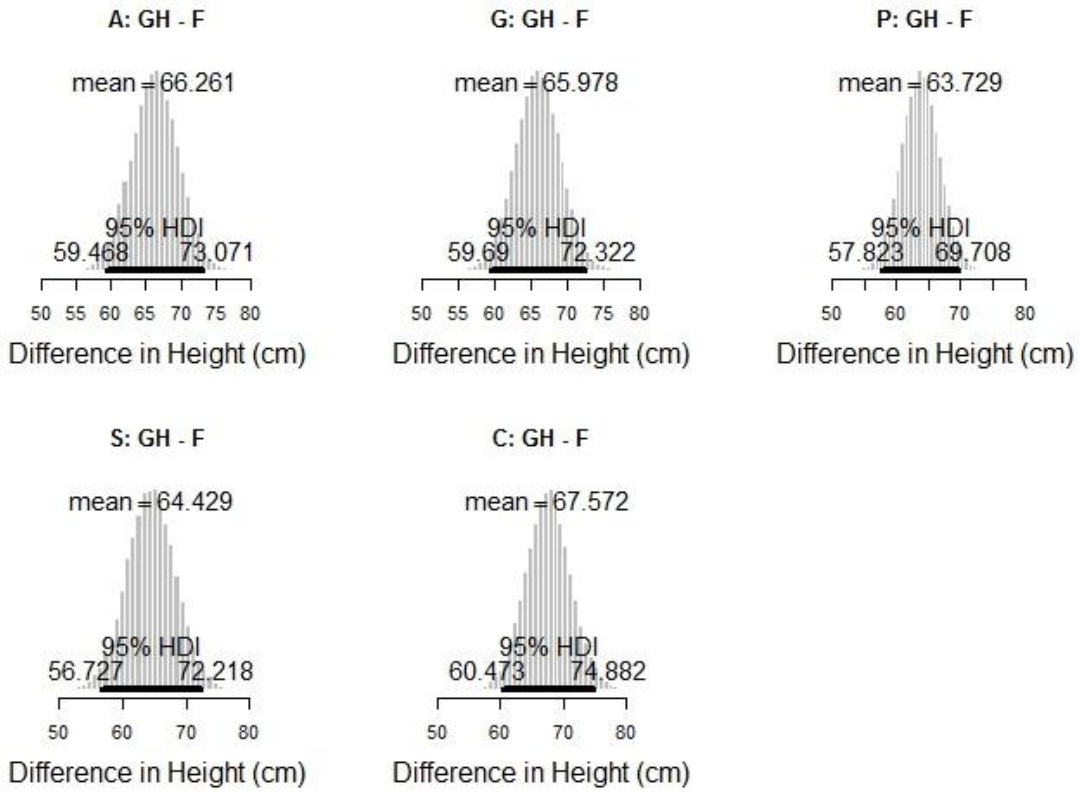

Figure 4-14. Effect of growth-promoter treatments on plant height between experiments. Included are the differences between posterior distributions for plant height (cm) per plant for all treatments. A = *A. brasilense* N8; G = *G. diazotrophicus* PAL5T; P = *P. bilaii*; S = Seaweed extract (*A. nodosum*); C = Control.

4.2.3.2.2 Number of Shoots

The number of shoots (≥ 10 cm) per plant were recorded at harvest time. The estimate for the average shoots per plant was 5.8131 (95 % HDI 5.6197 – 6.0172). From the posterior distribution plots of the differences between experiments, the greatest difference in number of shoots between experiments was the “C” treatment, and the least difference was the “G” treatment. The posterior distributions showed credible nonzero differences between plant height in the greenhouse and the field in all treatments (Figure 4-15).

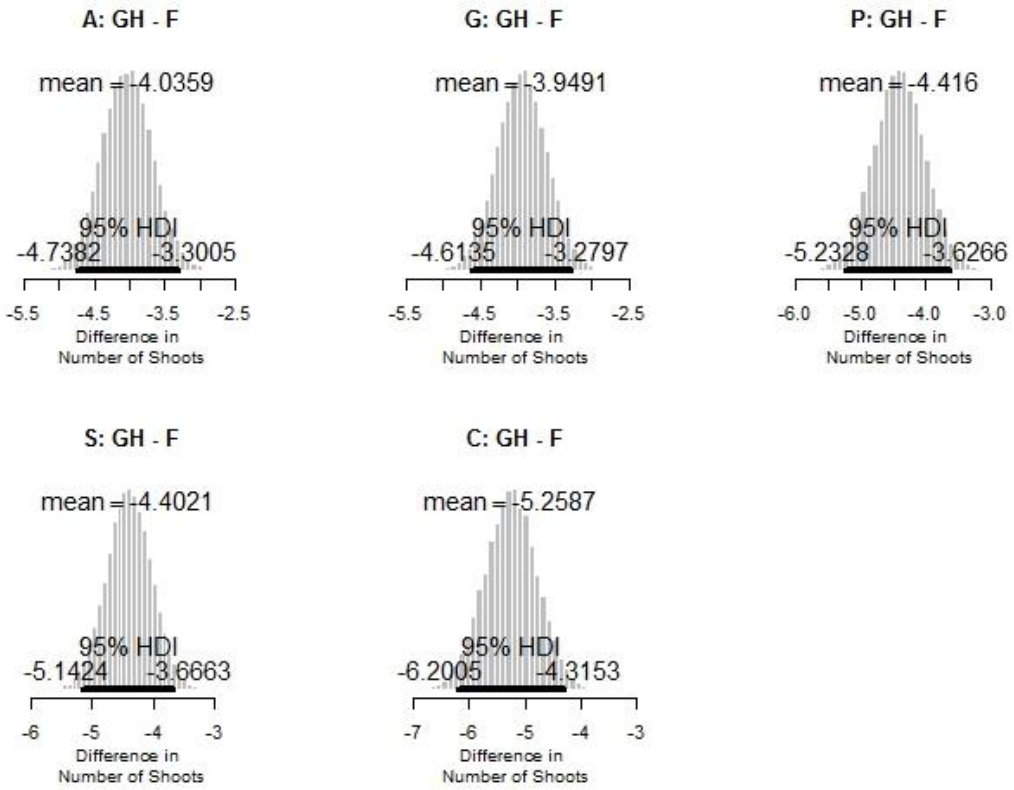

Figure 4-15. Effect of growth-promoter treatments on number of shoots per plant between experiments. Included are the differences between posterior distributions for number of shoots per plant for all treatments. A = *A. brasilense* N8; G = *G. diazotrophicus* PAL5T; P = *P. bilaii*; S = Seaweed extract (*A. nodosum*); C = Control.

4.3 Experiment 3 – Greenhouse Soil Drench

NileFiber™ treated with fifteen growth-promoter treatments through root soaking immediately after being removed from the growth chamber. Treatments included: A = *Azospirillum brasilense* N8; AL = *A. brasilense* + LCO; C = Control; D = *Gluconacetobacter azotocaptans* DS1; DL = *G. azotocaptans* DS1 + LCO; G = *Gluconacetobacter diazotrophicus* PAL5T; GL = *Gluconacetobacter diazotrophicus* PAL5T + LCO; PL = *Gluconacetobacter diazotrophicus* PAL5T LsdB++; PLL = *Gluconacetobacter diazotrophicus* PAL5T LsdB++ + LCO; GS = *Gluconacetobacter diazotrophicus* SRT4; GSL = *Gluconacetobacter diazotrophicus* SRT4 + LCO; P = *Penicillium bilaii*; S = Seaweed extract (*Ascophyllum nodosum*); VP = *Variovorax paradoxus* JM63; VPL = *Gluconacetobacter diazotrophicus* SRT4 + LCO.

4.3.1 Greenhouse Conditions

Mean hours of daily illumination recorded for Halifax, NS during the 2016 greenhouse experiment showed insignificant deviation from daily illumination records for the past twenty years (Figure 4-16). Supplemental lighting was used in the greenhouse to maintain a photoperiod of 16/8 hours (day/night), with minimum photosynthetic photon flux density (PPFD) at plant height of 300 μmol m-2 s-1.

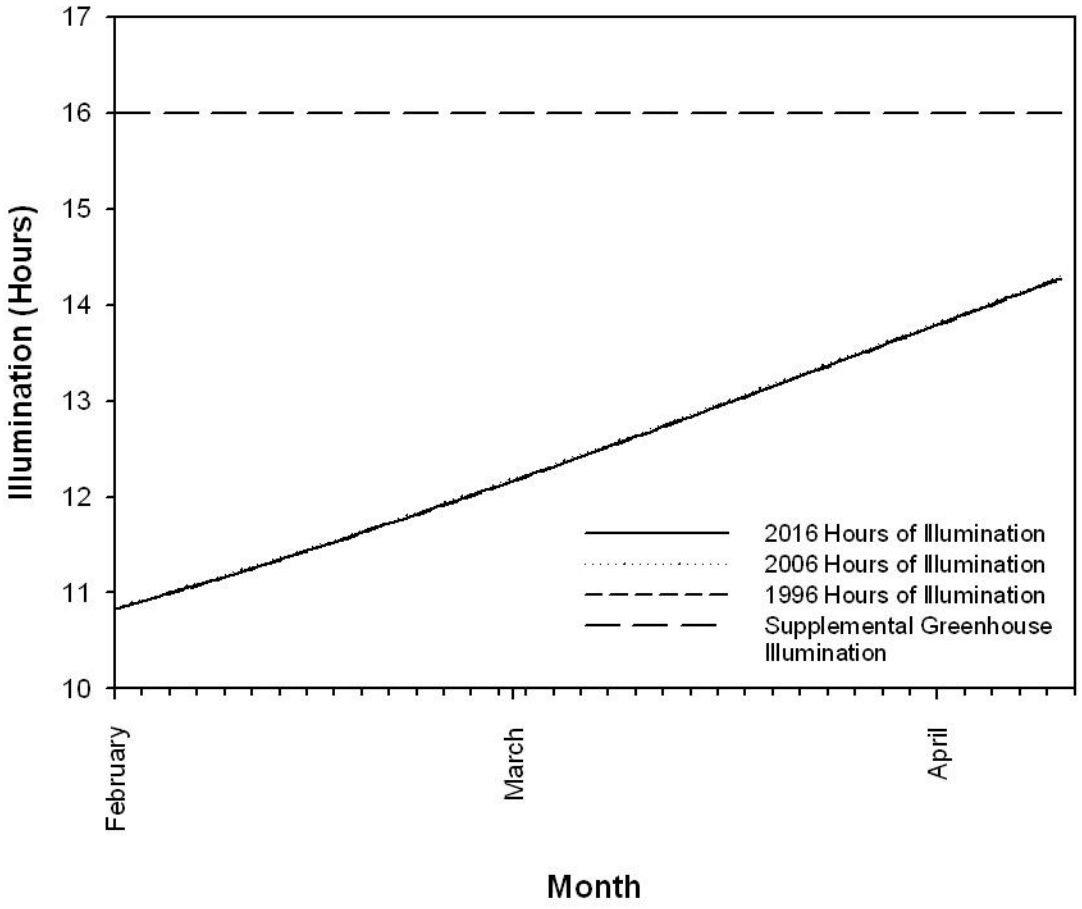

Figure 4-16. Mean hours of daily illumination for Halifax, NS for the duration of the second greenhouse experiment and two other time periods (1996, 2006 and 2016) (National Research Council, 2016) and the supplemental greenhouse illumination (16 hours day^{-1}).

The internal greenhouse temperature was set at 25/18 °C (day/night) and controlled by heating and ventilation. The internal temperature fluctuated consistently with external temperature (Figure 4-17), but the temperature difference is not as relevant as outside growth would not occur in this season.

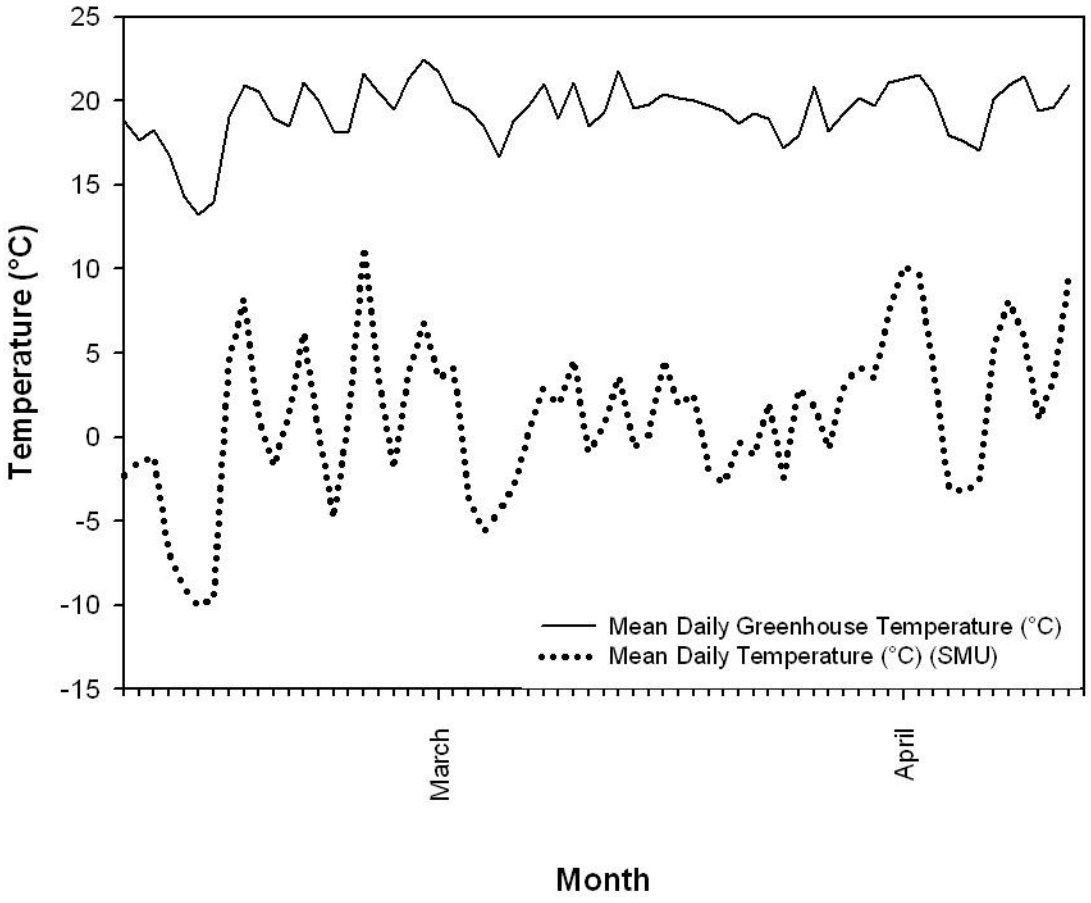

Figure 4-17. Mean daily internal temperature of the greenhouse (HOBO® Relative Humidity / Temperature / Light / External Data Logger) and mean daily external greenhouse temperature (SMU Observatory / Environmental Science **INOVASCO115**, 2016) during the second greenhouse experiment (2016).

4.3.2 Results & Statistical Analysis – Frequentist Approach

4.3.2.1 Plant Height

The height of NileFiber™ was measured from the soil surface to the highest leaf tip on the main stem. Mean height per plant ranged from 33.07 cm (GL and GSL) to 37 cm (GP) (Table 4-3). Of the combination treatments (commercial LCO), GL and GSL were significantly shorter than their single growth-promoter counterparts (G and GS respectively) (Figure 4-18). Treatment DL was significantly greater than its single growth-promoter counterpart (D). NileFiber™ plants treated with G were the only plants significantly taller than the untreated control (C) plants. The one-way ANOVA P-value was $P < 0.05$ (Appendix E).

Table 4-3. Greenhouse Experiment #2: results of fourteen growth-promoter treatments applied to NileFiber™ against an untreated control. Mean plant height (cm), number of shoots per plant, biomass fresh weight per plant (g) are reported. Treatments which were significantly different than the untreated control at $P \leq 0.05$ are indicated in bold with an asterisk (*). Standard errors are reported in brackets.

Treatment		Mean Height (cm)	Number of Shoots per Plant	Mean Above-Ground Biomass Fresh Weight (g)
A. brasilense N8	A	34.8 (4.2)	2.8 (0.77)	3.2 (0.90)
A. brasilense N8 + LCO	AL	36.07 (4.2)	**3.267* (0.88)**	**4.067* (0.78)**
G. azotocaptans DS1	D	33.13 (4.2)	**3.067* (0.70)**	2.867 (0.58)
G. azotocaptans DS1 + LCO	DL	35.4 (3.8)	**3* (0.65)**	**3.633* (0.74)**
G. diazatrophicus PAL5T	G	**37* (3.7)**	2.667 (0.90)	**3.633* (0.77)**
G. diazatrophicus PAL5T + LCO	GL	33.07 (2.4)	2.867 (0.74)	**3.6* (1.11)**
G. diazatrophicus PAL5T LsdB++	PL	36.07 (3.3)	**3.067* (0.80)**	3.033 (0.88)
G. diazatrophicus PAL5T LsdB++ + LCO	PLL	34.6 (2.7)	**2.933* (0.70)**	3.367 (0.58)
G. diazatrophicus SRT4	GS	34.73 (3.7)	2.4 (0.51)	**3.567* (0.46)**
G. diazatrophicus SRT4 + LCO	GSL	33.07 (4.6)	2.733 (0.59)	2.9 (0.51)
P. bilaii	P	36.33 (4.0)	2.8 (0.68)	**3.733* (0.98)**
A. nodosum	S	33.8 (3.1)	2.4 (0.63)	**2.3* (0.68)**
V. paradoxus JM63	VP	34.93 (4.1)	2.867 (0.64)	**3.633* (0.67)**
V. paradoxus JM63 + LCO	VPL	36.13 (3.2)	**3* (0.76)**	**3.733* (0.59)**
Control	C	34.13 (4.4)	2.467 (0.92)	3 (0.91)

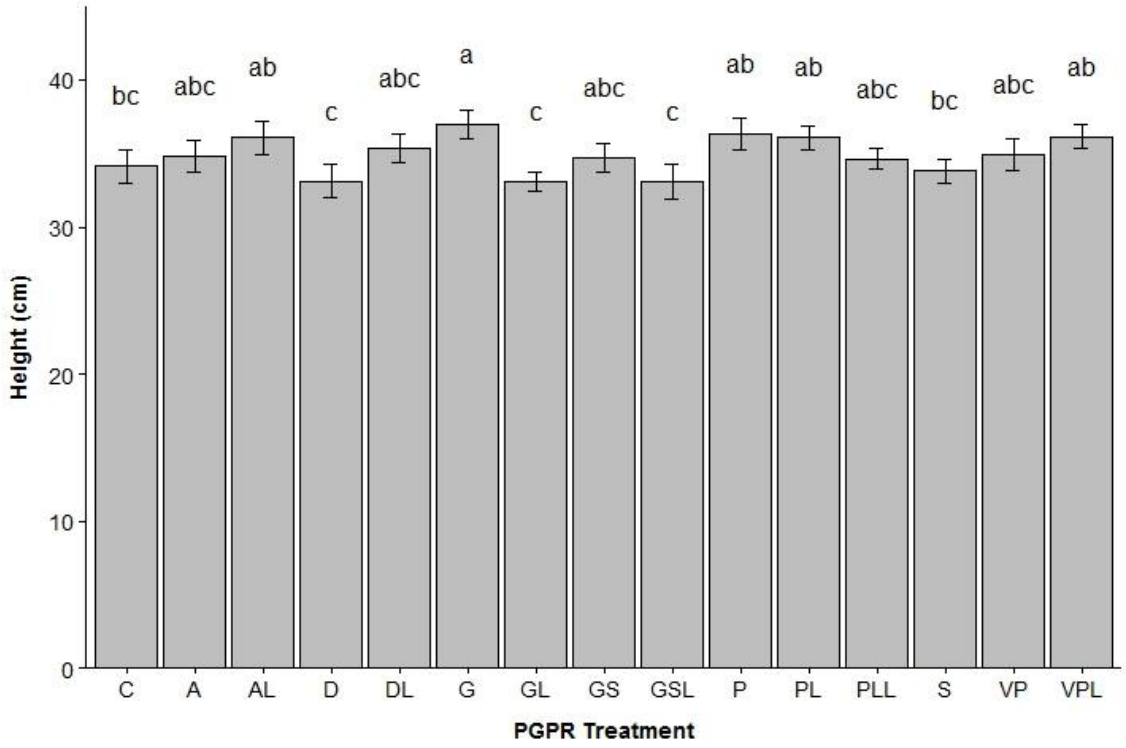

Figure 4-18. Mean NileFiber™ height per plant measured on 11 April 2016. C = Control; A = *Azospirillum brasilense* N8; AL = *A. brasilense* N8 + LCO; D = *Gluconacetobacter azotocaptans* DS1; DL = *G. azotocaptans* DS1 + LCO; G = *Gluconacetobacter diazotrophicus* PAL5T; GL = *Gluconacetobacter diazotrophicus* PAL5T + LCO; PL = *Gluconacetobacter diazotrophicus* PAL5T LsdB++; PLL = *Gluconacetobacter diazotrophicus* PAL5T LsdB++ + LCO; GS = *Gluconacetobacter diazotrophicus* SRT4; GSL = *Gluconacetobacter diazotrophicus* SRT4 + LCO; P = *Penicillium bilaii*; S = Seaweed extract (*Ascophyllum nodosum*); VP = *Variovorax paradoxus* JM63; VPL = *V. paradoxus* JM63 + LCO. Different letters indicate significant difference at $P < 0.05$. Bars indicate standard error. (n = 15).

4.3.2.2 Number of Shoots

Shoot number per plant was recorded at the time of harvest, as all shoots greater than 5 cm in height were included. Mean shoot number per plant ranged from 2.4 (GS & S) to 3.267 (AL) (Table 4-3). Of the five treatments with significantly greater shoots per plant compared to the untreated control (C), three of these treatments were combination treatments (AL, DL & VPL) (Figure 4-19). The one-way ANOVA P-value was $P < 0.01$ (Appendix E).

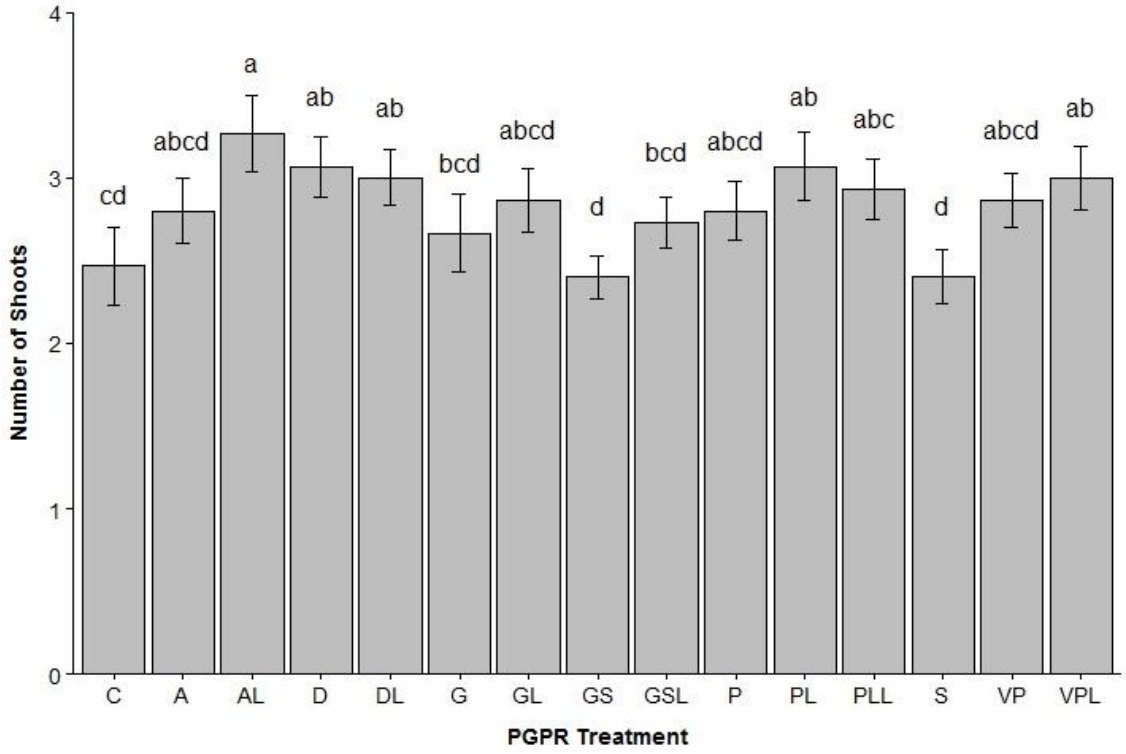

Figure 4-19. Mean NileFiber™ shoots per plant measured on 11 April 2016. C = Control; A = *Azospirillum brasilense* N8; AL = *A. brasilense* N8 + LCO; D = *Gluconacetobacter azotocaptans* DS1; DL = *G. azotocaptans* DS1 + LCO; G = *Gluconacetobacter diazotrophicus* PAL5T; GL = *Gluconacetobacter diazotrophicus* PAL5T + LCO; PL = *Gluconacetobacter diazotrophicus* PAL5T LsdB++; PLL = *Gluconacetobacter diazotrophicus* PAL5T LsdB++ + LCO; GS = *Gluconacetobacter diazotrophicus* SRT4; GSL = *Gluconacetobacter diazotrophicus* SRT4 + LCO; P = *Penicillium bilaii*; S = Seaweed extract (*Ascophyllum nodosum*); VP = *Variovorax paradoxus* JM63; VPL = *V. paradoxus* JM63 + LCO. Different letters indicate significant difference at $P < 0.05$. Bars indicate standard error. (n = 15).

4.3.2.3 Above-Ground Biomass Fresh Weight

NileFiber™ above-ground biomass fresh weight was weighed immediately after being harvested. The combination treatment AL had the greatest mean above-ground biomass fresh weight per plant (4.067 g) and the S treatment had the lowest (2.3 g) (Table 4-3). NileFiber™ plants treated with the commercial LCO combination treatments (except GL & GSL) had greater mean fresh weights than their corresponding single treatment, AL and DL weighing significantly greater than A and D respectively (Figure 4-20). Of the seven treatments with significantly greater biomass fresh weight per plant compared to the untreated control (C), four of these treatments were combination treatments (AL, DL, GL & VPL). The one-way ANOVA P-value was $P < 0.0001$. (Appendix E).

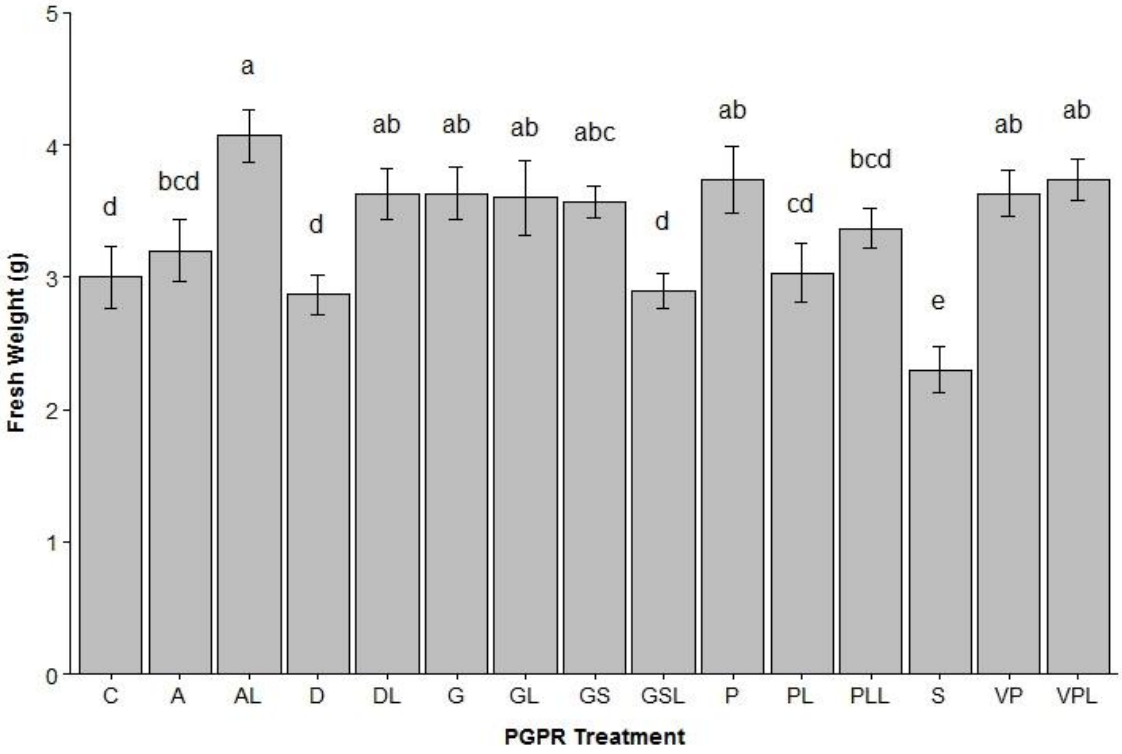

Figure 4-20. Mean NileFiber™ above-ground biomass fresh weight (g) per plant measured on 11 – 12 April 2016. C = Control; A = *Azospirillum brasilense* N8; AL = *A. brasilense* N8 + LCO; D = *Gluconacetobacter azotocaptans* DS1; DL = *G. azotocaptans* DS1 + LCO; G = *Gluconacetobacter diazotrophicus* PAL5T; GL = *Gluconacetobacter diazotrophicus* PAL5T + LCO; PL = *Gluconacetobacter diazotrophicus* PAL5T LsdB++; PLL = *Gluconacetobacter diazotrophicus* PAL5T LsdB++ + LCO; GS = *Gluconacetobacter diazotrophicus* SRT4; GSL = *Gluconacetobacter diazotrophicus* SRT4 + LCO; P = *Penicillium bilaii*; S = Seaweed extract (*Ascophyllum nodosum*); VP = *Variovorax paradoxus* JM63; VPL = *V. paradoxus* JM63 + LCO. Different letters indicate significant difference at $P < 0.05$. Bars indicate standard error. (n = 15).

4.3.2.4 Root Dry Weight

NileFiber™ roots were dried in an oven and weighed eight days post-harvest. Mean root dry weight per plant ranged from 0.41 g (D) to 0.6253 g (PL) (Table 4-4). Only one growth-promoter treatment (PL) showed significantly greater root dry weight than the untreated control (C), and two treatments (D & S) were significantly lighter (Figure 4-21). Three of the six combination treatments had greater root dry weights than their corresponding single treatment (AL, DL & GSL) with only DL showing statistical significance. The combination treatment PLL showed significantly lighter root dry weight than its corresponding single treatment (PL). The one-way ANOVA P-value was $P < 0.0001$ (Appendix E).

Table 4-4. Greenhouse Experiment #2: results of fourteen growth-promoter treatments applied to NileFiber™ against an untreated control. Mean root dry weight (g), biomass dry weight per plant (g) and dry matter content (%) are reported. Treatments which were significantly different than the untreated control at $P \leq 0.05$ are indicated in bold with an asterisk (*). Standard errors are reported in brackets.

Treatment		Mean Root Dry Weight (g)	Mean Above-Ground Biomass Dry Weight (g)	Mean Above-Ground Biomass Dry Matter Content (%)
A. brasilense N8	A	0.5167 (0.13)	**1.058* (0.23)**	**35.31* (12.23)**
A. brasilense N8 + LCO	AL	0.5207 (0.09)	**1.216* (0.19)**	30.16 (2.91)
G. azotocaptans DS1	D	**0.41* (0.08)**	**1.003* (0.21)**	**35.04* (2.61)**
G. azotocaptans DS1 + LCO	DL	0.49 (0.08)	**1.095* (0.23)**	30.45 (3.94)
G. diazatrophicus PAL5T	G	0.5427 (0.07)	**1.111* (0.20)**	31.19 (4.74)
G. diazatrophicus PAL5T + LCO	GL	0.546 (0.09)	**0.9913* (0.27)**	28.64 (6.62)
G. diazatrophicus PAL5T LsdB++	PL	**0.6253* (0.14)**	**0.938* (0.19)**	**33.79* (14.45)**
G. diazatrophicus PAL5T LsdB++ + LCO	PLL	0.4853 (0.11)	**1.028* (0.15)**	31.03 (5.30)
G. diazatrophicus SRT4	GS	0.4827 (0.08)	**1.016* (0.15)**	28.53 (2.65)
G. diazatrophicus SRT4 + LCO	GSL	0.4833 (0.09)	0.842 (0.16)	29.1 (3.04)
P. bilaii	P	0.4733 (0.10)	**1.067* (0.24)**	29.11 (3.64)
A. nodosum	S	**0.4313* (0.07)**	0.7293 (0.18)	32.39 (5.31)
V. paradoxus JM63	VP	0.5727 (0.08)	**1.133* (0.18)**	31.8 (5.53)
V. paradoxus JM63 + LCO	VPL	0.5327 (0.13)	**1.134* (0.15)**	30.62 (3.03)
Control	C	0.538 (0.11)	0.8067 (0.20)	28.46 (8.49)

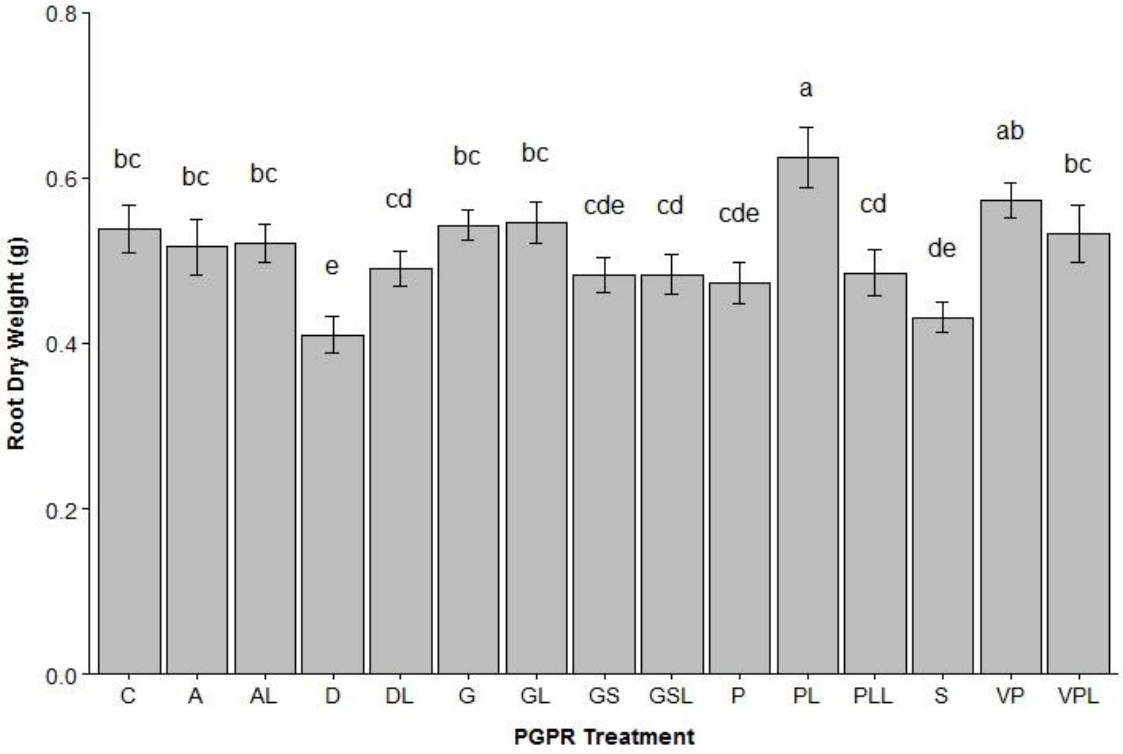

Figure 4-21. Mean NileFiber™ root dry weight (g) per plant weighed on 19 April 2016. C = Control; A = *Azospirillum brasilense* N8; AL = *A. brasilense* N8 + LCO; D = *Gluconacetobacter azotocaptans* DS1; DL = *G. azotocaptans* DS1 + LCO; G = *Gluconacetobacter diazotrophicus* PAL5T; GL = *Gluconacetobacter diazotrophicus* PAL5T + LCO; PL = *Gluconacetobacter diazotrophicus* PAL5T LsdB++; PLL = *Gluconacetobacter diazotrophicus* PAL5T LsdB++ + LCO; GS = *Gluconacetobacter diazotrophicus* SRT4; GSL = *Gluconacetobacter diazotrophicus* SRT4 + LCO; P = *Penicillium bilaii*; S = Seaweed extract (*Ascophyllum nodosum*); VP = *Variovorax paradoxus* JM63; VPL = *V. paradoxus* JM63 + LCO. Different letters indicate significant difference at $P < 0.05$. Bars indicate standard error. (n = 15).

4.3.2.5 Above-Ground Biomass Dry Weight

NileFiber™ above-ground biomass was dried in an oven and weighed eight days post-harvest. Mean above-ground biomass dry weight per plant ranged from 0.7293 g (S) to 1.216 g (AL) (Table 4-4). Of twelve growth-promoter treatments that showed significantly greater dry weights than the untreated control (C), five of these treatments were combination treatments (AL, DL, GL, VPL & PLL) (Figure 4-22). The dry weights of four of these five combination treatments was also greater than their corresponding single treatment, but none showed statistical significance. Additionally, the dry weights of two of the six combination treatments were less than their corresponding single treatment (GL & GSL), with GSL showing statistical significance. The one-way ANOVA P-value was $P < 0.0001$ (Appendix E).

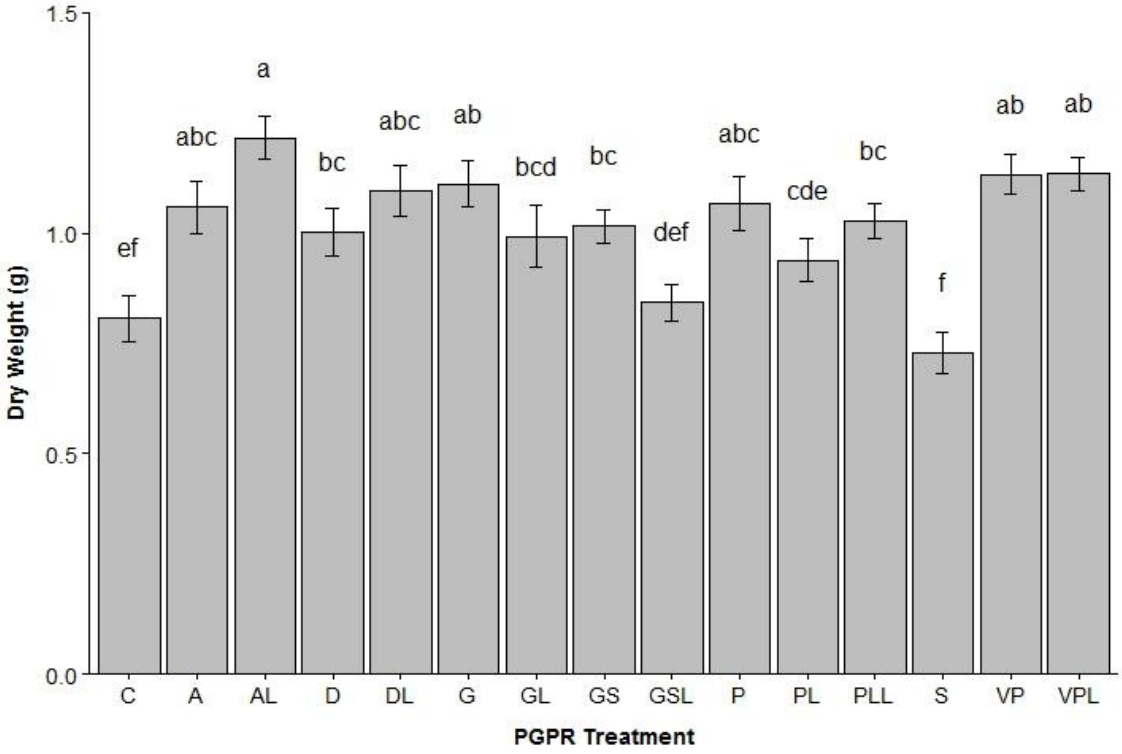

Figure 4-22. Mean NileFiber™ above-ground biomass dry weight (g) per plant weighed on 19 April 2016. C = Control; A = *Azospirillum brasilense* N8; AL = *A. brasilense* N8 + LCO; D = *Gluconacetobacter azotocaptans* DS1; DL = *G. azotocaptans* DS1 + LCO; G = *Gluconacetobacter diazotrophicus* PAL5T; GL = *Gluconacetobacter diazotrophicus* PAL5T + LCO; PL = *Gluconacetobacter diazotrophicus* PAL5T LsdB++; PLL = *Gluconacetobacter diazotrophicus* PAL5T LsdB++ + LCO; GS = *Gluconacetobacter diazotrophicus* SRT4; GSL = *Gluconacetobacter diazotrophicus* SRT4 + LCO; P = *Penicillium bilaii*; S = Seaweed extract (*Ascophyllum nodosum*); VP = *Variovorax paradoxus* JM63; VPL = *V. paradoxus* JM63 + LCO. Different letters indicate significant difference at $P < 0.05$. Bars indicate standard error. (n = 15).

4.3.2.6 Above-Ground Biomass Dry Matter Content

The mean dry matter content per NileFiber™ plant ranged from 28.46 % (C) to 35.31 % (A) (Table 4-4). Only three growth-promoter treatments (A, D & PL) showed significantly greater dry matter contents than the untreated control (C) (Figure 4-16). Of six combination growth-promoter treatments, five of these treatments had lower dry matter contents than their corresponding single treatments (AL, DL, GL, PLL & VPL) (Figure 4-23). The one-way ANOVA P-value was $P < 0.001$ (Appendix E).

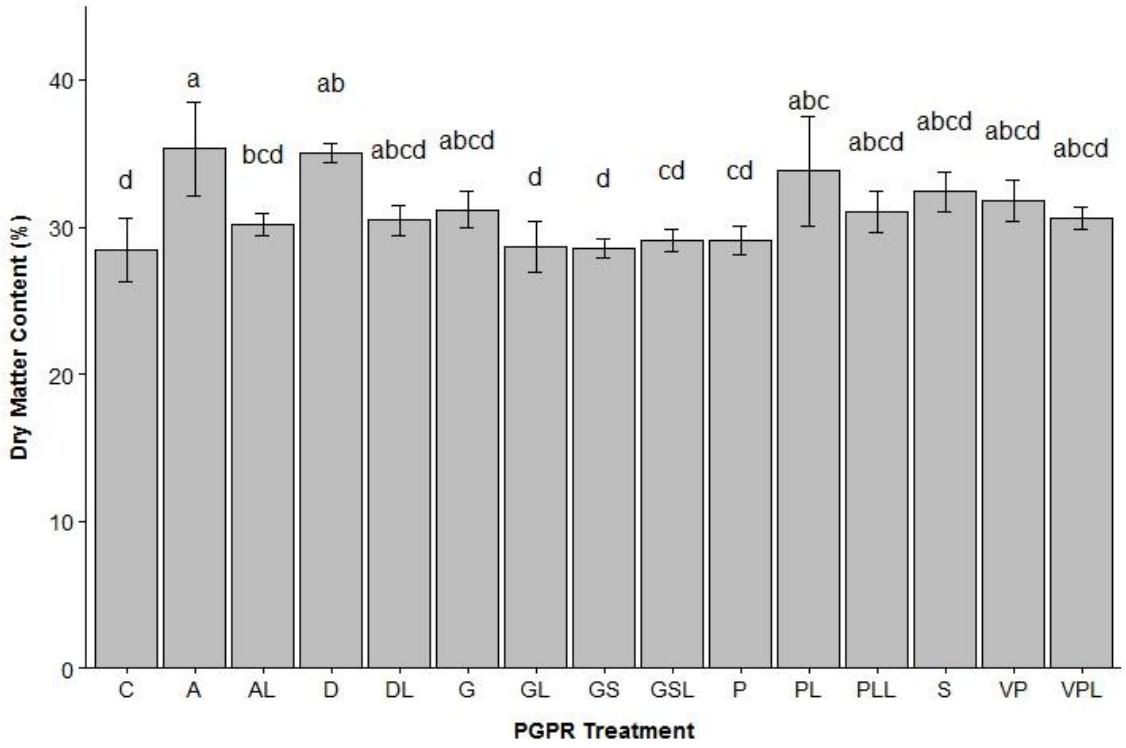

Figure 4-23. Mean NileFiber™ dry matter content (%) per plant calculated on 19 April 2016. C = Control; A = *Azospirillum brasilense* N8; AL = *A. brasilense* N8 + LCO; D = *Gluconacetobacter azotocaptans* DS1; DL = *G. azotocaptans* DS1 + LCO; G = *Gluconacetobacter diazotrophicus* PAL5T; GL = *Gluconacetobacter diazotrophicus* PAL5T + LCO; PL = *Gluconacetobacter diazotrophicus* PAL5T LsdB++; PLL = *Gluconacetobacter diazotrophicus* PAL5T LsdB++ + LCO; GS = *Gluconacetobacter diazotrophicus* SRT4; GSL = *Gluconacetobacter diazotrophicus* SRT4 + LCO; P = *Penicillium bilaii*; S = Seaweed extract (*Ascophyllum nodosum*); VP = *Variovorax paradoxus* JM63; VPL = *V. paradoxus* JM63 + LCO. Different letters indicate significant difference at $P < 0.05$. Bars indicate standard error. (n = 15).

4.3.3 Results & Statistical Analysis – Bayesian Approach

4.3.3.1 Metric Predicted Variable with One Nominal Predictor Variable

4.3.3.1.1 Plant Height

The height of NileFiber™ was measured from the soil surface to the highest leaf tip on the main stem. The estimate for average plant height was 34.9 cm (95 % HDI 34.371 – 35.411 cm). The G treatment showed the greatest increase in plant height while its corresponding combination treatment (GL) showed the greatest decline. The posterior distributions showed no credible nonzero differences between the treatments.

4.3.3.1.2 Number of Shoots

The number of shoots (≥ 5 cm) per plant were recorded at harvest time. The estimate for the average shoots per plant was 2.7903 (95 % HDI 2.6858 – 2.8981). The AL treatment showed the greatest increase in shoots per plant while the S treatment showed the greatest decline. The posterior distributions showed no credible nonzero differences between the treatments.

4.3.3.1.3 Above-Ground Biomass Fresh Weight

NileFiber™ above-ground biomass fresh weight was weighed immediately after being harvested. The estimate for the average above-ground biomass fresh weight per plant was 3.3532 g (95 % HDI 3.247 – 3.4646 g). Three treatments showed a credible negative effect (D, GSL & S) and one treatment showed a credible positive effect (AL) from the average value. The GSL treatment showed the greatest decline in above-ground biomass fresh weight out of all treatments, while the AL treatment showed the greatest increase. The posterior distribution shows credible nonzero differences among treatments AL, D, GSL and S (given zero is not within the 95 % HDI). Upon further evaluation of these treatments in comparison to the untreated control treatment (C), there is a non-credible difference between D and C (Appendix F) and GSL and C (Appendix F). A credible nonzero difference is found through the comparison of the AL treatment and the S treatment to the C treatment (Figures 4-24 & 4-25).

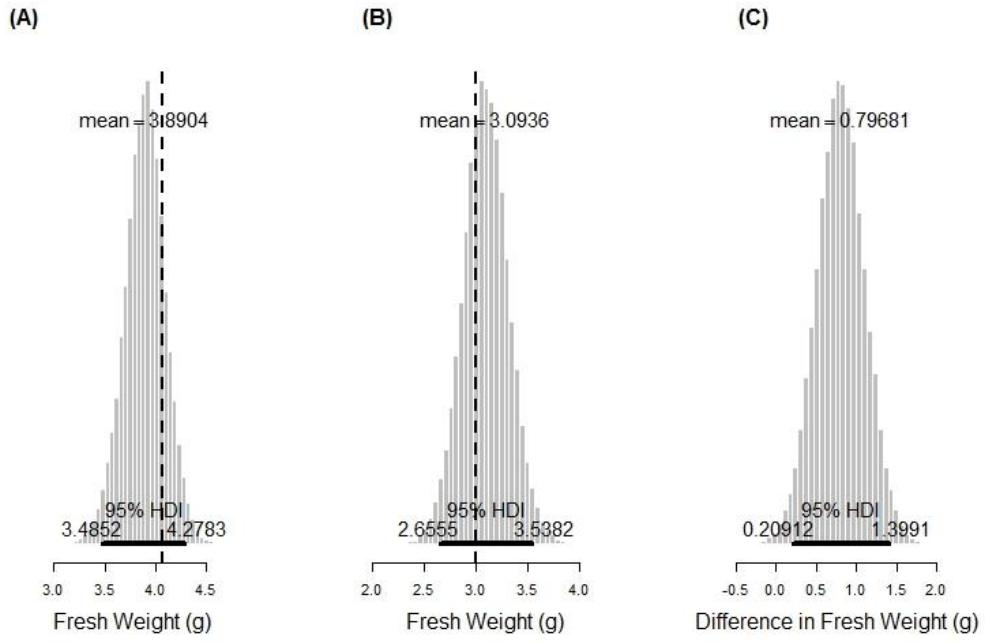

Figure 4-24. Effect of AL treatment on above-ground biomass fresh weight. Included are the posterior distributions for fresh weight (g) per plant under conditions: **(A)** AL treatment (mean = 4.067); **(B)** C treatment (mean = 3.00); and **(C)** the difference between posterior distributions of the AL and C treatments. AL treatment = *A. brasilense* N8 + LCO; C = Untreated Control.

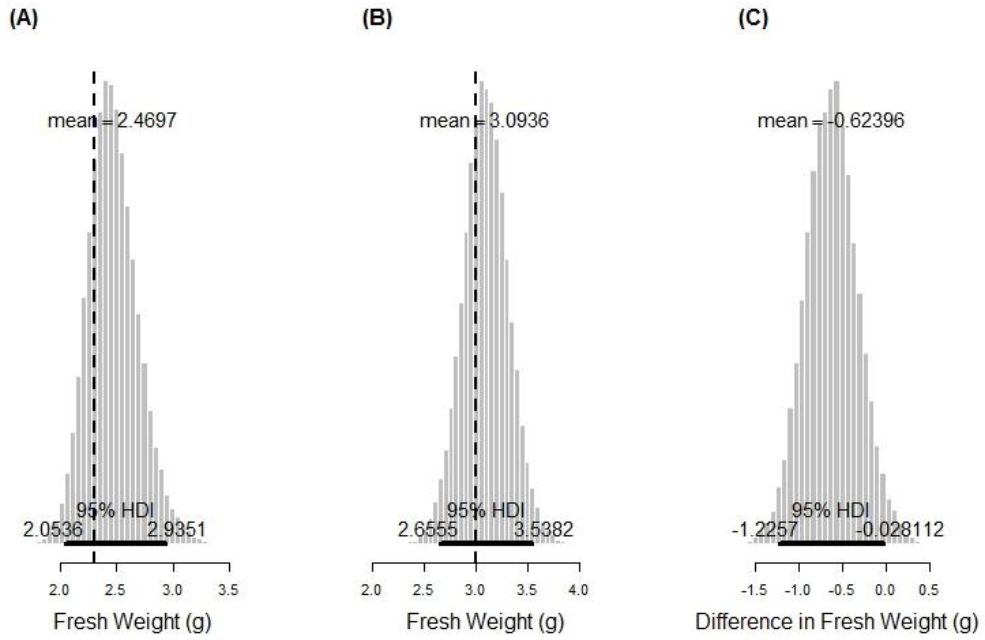

Figure 4-25. Effect of S treatment on above-ground biomass fresh weight. Included are the posterior distributions for fresh weight (g) per plant under conditions: **(A)** S treatment (mean = 2.3); **(B)** C treatment (mean = 3.00); and **(C)** the difference between posterior distributions of the S and C treatments. S treatment = *A. nodosum*; C = Untreated Control

4.3.3.1.4 Root Dry Weight

NileFiber™ roots were oven-dried and weighed eight days post-harvest. The estimate for the average root dry weight per plant was 0.50342 g (95% HDI 0.48898 – 0.51789 g). Two treatments showed a credible negative effect (D & S) and one treatment showed a credible positive effect (VP) from the average value. The D treatment showed the greatest decline in root dry weight out of all treatments, while the PL treatment showed the greatest increase. The posterior distribution shows credible nonzero differences among treatments D, S and VP (given zero is not within the 95 % HDI). Upon further evaluation of these treatments in comparison to the untreated control treatment (C), a credible nonzero difference is found through the comparison of the D treatment and the S treatment to the C treatment (Figures 4-26 & 4-27).

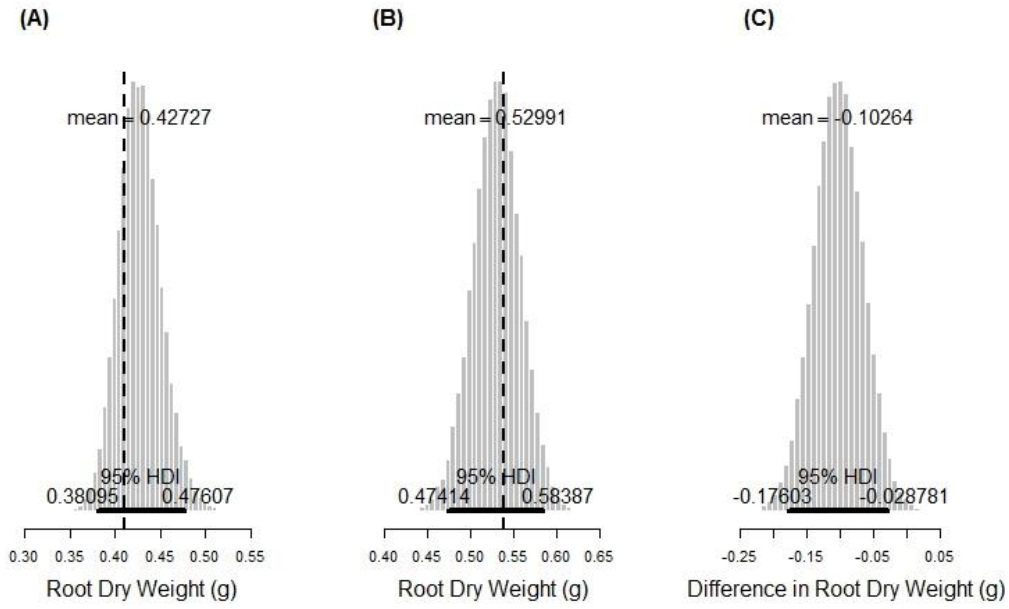

Figure 4-26. Effect of D treatment on root dry weight. Included are the posterior distributions for root dry weight (g) per plant under conditions: **(A)** D treatment (mean = 0.41); **(B)** C treatment (mean = 0.538); and **(C)** the difference between posterior distributions of the D and C treatments. D treatment = *G. azotocaptans* DS1; C = Untreated Control.

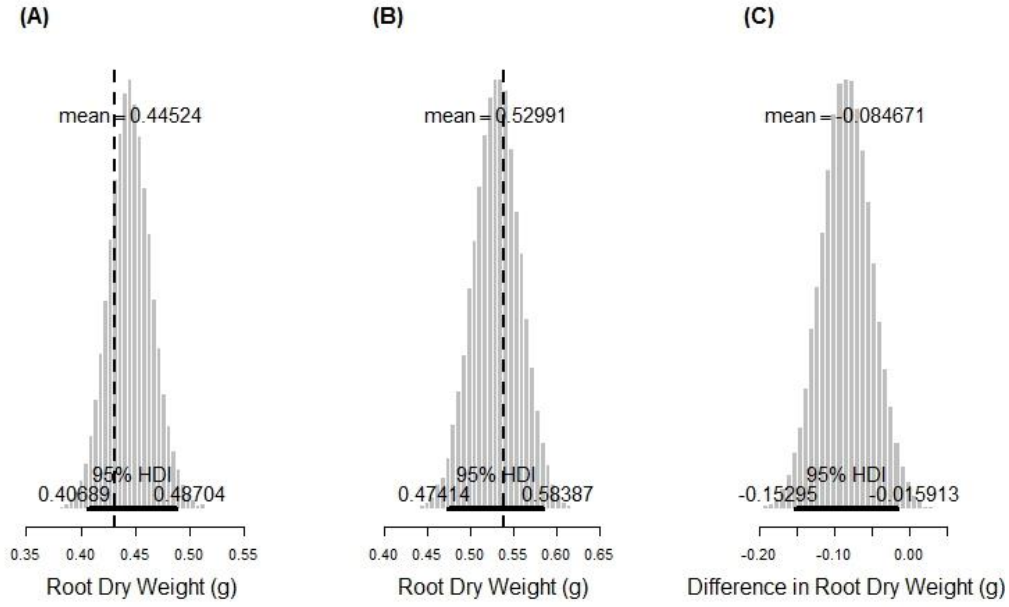

Figure 4-27. Effect of S treatment on root dry weight. Included are the posterior distributions for root dry weight (g) per plant under conditions: **(A)** S treatment (mean = 0.4313); **(B)** C treatment (mean = 0.538); and **(C)** the difference between posterior distributions of the S and C treatments. S treatment = Seaweed Extract (*A. nodosum*); C = Untreated Control.

4.3.3.1.5 Above-Ground Biomass Dry Weight

NileFiber™ above-ground biomass was oven-dried and weighed eight days post-harvest. The estimate for the average above-ground biomass dry weight per plant was 1.0072 g (95% HDI 0.97828 – 1.0357 g). Three treatments showed a credible positive effect (AL, VP & VPL) and two treatments showed a credible neagtive effect (S & GSL) from the average value. The S treatment showed the greatest decline in above-ground biomass dry weight out of all treatments, while the AL treatment showed the greatest increase. The posterior distribution shows credible nonzero differences among treatments AL, GSL, S, VP and VPL (given zero is not within the 95 % HDI). Upon further evaluation of these treatments in comparison to the untreated control treatment (C), there are non-credible differences between GSL and C and S and C (Appendix F). Credible nonzero differences are found through the comparison of AL, VP and VPL treatments to the C treatment (Figures 4-28 – 4-30).

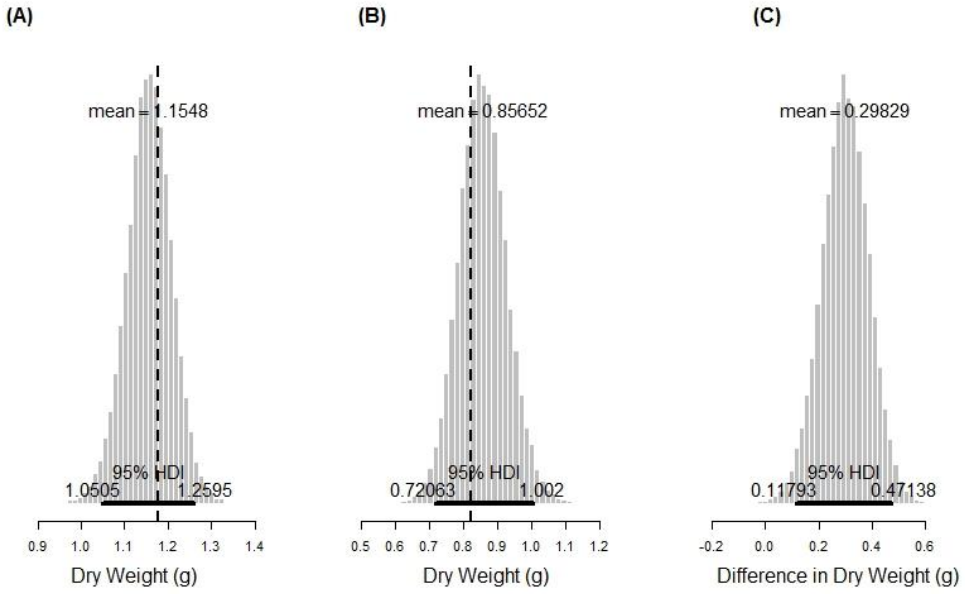

Figure 4-28**.** Effect of AL treatment on above-ground biomass dry weight. Included are the posterior distributions of dry weight (g) per plant under conditions: **(A)** AL treatment (mean = 1.216); **(B)** C treatment (mean = 0.8067); and **(C)** the difference between posterior distributions of the AL and C treatments. AL treatment = *A. brasilense* N8 + LCO; C = Untreated Control.

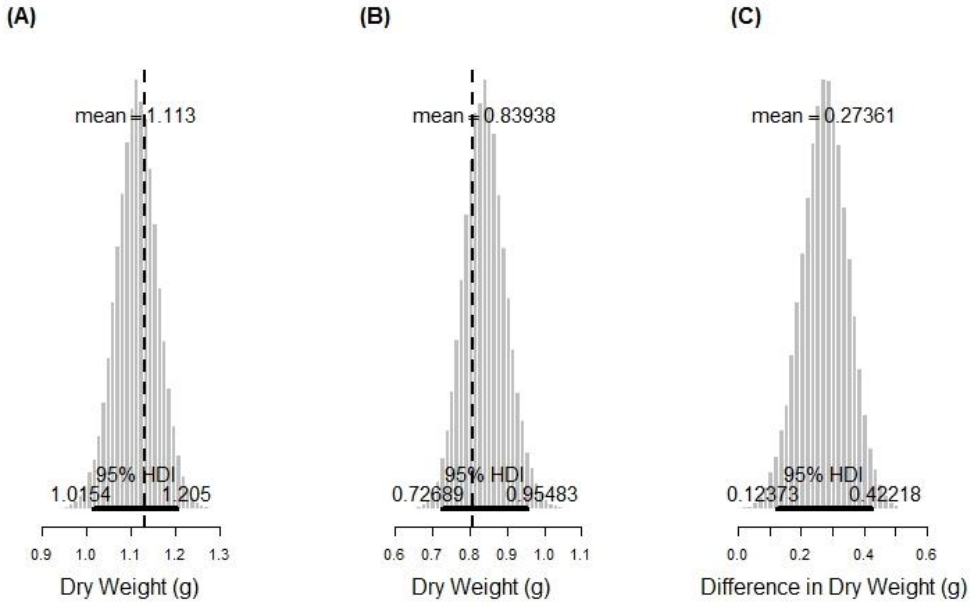

Figure 4-29. Effect of VP treatment on above-ground biomass dry weight. Included are the posterior distributions of dry weight (g) per plant under conditions: **(A)** VP treatment (mean = 1.133); **(B)** C treatment (mean = 0.8067); and **(C)** the difference between posterior distributions of the VP and C treatments. VP treatment = *Variovorax paradoxus* JM63 + LCO; C = Untreated Control.

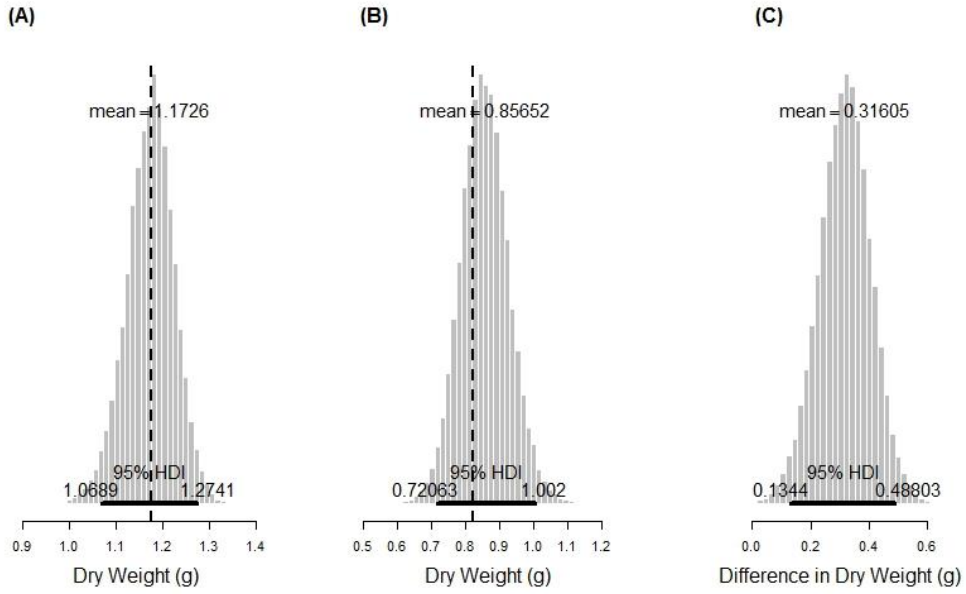

Figure 4-30. Effect of VPL treatment on above-ground biomass dry weight. Included are the posterior distributions of dry weight (g) per plant under conditions: **(A)** VPL treatment (mean = 1.134); **(B)** C treatment (mean = 0.8067); and **(C)** the difference between posterior distributions of the VPL and C treatments. VPL treatment = *V. paradoxus* JM63 + LCO; C = Untreated Control.

4.3.3.2 Above-Ground Biomass Dry Matter Content

The estimate for the average dry matter content was 29.929 % (95 % HDI 29.325 – 30.552 %). The D treatment showed a credible positive effect and the GS treatment showed a credible negative effect from the average value. The C treatment showed the greatest decline in dry matter content, while the D treatment showed the greatest increase. The posterior distribution shows credible nonzero differences among treatments D and GS (given zero is not within the 95 % HDI). Upon further evaluation of these treatments in comparison to the untreated control treatment (C), there are non-credible differences between the GS treatment and the C treatment (Appendix F). A credible nonzero difference was found through the comparison of D treatment to the C treatment (Figure 4-31).

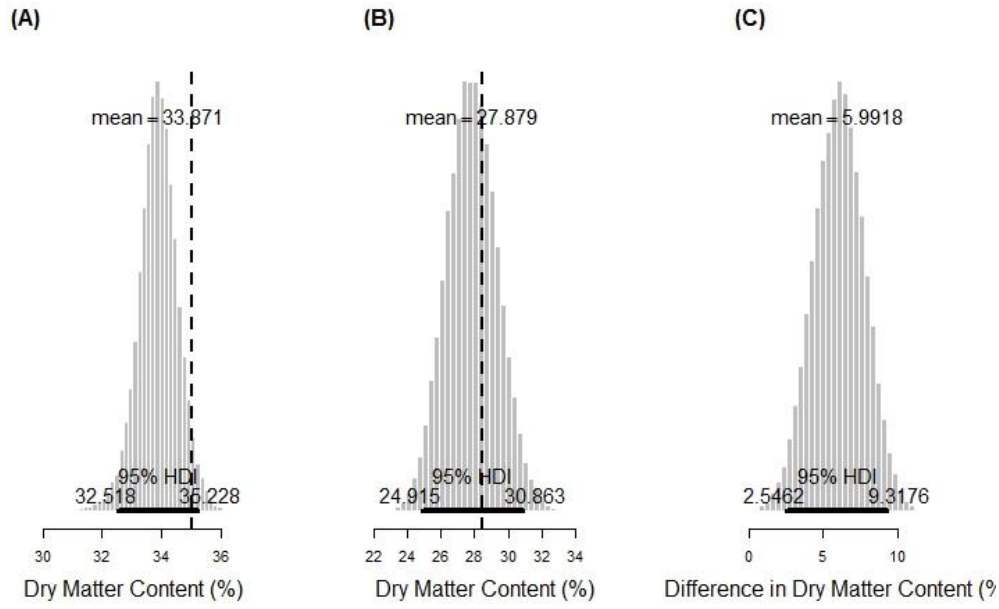

Figure 4-31. Effect of D treatment on dry matter content. Included are the posterior distributions of dry matter content (%) per plant under conditions: **(A)** D treatment (mean = 35.04); **(B)** C treatment (mean = 28.46); and **(C)** the difference between posterior distributions of the G and C treatments. G treatment = *G. azotocaptans* DS1; C = Untreated Control.

5.0 DISCUSSION

In this study, *Arundo donax* L. (genotype NileFiber™) was evaluated for its potential as a purpose-grown biofuel feedstock in Nova Scotia, Canada. Additionally, effects of growth-promoter microorganisms and stimulants on NileFiber™ growth were also investigated.

A major finding of this research illustrates the potential for growth-promoter microorganisms and supplements to enhance growth and subsequent biomass productivity of *A. donax*. However, field experimentation resulted in unsuccessful *A. donax* overwintering in Nova Scotia climatic conditions despite the beneficial effects of growth-promoters noted in greenhouse experimentation.

5.1 Unsuccessful First Planting

The initial planting of NileFiber™ at the field site occurred in July 2014, using rhizomatous materials from Nile Fiber Atlantic Canada Inc. This planting was deemed unsuccessful based on low emergence (~ 30 %) in the establishment year. The nature of the research required successful plant growth, so rhizomes were unearthed to collect dormant buds for *in vitro* tissue culture propagation.

There are various reasons for the lack of emergence and success of establishment of NileFiber™ after the 2014 planting including 1) time of planting and 2) seasonal temperature variation. NileFiber™ rhizome fragments were planted at the end of July, which is extremely late into the growing season: the recommendation for *Arundo donax* planting to increase biomass yield is early spring (Cavallaro et al., 2014; Copani et al., 2013).

Hardiness zones are defined by climate variables and the relative probability of successful survival of a plant species based on those variables (Ouellet and Sherk, 1967; McKenney et al., 2006; McKenney et al., 2001). Since the introduction of the original Canadian hardiness zones in 1967 by Ouellet and Sherk, these models have been modified to consolidate the systems used by the United States Department of Agriculture and Agriculture & Agri-Food Canada by incorporating extreme minimum temperature (McKenney et al., 2006). Additionally, through this consolidation, the Canadian Plant Hardiness zones can be compared to numerous other countries whose systems are also based around the USDA system.

Arundo donax, a species that thrives in a Mediterranean climate (Lambert et al., 2014) is also used as a purpose-grown feedstock for bioethanol in Vercelli, Italy (Bomgardner, 2013; Palmqvist and Lidén, 2014). Based on a similar system to the USDA Plant Hardiness Zone (extreme minimum temperature), the location of the bioethanol plant falls into Zone 8b (-9.4 to -6.7 °C) (PlantMaps, 2016a). In the Southern United States, where *A. donax* has been introduced and in some states has become invasive, these areas fall into Zones 8 through 10 (-12.2 to 4.4 °C) (USDA, 2012; USDA, 2016). When classified using the extreme minimum temperature approach, Nova Scotia falls into Zones 5 through 7a (-28.9 to -15.0 °C) (PlantMaps, 2016b; NRC, 2014a; NRC, 2014b).

During the 2014 – 15 winter season, the field site experienced below average temperatures while in the 2015 – 16 winter season, the field site experienced above average temperatures (Figure 5-1).

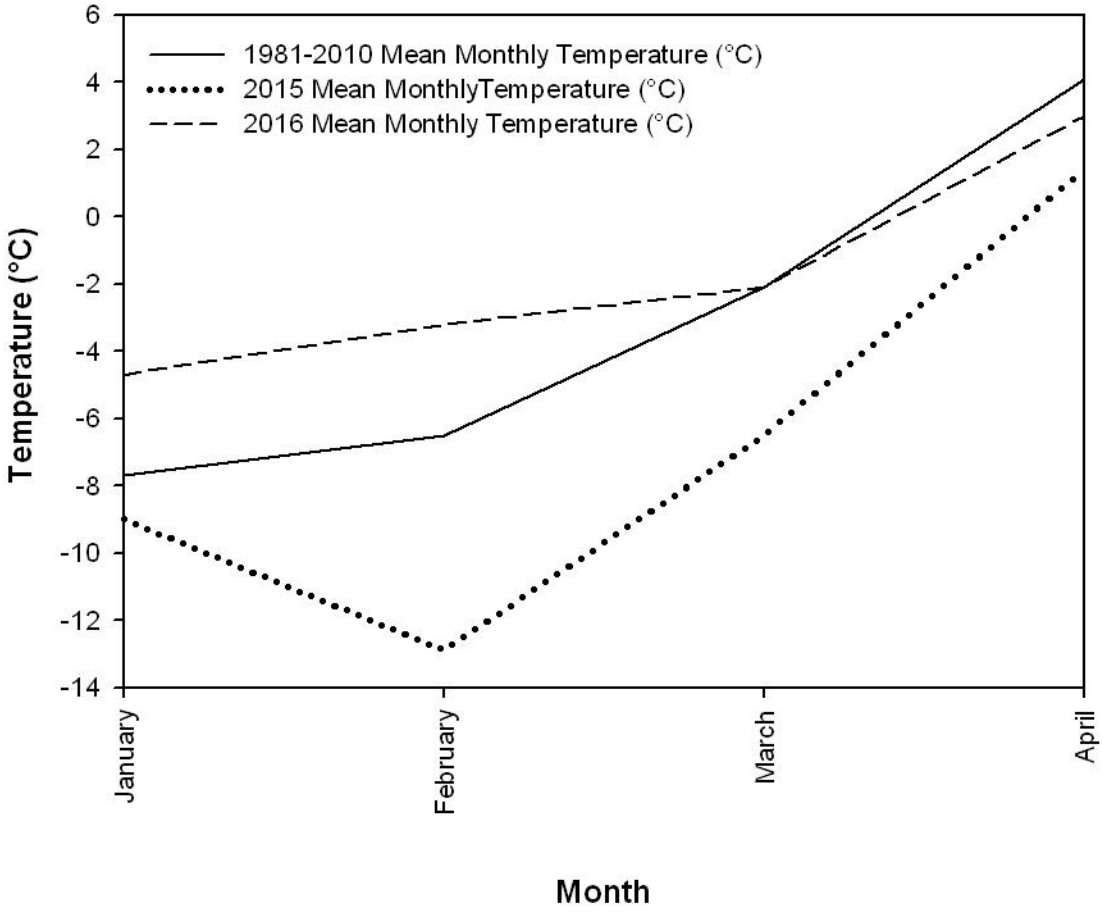

Figure 5-1. Mean monthly field temperatures (°C) at Nappan, NS in 2015 and 2016 compared to the average temperature (°C) from 1981 – 2010 (Environment Canada, 2015a; Environment Canada, 2015b).

This variation in temperature over the two winter seasons could have contributed to the survival of NileFiber™ in 2016 and lack of survival in 2015, as Nova Scotia winter temperatures in 2015 were more characteristic of Zone 3a (i.e. Fort McMurray, AB) than Zones 5 through 7 (Environment Canada, 2015a). Although Nova Scotia experiences more extreme minimum temperatures than areas of successful *A. donax* establishment, *A.*

donax could survive in average Nova Scotia climatic conditions based on its proximity to successful conditions. An estimated 2 % of Canada is suitable for *A. donax* establishment including Nova Scotia, based on successful establishment in Plant Hardiness Zones greater than Zone 6 (USDA, 2012). Unfortunately, this *A. donax* genotype did not survive the warmer of the two winter seasons.

5.2 Experiment 1 – Greenhouse Soil Drench

The first greenhouse experiment evaluated the performance of nine growth-promoter treatments on NileFiber™ growth and biomass productivity. The soil drench growth-promoter application occurred thirty-five days after the plantlets were transplanted into the greenhouse from the growth chamber. Plants were grown in the greenhouse for four months (mimicking a typical Nova Scotia growing season), measured and harvested for further analysis.

5.2.1 Greenhouse Conditions

The shading cloth was applied to the greenhouse during the first greenhouse experiment to regulate the internal greenhouse temperature to better mimic a typical growing season in Nova Scotia. The shading cloth reduced the margin of variation between the greenhouse temperature and the field site temperature (Figure 5-2) however the shading cloth also posed potential growth inhibition risks. The Saint Mary's greenhouse is set among many buildings, disabling access to sunlight before and after midday. In addition, the shading cloth reduces temperature by reducing solar penetration, and irradiance in the greenhouse was reduced to approximately 20 % of irradiance outside the greenhouse (Zhongmin Dong [Saint Mary's University], personal

communication). To compensate for these possible constraining conditions, supplemental lighting was used to ensure a minimum photoperiod of 16/8 hours (day/night). These potted plants could have been moved outside the greenhouse upon application of the growth-promoter treatments to reduce any growth inhibition in the greenhouse.

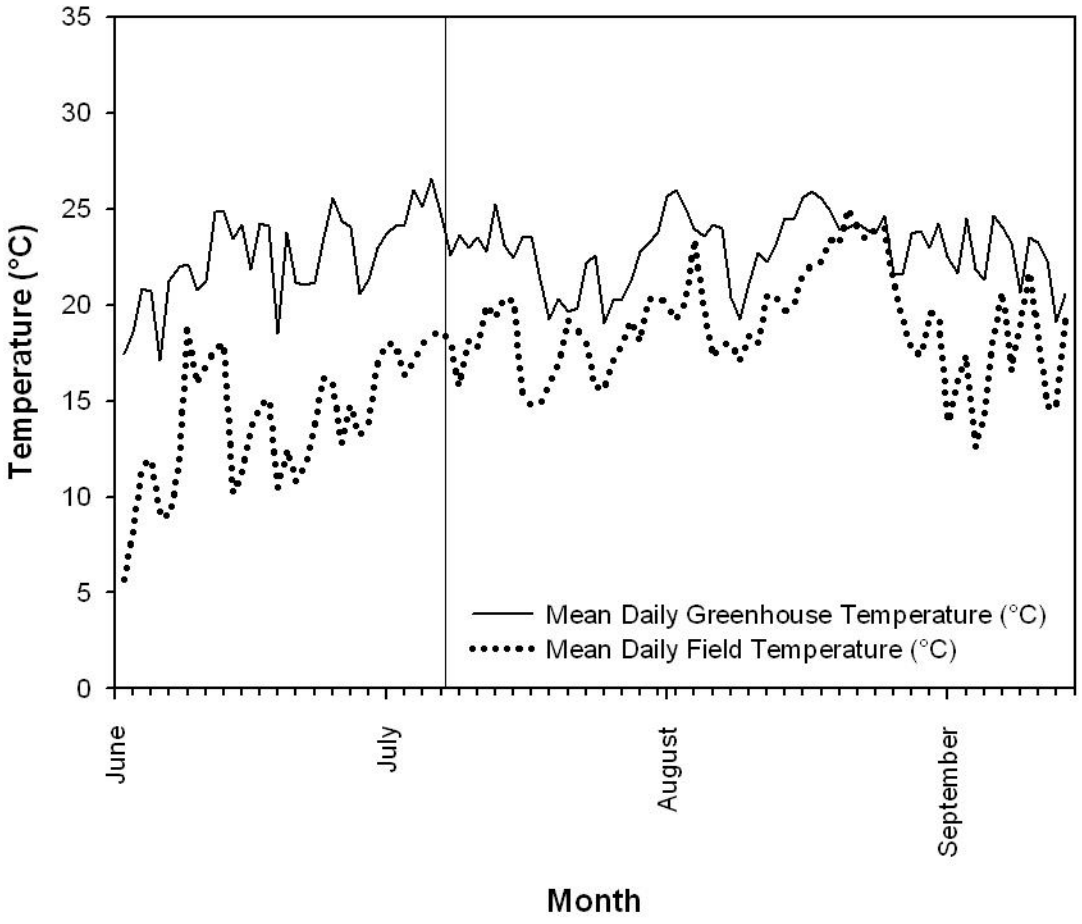

Figure 5-2. Mean daily internal SMU greenhouse temperature (SMU, Halifax, NS) (HOBO® Relative Humidity / Temperature / Light / External Data Logger) and mean daily field temperature (Nappan, NS) (Environment Canada, 2016). The vertical line indicates the date in which the shading cloth was applied to the greenhouse.

5.2.2 Growth Parameters

Of the growth-promoter treatments applied to NileFiber™ in the first greenhouse experiment, there was very little positive impact measured in growth parameters compared to the untreated control. The GL treatment (*G. diazotrophicus* PAL5T + LCO) showed an overwhelmingly negative trend in fresh and dry above-ground biomass yield and root dry weight compared to the untreated control (C). In addition, the other L treatments (AL (*A. brasilense* N8 + LCO) & L (LCO)) also exhibited negative effects on plant growth. The PL treatment (*P. bilaii* + LCO) exhibited similar trends in effects on plant growth as the untreated control (C).

The negative effects of LCO treatments on *A. donax* growth may have occurred because of the prior colonization of the plant rhizosphere. If *A. donax* had already formed a relationship with rhizospheric organisms, these organisms may have identified the exogenous application of LCO as a pathogenic substance, rendering it ineffective to the plant. Alternatively, the exogenous LCO may not have been identified at all: in this experiment, LCO treatments were diluted with distilled water, giving a final concentration of 10^{-12} M LCO where the most commonly studied concentration with growth-promoting activity is between $10^{-6} – 10^{-8}$ M (Prithiviraj et al., 2000; Schwinghamer et al., 2015; Smith et al., 2005).

The PL treatment (*P. bilaii* + LCO) exhibited effects on plant growth unlike the other LCO treatments, more similar to the positive effect of the untreated control. Interestingly, there is a commercial inoculant (TagTeam®LCO, Novozymes®) that combines *P. bilaii* and LCO with a rhizobium species to form a single inoculant to

improve plant growth. Firstly, *P. bilaii* mobilizes plant unavailable phosphorus increasing microbial phosphorus (Shen et al., 2011; Wakelin et al., 2007). Secondly, the combination of LCO and the rhizobia create the signalling cycle necessary to fix more nitrogen for plant use (Novozymes, 2011; Prithiviraj et al., 2003). This inoculant exhibits plant growth-promoting effects based on the presence of the rhizobium: although the PL treatment did not contain a bacterial species, there could have been a similar interactive process between the PL treatment and some native soil bacteria, or the positive effects of this treatment could be based solely on the presence of *P. bilaii*.

5.3 Experiment 2 – Field Soil Drench

The field experiment evaluated a) the performance of six growth-promoter treatments on NileFiber™ growth and biomass productivity and b) the survival of NileFiber™ through a Nova Scotian winter. The soil drench growth-promoter application occurred seventy days after the plantlets were transplanted into the greenhouse from the growth chamber. Plants grew for fourth months (mimicking a typical Nova Scotia growing season), and were measured and harvested for further analysis.

5.3.1 Plant Spacing

NileFiber™ plantlet density in the field was 6.25 plants m^{-2} with dry biomass yields in the establishment year (2015) ranging from 1.68 – 1.85 Mg ha^{-1}. This planting density is comparable with other studies reporting much lower planting densities with increasing biomass yields in subsequent post-establishment harvests (Cosentino et al., 2006; Pari et al., 2016; Testa et al., 2016). Although comparable, future yields may

decline with this relatively high planting density, as previous studies have shown that after the establishment season, lower planting densities prevailed with higher yields than higher planting densities (Angelini et al., 2005; Smith et al., 2015). *Arundo donax* L. has a reportedly long establishment time in ideal conditions, reaching stabilized dry matter yields three years post-planting (Cavallaro et al., 2014; Cosentino et al., 2014; Lewandowski et al., 2003) perhaps based on its perennial nature (Smith et al., 2015).

5.3.2 Growth Parameters

There is less variance between growth parameter measurements in the field compared with the first greenhouse experiment, creating less distinction between treatments. NileFiber™ plants treated with the P treatment (*P. bilaii*) showed similarities to untreated control plants while the A (*A. brasilense* N8) and G (*G. diazotrophicus* PAL5T) treated plants were less similar to the untreated control. The A treatment (*A. brasilense* N8) showed negative effects on shoots per plant and dry matter content.

5.4 First Greenhouse Experiment and Field Experiment – Similarities

The first greenhouse experiment and the field experiment were run concurrently to extrapolate results between similar experimental conditions. Similar aspects of these two experiments will be discussed together, whereas dissimilar aspects will follow.

5.4.1 Growth-Promoter Application Method

The soil drench growth-promoter application method was chosen to mimic a realistic agricultural situation, in which a producer would most easily apply growth-promoter treatments upon planting. In combination with the plants being well established

(delayed treatment), this simplistic, passive treatment application method did not ensure that the promoters reached the NileFiber™ root systems.

5.4.2 Soil Properties

It is very likely the combination of delayed treatment and non-sterile soil used in the first greenhouse experiment and the field experiment could have impacted the effectiveness of the growth-promoter treatments. Root colonization is the most basic yet important step in establishing a beneficial relationship between plants and promoters (Ahmad et al., 2011; Mangmang et al., 2015). The delayed treatment (thirty-five and seventy days) of NileFiber™ enabled native microfauna to colonize root systems and establish their rhizospheric role prior to the supplemental exogenous growth-promoter introduction (Requena et al., 1997; Schippers et al., 1987).

5.4.3 Growth Parameters

The evident trend throughout the first greenhouse experiment and the field experiment with the P treatment (*P. bilaii*) showing a similar effect as the untreated control on *A. donax* growth could be indicative of the macronutrient most lacking in these edaphic conditions or simply that *A. donax* exhibits high phosphorus uptake and accumulation (Kering et al., 2012; Sagehashi et al., 2009).

5.5 Experiment 3 – Greenhouse Root Soaking

The second greenhouse experiment evaluated the performance of fifteen growth-promoter treatments on NileFiber™ growth and biomass productivity. Growth-promoter application occurred through root soaking immediately after plantlets were removed from the growth chamber (immediately before being transplanted into the greenhouse). Plants

were grown in the greenhouse for two months, measured and harvested for further analysis.

5.5.1 Growth Parameters

Unlike the results from the first greenhouse experiment, the NileFiber™ plants treated with the combinatory AL treatment (*A. brasilense* N8 + LCO) had a positive impact on measured growth parameters compared to the untreated control treatment (C). Another combinatory treatment, GL (*G. diazotrophicus* PAL5T + LCO), and the commercial seaweed treatment, *A. nodosum* (S) showed negative impacts compared to the untreated control.

The positive effect of the AL treatment (*A. brasilense* N8 + LCO) on *A. donax* growth measured through shoots per plant and above-ground fresh and dry weight per plant could be attributed to a similar signalling cycle between the bacteria and the LCO to the cycle between rhizobium and LCO.

5.6 First Greenhouse Experiment versus Second Greenhouse Experiment

The objective of the greenhouse experiments was to investigate any relationships between growth-promoter treatments and *A. donax* plant growth. Results from the first greenhouse experiment did not support the hypothesis that the growth-promoter treatments would positively effect *A. donax* growth, so a more aggressive inoculation method combined with sterilized soil were utilized in the second greenhouse experiment to increase the potential of a beneficial relationship between the growth-promoters and *A. donax*, thus supporting the hypothesis.

5.6.1 Growth-Promoter Application Method

The uncertainty of exposure of plant roots to growth-promoter treatments through the soil drench method lead to the change of inoculation method in the second greenhouse experiment. The root soaking inoculation method was more beneficial for plant-promoter interaction because a) the roots were in direct contact with the promoters for 30 minutes prior to being planted, making root colonization very simple (Schloter and Hartmann, 1998) and b) the plants were inoculated immediately before being planted, leaving virtually no chance for other rhizospheric species to colonize before the intended promoters.

5.6.2 Soil Properties

Sterilized soil was used in the second greenhouse experiment to further initiate root colonization by the intended growth-promoters. Unfortunately, this is not a realistic agricultural approach as soil sterilization is a highly intensive procedure in terms of time, energy and resources. Sterilized soil can promote a greater colonization of the host plant by growth-promoters due to less microfloral competition: there was a greater *A. brasilense* concentration in axenic systems of wheat (*Tritucum aestivum*) as compared to natural soil (Schloter and Hartmann, 1998). but also may allow for a potentially inhibitory growth-promoter concentration as it would be higher under these conditions (Requena et al., 1997).

6.0 CONCLUSION

There were two objectives of this research: 1) to evaluate the growth potential of a proprietary genotype of *A. donax* L. (NileFiber™) for use as a purpose-grown feedstock for advanced biofuel production in Nova Scotia and 2) to investigate the effects of plant growth-promoters on NileFiber™. The results of this research show that this genotype cannot survive the climatic conditions of Nova Scotia and that there are some interesting effects of growth-promoter treatments on NileFiber™ growth.

A. donax (NileFiber™) did not survive the climatic conditions at the field site through two different plantings: 2014 – rhizome planting and 2015 – propagated plantlet planting. Upon each summer planting of NileFiber™, the subsequent winter season through which the planting was set to survive was different, illustrating that under various conditions, Nova Scotia's winter climate is not suitable for the overwintering of this *A. donax* genotype.

Effectiveness of growth-promoter treatments vary with edaphic and climatic conditions, however, to ensure a direct relationship between growth-promoter treatments and host plant roots prior to native soil organisms, roots should be soaked in growth-promoter treatments immediately before planting rather than treating with a delayed soil drench method.

The findings of this research are helpful in many ways. Primarily, there is now evidence of experimentation with *A. donax* in a northern climate. Secondly, from the growth-promoter experimentation, it is evident that *A. donax* growth is not extensively impacted by the presence or absence of growth-promoters, enhancing the viability of this

grass as a low-carbon (low input) biomass feedstock. Biomass producers in Nova Scotia should be encouraged to investigate other potential biomass feedstocks as *A. donax* (NileFiber™) does not have the reliability to establish or to produce substantial yields in this province.

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8.0 APPENDICES

APPENDIX A: LABORATORY PROTOCOL I

Table A1. Concentrations (mg L^{-1}; g 100 mL^{-1}; mL L^{-1}) of Murashige and Skoog macro-nutrients in base propagation medium. (Adapted from Murashige and Skoog, 1962).

Molecular Compound	**Molecular Formula**	**Concentration (mg L^{-1})**	**Stock Solution (20x) (g 100 mL^{-1})**	**Amount per Litre of Medium (mL L^{-1})**
Ammonium nitrate	NH_4NO_3	1,650	33.00	5
Calcium chloride	$CaCl_2 \bullet 2H_2O$	440	8.80	5
Magnesium sulfate	$MgSO_4 \bullet 7H_2O$	370	7.40	5
Potassium phosphate	KH_2PO_4	170	3.40	5
Potassium nitrate	KNO_3	1,900	38.0	5

Table A2. Sources of MS macro-nutrients used in MS base propagation medium.

Chemical	Source
Ammonium nitrate	Caledon Laboratory Chemicals
Calcium chloride	Fisher Scientific
Magnesium sulfate	Caledon Laboratory Chemicals
Potassium phosphate	Fisher Scientific
Potassium nitrate	Fisher Scientific

Table A3. Concentrations (mg L^{-1}; g 100 mL^{-1}; mL L^{-1}) of Murashige and Skoog micro-nutrients in base propagation medium. (Adapted from Murashige and Skoog, 1962).

Molecular Compound	**Molecular Formula**	**Concentration (mg L^{-1})**	**Stock Solution (100x) (mg 100 mL^{-1})**	**Amount per Litre of Medium (mL L^{-1})**
Boric acid	H_3Bo_3	6.200	620.00	
Cobalt Chloride	$CoCl_2 \cdot 6H_2O$	0.025	2.50	
Cupric sulfate	$CuSO_4 \cdot 5H_2O$	0.025	2.50	
Manganese sulfate	$MnSO_4 \cdot 4H_2O$	22.300	2.23	1
Potassium iodide	KI	0.830	83.00	
Sodium molybdate	$Na_2MoO_4 \cdot 2H_2O$	0.250	25.00	
Zinc sulfate	$ZnSO_4 \cdot 7H_2O$	8.600	860.00	
Ferrous sulfate	$FeSO_4 \cdot 7H_2O$	27.800	2,780.00	1
Na_2-EDTA	Na_2-EDTA $\cdot 2H_2O$	37.200	3,720.00	

Table A4. Sources of MS micro-nutrients used in MS base propagation medium.

Chemical	Source
Boric acid	Caledon Laboratory Chemicals
Cobalt Chloride	Fisher Scientific
Cupric sulfate	Caledon Laboratory Chemicals
Manganese sulfate	Sigma-Aldrich Inc.
Potassium iodide	
Sodium molybdate	Fisher Scientific
Zinc sulfate	Fisher Scientific
Ferrous sulfate	Fisher Scientific
Na_2-EDTA	Fisher Scientific

Table A5. Concentrations (mg L^{-1}; g 100 mL^{-1}; mL L^{-1}) of Morel and Wetmore's vitamins and Murashige and Skoog organic constituents in base propagation medium. (Adapted from Morel and Wetmore, 1951 and Murashige and Skoog, 1962).

Molecular Compound	**Molecular Formula**	**Concentration (mg L^{-1})**	**Stock Solution (100x) (mg 100 mL^{-1})**	**Amount per Litre of Medium (mL L^{-1})**
i-Inositol	$C_6H_{12}O_6$	100.00	10,000	1
Biotin	$C_{10}H_{16}N_2O_3S$	0.01	1	1
Calcium pantothenate	$C_{18}H_{32}CaN_2O_{10}$	1.00	100	1
Folic acid	$C_{19}H_{19}N_7O_6$	10.00	500[1]	2
Glycine (recrystallized)	$C_2H_5NO_2$	2.00	200	1
Nicotinic acid	$C_6H_5NO_2$	0.50	50	1
Pyridoxine • HCl	$C_8H_{12}ClNO_3$	0.50	50	1
Thiamine • HCl	$C_{12}H_{18}Cl_2N_4OS$	0.10	10	1

[1]the stock solution is 50x

Table A6. Sources of Morel and Wetmore's vitamins and MS organic constituents used in base propagation medium.

Chemical	Source
i-Inositol	MP Biomedicals
Biotin	Sigma Aldrich Inc.
Calcium pantothenate	Acros Organics
Folic acid	Sigma-Aldrich Inc.
Glycine (recrystallized)	
Nicotinic acid	
Pyridoxine • HCl	Sigma-Aldrich Inc.
Thiamine • HCl	Sigma-Aldrich Inc.

Table A7. Concentrations (mg L^{-1}; µL L^{-1}) of additional constituents in shoot proliferation propagation medium. (Adapted from Cavallaro et al., 2011).

Compound	**Abbreviation**	**Molecular Formula**	**Stock Solution (mg L^{-1})**	**Amount per Litre of Medium (µL L^{-1})**
Benzylaminopurine	BA	$C_{12}H_{11}N_5$	3.00	3.0
Indole-3-butyric acid	IBA	$C_{12}H_{13}NO_2$	1.00	1.0
Thidiazuron	TDZ	$C_9H_8N_4OS$	-	0.1[1]
Gibberellic Acid	GA_3	$C_{19}H_{22}O_6$	0.05	0.5
Plant Preservative Mixture	PPM	C_4H_4ClNOS C_4H_5NOS	-	0.2[2]
Sucrose	-	$C_{12}H_{22}O_{11}$	-	30[3]
Gelrite®	-	-	-	2.5[3]

[1]uM, [2]%, [3]g L^{-1}

Table A8. Concentrations (mg L^{-1}; µL L^{-1}) of additional constituents in shoot growth propagation medium. (Adapted from).

Compound	Abbreviation	Molecular Formula	Stock Solution (mg L^{-1})	Amount per Litre of Medium (µL L^{-1})
Benzylaminopurine	BA	$C_{12}H_{11}N_5$	1.00	3.0
Indole-3-butyric acid	IBA	$C_{12}H_{13}NO_2$	1.00	1.0
Thidiazuron	TDZ	$C_9H_8N_4OS$	-	0.1[1]
Gibberellic Acid	GA_3	$C_{19}H_{22}O_6$	0.05	0.5
Plant Preservative Mixture	PPM	C_4H_4ClNOS C_4H_5NOS	-	0.2[2]
Sucrose	-	$C_{12}H_{22}O_{11}$	-	30[3]
Gelrite®	-	-	-	2.5[3]

[1]uM, [2]%, [3]g L^{-1}

Table A9. Concentrations (mg L^{-1}; µL L^{-1}) of additional constituents in rooting (plantlet generation) propagation medium. (Adapted from Cavallaro et al., 2011).

Compound	Abbreviation	Molecular Formula	Stock Solution (mg L^{-1})	Amount per Litre of Medium (µL L^{-1})
Naphthalenacetic acid	NAA	$C_{12}H_{10}O_2$	2	2
Sucrose	-	$C_{12}H_{22}O_{11}$	-	20[1]
Gelrite®	-	-	-	2.5[1]

[1]g L^{-1}

Table A10. Sources of additional constituents used in shoot proliferation, shoot growth and rooting (plantlet generation) propagation media.

Chemical	Source
Benzylaminopurine	PhytoTechnology Laboratories
Indole-3-butyric acid	Sigma-Aldrich Inc.
Thidiazuron	
Gibberellic Acid	PhytoTechnology Laboratories
Plant Preservative Mixture	Plant Cell Technology
Sucrose	Fisher Scientific
Gelrite®	Sigma-Aldrich Inc.
Naphthalenacetic acid	PhytoTechnology Laboratories

APPENDIX B: LABORATORY PROTOCOL II

Table B1. Concentrations (g L^{-1}; g 100 mL^{-1}; mL L^{-1}) of constituents in LGI-P liquid medium. (Adapted from Cavalcante and Dobereiner, 1988 and Pan and Vessey, 2001).

Molecular Compound	Molecular Formula	Concentration (g L^{-1})	Stock Solution (g 100 mL^{-1})	Amount per Litre of Medium (mL L^{-1})
Potassium phosphate dibasic	K_2HPO_4	0.200	10.000	2
Potassium phosphate monobasic	KH_2PO_4	0.600	12.000	5
Magnesium sulfate heptahydrate	$MgSO_4 \bullet 7H_2O$	0.200	20.000	1
Calcium chloride dihydrate	$CaCl_2 \bullet 2H_2O$	0.020	2.000	1
Sodium molybdate dihydrate	$NaMoO_4 \bullet 2H_2O$	0.002	0.200	1
Iron (III) chloride hexahydrate	$FeCl_3 \bullet 6H_2O$	0.010	1.00	1
0.5% bromothymol blue solution in 0.2 M KOH	-	-	-	5
Biotin	$C_{10}H_{16}N_2O_3S$	0.1^1	10^2	1
Pyridoxal HCl	$C_8H_{12}ClNO_3$	0.2^1	20^2	
Ammonium sulfate	$(NH_4)_2SO_4$	1.320	33.00	4
Sucrose	$C_{12}H_{22}O_{11}$	100.000	-	100^3

[1]mg L^{-1}; [2]mg 100 mL^{-1}; [3]g L^{-1}

Table B2. Sources of constituents used in LGI-P liquid medium.

Chemical	Source
Potassium phosphate dibasic	Fisher Scientific
Potassium phosphate monobasic	Fisher Scientific
Magnesium sulfate heptahydrate	Caledon Laboratory Chemicals
Calcium chloride dihydrate	Fisher Scientific
Sodium molybdate dihydrate	Fisher Scientific
Iron (III) chloride hexahydrate	Fisher Scientific
0.5% bromothymol blue solution in 0.2 M KOH	Fisher Scientific
Biotin	Sigma-Aldrich Inc.
Pyridoxal HCl	Sigma Aldrich Inc.
Ammonium sulfate	Caledon Laboratory Chemicals
Sucrose	Fisher Scientific

Table B3. Concentrations (g L^{-1}; g 100 mL^{-1}; mL L^{-1}) of constituents in Lysogeny Broth (L.B.) liquid medium. (Adapted from Bertani, 1951).

Molecular Compound	Molecular Formula	Concentration (g L^{-1})
Tryptone	-	10.000
Yeast Extract	-	5.000
Sodium Chloride	NaCl	10.000

Table B4. Sources of constituents used in L.B. liquid medium.

Chemical	Source
Tryptone	Fisher Scientific
Yeast Extract	Fluka BioChemika
Sodium Chloride	Anachemia Science

APPENDIX C: GREENHOUSE MAINTENANCE LOG

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
					1 Plantlets moved into greenhouse; Domes sealed;	2
3	4	5 Dome-tray seal broken;	6	7 Domes removed from trays;	8	9 Water[1]
10	11	12 Water[1]	13	14	15	16 Fertilizer (2 L/ tray)
17	18	19 Water[1]	20	21	22	23
24	25	26	27 Plantlets moved outside; Water[1]	28 Water[1] (outside)	29 Fertilizer (2 L/ tray)	30
31	**Notes:** 28 May: Plantlets for greenhouse experiment were potted and put into the greenhouse. [1]Seed trays were filled with water to an approximate depth of 1", approximately 3.83 L $tray^{-1}$					

Figure C1. Greenhouse maintenance schedule for May 2015.

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
	1 Water[1]	2	3 Water[1]	4	5 Growth-promoter treatments applied in greenhouse;	6
7	8 Water[1]	9 Water[1]	10	11	12 Water[1]	13
14 Water[1]	15	16 Water[1]	17 Water[1]	18 Water[2]	19 Water[3] Randomized;	20
21	22 Water[3]	23	24	25	26 Water[2]	27 Water[2]
28	29	30 Water[2]	**Notes:**			

[1] 250 mL plant^{-1} with watering can; [2] 500 mL plant^{-1}; [3] 250 mL plant^{-1}

Figure C2. Greenhouse maintenance schedule for June 2015.

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
			1	2 Water[2]	3	4 Water[2]
5	6	7 Shade net put on greenhouse; Randomized;	8 Water[2]	9 Water[2]	10	11
12	13 Water[3]	14	15	16 Water[2]	17	18
19	20 Water[2]	21	22	23 Water[2]	24	25
26	27	28	29 Water[3] Randomized;	30	31 Water[3]	

[2] 500 mL plant^{-1}; [3] 250 mL plant^{-1}

Figure C3. Greenhouse maintenance schedule for July 2015.

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
						1
2	3	4 Water[2]	5	6	7 Water[2]	8
9	10	11	12 Water[2]	13	14 Water[3]	15
16	17 Water[2]	18	19	20 Water[2]	21	22
23	24	25 Water[2]	26	27	28	29
30 Water[3]	31	**Notes:** [2] 500 mL plant^{-1}; [3] 250 mL plant^{-1}				

Figure C4. Greenhouse maintenance schedule for August 2015.

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
		1	2	3 Water[2]	4	5
6	7	8 Water[3]	9	10 Water[3]	11	12
13	14 Greenhouse harvest	15 Greenhouse harvest	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30			

[2] 500 mL plant^{-1}; [3] 250 mL plant^{-1}

Figure C5. Greenhouse maintenance schedule for September 2015.

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
	1	2	3	4	5	6
7	8 Plantlets treated and moved into greenhouse;	9	10 Domes lifted slightly off trays;	11 Phosphate buffer application;	12 Domes removed from trays;	13
14 Water[4]	15	16	17 Water[4]	18	19	20
21 Water[4]	22	23	24	25 Water[4]	26	27 Water[4]
28	29 Water[4]					

[4] 1000 mL $tray^{-1}$

Figure C6. Greenhouse maintenance schedule for February 2016.

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
		1	2	3 Water[4] Trays rotated 180°;	4	5 Water[4]
6	7 Water[4]	8	9 Water[4]	10 SE treatment (2 mL L^{-1});	11 Water[4]	12
13	14 Water[4]	15	16	17 Water[4]	18	19
20	21 Water[4]	22	23	24 Water[4]	25	26
27 Water[4]	28	29	30 Water[4]	31 SE treatment (2 mL L^{-1});		

[4] 1000 mL $tray^{-1}$

Figure C7. Greenhouse maintenance schedule for March 2016.

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
					1	2
3	4 Water[4]	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30

[4] 1000 mL $tray^{-1}$

Figure C8. Greenhouse maintenance schedule for April 2016

APPENDIX D: EXPERIMENTAL SITE MAP

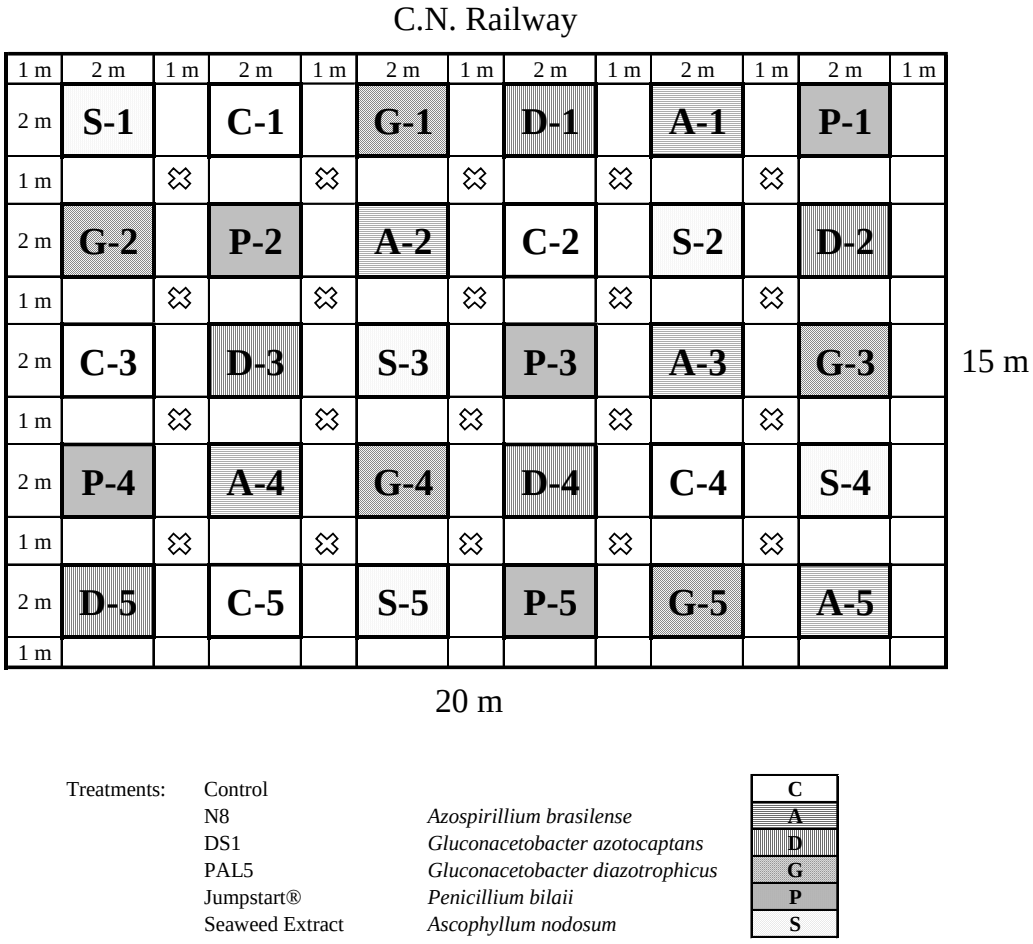


Figure D1. *A. donax* field trial map (Field C1 at Nappan Research Farm (AAFC)), Nappan, NS, Canada. The experimental plots cover an approximate area of 300 m^2 in the west end of the field. Letter and number combinations within plots indicate the growth-promoter treatment and the plot replicate. The "X" symbols represent the locations of soil sample collection (2 soil cores per location, 1 × 0 – 15 cm core and 1 × 15 – 30 cm core).

APPENDIX E: FREQUENTIST STATISTICAL ANALYSES

Data was analyzed using one-way analysis of variance testing (ANOVA) using RStudio Version 0.99.484. One-way ANOVA testing was performed on all variables in which data was collected. When the F-statistic was significant, the treatment means of each variable were analyzed using various Post-Hoc analyses, including least significant difference (LSD) tests and pairwise comparisons (RStudio Version 0.99.484).

Greenhouse Experiment #1

Table E1. ANOVA results of mean plant height (cm) measured on 14 September 2015 prior to biomass harvest. Eight growth-promoter treatments applied to NileFiber™ compared to an untreated control.

Source	***df***	***SS***	***MS***	***F***	***Pr(>F)***
PGPR	8	3098	387.3	2.859	0.00667**
Residuals	99	13410	135.5		
Total	107	16508			

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table E2. LSD results of mean plant height (cm) with no adjustment to the P-value. Treatments which were significantly different at $P \leq 0.05$ are indicated by different letters. Standard errors are reported in brackets.

Treatment	**Mean Height (cm)**	**LSD (p.adj = none)**
PL	119.2 (6.4)	a
C	118.8 (13.3)	a
S	115.7 (14.8)	ab
A	115.6 (11.5)	ab
G	115.2 (10.2)	ab
P	114.5 (9.1)	ab
GL	107.8 (17.9)	bc
L	107.6 (7.6)	bc
AL	102.6 (9.2)	c

Table E3. Pairwise comparisons using t-tests with pooled standard deviation of mean plant height (cm) between eight growth-promoter treatments and an untreated control treatment applied to NileFiber™.

	A	AL	C	G	GL	L	P	PL
AL	< 0.05	-	-	-	-	-	-	-
C	ns	< 0.05	-	-	-	-	-	-
G	ns	< 0.05	ns	-	-	-	-	-
GL	ns	ns	< 0.05	ns	-	-	-	-
L	ns	ns	< 0.05	ns	ns	-	-	-
P	ns	< 0.05	ns	ns	ns	ns	-	-
PL	ns	< 0.05	ns	ns	< 0.05	< 0.05	ns	-
S	ns	< 0.05	ns	ns	ns	ns	ns	ns

Table E4. ANOVA results of mean number of shoots per plant measured on 14 September 2015 prior to biomass harvest. Eight growth-promoter treatments applied to NileFiber™ against an untreated control.

Source	***df***	***SS***	***MS***	***F***	***Pr(>F)***
PGPR	8	3.91	0.4884	0.861	0.552
Residuals	99	56.17	0.5673		
Total	107	60.08			

Table E5. ANOVA results of mean number of visible buds per plant measured on 14 September 2015 prior to biomass harvest. Eight growth-promoter treatments applied to NileFiber™ against an untreated control.

Source	***df***	***SS***	***MS***	***F***	***Pr(>F)***
PGPR	8	2.33	0.2917	0.795	0.609
Residuals	99	36.33	0.3670		
Total	107	38.66			

Table E6. ANOVA results of mean above-ground biomass fresh weight per plant (g) measured 14 September 2015 during biomass harvest. Eight growth-promoter treatments applied to NileFiber™ against an untreated control.

Source	***df***	***SS***	***MS***	***F***	***Pr(>F)***
PGPR	8	701.9	87.73	2.922	0.00571**
Residuals	99	2972.9	30.03		
Total	107	3674.8			

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table E7. LSD results of mean above-ground biomass fresh weight per plant (g) with no adjustment to the P-value. Treatments which were significantly different at $P \leq 0.05$ are indicated by different letters. Standard errors are reported in brackets.

Treatment	**Mean Above-Ground Biomass Fresh Weight (g)**	**LSD (p.adj = none)**
C	47 (5)	a
G	45 (5)	ab
A	45 (6)	ab
P	43 (5)	abc
PL	43 (4)	abc
L	42 (4)	bcd
S	42 (6)	bcd
AL	39 (5)	cd
GL	38 (7)	d

Table E8. Pairwise comparisons using t-tests with pooled standard deviation of mean above-ground biomass fresh weight per plant (g) between eight growth-promoter treatments and an untreated control treatment applied to NileFiber™.

	A	AL	C	G	GL	L	P	PL
AL	< 0.05	-	-	-	-	-	-	-
C	ns	< 0.05	-	-	-	-	-	-
G	ns	< 0.05	ns	-	-	-	-	-
GL	< 0.05	ns	< 0.05	< 0.05	-	-	-	-
L	ns	ns	< 0.05	ns	ns	-	-	-
P	ns	ns	ns	ns	< 0.05	ns	-	-
PL	ns	ns	ns	ns	< 0.05	ns	ns	-
S	ns	ns	< 0.05	ns	ns	ns	ns	ns

Table E9. ANOVA results of mean root dry weight per plant (g) measured 22 September 2015 (post biomass harvest). Eight growth-promoter treatments applied to NileFiber™ against an untreated control.

Source	***df***	***SS***	***MS***	***F***	***Pr(>F)***
PGPR	8	12.76	1.5952	4.648	7.6×10^{-5}***
Residuals	99	33.98	0.3432		
Total	107	46.74			

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table E10. LSD results of mean root dry weight per plant (g) with no adjustment to the P-value. Treatments which were significantly different at $P \leq 0.05$ are indicated by different letters. Standard errors are reported in brackets.

Treatment	**Mean Root Dry Weight (g)**	**LSD (p.adj = none)**
C	4.11 (0.63)	a
A	4.00 (0.59)	ab
P	3.85 (0.63)	abc
S	3.75 (0.76)	abc
PL	3.60 (0.54)	bc
G	3.56 (0.53)	bcd
L	3.44 (0.48)	cde
AL	3.10 (0.57)	de
GL	3.07 (0.49)	e

Table E11. Pairwise comparisons using t-tests with pooled standard deviation of mean root dry weight per plant (g) between eight growth-promoter treatments and an untreated control treatment applied to NileFiber™.

	A	AL	C	G	GL	L	P	PL
AL	< 0.05	-	-	-	-	-	-	-
C	ns	< 0.05	-	-	-	-	-	-
G	ns	0.05	< 0.05	-	-	-	-	-
GL	< 0.05	ns	< 0.05	< 0.05	-	-	-	-
L	< 0.05	ns	< 0.05	ns	ns	-	-	-
P	ns	< 0.05	ns	ns	< 0.05	ns	-	-
PL	ns	< 0.05	< 0.05	ns	< 0.05	ns	ns	-
S	ns	< 0.05	ns	ns	< 0.05	ns	ns	ns

Table E12. ANOVA results of mean above-ground biomass dry weight per plant (g) measured 22 September 2015 (post biomass harvest). Eight growth-promoter treatments applied to NileFiber™ against an untreated control.

Source	***df***	***SS***	***MS***	***F***	***Pr(>F)***
PGPR	8	94.4	11.80	0.804	0.6
Residuals	99	1451.9	14.66		
Total	107	1546.3			

Table E13. ANOVA results of mean dry matter content (%) calculated 22 September 2015 (post biomass harvest). Eight growth-promoter treatments applied to NileFiber™ against an untreated control.

Source	***df***	***SS***	***MS***	***F***	***Pr(>F)***
PGPR	8	317	39.58	1.07	0.39
Residuals	99	3663	37.00		
Total	107	3980			

Table E14. Greenhouse Experiment #1: results of eight growth-promoter treatments applied to NileFiber™ against an untreated contr ol.
Mean plant height (cm), biomass fresh weight (g) and root dry weight (g) are reported. Treatments which were significantly different than the untreated control at $P \leq 0.05$ are indicated in bold with an asterisk (*). Standard errors are reported in brackets.

Treatment		**Mean Height (cm)**	**Mean Biomass Fresh Weight (g)**	**Mean Root Dry Weight (g)**
A. brasilense N8	A	115.6 (11.5)	45 (6)	4.00 (0.59)
A. brasilense N8 + LCO	AL	**102.6* (9.2)**	**39* (5)**	**3.10* (0.57)**
G. diazatrophicus PAL5T	G	115.2 (10.2)	45 (5)	**3.56* (0.53)**
G. diazatrophicus PAL5T + LCO	GL	**107.8* (17.9)**	**38* (7)**	**3.07* (0.49)**
P. bilaii	P	114.5 (9.1)	43 (5)	3.85 (0.63)
P. bilaii + LCO	PL	119.2 (6.4)	43 (4)	**3.60* (0.54)**
A. nodosum	S	115.7 (14.8)	**42* (6)**	3.75 (0.76)
LCO	L	**107.6* (7.6)**	**42* (4)**	**3.44* (0.48)**
Control	C	118.8 (13.3)	47 (5)	4.11 (0.63)

Field Experiment

Table E15. ANOVA results of mean plant height (cm) measured on 8 October 2015 prior to biomass harvest. Five growth-promoter treatments applied to NileFiber™ compared to an untreated control.

Source	***df***	***SS***	***MS***	***F***	***Pr(>F)***
PGPR	5	477	95.4	0.891	0.487
Residuals	264	28256	107.0		
Total	269	28733			

Table E16. ANOVA results of mean number of shoots per plant measured on 8 October 2015 prior to biomass harvest. Five growth-promoter treatments applied to NileFiber™ compared to an untreated control.

Source	***df***	***SS***	***MS***	***F***	***Pr(>F)***
PGPR	5	114.2	22.836	3.999	0.00163**
Residuals	264	1507.7	5.711		
Total	269	1621.9			

Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘’ 1

Table E17. LSD results of mean number of shoots per plant with no adjustment to the P-value. Treatments which were significantly different at $P \leq 0.05$ are indicated by different letters. Standard errors are reported in brackets.

Treatment	**Mean Number of Shoots (g)**	**LSD (p.adj = none)**
C	9.3 (2.3)	a
D	9.0 (2.3)	a
P	8.4 (2.6)	ab
S	8.0 (2.5)	b
G	7.8 (2.2)	b
A	7.5 (2.4)	b

Table E18. Pairwise comparisons using t-tests with pooled standard deviation of mean number of shoots per plant between five growth-promoter treatments and an untreated control treatment applied to NileFiber™.

	A	C	D	G	P
C	< 0.05	-	-	-	-
D	< 0.05	ns	-	-	-
G	ns	< 0.05	< 0.05	-	-
P	0.05	ns	ns	ns	-
S	ns	< 0.05	< 0.05	ns	ns

Table E19. ANOVA results of mean above-ground biomass fresh weight per plot (g) measured on 8 October 2015 during biomass harvest. Five growth-promoter treatments applied to NileFiber™ compared to an untreated control.

Source	***df***	***SS***	***MS***	***F***	***Pr(>F)***
PGPR	5	113549	22710	0.222	0.949
Residuals	24	2454293	102262		
Total	29	2567842			

Table E20. ANOVA results of mean above-ground biomass dry weight per plot (g) measured on 15 October 2015 (post biomass harvest). Five growth-promoter treatments applied to NileFiber™ compared to an untreated control.

Source	***df***	***SS***	***MS***	***F***	***Pr(>F)***
PGPR	5	14288	2858	0.3	0.908
Residuals	24	228860	9536		
Total	29	243148			

Table E21. ANOVA results of mean dry matter content (%) calculated 15 October 2015 (post biomass harvest). Five growth-promoter treatments applied to NileFiber™ against an untreated control.

Source	***df***	***SS***	***MS***	***F***	***Pr(>F)***
PGPR	5	3.896	0.7792	1.661	0.182
Residuals	24	11.257	0.4690		
Total	29	15.153			

Greenhouse Experiment #2

Table E22. ANOVA results of mean plant height (cm) measured on 11 April 2016 prior to biomass harvest. Fourteen growth-promoter treatments applied to NileFiber™ compared to an untreated control.

Source	***df***	***SS***	***MS***	***F***	***Pr(>F)***
PGPR	14	340.9	24.35	1.717	0.0541 .
Residuals	210	2978.1	14.18		
Total	224	3319			

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table E23. LSD results of mean plant height (cm) with no adjustment to the P-value. Treatments which were significantly different at $P \leq 0.05$ are indicated by different letters. Standard errors are reported in brackets.

Treatment	**Mean Height (cm)**	**LSD (p.adj = none)**
G	37 (3.7)	a
P	36.33 (4.0)	ab
VPL	36.13 (3.2)	ab
AL	36.07 (4.2)	ab
PL	36.07 (3.3)	ab
DL	35.4 (3.8)	abc
VP	34.93 (4.1)	abc
A	34.8 (4.2)	abc
GS	34.73 (3.7)	abc
PLL	34.6 (2.7)	abc
C	34.13 (4.4)	bc
S	33.8 (3.1)	bc
D	33.13 (4.2)	c
GL	33.07 (2.4)	c
GSL	33.07 (4.6)	c

Table E24. Pairwise comparisons using t-tests with pooled standard deviation of mean plant height (cm) between fourteen growth-promoter treatments and an untreated control treatment applied to NileFiber™.

	A	AL	C	D	DL	G	GL	GS	GSL	P	PL	PLL	S	VP	VPL
AL	ns	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	ns	ns	-	-	-	-	-	-	-	-	-	-	-	-	-
D	ns	< 0.05	ns	-	-	-	-	-	-	-	-	-	-	-	-
DL	ns	ns	ns	ns	-	-	-	-	-	-	-	-	-	-	-
G	ns	ns	< 0.05	< 0.05	ns	-	-	-	-	-	-	-	-	-	-
GL	ns	< 0.05	ns	ns	ns	< 0.05	-	-	-	-	-	-	-	-	-
GS	ns	ns	ns	ns	ns	ns	ns	-	-	-	-	-	-	-	-
GSL	ns	< 0.05	ns	ns	ns	< 0.05	ns	ns	-	-	-	-	-	-	-
P	ns	ns	ns	< 0.05	ns	ns	< 0.05	ns	< 0.05	-	-	-	-	-	-
PL	ns	ns	ns	< 0.05	ns	ns	< 0.05	ns	< 0.05	ns	-	-	-	-	-
PLL	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-	-	-	-
S	ns	ns	ns	ns	ns	< 0.05	ns	ns	ns	ns	ns	ns	-	-	-
VP	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-	-
VPL	ns	ns	ns	< 0.05	ns	ns	< 0.05	ns	< 0.05	ns	ns	ns	ns	ns	-

Table E25. ANOVA results of mean number of shoots per plant measured on 11 April 2016 prior to biomass harvest. Fourteen growth-promoter treatments applied to NileFiber™ compared to an untreated control.

Source	***df***	***SS***	***MS***	***F***	***Pr(>F)***
PGPR	14	13.69	0.9778	1.814	0.0382*
Residuals	210	113.20	0.5390		
Total	224	126.89			

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table E26. LSD results of mean number of shoots per plant with no adjustment to the P-value. Treatments which were significantly different at $P \leq 0.05$ are indicated by different letters. Standard errors are reported in brackets.

Treatment	**Mean Number of Shoots (per plant)**	**LSD (p.adj = none)**
AL	3.267 (0.88)	a
D	3.067 (0.70)	ab
PL	3.067 (0.80)	ab
DL	3 (0.65)	ab
VPL	3 (0.76)	ab
PLL	2.933 (0.70)	abc
GL	2.867 (0.74)	abcd
VP	2.867 (0.64)	abcd
A	2.8 (0.77)	abcd
P	2.8 (0.68)	abcd
GSL	2.733 (0.59)	bcd
G	2.667 (0.90)	bcd
C	2.467 (0.92)	cd
GS	2.4 (0.51)	d
S	2.4 (0.63)	d

Table E27. Pairwise comparisons using t-tests with pooled standard deviation of mean number of shoots per plant between fourteen growth-promoter treatments and an untreated control treatment applied to NileFiber™.

	A	AL	C	D	DL	G	GL	GS	GSL	P	PL	PLL	S	VP	VPL
AL	ns	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	ns	< 0.05	-	-	-	-	-	-	-	-	-	-	-	-	-
D	ns	ns	< 0.05	-	-	-	-	-	-	-	-	-	-	-	-
DL	ns	ns	< 0.05	ns	-	-	-	-	-	-	-	-	-	-	-
G	ns	< 0.05	ns	ns	ns	-	-	-	-	-	-	-	-	-	-
GL	ns	ns	ns	ns	ns	ns	-	-	-	-	-	-	-	-	-
GS	ns	< 0.05	ns	< 0.05	< 0.05	ns	ns	-	-	-	-	-	-	-	-
GSL	ns	< 0.05	ns	ns	ns	ns	ns	ns	-	-	-	-	-	-	-
P	ns	ns	ns	ns	ns	ns	ns	ns	ns	-	-	-	-	-	-
PL	ns	ns	< 0.05	ns	ns	ns	ns	< 0.05	ns	ns	-	-	-	-	-
PLL	ns	ns	ns	ns	ns	ns	ns	< 0.05	ns	ns	ns	-	-	-	-
S	ns	< 0.05	ns	< 0.05	< 0.05	ns	ns	ns	ns	ns	< 0.05	< 0.05	-	-	-
VP	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	< 0.05	-	-
VPL	ns	ns	< 0.05	< 0.05	ns	ns	ns	< 0.05	ns	ns	ns	ns	< 0.05	ns	-

Table E28. ANOVA results of mean above-ground biomass fresh weight per plant (g) measured on 11 April 2016 prior to biomass harvest. Fourteen growth-promoter treatments applied to NileFiber™ compared to an untreated control.

Source	***df***	***SS***	***MS***	***F***	***Pr(>F)***
PGPR	14	44.13	3.1521	5.42	8.02×10^{-9}***
Residuals	210	122.13	0.5816		
Total	224	166.26			

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table E29. LSD results of mean above-ground biomass fresh weight per plant (g) with no adjustment to the P-value. Treatments which were significantly different at $P \leq 0.05$ are indicated by different letters. Standard errors are reported in brackets.

Treatment	**Mean Above-Ground Biomass Fresh Weight (g)**	**LSD (p.adj = none)**
AL	4.067 (0.78)	a
P	3.733 (0.98)	ab
VPL	3.733 (0.59)	ab
DL	3.633 (0.74)	ab
G	3.633 (0.77)	ab
VP	3.633 (0.67)	ab
GL	3.6 (1.11)	ab
GS	3.567 (0.46)	abc
PLL	3.367 (0.58)	bcd
A	3.2 (0.90)	bcd
PL	3.033 (0.88)	cd
C	3 (0.91)	d
GSL	2.9 (0.51)	d
D	2.867 (0.58)	d
S	2.3 (0.68)	e

Table E30. Pairwise comparisons using t-tests with pooled standard deviation of mean above-ground biomass fresh weight per plant (g) between fourteen growth-promoter treatments and an untreated control treatment applied to NileFiber™.

	A	AL	C	D	DL	G	GL	GS	GSL	P	PL	PLL	S	VP	VPL
AL	< 0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	ns	< 0.05	-	-	-	-	-	-	-	-	-	-	-	-	-
D	ns	< 0.05	ns	-	-	-	-	-	-	-	-	-	-	-	-
DL	ns	ns	< 0.05	< 0.05	-	-	-	-	-	-	-	-	-	-	-
G	ns	ns	< 0.05	< 0.05	ns	-	-	-	-	-	-	-	-	-	-
GL	ns	ns	< 0.05	< 0.05	ns	ns	-	-	-	-	-	-	-	-	-
GS	ns	ns	< 0.05	< 0.05	ns	ns	ns	-	-	-	-	-	-	-	-
GSL	ns	< 0.05	ns	ns	< 0.05	< 0.05	< 0.05	< 0.05	-	-	-	-	-	-	-
P	ns	ns	< 0.05	< 0.05	ns	ns	ns	ns	< 0.05	-	-	-	-	-	-
PL	ns	< 0.05	ns	ns	< 0.05	< 0.05	< 0.05	< 0.05	ns	< 0.05	-	-	-	-	-
PLL	ns	< 0.05	ns	ns	ns	ns	ns	ns	ns	ns	ns	-	-	-	-
S	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	-	-	-
VP	ns	ns	< 0.05	< 0.05	ns	ns	ns	ns	< 0.05	ns	< 0.05	ns	< 0.05	-	-
VPL	ns	ns	< 0.05	< 0.05	ns	ns	ns	ns	< 0.05	ns	< 0.05	ns	< 0.05	ns	-

Table E31. ANOVA results of mean root dry weight per plant (g) weighed on 19 April 2016 prior to biomass harvest. Fourteen growth-promoter treatments applied to NileFiber™ compared to an untreated control.

Source	***df***	***SS***	***MS***	***F***	***Pr(>F)***
PGPR	14	0.6157	0.04398	4.312	1.04×10^{-6}***
Residuals	210	2.1420	0.01020		
Total	224	2.7577			

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table E32. LSD results of mean root dry weight per plant (g) with no adjustment to the P-value. Treatments which were significantly different at $P \leq 0.05$ are indicated by different letters. Standard errors are reported in brackets.

Treatment	**Mean Root Dry Weight (g)**	**LSD (p.adj = none)**
PL	0.6253 (0.14)	a
VP	0.5727 (0.08)	ab
GL	0.546 (0.09)	bc
G	0.5427 (0.07)	bc
C	0.538 (0.11)	bc
VPL	0.5327 (0.13)	bc
AL	0.5207 (0.09)	bc
A	0.5167 (0.13)	bc
DL	0.49 (0.08)	cd
PLL	0.4853 (0.11)	cd
GSL	0.4833 (0.09)	cd
GS	0.4827 (0.08)	cde
P	0.4733 (0.10)	cde
S	0.4313 (0.07)	de
D	0.41 (0.08)	e

Table E33. Pairwise comparisons using t-tests with pooled standard deviation of mean root dry weight per plant (g) between fourteen growth-promoter treatments and an untreated control treatment applied to NileFiber™.

	A	AL	C	D	DL	G	GL	GS	GSL	P	PL	PLL	S	VP	VPL
AL	ns	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	ns	ns	-	-	-	-	-	-	-	-	-	-	-	-	-
D	< 0.05	< 0.05	< 0.05	-	-	-	-	-	-	-	-	-	-	-	-
DL	ns	ns	ns	< 0.05	-	-	-	-	-	-	-	-	-	-	-
G	ns	ns	ns	< 0.05	ns	-	-	-	-	-	-	-	-	-	-
GL	ns	ns	ns	< 0.05	ns	ns	-	-	-	-	-	-	-	-	-
GS	ns	ns	ns	< 0.05	ns	ns	ns	-	-	-	-	-	-	-	-
GSL	ns	ns	ns	< 0.05	ns	ns	ns	ns	-	-	-	-	-	-	-
P	ns	ns	ns	ns	ns	ns	< 0.05	ns	ns	-	-	-	-	-	-
PL	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	-	-	-	-	-
PLL	ns	ns	ns	< 0.05	ns	ns	ns	ns	ns	ns	< 0.05	-	-	-	-
S	< 0.05	< 0.05	< 0.05	ns	ns	< 0.05	< 0.05	ns	ns	ns	< 0.05	ns	-	-	-
VP	ns	ns	ns	< 0.05	< 0.05	ns	ns	< 0.05	< 0.05	< 0.05	ns	< 0.05	< 0.05	-	-
VPL	ns	ns	ns	< 0.05	ns	ns	ns	ns	ns	ns	< 0.05	ns	< 0.05	ns	-

Table E34. ANOVA results of mean above-ground biomass dry weight per plant (g) weighed on 19 April 2016 prior to biomass harvest. Fourteen growth-promoter treatments applied to NileFiber™ compared to an untreated control.

Source	***df***	***SS***	***MS***	***F***	***Pr(>F)***
PGPR	14	4.746	0.3390	6.953	1.12×10^{-11}***
Residuals	210	10.240	0.0488		
Total	224	14.986			

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table E35. LSD results of mean above-ground biomass dry weight per plant (g) with no adjustment to the P-value. Treatments which were significantly different at $P \leq 0.05$ are indicated by different letters. Standard errors are reported in brackets.

Treatment	**Mean Above-Ground Biomass Dry Weight (g)**	**LSD (p.adj = none)**
AL	1.216 (0.19)	a
VPL	1.134 (0.15)	ab
VP	1.133 (0.18)	ab
G	1.111 (0.20)	ab
DL	1.095 (0.23)	abc
P	1.067 (0.24)	abc
A	1.058 (0.23)	abc
PLL	1.028 (0.15)	bc
GS	1.016 (0.15)	bc
D	1.003 (0.21)	bc
GL	0.9913 (0.27)	bcd
PL	0.938 (0.19)	cde
GSL	0.842 (0.16)	def
C	0.8067 (0.20)	ef
S	0.7293 (0.18)	f

Table E36. Pairwise comparisons using t-tests with pooled standard deviation of mean above-ground biomass dry weight per plant (g) between fourteen growth-promoter treatments and an untreated control treatment applied to NileFiber™.

	A	AL	C	D	DL	G	GL	GS	GSL	P	PL	PLL	S	VP	VPL
AL	< 0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	< 0.05	< 0.05	-	-	-	-	-	-	-	-	-	-	-	-	-
D	ns	< 0.05	< 0.05	-	-	-	-	-	-	-	-	-	-	-	-
DL	ns	ns	< 0.05	ns	-	-	-	-	-	-	-	-	-	-	-
G	ns	ns	< 0.05	ns	ns	-	-	-	-	-	-	-	-	-	-
GL	ns	< 0.05	< 0.05	ns	ns	ns	-	-	-	-	-	-	-	-	-
GS	ns	< 0.05	< 0.05	ns	ns	ns	ns	-	-	-	-	-	-	-	-
GSL	< 0.05	< 0.05	ns	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	-	-	-	-	-	-	-
P	ns	< 0.05	< 0.05	ns	ns	ns	ns	ns	< 0.05	-	-	-	-	-	-
PL	ns	< 0.05	ns	ns	< 0.05	< 0.05	ns	ns	ns	ns	-	-	-	-	-
PLL	ns	< 0.05	< 0.05	ns	ns	ns	ns	ns	< 0.05	ns	ns	-	-	-	-
S	< 0.05	< 0.05	ns	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	ns	< 0.05	< 0.05	< 0.05	-	-	-
VP	ns	ns	< 0.05	ns	ns	ns	< 0.05	ns	< 0.05	ns	< 0.05	ns	< 0.05	-	-
VPL	ns	ns	< 0.05	ns	ns	ns	< 0.05	ns	< 0.05	ns	< 0.05	ns	< 0.05	ns	-

Table E37. ANOVA results of mean dry matter content per plant (%) calculated on 19 April 2016 prior to biomass harvest. Fourteen growth-promoter treatments applied to NileFiber™ compared to an untreated control.

Source	***df***	***SS***	***MS***	***F***	***Pr(>F)***
PGPR	14	1652	118.01	2.312	0.00564**
Residuals	210	10719	51.04		
Total	224	12371			

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table E38. LSD results of mean dry matter content per plant (%) with no adjustment to the P-value. Treatments which were significantly different at $P \leq 0.05$ are indicated by different letters. Standard errors are reported in brackets.

Treatment	**Mean Dry Matter Content (%)**	**LSD (p.adj = none)**
A	35.31 (12.23)	a
G	35.04 (2.61)	ab
PL	33.79 (14.45)	abc
S	32.39 (5.31)	abcd
VP	31.8 (5.53)	abcd
G	31.19 (4.74)	abcd
PLL	31.03 (5.30)	abcd
VPL	30.62 (3.03)	abcd
DL	30.45 (3.94)	abcd
AL	30.16 (2.91)	bcd
P	29.11 (3.64)	cd
GSL	29.1 (3.04)	cd
GL	28.64 (6.62)	d
GS	28.53 (2.65)	d
C	28.46 (8.49)	d

Table E39. Pairwise comparisons using t-tests with pooled standard deviation of mean dry matter content per plant (%) between fourteen growth-promoter treatments and an untreated control treatment applied to NileFiber™.

	A	AL	C	D	DL	G	GL	GS	GSL	P	PL	PLL	S	VP	VPL
AL	< 0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	< 0.05	ns	-	-	-	-	-	-	-	-	-	-	-	-	-
D	ns	< 0.05	< 0.05	-	-	-	-	-	-	-	-	-	-	-	-
DL	< 0.05	ns	ns	< 0.05	-	-	-	-	-	-	-	-	-	-	-
G	ns	ns	ns	ns	ns	-	-	-	-	-	-	-	-	-	-
GL	< 0.05	ns	ns	< 0.05	ns	ns	-	-	-	-	-	-	-	-	-
GS	< 0.05	ns	ns	< 0.05	ns	ns	ns	-	-	-	-	-	-	-	-
GSL	< 0.05	ns	ns	< 0.05	ns	ns	ns	ns	-	-	-	-	-	-	-
P	< 0.05	ns	ns	< 0.05	ns	ns	ns	ns	ns	-	-	-	-	-	-
PL	ns	ns	< 0.05	ns	ns	ns	< 0.05	< 0.05	< 0.05	< 0.05	-	-	-	-	-
PLL	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-	-	-	-
S	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-	-	-
VP	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-	-
VPL	< 0.05	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-

APPENDIX F: BAYESIAN STATISTICAL ANALYSES

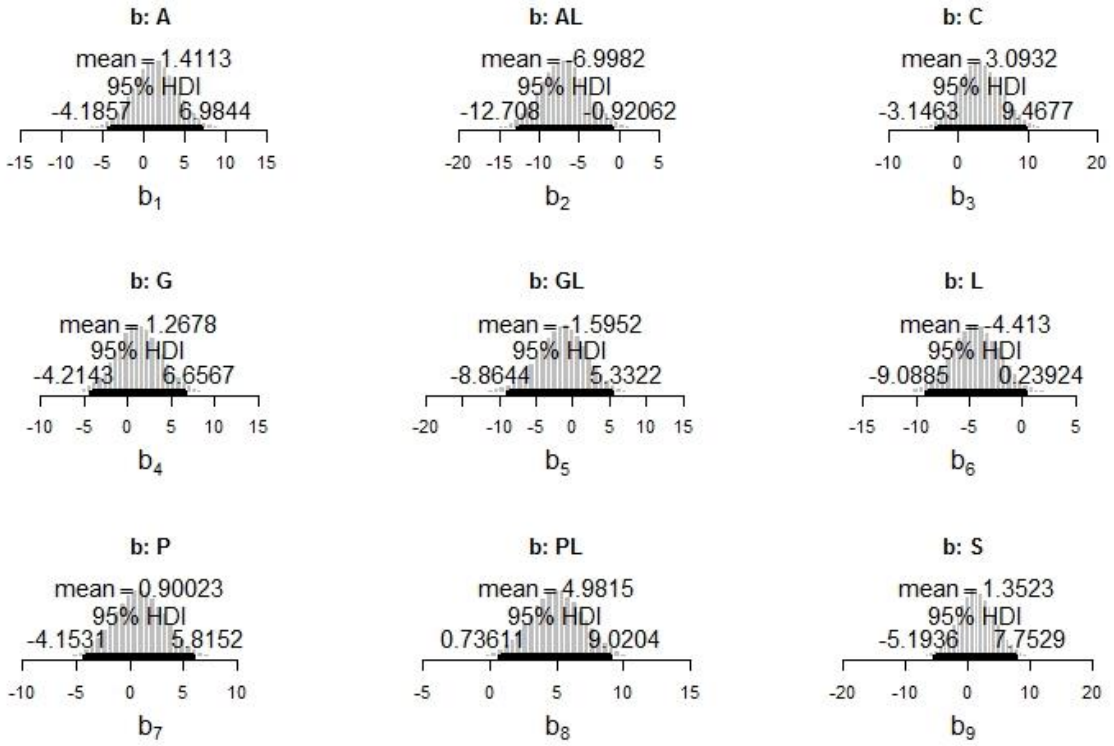


Figure F1. Effect of growth-promoter treatments on plant height (cm) in the first greenhouse experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 9$.

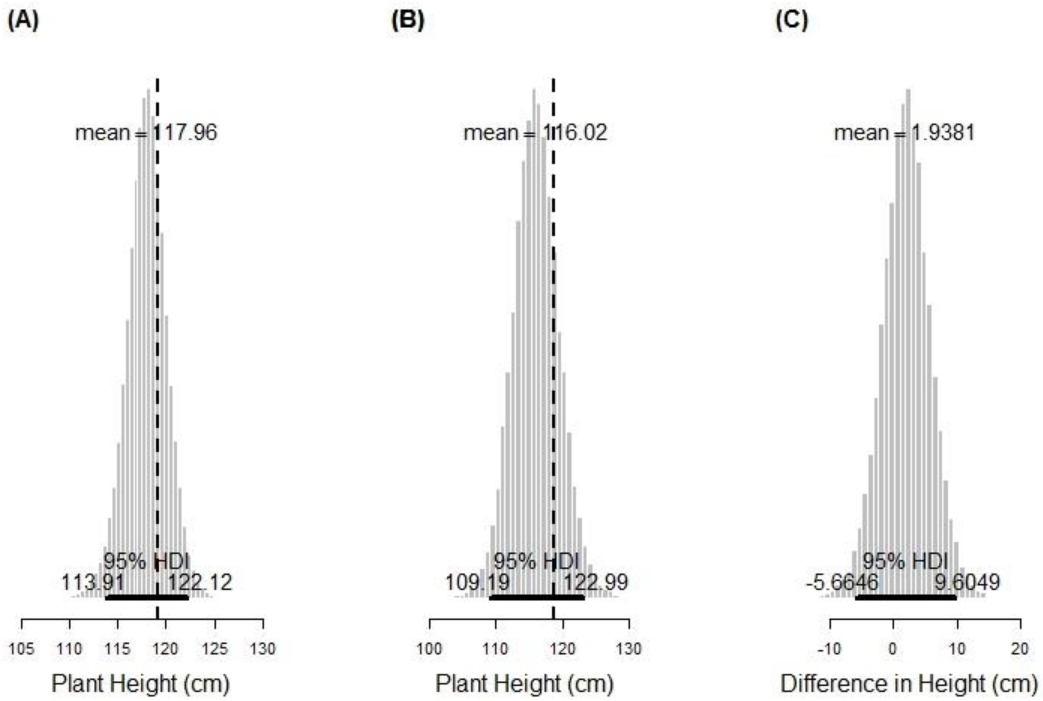


Figure F2. Effect of PL treatment on plant height. Included are the posterior distributions for plant heights (cm) under conditions: **(A)** PL treatment (dashed line represents the observed value of 119.2 cm); **(B)** C treatment (mean = 118.8 cm); and **(C)** the difference between posterior distributions of the PL and C treatments. PL = *P. bilaii* + LCO; C = Control.

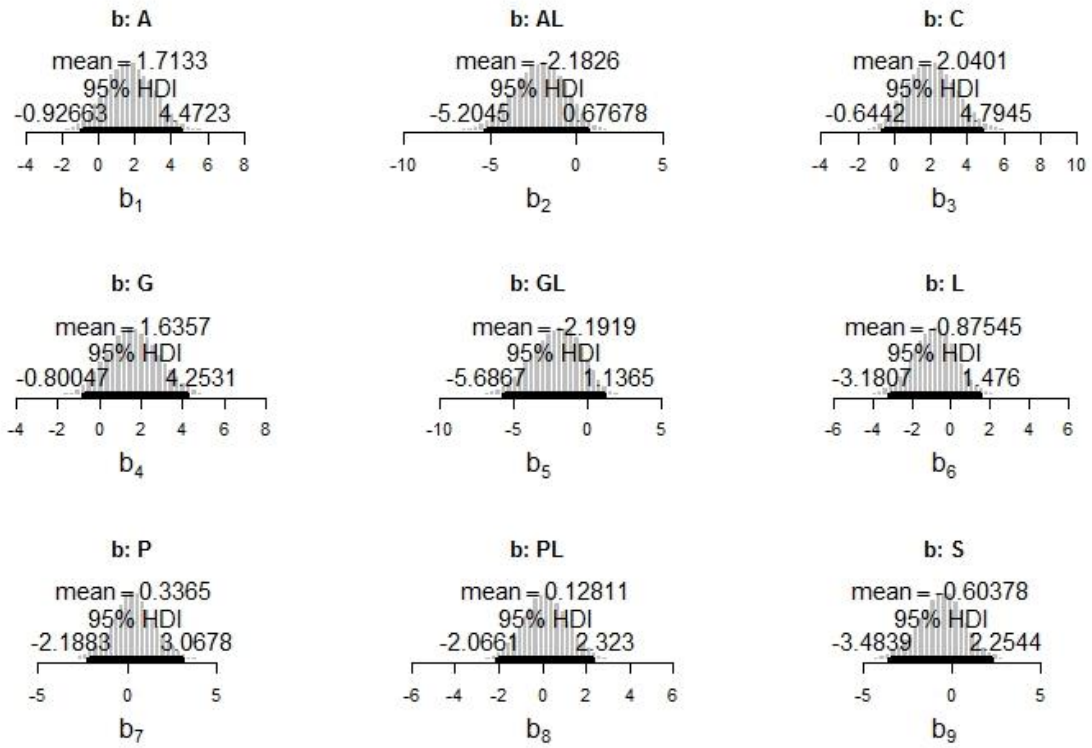


Figure F3. Effect of growth-promoter treatments on above-ground biomass fresh weight (g) per plant in the first greenhouse experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 9$.

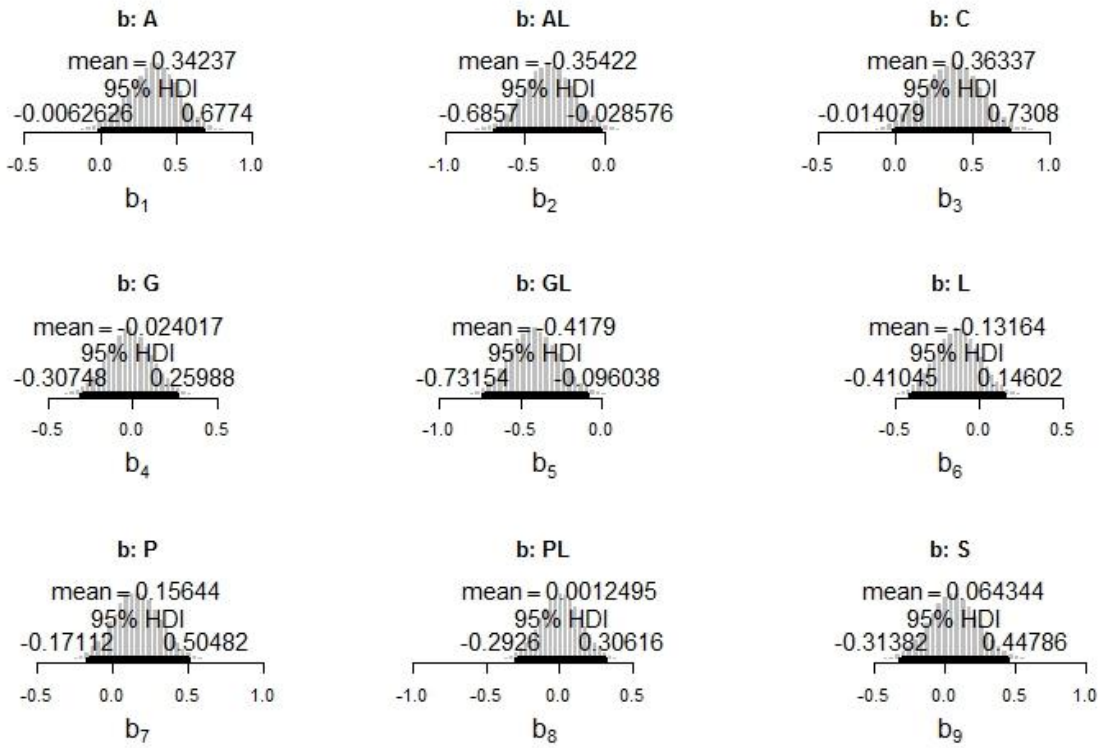


Figure F4. Effect of growth-promoter treatments on root dry weight (g) per plant in the first greenhouse experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 9$.

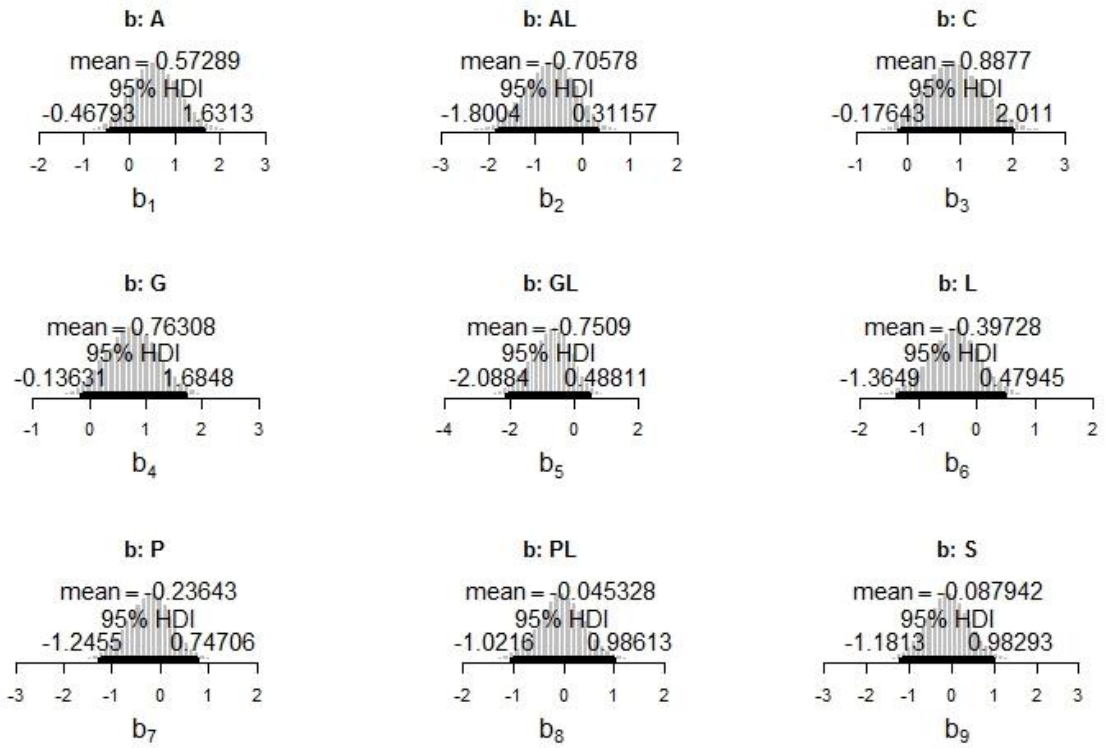


Figure F5. Effect of growth-promoter treatments on above-ground biomass dry weight (g) per plant in the first greenhouse experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 9$.

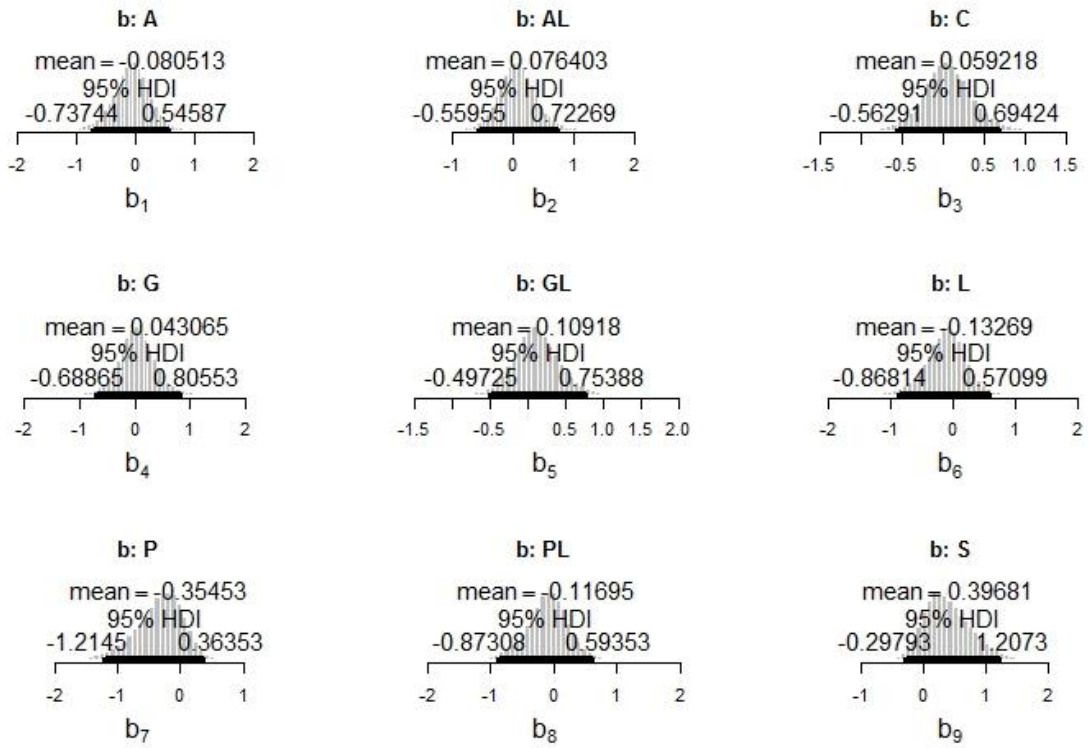


Figure F6. Effect of growth-promoter treatments on dry matter content (%) in the first greenhouse experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 9$.

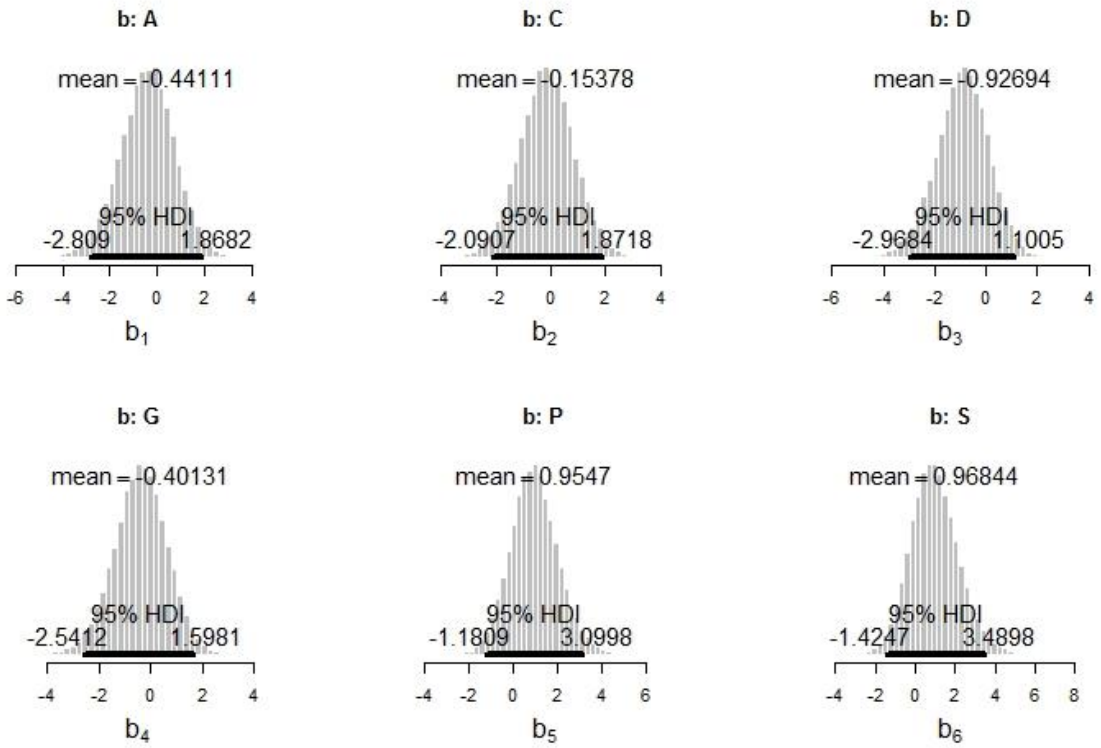


Figure F7. Effect of growth-promoter treatments on plant height (cm) in the field experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 6$.

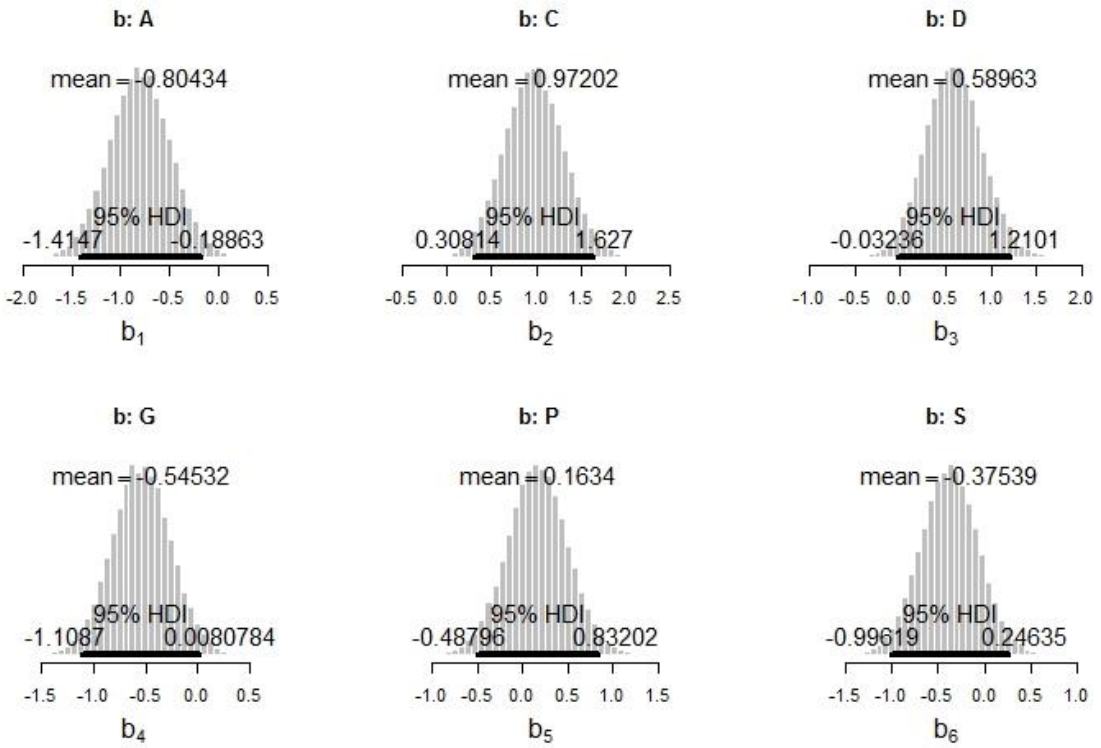


Figure F8. Effect of growth-promoter treatments on number of shoots per plant in the field experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 6$.

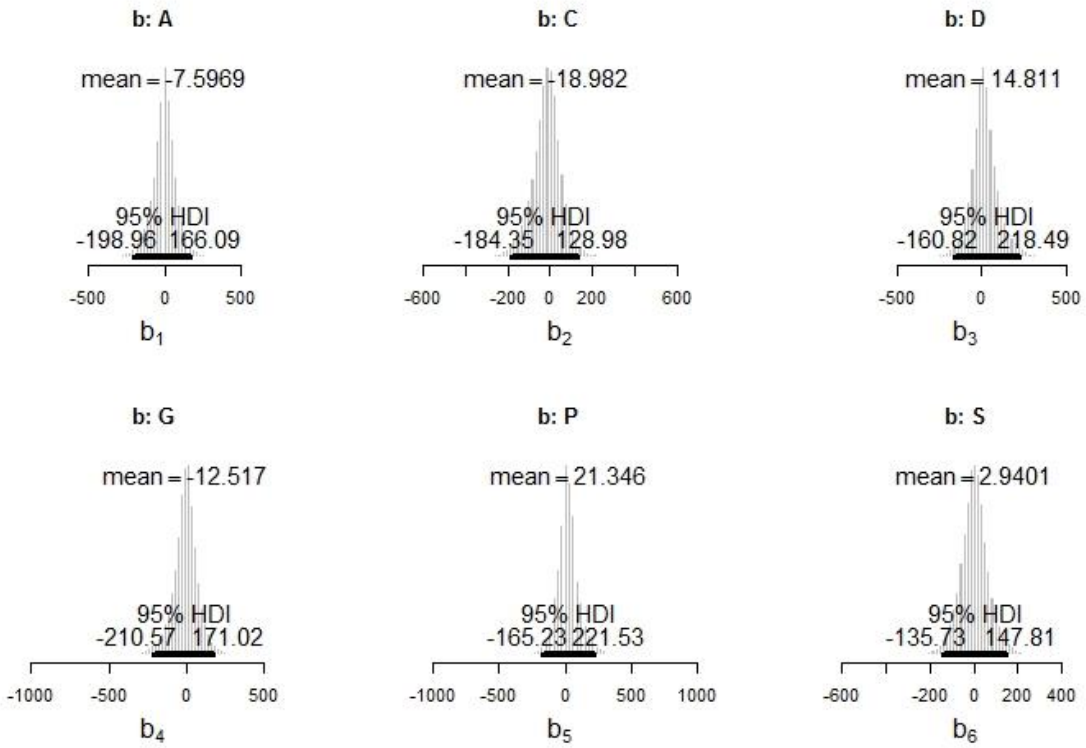


Figure F9. Effect of growth-promoter treatments on above-ground biomass fresh weight (g) per plot in the field experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 6$.

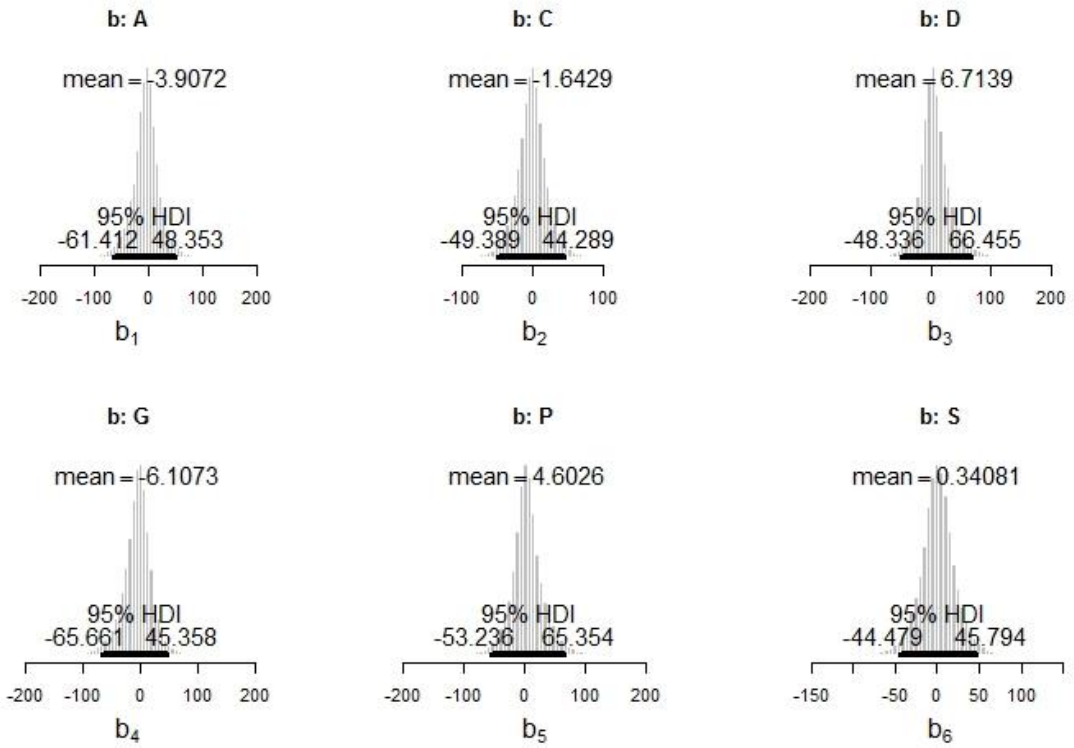


Figure F10. Effect of growth-promoter treatments on above-ground biomass dry weight (g) per plot in the field experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 6$.

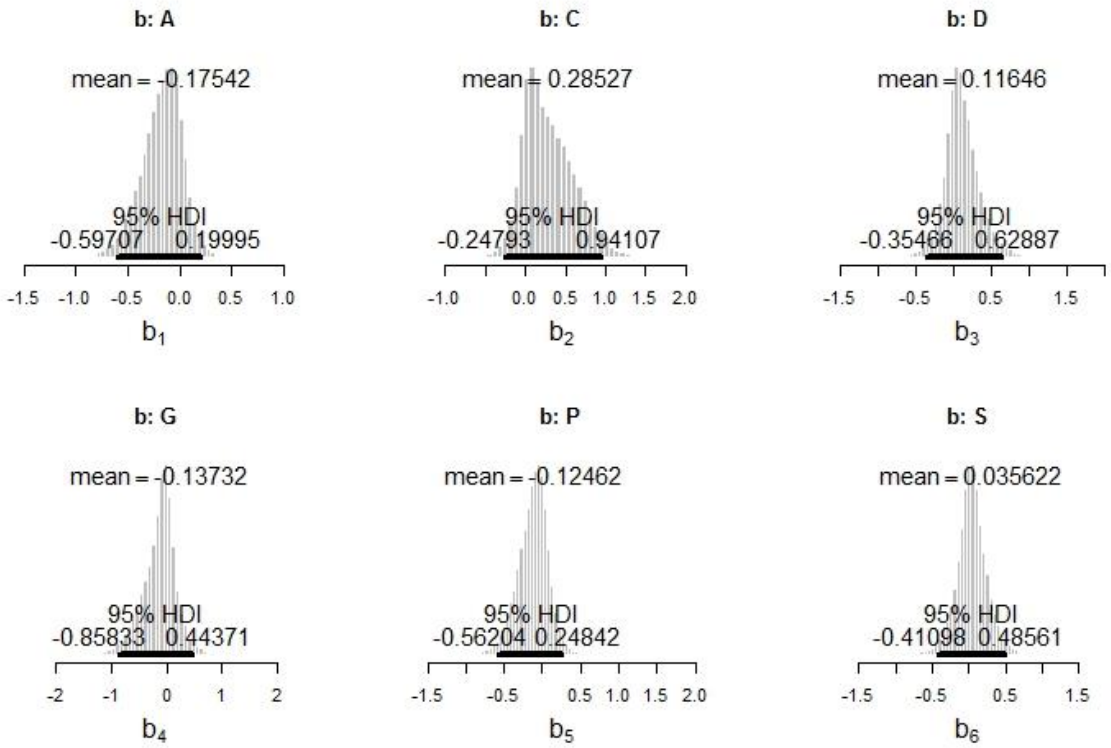


Figure F11. Effect of growth-promoter treatments on dry matter content (%) in the field experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 6$.

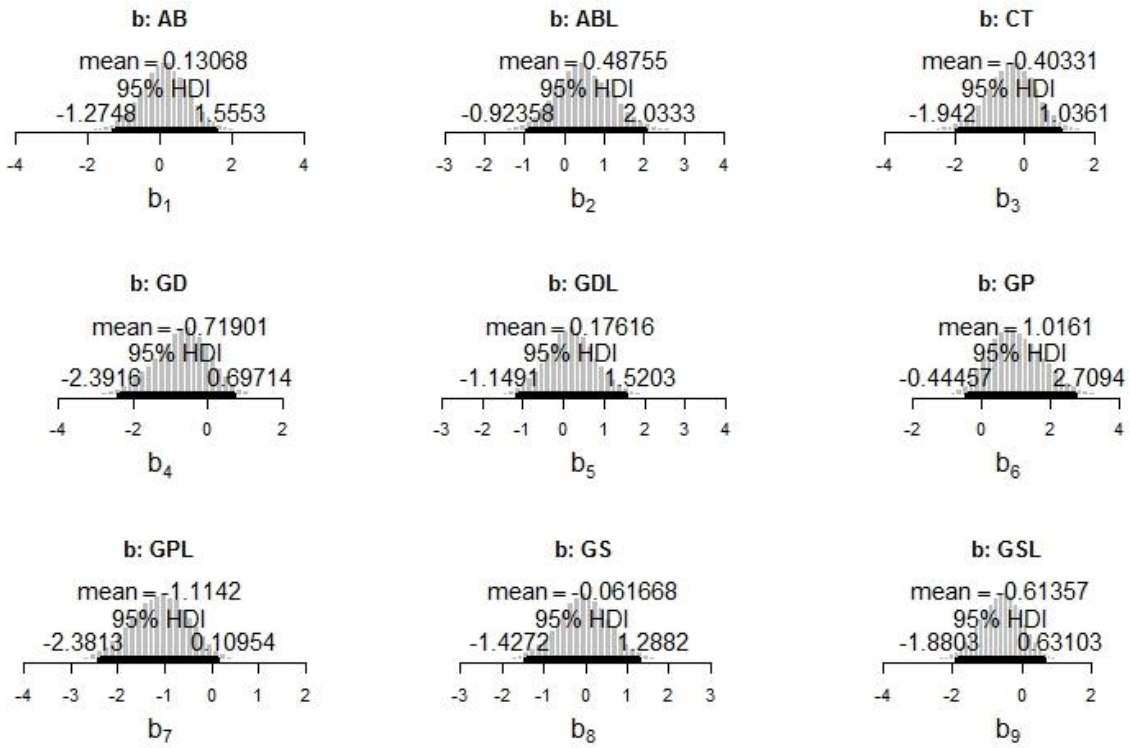


Figure F12a. Effect of growth-promoter treatments on plant height (cm) in the second greenhouse experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 15$.

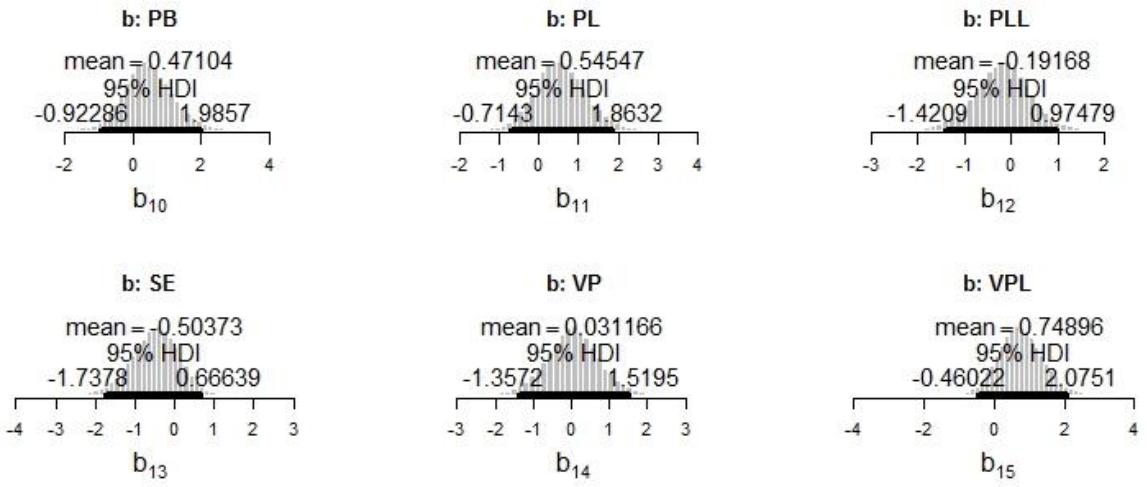


Figure F12b. Effect of growth-promoter treatments on plant height (cm) in the second greenhouse experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 15$.

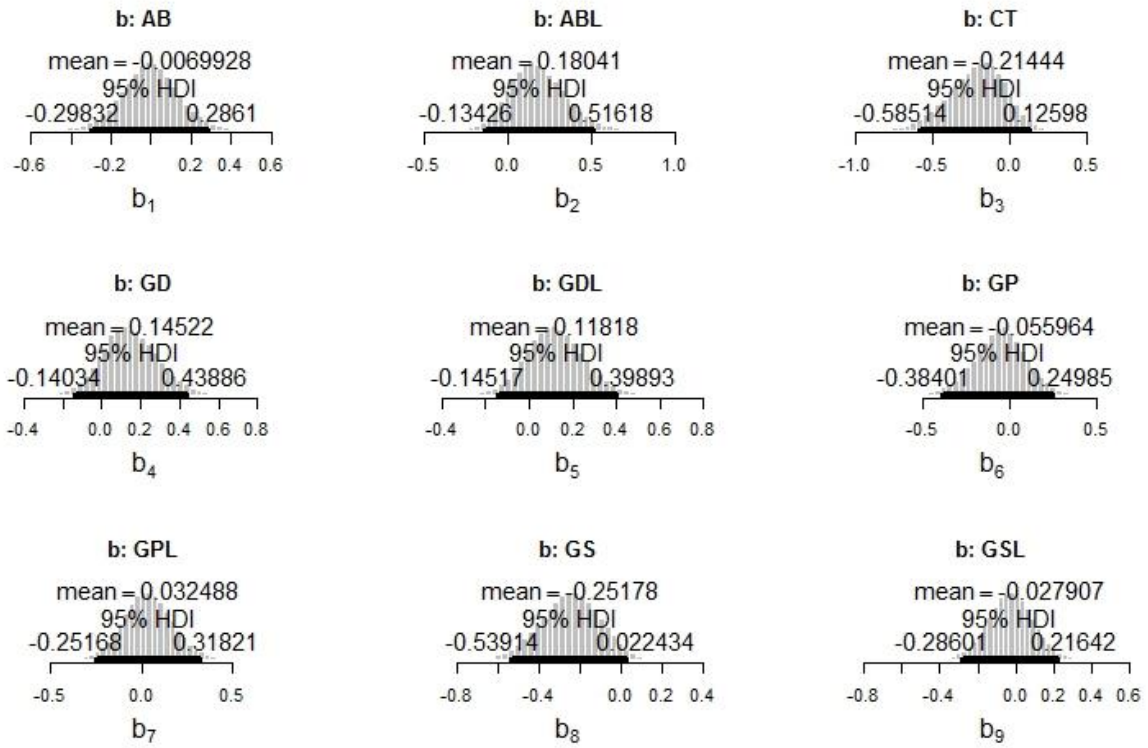


Figure F13a. Effect of growth-promoter treatments on number of shoots per plant in the second greenhouse experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 15$.

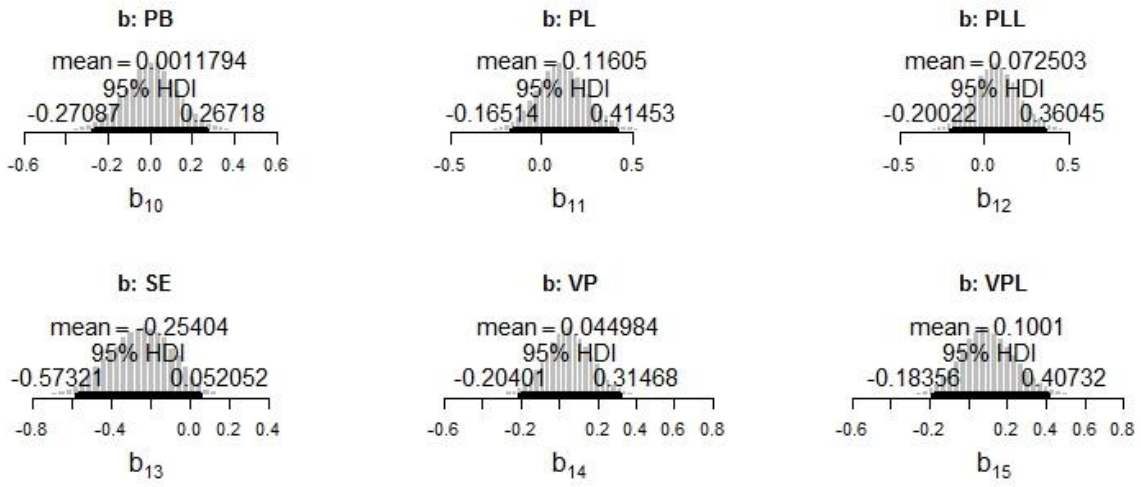


Figure F13b. Effect of growth-promoter treatments on number of shoots per plant in the second greenhouse experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 15$.

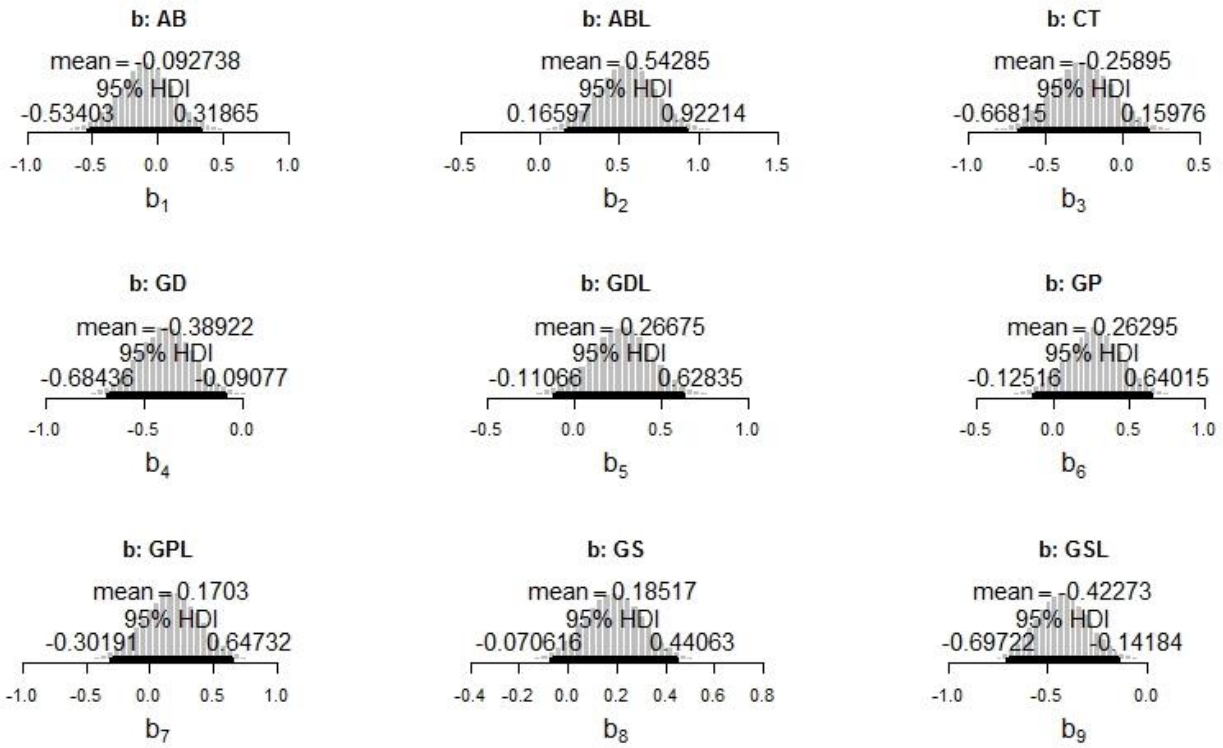


Figure F14a. Effect of growth-promoter treatments on above-ground biomass fresh weight (g) per plant in the second greenhouse experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 15$.

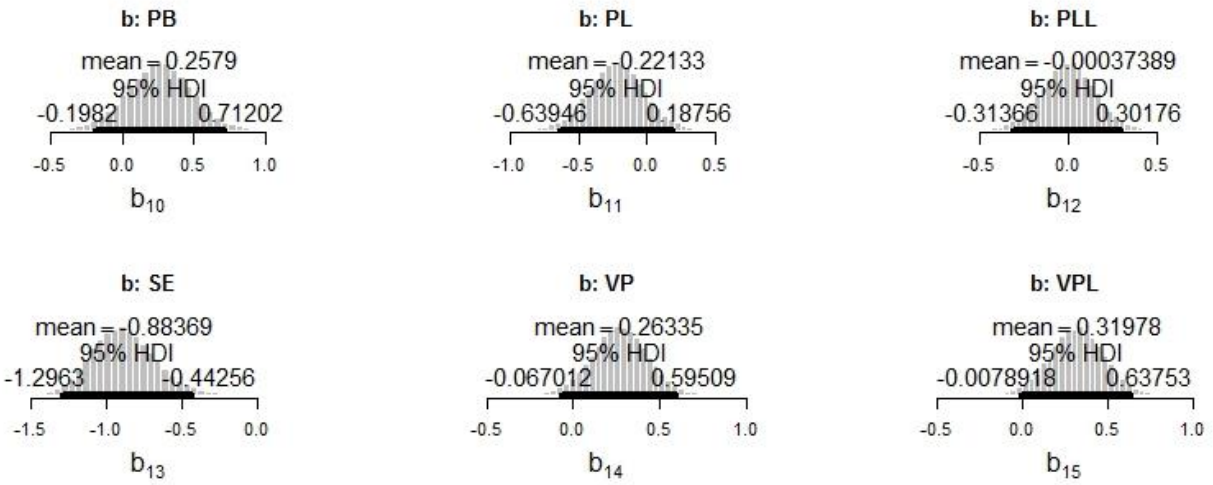


Figure F14b. Effect of growth-promoter treatments on above-ground biomass fresh weight (g) per plant in the second greenhouse experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 15$.

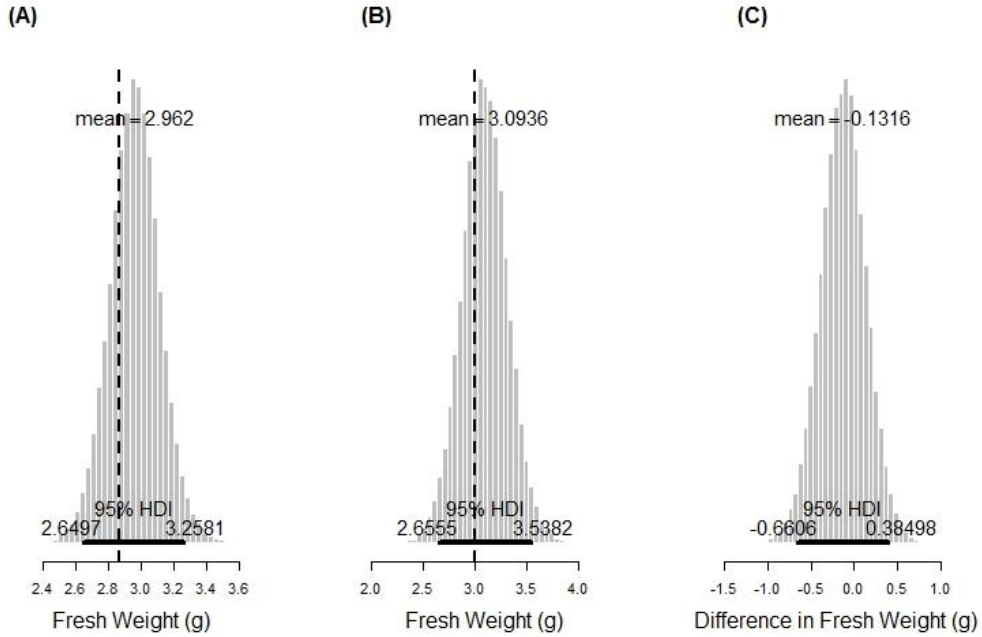


Figure F15. Effect of D treatment on above-ground biomass fresh weight. Included are the posterior distributions for fresh weight (g) per plant under conditions: **(A)** D treatment (mean = 2.867); **(B)** C treatment (mean = 3.00); and **(C)** the difference between posterior distributions of the D and C treatments. D treatment = *G. azotocaptans* DS1; C = Untreated Control.

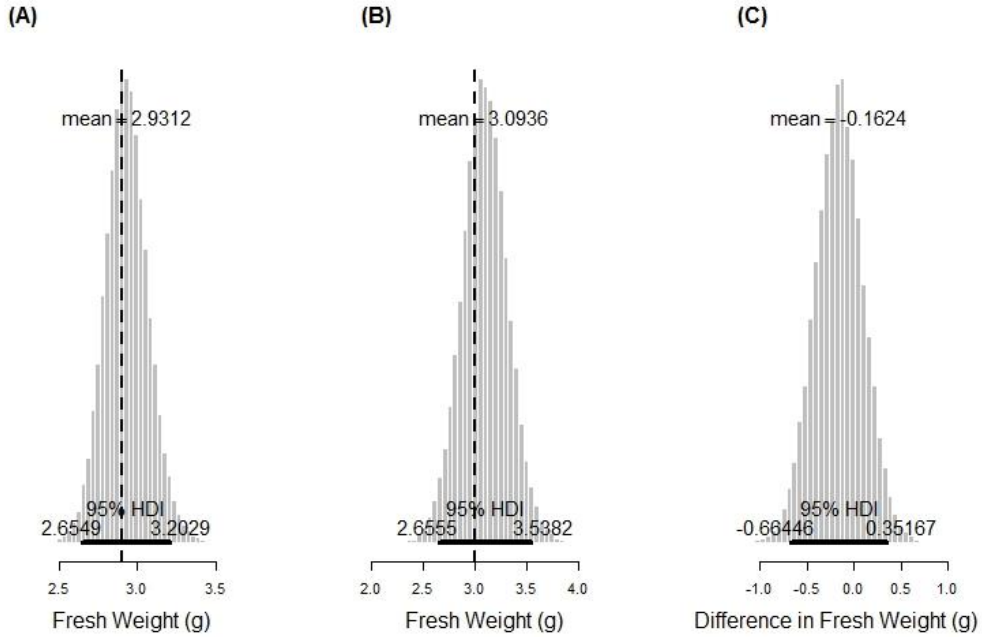


Figure F16. Effect of GSL treatment on above-ground biomass fresh weight. Included are the posterior distributions for fresh weight (g) per plant under conditions: **(A)** GSL treatment (mean = 2.9); **(B)** C treatment (mean = 3.00); and **(C)** the difference between posterior distributions of the GSL and C treatments. GSL treatment = *G. diazotrophicus* SRT4 + LCO; C = Untreated Control.

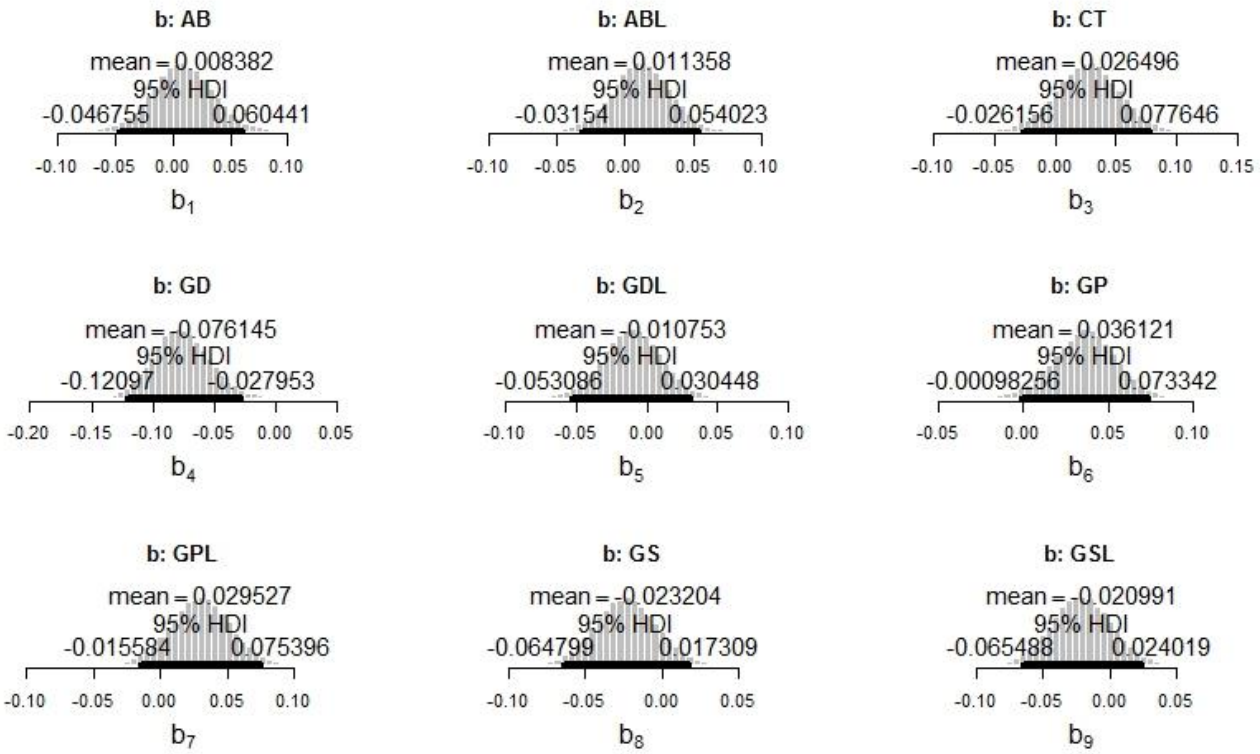


Figure F17a. Effect of growth-promoter treatments on root dry weight (g) per plant in the second greenhouse experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 15$.

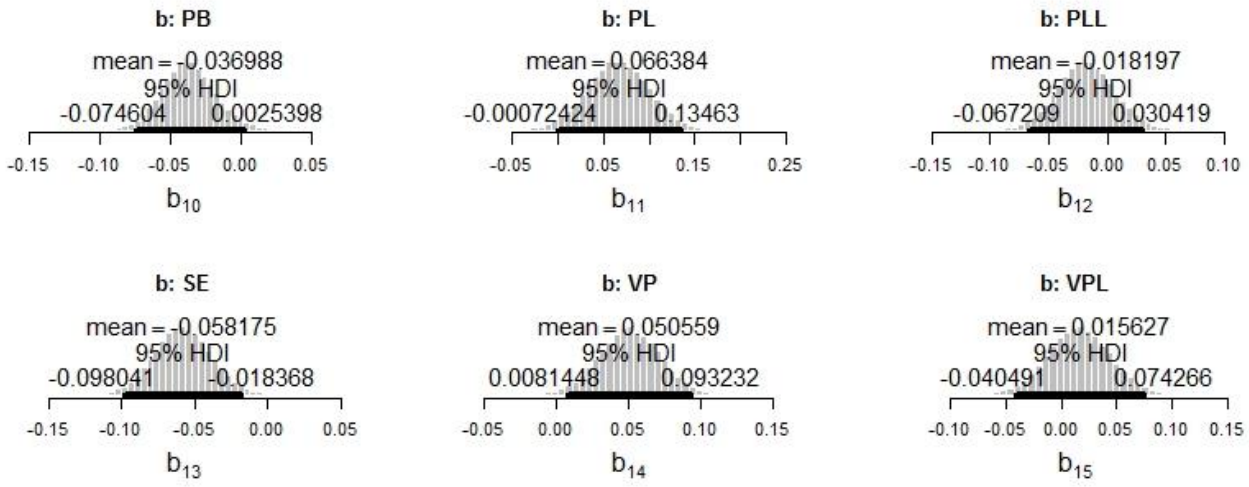


Figure F17b. Effect of growth-promoter treatments on root dry weight (g) per plant in the second greenhouse experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 15$.

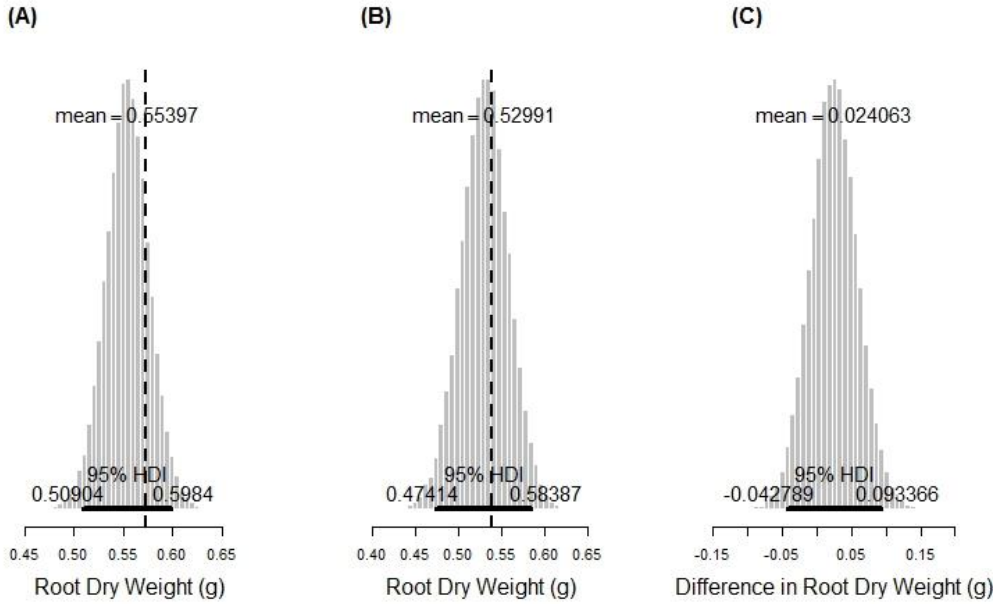


Figure F18. Effect of VP treatment on root dry weight. Included are the posterior distributions for root dry weight (g) per plant under conditions: **(A)** VP treatment (mean = 0.5727); **(B)** C treatment (mean = 0.538); and **(C)** the difference between posterior distributions of the VP and C treatments. VP treatment = *V. paradoxus* JM63; C = Untreated Control.

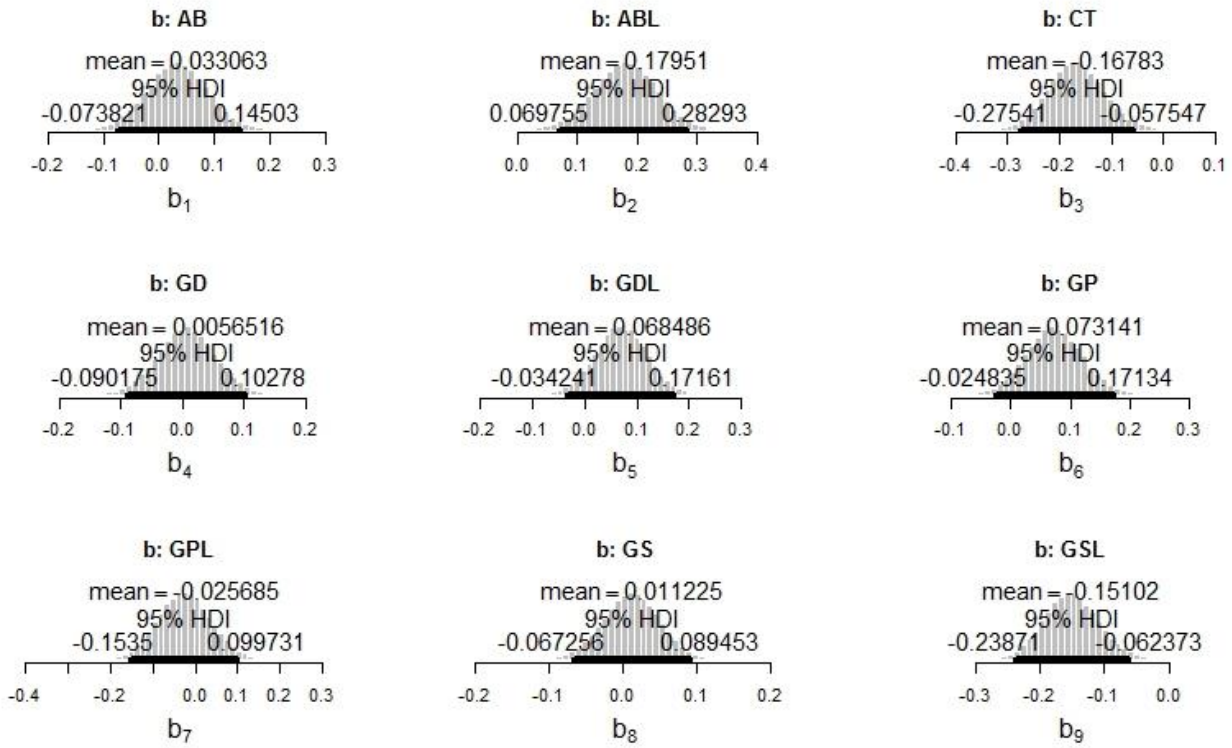


Figure F19a. Effect of growth-promoter treatments on above-ground biomass dry weight (g) per plant in the second greenhouse experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 15$.

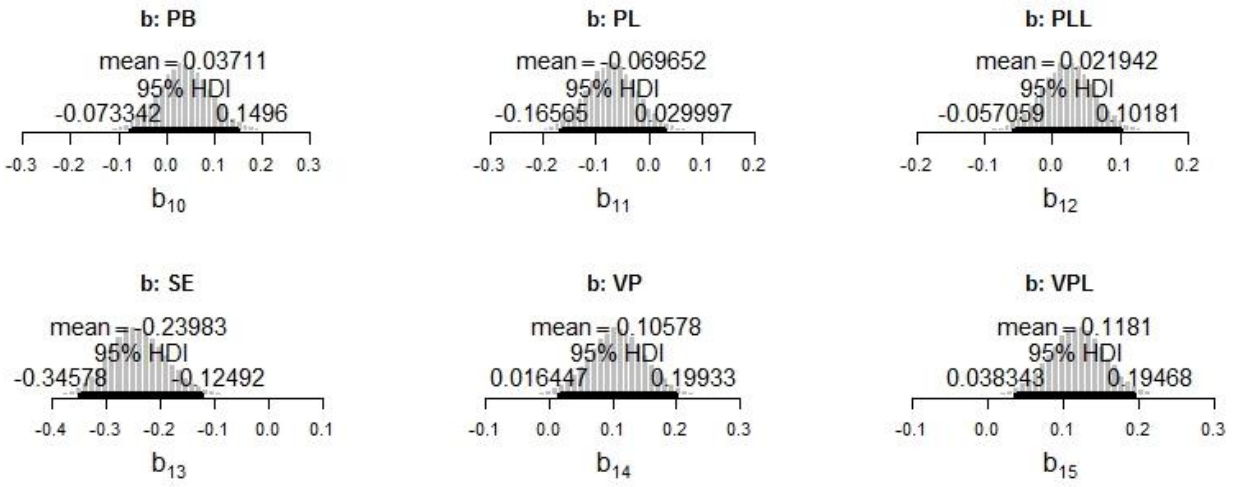


Figure F19b. Effect of growth-promoter treatments on above-ground biomass dry weight (g) per plant in the second greenhouse experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 15$.

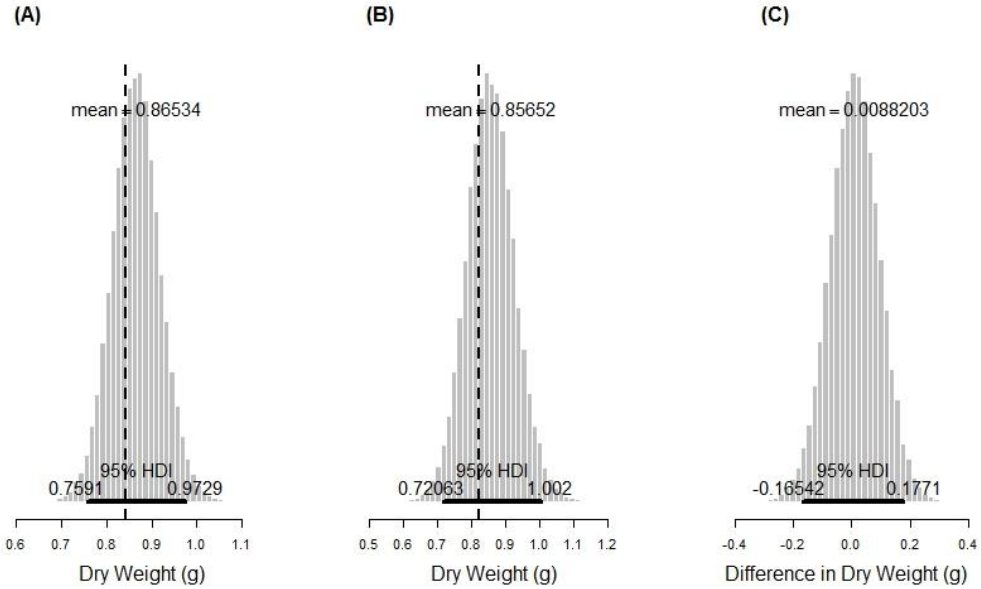


Figure F20. Effect of GSL treatment on above-ground biomass dry weight. Included are the posterior distributions of dry weight (g) per plant under conditions: **(A)** GSL treatment (mean = 0.842); **(B)** C treatment (mean = 0.8067); and **(C)** the difference between posterior distributions of the GSL and C treatments. GSL treatment = *G. diazotrophicus* SRT4 + LCO; C = Untreated Control.

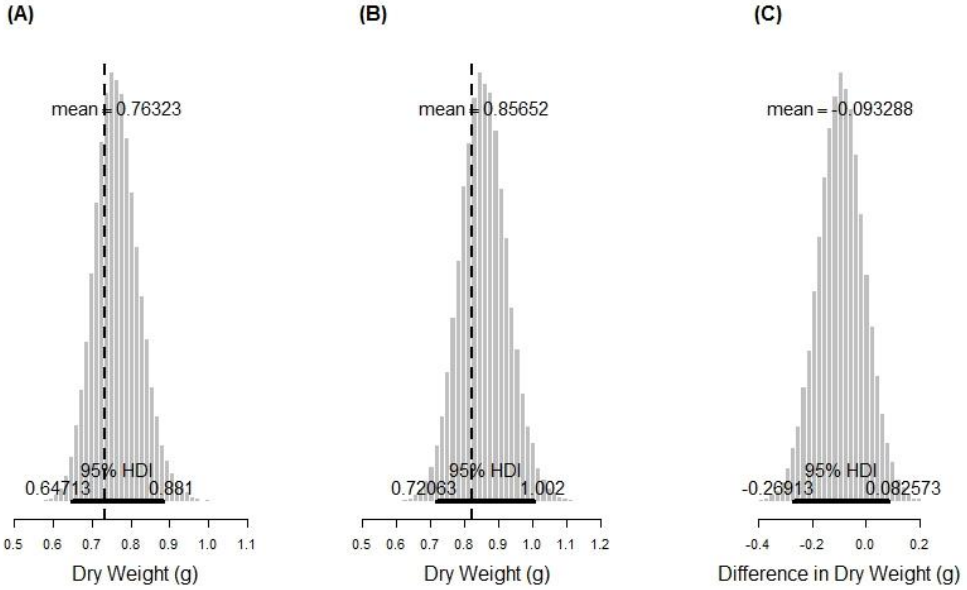


Figure F21. Effect of S treatment on above-ground biomass dry weight. Included are the posterior distributions of dry weight (g) per plant under conditions: **(A)** S treatment (mean = 0.7293); **(B)** C treatment (mean = 0.8067); and **(C)** the difference between posterior distributions of the S and C treatments. S treatment = *A. nodosum*; C = Untreated Control.

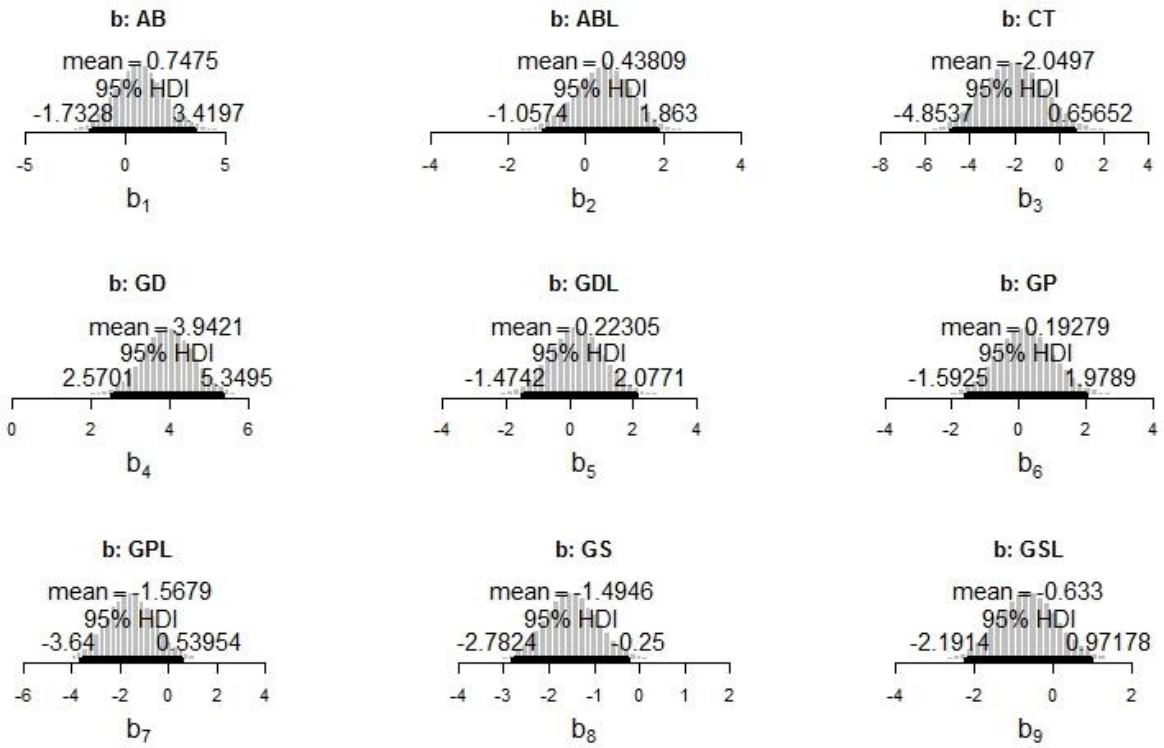


Figure F22a. Effect of growth-promoter treatments on dry matter content (%) in the second greenhouse experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 15$.

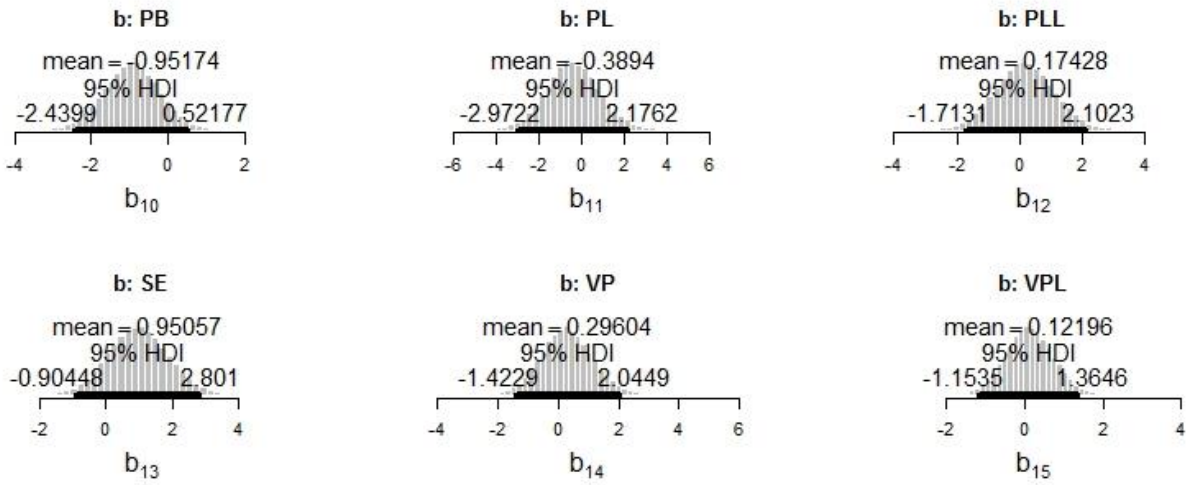


Figure F22b. Effect of growth-promoter treatments on dry matter content (%) in the second greenhouse experiment. Credible nonzero differences observed when 0 does not fall within the 95 % HDI. Plot titles represent the growth-promoter treatment, *x*-axis titles represent the category of β_j, where $x = 15$.

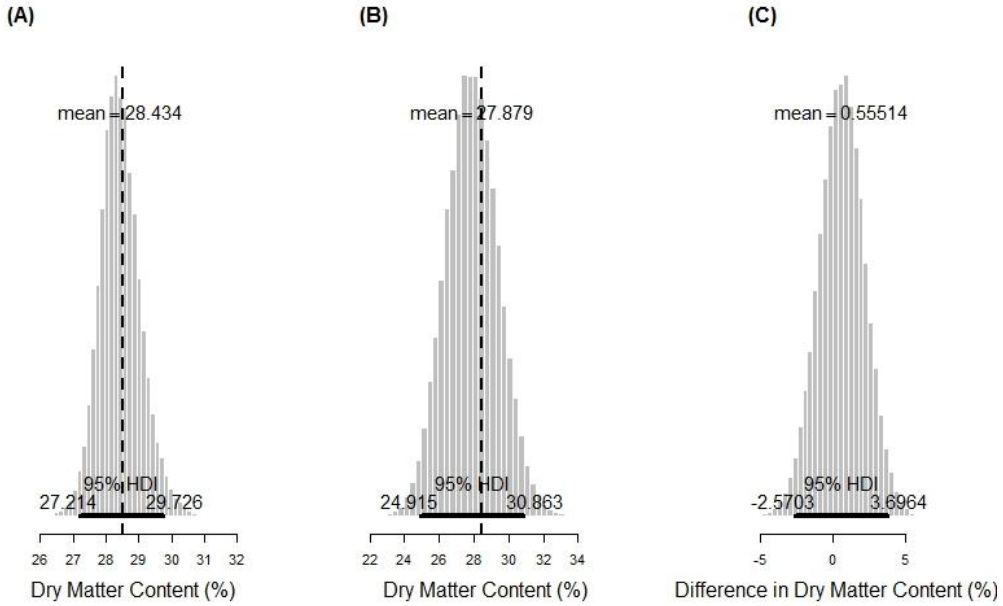


Figure F23. Effect of GS treatment on root dry weight. Included are the posterior distributions of dry matter content (%) per plant under conditions: **(A)** GS treatment (mean = 28.53); **(B)** C treatment (mean = 28.46); and **(C)** the difference between posterior distributions of the GS and C treatments. GS treatment = *G. diazotrophicus* SRT4; C = Untreated Control.

APPENDIX G: NUTRIENT ANALYSIS

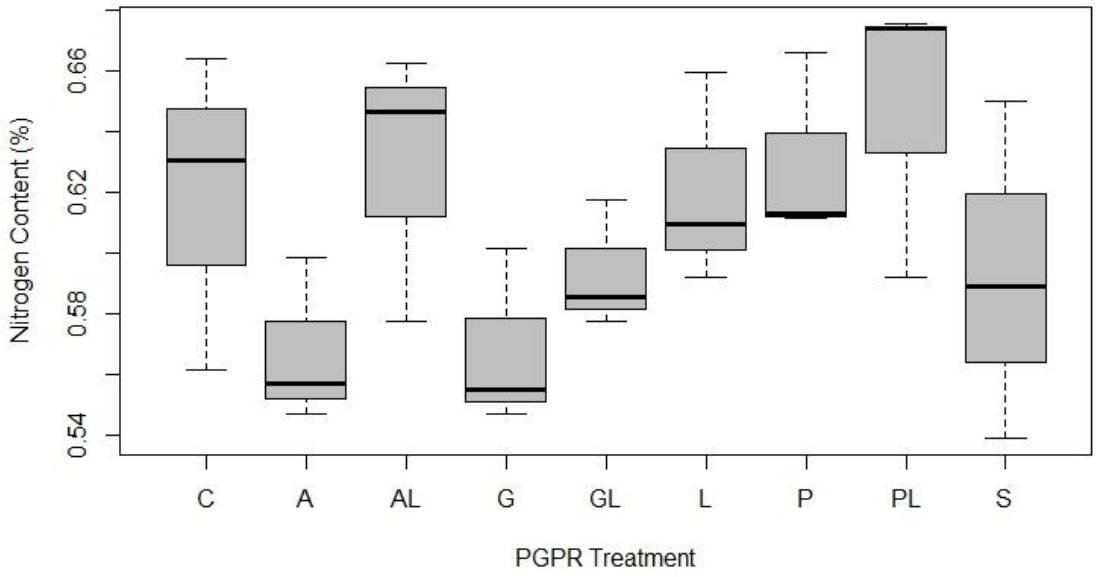
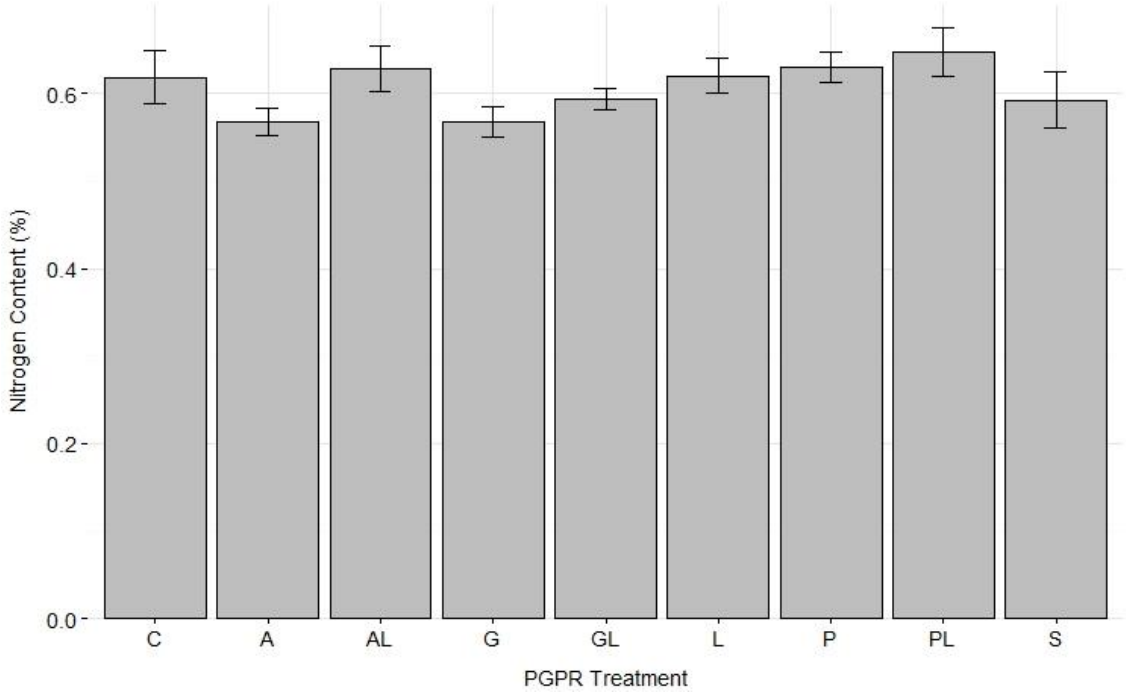


Figure G1. Nitrogen content (%) of *A. donax* (NileFiber™) above-ground biomass from the first greenhouse experiment. (n = 3).



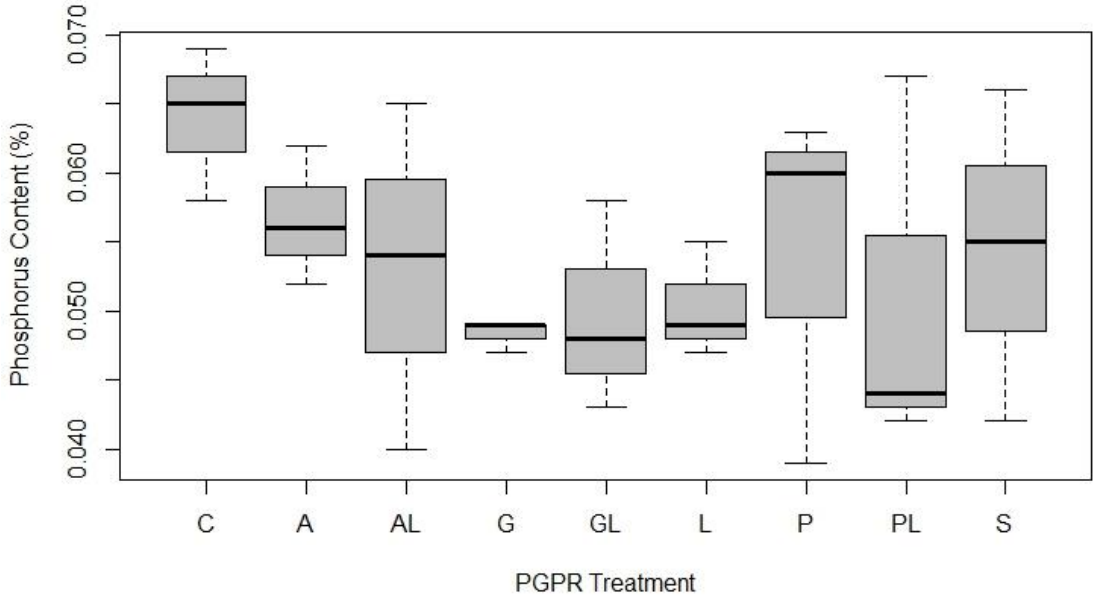
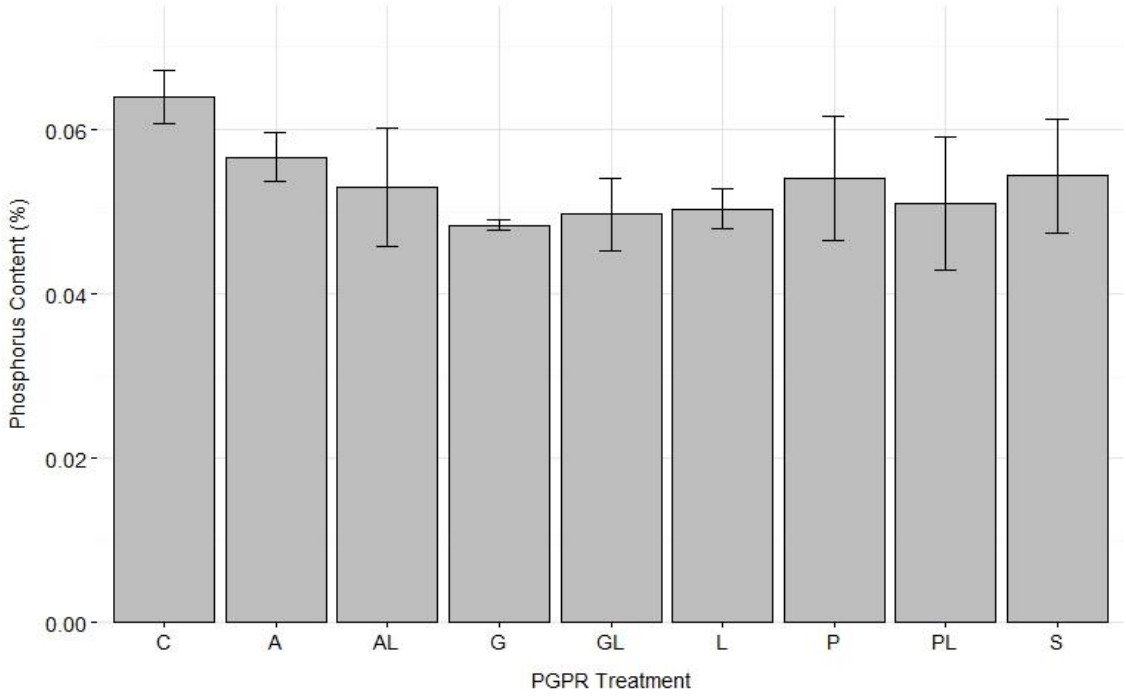


Figure G2. Phosphorus content (%) of *A. donax* (NileFiber™) above-ground biomass from the first greenhouse experiment. (n = 3).



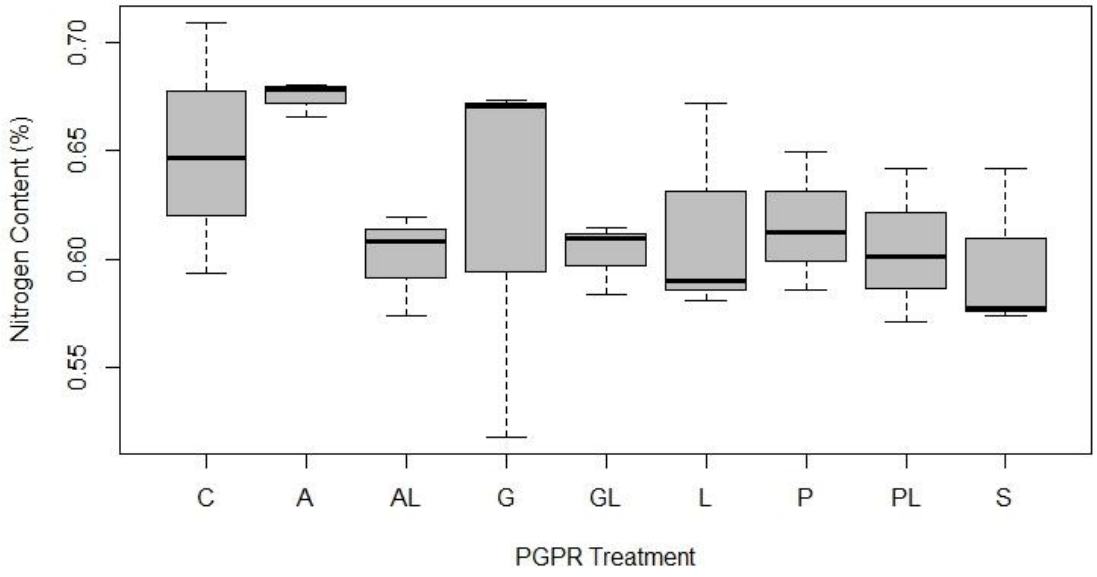
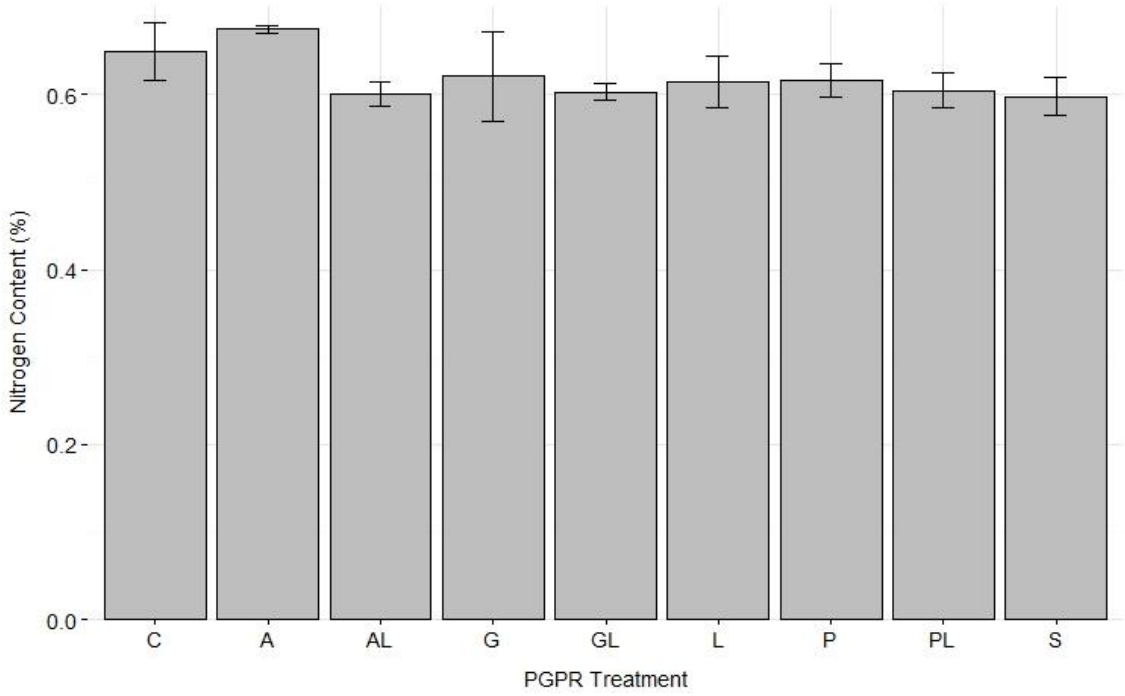


Figure G3. Nitrogen content (%) of *A. donax* (NileFiber™) root biomass from the first greenhouse experiment. (n = 3).



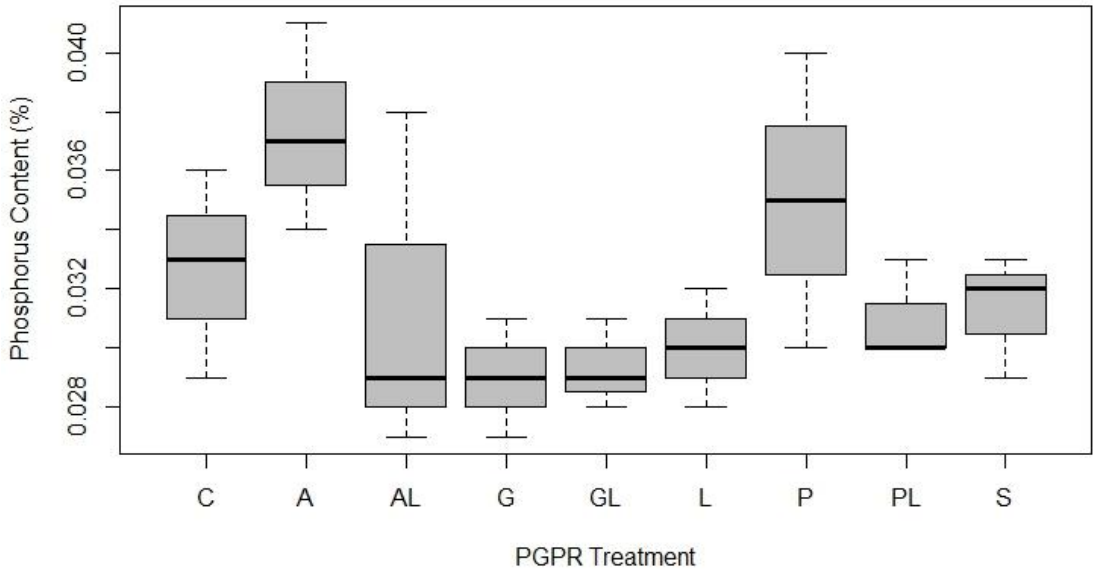
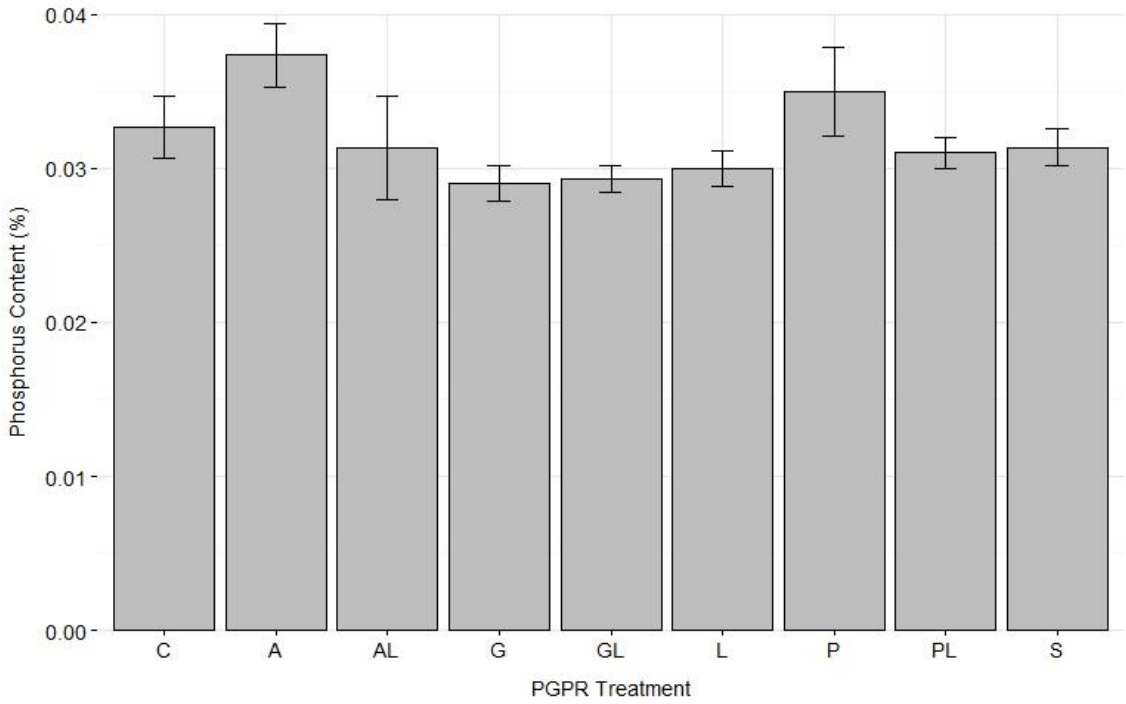


Figure G4. Phosphorus content (%) of *A. donax* (NileFiber™) root biomass from the first greenhouse experiment. (n = 3).



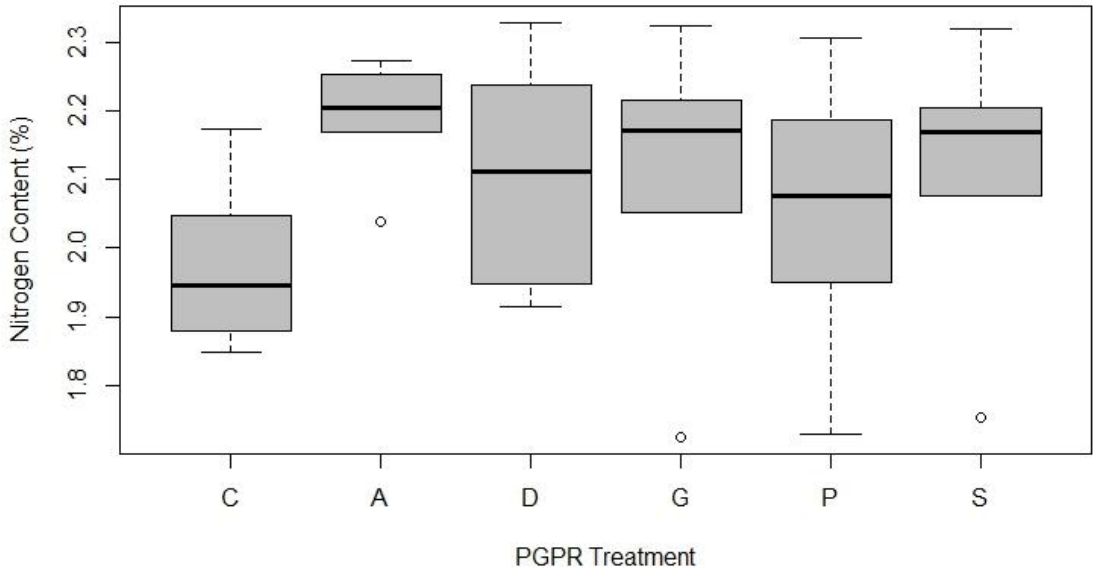
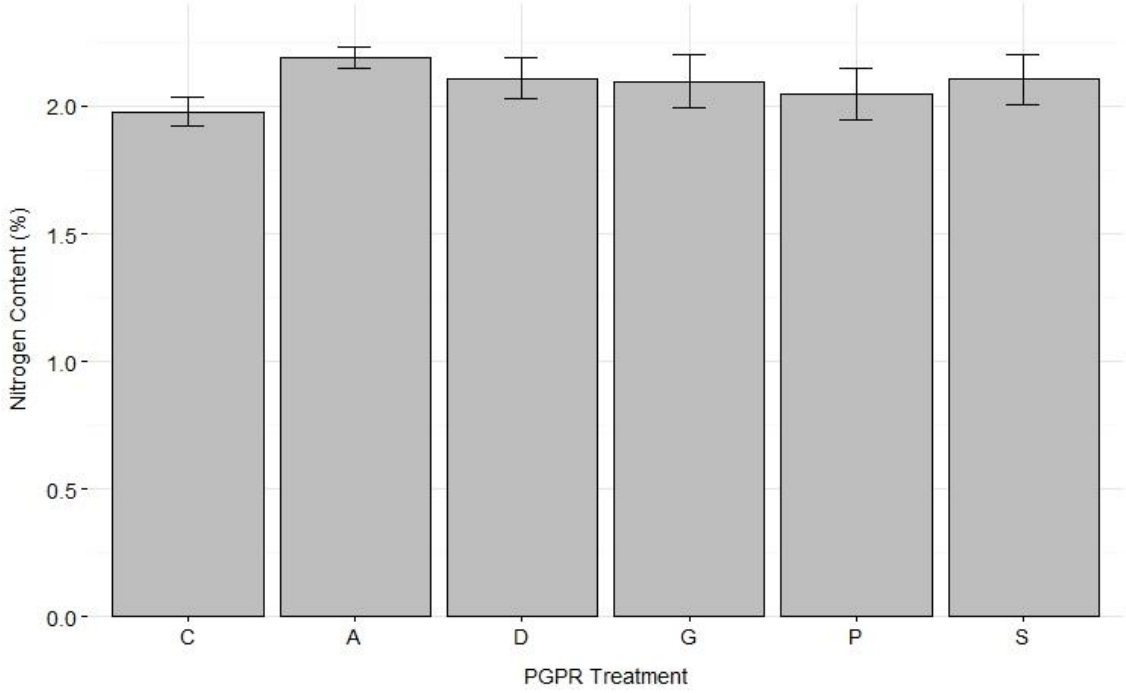


Figure G5. Nitrogen content (%) of *A. donax* (NileFiber™) above-ground biomass from the field experiment. (n = 3).



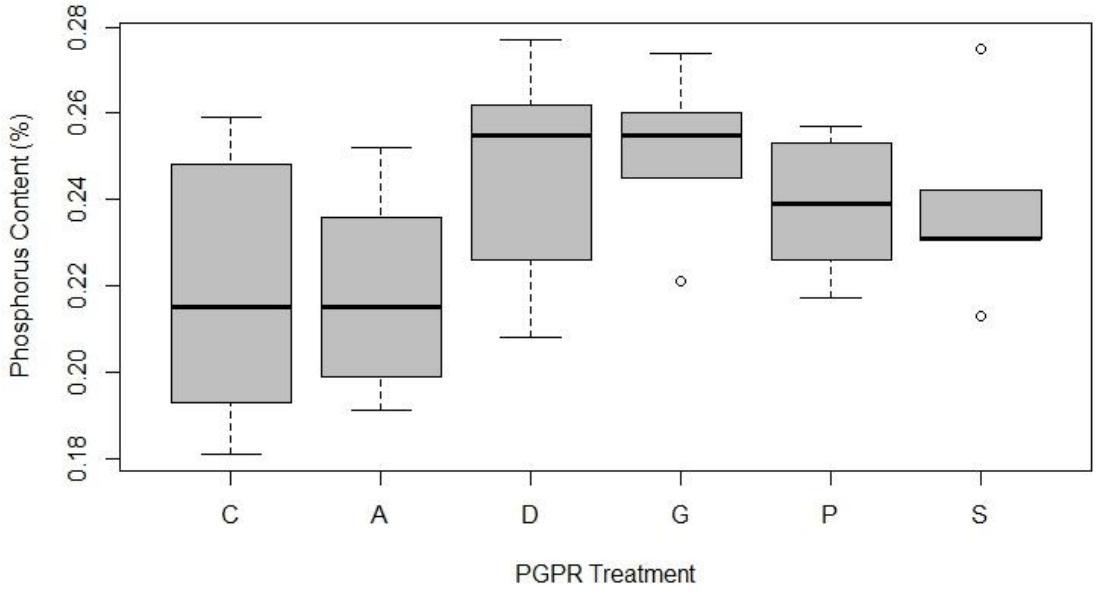


Figure G6. Phosphorus content (%) of *A. donax* (NileFiber™) above-ground biomass from the field experiment. (n = 3).