Biofertilizers for the sustainable production of herbaceous biomass crops in southern Ontario

by

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ABSTRACT

BIOFERTILIZERS FOR THE SUSTAINABLE PRODUCTION OF HERBACEOUS BIOMASS CROPS IN SOUTHERN ONTARIO

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Cultivation of switchgrass (*Panicum virgatum*) and miscanthus (*Miscanthus* spp.) as dedicated biomass crops on Ontario's marginal agricultural lands is increasing, and producers are seeking opportunities to enhance the sustainability of their operations. Therefore, we conducted a field study addressing the knowledge gap regarding field scale agronomic and environmental impact of four biofertilizers compared to a synthetic nitrogen fertilizer and a control for mature switchgrass and miscanthus. Biomass yield, plant morphology, soil fertility and biological health, and greenhouse gas fluxes were measured. Synthetic nitrogen and AGTIV® biofertilizer produced the highest yield for switchgrass and miscanthus, respectively. AGTIV® and Optimyc + MooR also increased bacterial and fungal gene abundance in the top 10 cm of soil under switchgrass cultivation in 2020. All fertilizers increased the release of key macronutrients under controlled conditions. In conclusion, this research shows that certain biofertilizers may be an alternative option to synthetic fertilizers for biomass crop production.

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LIST OF SYMBOLS AND ABBREVIATIONS

α	Threshold value used to judge whether a test statistic is statistically significant
AG	AGTIV® biofertilizer treatment
AMF	Arbuscular mycorrhizal fungus
ANOVA	Analysis of variance
BIO	All biofertilizer treatments
BM	Burlington miscanthus field site
BS	Burlington switchgrass field site
˚C	Degrees Celsius
C	Carbon
Ca	Calcium
CFX96™	CFX96™ Real-Time PCR Detection System
CH_4	Methane
SYN	Synthetic N fertilizer treatment
CLI	Canada Land Inventory
cm	Centimeters
CO_2	Carbon dioxide
CO_2e	Carbon dioxide equivalent
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
g	Grams
GHG	Greenhouse gas
GIS	Geographic information system(s)
GS	Guelph switchgrass field site
GWP	Global warming potential
ha	Hectares
ICP-OES	Inductively coupled plasma - optical emission spectrometry
JS	JumpStart® biofertilizer treatment
K	Potassium
kg	Kilograms
L	Liter
LG	LysteGro biofertilizer treatment
m	Meters
MES	Master of Environmental Sciences
mg	Milligrams
Mg	Magnesium
mL	Milliliters
mm	Millimeters
MP	MYKE® Pro biofertilizer treatment

N	Nitrogen
nm	Nanometers
N_2O	Nitrous oxide
NH_4^+	Ammonium
NO_3^-	Nitrate
O_2	Oxygen gas
OBPC	Ontario Biomass Producers Co-operative
OM	Optimyc and MooR biofertilizer treatment
ON	Ontario, province of Canada
P	Phosphorus
PGPR	Plant-growth promoting rhizobacteria
pmol	picomole
ppm	Parts per million
proc GLIMMIX	Generalized mixed model procedure in SAS® OnDemand for Academics
qPCR	Quantitative polymerase chain reaction
RCBD	Randomized complete block design
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
RNase	Ribonuclease
SOC	Soil organic carbon
SOM	Soil organic matter
T_0	Time equals 0 minutes
T_1	Time equals 15 minutes
T_2	Time equals 30 minutes
TEP	Theoretical ethanol potential
TEY	Theoretical ethanol yield
µL	Microliter
USA	United States of America

LIST OF APPENDICES

Chapter 1: Introduction

Biomass crops are any purpose-grown crop whose aboveground biomass is harvested for use in value-added industries, including biofuels, bioplastics, livestock feed and bedding, and garden mulch (Oo et al., 2012; Samson et al., 2018; Withers et al., 2016). Switchgrass (*Panicum virgatum*) and miscanthus (*Miscanthus* spp.) are two perennial C4 warm season grasses grown as biomass crops in the province of Ontario, both of which are being promoted by the provincial government and Ontario Biomass Producers Co-operative (OBPC) (Oo et al., 2012). Although no published data documenting change in land area being used to produce dedicated biomass crops in Ontario could be found, there is evidence that the bioproduct industry in Canada, particularly in Ontario, is growing.

Rancourt et al. (2017) summarizes results from an Agriculture and Agri-food Canada survey documenting activity in the "non-conventional bioproduct" (i.e. biofuels, bio-gas and bioenergy, organic chemicals, bio-pesticides, plant-made biologics, non-conventional construction materials and composites, intermediary biochemicals, and biomaterials) industry in Canada. This report states that of the 190 bioproduct establishments identified in Canada, 59 were found in Ontario; this is more than any other geographic region. Furthermore, 60% of the total 190 Canadian bioproduct establishments had begun their bioproduct operations within the last 10 years (Rancourt et al., 2017). Rancourt et al. (2017) also found that Ontario accounted for 44.4% of all Canadian bioproduct sales in 2015, again being higher than any other geographic region. Although bioproducts may be derived from any kind of organic materials, agricultural biomass was the second largest source of biomass used by bioproduct establishments (the largest source being forestry products), contributing a total of 8.8 million metric tonnes to the industry and being identified as the primary source of biomass by 42.1% of establishments (Rancourt et al.,

2017). Taken together, the findings of this report demonstrate that there is a growing bioproduct industry in Canada which is heavily represented by establishments in Ontario. Many of these establishments across the country are sourcing their biomass from agricultural operations, and although the vast majority of this agricultural biomass is currently represented by grains and oilseeds (Rancourt et al., 2017), there is certainly a demand for agricultural biomass that could be filled by dedicated biomass crops as production systems for these crops improve.

There are several reasons why these crops are gaining popularity in the province. First, switchgrass and miscanthus biomass is currently being used in various industries in Ontario, such as livestock (livestock fodder and bedding) and green technologies (bioplastics and biofuels) (Oo et al., 2012; Samson et al., 2018; Withers et al., 2016), thus contributing to the development of a more sustainable economy by reducing dependency on fossil fuels and petro-chemicals (Valentine et al., 2011). Secondly, switchgrass and miscanthus can grow on lands which are unsuitable for more intensive field crops (Cobuloglu and Büyüktahtak, 2015; Tilman et al., 2009). This means they can be grown without competing with food crops for prime agricultural land (Cobuloglu and Büyüktahtak, 2015). Finally, these crops can enhance environmental quality on multiple scales. Locally, switchgrass and miscanthus improve soil quality by improving soil physical structure as well as enhancing the soil microbial community, carbon mineralization, and soil organic carbon (SOC) levels, all of which contributes to improved soil stability and nutrient cycling rates (Blanco-Canqui, 2010; Graham et al., 2019; Schloter et al., 2018; Simpson, 2018). These crops also improve local water quality by reducing erosion, nutrient leaching, and non-point source pollution (Blanco-Canqui, 2010; Feng et al., 2015). Globally, the cultivation of switchgrass and miscanthus on marginal lands may contribute to combating climate change by enhancing soil carbon storage on agricultural lands; this may help to off-

set greenhouse gasses (GHGs) emitted from the agricultural sector (Agostini et al., 2015; Eichelmann et al., 2016).

Of the environmental benefits described above, the soil quality benefits associated with switchgrass and miscanthus are particularly valuable for landowners. This is because improving soil quality (or soil health) contributes to increased crop productivity and, in the long term, this may facilitate the reclamation of degraded land for more intensive and profitable crop production. Although the concept is difficult to define, soil health is often used to describe the combination of soil ecological processes that contribute to the soil's ability to support ecosystem functions, including but not limited to plant growth and productivity (Slater, 2018). While these ecological processes are driven by the biotic components of the soil (i.e. soil micro-, meso- and macro-fauna), the ability of these biota to carry out their associated processes will be influenced by abiotic conditions (Kibblewhite et al., 2008). Some of the most commonly measured indicators of soil health include pH, concentrations of key nutrients and other chemical compounds, carbon-nitrogen ratio, cation exchange capacity, electrical conductivity, texture, structure, bulk density, porosity, microbial biomass, microbial carbon and nitrogen pools, respiration rate, and soil organic matter (SOM) fractions (Slater, 2018). SOM fractions and soil microbial communities are two important indicators because they can influence many of the other indicators, and both are heavily influenced by plant communities and land management practices (Arriaga et al., 2017). In the context of agricultural ecosystems, like crop fields, the availability of key plant nutrients is also a particularly important indicator of soil health contributing to productivity which can be influenced through management practices like fertilization (Arriaga et al., 2017; Slater, 2018). Because of this, land management

recommendations for switchgrass and miscanthus production should focus on practices that will enhance their effects on dynamic indicators of soil health, or at least not contradict them.

While it may seem that switchgrass and miscanthus are able to address some of the major environmental challenges that exist within and beyond the agricultural sector, affordable methods of increasing yields for both species must be established if they are to be economically viable while offering competitive prices for their products (Oo et al., 2012; Cobuloglu and Büyüktahtak, 2015). Synthetic nitrogen fertilizer has significantly increased yields for both species (Marsal et al., 2016), however, these fertilizers have adverse environmental impacts when excess nutrients enter adjacent ecosystems or are released from the soils as GHGs (Ashworth et al., 2015; Steffen et al. 2015). Synthetic fertilizers can also negatively impact soil microbial communities which are vital to several ecosystem functions, including nutrient cycling (Oates et al., 2016). These direct negative impacts, in addition to the large amount of energy and non-renewable resources required to produce synthetic fertilizers, make it important to minimize their use in the expanding biomass production industry (Ashworth et al., 2015).

The alternative to synthetic fertilizers is biofertilizers. The term "biofertilizer" describes inoculants of plant-growth promoting rhizobacteria (PGPR) or mycorrhizal fungi (hereafter, "microbial inoculants" or simply "inoculants") or organic fertilizers which may be derived from a variety of materials (Jacoby et al., 2017; Tailor and Joshi, 2014). Microbial inoculants may be particularly valuable for switchgrass and miscanthus production because these crops rely heavily on soil microbial relations to acquire soil nutrients (Ker et al., 2012; Li et al., 2015). Several microbial inoculants have been tested in switchgrass and miscanthus cropping systems with some success (Clark, 2007; Pogrzeba et al., 2017; Shanta et al., 2016; Simpson, 2018), but further study is required to determine which options can consistently enhance yields for these

crops. Three promising microbial inoculants for use in switchgrass and miscanthus production systems are JumpStart® by Novozymes (inoculant of the phosphorus-solubilizing fungus *Penicillium bilaiae*), MYKE® Pro or AGTIV® by Premier Tech (inoculant of the arbuscular mycorrhizal fungus, or AMF, *Glomus intraradices*), and a combined application of Optimyc and MooR by VisscherHolland (inoculants of endomycorrhizae and rhizobacteria consortia, respectively). These inoculants will be discussed in depth in the Chapter 2 Literature Review. As mentioned, organic biofertilizers can be derived from various materials, including livestock manure, plant matter, or municipal sewage (Amundson et al., 2015; Tailor and Joshi, 2014). Municipal sewage (or biosolids) fertilizers are of interest for biomass crop production because any trace toxic elements in the biosolids that may be taken up by the plants will not pose a health concern since the crops are not used for human consumption (Kołodziej et al., 2016). Research regarding the use of biosolids for biomass crop production and has demonstrated success with both miscanthus and switchgrass (Kołodziej et al., 2016; Liu et al., 2013; Liu et al., 2014). To my knowledge, no study has simultaneously compared the agronomic and environmental impacts of a microbial inoculant, a biosolids fertilizer, and a synthetic fertilizer under field conditions.

The following study has been designed to address the knowledge gap surrounding field scale use of various fertilizer products to enhance the yields and environmental benefits of switchgrass and miscanthus production in southern Ontario. This research will examine and compare the plant morphology and yield, soil health, and GHG emissions impacts of four biofertilizer products, a traditional synthetic fertilizer, and a zero-input control at three different field sites over two growing seasons. One of the field sites used for this study is the Guelph Turfgrass Institute (328 Victoria Rd S, Guelph, ON), a University of Guelph research centre. The other two sites are landowner fields volunteered by members of the Ontario Biomass Producers Co-

operative (OBPC). Written letters of support for this project have been provided by the OBPC, the Ontario Soil and Crop Improvement Association, the Biomass North Development Centre, the Canadian Wood Fibre Centre of Natural Resources Canada, REDICan Bioenergy, Lystek International Incorporated, and AllSys Biogenics Incorporated. This demonstrates the broad range of support for this project and strong interest in developing best management practices regarding the fertilization of switchgrass and miscanthus grown as biomass crops. Working collaboratively with biomass growers in the OBPC and the various fertilizer companies has also allowed for improved dissemination of the results from this study and stakeholder engagement throughout the project.

Chapter 2: Literature Review

2.1 Biomass Crops

2.1.1 Biomass Economy in Ontario

Biomass crops include any purpose-grown (dedicated) crop where the aboveground biomass is harvested as renewable feedstocks for various value-added industries including biofuels, bioplastics, livestock feed and bedding, and garden mulch (Oo et al., 2012; Samson et al., 2018; Withers et al., 2016). These industries are expanding, and biomass crops are occupying more of the agricultural landscape as people and governments place more value on the sustainability of the products and services they endorse (Oo et al., 2012). While interest in sustainable energy and other products is certainly growing, the economic sustainability of any industry requires that producers and all related value-added industries are making enough of a profit to justify the endeavor. At the level of the producer, the biomass crop growers, profit is defined as the value of the products (in this case, biomass) minus the costs of required inputs (Zering, 2014). Inputs may include seeds or seedlings, fertilizers, irrigation, pesticides, and herbicides, as well as the purchase of equipment and associated fuel and maintenance, among others (Zering, 2014). Optimizing the various inputs to maximize yield is one way to control profits, however biomass yields are also affected by topography, soil type, weather patterns, and other environmental variables along with market conditions that the producer may or may not be able to control (Zering, 2014). Producers must therefore use their knowledge of the land and growth requirements of various crop types to determine which combination of inputs and management decisions will sustainably maximize their profits from any given piece of land (Zering, 2014).

Switchgrass (*Panicum virgatum*) and miscanthus (*Miscanthus* spp.) are the two most common herbaceous biomass crops in Ontario; these herbaceous crops are preferred by growers

over woody species (such as poplar or willow) because many of these growers already have experience with hay and other grass crops (Oo et al. 2012). Both species are perennial and, once established, can maintain productivity for 15-20 years (Oo et al., 2012). Miscanthus is the higher yielding species per dollar spent on inputs (namely, fertilizers), however, switchgrass is easier to establish which can reduce initial investment costs and is less sensitive to soil quality because it is native to North American prairies (Oo et al. 2012). It is also important to note that because these crops can be grown successfully on marginal (less agriculturally productive) lands, they can improve the land quality and diversify producers' income (Valentine et al., 2011).

As mentioned above, biomass from purpose-grown biomass crops may be sold into a variety of markets. One market which has been of particular interest to several governments around the globe is the renewable energy sector, as bioenergy can help reduce carbon emissions, support energy independence, and meet sustainable development targets (Cosentino et al., 2018; Oo et al., 2012). Raw harvested biomass can undergo various biochemical or thermochemical procedures to produce energy products such as bioethanol, biogas, torrefied biomass, bio-oil, syngas, and combustion heat and energy (Alexopoulou et al., 2018; Lewandowski et al., 2018). Oo et al. (2012) examined the business case for biomass crop production for heat and power in Ontario. This study determined that there is a case in favour of developing a bioenergy economy at a margin comparable to cash crops. However, this case would improve significantly with the development of higher yielding biomass species and varieties and more yield-efficient agronomic practices (Oo et al., 2012). Furthermore, Calvert and Mabee (2015) used geographic information systems (GIS) and energy production potential analyses to conclude that, if properly coordinated, solar photovoltaic and biomass energy could meet almost 100% of peak energy demand in eastern Ontario.

Other marketable uses for switchgrass and miscanthus biomass being promoted and investigated by the OBPC include livestock bedding, feed for dairy cattle, compost for mushroom production, horticultural mulch, hydro-mulch, erosion control logs, and feedstocks for various biomaterials (i.e. bio-plastics, biopolymers, bio-composite materials) (Samson et al., 2019; Withers et al., 2016). Furthermore, non-energy markets being investigated for perennial grass biomass in Europe, including switchgrass and miscanthus biomass, include building materials (i.e. bricks, fibreboards, aggregate for lightweight concrete mixtures), compostable packaging, paper pulp, and pectin, in addition to the markets currently being explored in Ontario (Alexopoulou et al., 2018; Lewandowski et al., 2018). These non-energy products, along with the various energy pathways, show that there are numerous potential markets available for purpose-grown biomass in Ontario and around the world.

In order to meet the growing demands of the variety of industries reliant on feedstocks from herbaceous biomass crops, it is important to conduct research in both agronomic and genetic aspects of switchgrass and miscanthus to enhance yield potential. Genetic development of higher yielding varieties is time and labour intensive, therefore it is important to optimize agronomic practices, such as fertilizer use, to sustainably enhance yields of existing varieties and further improve the yields in any future varieties. Nitrogen (N) fertilizer represents the most significant on-farm cost associated with switchgrass production (Hall et al., 2011) and miscanthus N fertility requirements require further research (Brancourt-Hulmel et al., 2014). Therefore, optimizing yield per dollar spent on fertilizers is of great interest in the industry.

2.1.2 Switchgrass

Switchgrass is native to North America (including Ontario's native tallgrass prairies), is very tolerant of abiotic stress, and requires minimal management or inputs for production as a

biomass crop (Mitchell et al., 2014; Samson et al., 2018). Current management recommendations for this crop are to apply a broad-spectrum herbicide with no fertilizer during the establishment year, followed by N fertilization in subsequent years to maintain yield over time (Mitchell et al., 2014; Samson et al., 2018). The optimal N fertilization rate varies depending on the cultivar, existing soil N availability, and harvesting time (Mitchell et al., 2014), but the OBPC recommends applying 55-65 kg N/ha when the grass has grown 15-25 cm high (Samson et al., 2018). Phosphorus (P) and potassium (K) may also be applied but are only recommended for Ontario growers if soil levels are below 10 and 81 ppm, respectively (Samson et al., 2018). Mitchell et al. (2014) have reported an average annual yield of 7.3 ± 3.1 tonnes ha^{-1} for switchgrass grown in US Plant Hardiness Zones 3 and 4, matching well with the OBPC expectations ranging from 7.4-12.4 tonnes ha^{-1} yr^{-1} (Samson et al., 2018). Marsal et al. (2016) report a slightly lower annual yield of 5.99 ± 0.46 on a site in Guelph, ON labeled as class 4 soils (severe limitations restricting the range of crops or requiring special conservation practices) according to the Canada Land Inventory (CLI) classification system.

Because N fertilizer is the largest on-farm cost associated with switchgrass production (Hall et al., 2011), there are numerous studies that have investigated optimal N application rates. Switchgrass yield response to N application and fertilizer N recovery rates may vary according to initial soil N availability, annual rainfall, soil microbial activity and symbioses, and atmospheric N deposition rates (Owens et al., 2013; Parrish and Fike, 2005). The final product being produced from the biomass and the harvesting time and frequency will also influence the appropriate N application rate; switchgrass grown for forage and being harvested multiple times per year will require more N than switchgrass grown as feedstock for biofuels and harvested only once per year after senescence (Parrish and Fike, 2005). Overall, there may be no significant

effect of N fertilizer on yield in the first year or two after planting (Fike et al., 2017; Owens et al., 2013), but more consistent significant positive yield effects are observed in mature systems (Fike et al., 2017; Guretzky et al., 2011; Lemus et al., 2008a; Lemus et al., 2008b; Sanderson and Reed, 2000; Vogel et al., 2002). Conversely, Marsal et al. (2016) found that N fertilizer applied to established stands of switchgrass var. Cave-in-Rock at 60 kg N ha^{-1} did not significantly increase yields on marginal land in southern Ontario ($p = 0.07$). Palmer et al. (2014) also report no consistent significant yield response of switchgrass var. Alamo to N applications at field sites in North Carolina. Where significant responses to N application were observed, Palmer et al. (2014) note that the magnitude of this response was very small compared to the effect of annual and site-specific environmental conditions. In four-year-old switchgrass stands established in Ohio, Jung and Lal (2011) observed no significant yield response to N application across four field sites. In the following year, only one of the four study sites demonstrated a significant positive yield response to N application at 200 kg N ha^{-1} compared to their 0, 50, and 100 kg N ha^{-1} treatments (Jung and Lal, 2011).

Furthermore, Lemus et al. (2008b) did not observe significant positive effects on yield in the year their N treatments were applied to mature switchgrass plots, but rather in the two following seasons when no additional N was applied to any of the plots. This study found that applications of 90-270 kg N ha^{-1} to 5-year-old stands of switchgrass var. Cave-in-Rock in 2001 produced significant residual yield increases one year after application, with the 270 kg N ha^{-1} treatment having significant residual effects lasting two years after application. It is notable, however, that the researchers used a single end-of-season harvest in the year that the N treatments were applied, but a two-harvest system in the subsequent two years which contribute to the yield increases becoming significant in the following years (Lemus et al., 2008b). This may be due to

the increased N removal rate and reduced N recycling in two-harvest systems, as observed by Pedroso et al. (2013), which could improve crop response to N additions. Regardless, the Lemus et al. (2008b) study suggests that there may be residual benefits of N fertilizers up to two years after application.

Taken together, the existing literature indicates that synthetic N fertilization is a reliable means of enhancing and maintaining switchgrass yields in North America, however, the degree to which yields are affected is variable. This variability, in addition to the negative environmental effects of synthetic N fertilizers which is discussed below, warrants an investigation into alternative means of fertilizing these crops to maximize sustainable yields while minimizing environmental damage.

2.1.3 Miscanthus

Miscanthus is not native to North America, however, *Miscanthus* x *giganteus*, which is commonly used in biomass production systems, is sterile and has been classified as non-invasive (Brancourt-Hulmel et al., 2014). Two other species investigated for biomass production purposes in North America are *Miscanthus sinensis* and *Miscanthus sacchariflorus*, although they are less common and can be considered invasive (Brancourt-Hulmel et al., 2014). As with switchgrass, it is not recommended that producers fertilize this crop in the first two years due to the low yields during establishment which results in nutrient loss (Cadoux et al., 2012; Withers et al., 2016). After the second year, the OBPC recommends applying 50-60 kg N ha^{-1} (Withers et al., 2016). P and K may also be applied but are only recommended for Ontario growers if soil levels are below 10 and 81 ppm, respectively (Withers et al., 2016). The OBPC reports annual yield expectations in Ontario to be 17-26 tonnes ha^{-1} (Withers et al., 2016). This aligns with a study conducted in Guelph which found mature stands producing 17.03 ± 8.10 tonnes ha^{-1} yr^{-1} on CLI

class 4 land (Marsal et al., 2016). Baute et al. (2018) reports annual dry matter yields at their southeastern Ontario field sites ranging from 12.8-24.3 tonnes ha^{-1}. Although the lower end of this range falls below the OBPC yield expectations, it is important to note that none of these field sites received any fertilization or pesticide inputs once the crops were established (Baute et al., 2018).

Until recently, much of the literature examining miscanthus yield response to fertilization has been conducted in Europe. The general conclusion from these European studies, however, is mixed. Christian, Riche, and Yates (2008) report that miscanthus had no significant yield response to N fertilization up to 120 kg N ha^{-1} at any point during the 14 years of study at their field site in England. That said, there have also been studies in England and Ireland that report a significant positive response of miscanthus yield to N fertilization (Finnan and Burke, 2016; Shield et al., 2014). Similarly, Cadoux et al. (2012) reviewed 11 studies related to the miscanthus yield response to N fertilizers across nine European countries and one American state. This review study also found mixed results, where five studies reported no significant response to N application and six studies reported some degree of significant positive response. The authors noted that only two of the six studies reporting a significant positive response to N fertilization had a large effect size, and both occurred at irrigated field sites (Cadoux et al., 2012). Among the studies reporting no significant yield response to N fertilization, two report high levels of existing soil mineral N stocks, and the remaining three report very high yields indicative of high existing soil N stocks although the authors did not measure soil fertility during their study period (Cadoux et al., 2012). Based on the combined results of these 11 studies, Cadoux et al. (2012) concluded that miscanthus may respond to N fertilization, but only on field sites with low initial soil mineral N. Lewandowski and Schmidt (2006) employed the boundary line approach to

model the functional response of miscanthus yield to total N availability (fertilizer plus existing soil mineral N) in southwest Germany and found a positive response up to a total N availability of about 110 kg N ha^{-1} yr^{-1}. Their model shows that this response inverts at 114 kg N ha^{-1} yr^{-1}, indicating that N application beyond this point may even become detrimental to miscanthus yields (Lewandowski and Schmidt, 2006). The authors note, however, that similar studies should be conducted in a range of environments rather than assuming this response curve is universal (Lewandowski and Schmidt, 2006).

Although the European studies indicate how miscanthus grown in North America may respond to fertilization, Arundale et al. (2014) note that findings from European trials are unlikely to be transferable to the North American context. This is due to lower observed yields and increased rates of N deposition observed in Europe which may weaken the N fertilization response compared to the American Midwest, where their study takes place (Arundale et al., 2014). Studies investigating the response of miscanthus to N fertilization in North America are less abundant than European studies but indicate similarly mixed responses. Arundale et al. (2014) and Lee et al. (2017) both studied N fertilizer response of miscanthus in Illinois, USA and both reported significant positive yield responses. Arundale et al. (2014) report a fairly small increase in mature (5+ years post-establishment) miscanthus yields (25% ± 11%) up to 202 kg N ha^{-1}, their highest N application rate, when averaged across all sites. When results were separated by site, however, the authors report much larger yield responses to N fertilization at the sites with the two lowest land capability classes than the site with the highest land capability class (Arundale et al., 2014). Lee et al. (2017) reported a significant positive yield response of a young (2-5 years post-establishment) miscanthus system at 60 kg N ha^{-1}, with no significant difference observed between 60 and 120 kg N ha^{-1}. Similarly, Marsal et al. (2016) report a significant

positive effect of N-P-K fertilizer applied at 74 kg N ha^{-1}, 42 kg P_2O_5 ha^{-1}, and 62 kg K_2O ha^{-1} on the yield of young miscanthus crops in a southern Ontario field trial. Finally, a study by Davis et al. (2014) also reports significantly higher yields in young miscanthus systems (1-4 years post-establishment) at 60 kg N ha^{-1} compared to unfertilized plots at their Illinois field site, with no significant difference in yield between their 60 and 120 kg N ha^{-1} treatments. However, Davis et al. (2014) report no significant yield response to N fertilization up to 120 kg N ha^{-1} at any of their other four field sites in Kentucky, Nebraska, New Jersey, and Virginia, USA. These sites were established at the same time as the Illinois field site.

This review suggests that the yield response of miscanthus to N fertilization is highly variable and strongly dependant on site conditions. While N fertilizers can create large and significant yield increases in some scenarios, more research is needed to determine the exact N rates that can be economically justified under a variety of climatic conditions and soil environments within the North American context. Given this demonstrated research gap, there is interest a need to investigate alternative fertilization techniques, such as biofertilizers, that can support both biomass yields and environmental benefits in a sustainable fashion.

2.1.4 Environmental Impacts

In addition to economic profits that can be derived from switchgrass and miscanthus biomass, these biomass crops can provide local and global environmental benefits. One of these benefits include improving soil properties and health by increasing soil porosity and reducing bulk density, continuously adding soil C through aboveground and belowground biomass production, and improved soil aggregation due to the extensive and dense fine root systems (Blanco-Canqui, 2010; Graham et al., 2019; Schloter et al., 2018). These crops also contribute to improving ground and surface water quality by reducing nutrient leaching and soil erosion

through improved infiltration of rainfall leading to reduced surface runoff resulting from increased soil porosity, improved water retention due to higher soil organic matter, and the provision of year-round soil cover (Blanco-Canqui, 2010; Feng et al., 2015; Skinner et al., 2012; Smith et al. 2013). Finally, these crops may contribute to offsetting GHG emissions by sequestering atmospheric carbon dioxide (CO_2) as soil organic C through above- and belowground biomass inputs, as well as reducing nitrous oxide (N_2O) emissions by reducing the risk of soils becoming anoxic due to saturation and their highly efficient uptake and cycling of soil N (Agostini et al., 2015; Eichelmann et al., 2016; Skinner et al., 2012; Smith et al., 2013) compared to conventional field crops. It is important that researchers prioritize the development of best management practices that support both the economic and environmental values of these crops so their expansion in the agricultural landscape can be sustainable. This is particularly true for biomass crop industries as they are being developed with the goal of reducing the negative impacts of human activities, including agriculture, on the environment.

As indicated in the previous two sections, synthetic N fertilizer is the most significant input for switchgrass and miscanthus production (Samson et al., 2018; Withers et al., 2016). Unfortunately, synthetic N fertilizers have negative environmental impacts, such as disrupting the N cycle, leaching into adjacent ecosystems causing N saturation and eutrophication, and enhancing emissions of the potent GHG, N_2O, through denitrification (Ashworth et al., 2015; Oates et al., 2016; Steffen et al. 2015). Bender et al. (2016) also report negative impacts of synthetic fertilizer and other agricultural inputs on soil biota ranging from macrofauna (i.e. earthworms, arthropods), to diverse microbial communities driving many ecosystem functions. Owens et al. (2013) observed that apparent fertilizer N recovery for switchgrass (based on aboveground biomass) may be as low as 10%, leaving 90% of fertilizer N unaccounted for. This

missing N may be sequestered into belowground pools but may also be susceptible to loss via nitrate leaching or denitrification (Owens et al., 2013). This finding aligns with results from Ruan et al. (2016) which reported an exponential increase in N_2O emissions and nitrate leaching from switchgrass fields in response to increasing N fertilization rates in the first three years after establishment. While Smith et al. (2013) did not observe increased N_2O release in the years they fertilized switchgrass plots compared to the years they did not, this could also be tied to differences in annual temperature and precipitation patterns which also significantly impact denitrification rates. In a study investigating N losses in fertilized miscanthus, Behnke et al. (2012) found that applications of 120 kg N ha^{-1} resulted in significant increases in the cumulative annual N_2O flux from the soil. Behnke et al. (2012) also report approximately twice as much N leaching in plots receiving 60 kg N ha^{-1} compared to the control, with the N leaching rate nearly doubling in plots receiving 120 kg N ha^{-1} compared to 60 kg N ha^{-1}. In addition to the negative environmental effects associated with synthetic N fertilizer application, the process of manufacturing these fertilizers through the Haber-Bosch process is extremely energy intensive which reduces the net energy efficiency and net carbon storage benefits of bioenergy crops (Ashworth et al., 2015; Huo et al., 2012; Woods et al. 2010).

The above studies show that there can be significant negative environmental impacts when applying synthetic N fertilizer to perennial biomass crops which is counterproductive to their numerous environmental benefits. Furthermore, extensive discussions in the previous two sections reveal that synthetic N fertilizers can be inconsistent in improving the yields for switchgrass and especially for miscanthus. For these reasons, it is imperative to research a variety of fertilization strategies to determine which will consistently improve yields and minimize negative environmental impacts to develop best management practices for these crops

that are both economically and environmentally sustainable. Alternatives to traditional synthetic fertilizers are often broadly referred to as biofertilizers. As described in the Introduction, "biofertilizers" encompass inoculants of plant growth-promoting rhizobacteria (PGPR) or mycorrhizal fungi or organic fertilizers derived from a variety of materials, including municipal sewage (biosolids) (Jacoby et al., 2017; Tailor and Joshi, 2014). In the next section, details on existing research investigating the use of microbial inoculants and biosolids as biofertilizers for switchgrass and miscanthus are discussed within the context of this study.

2.2 Biofertilizers

2.2.1 Microbial Inoculants

One alternative to synthetic fertilizers are inoculants of PGPR and/or mycorrhizal fungi that enhance plant growth by increasing plant nutrient availability and uptake and may also work by regulating or releasing plant growth hormones or by protecting the plant from pathogens (Jacoby et al., 2017). Inoculation of seeds, seedlings, and soils with plant beneficial microbes to enhance yields is already commonly practiced in subtropical regions where access to synthetic fertilizers is often limited (Bender et al., 2016). However, inoculation success in temperate regions is variable depending on plant species and soil type (Bender et al., 2016). As such, it is important to research the use of microbial inoculants to enhance temperate agricultural crop yields to better understand crop-specific interactions with inoculants under varying soil conditions. Microbial inoculants may be particularly valuable for switchgrass and miscanthus production because these crops rely heavily on interactions with soil microorganisms to acquire soil nutrients (Ker et al., 2012; Li et al., 2015; Parrish and Fike, 2005).

One biofertilizer that should be better researched with switchgrass and miscanthus is JumpStart® (Novozymes BioAg Ltd.), an inoculant containing phosphorus-solubilizing fungus

Penicillium bilaiae. This fungus promotes plant growth by releasing organic acids which release P from soils that is otherwise unavailable for plants (Asea et al., 1988; Leggett et al., 2015; Takeda and Knight, 2006; Wakelin et al., 2004). Binding of soil P in plant-unavailable inorganic forms is prevalent in calcareous soils with a neutral or basic pH, thus the effect of *P. bilaiae* is particularly beneficial under these conditions (Takeda and Knight, 2006). Inoculation with *P. bilaiae* has significantly increased yields for several food crops under greenhouse and field conditions, including field beans, maize, and wheat (Asea et al., 1988; Kucey, 1987; Leggett et al., 2015). Specific to bioenergy crops, Parrish and Fike (2005) report that switchgrass P uptake is strongly regulated by its interactions with soil fungi, as the plants were less responsive to P (as well as N) fertilizers when soil bacterial and fungal communities were present. This suggests that switchgrass, in particular, may respond well to JumpStart®. This is further confirmed in a small Ontario field trial where JumpStart® produced significantly greater yields compared to control and synthetic N fertilizer treatments in an established stand of Cave-in-Rock switchgrass (Simpson, 2018). Furthermore, Fei et al. (2019) reported significant a positive effect of *P. bilaiae* on the yield of one of two cultivars of *Miscanthus* × *giganteus* in their greenhouse trials, but this positive response did not remain significant under field conditions. Subsequent years of field study are therefore warranted to confirm these initial positive results of JumpStart® inoculation of biomass crops.

Another biofertilizer that could be used in herbaceous biomass production is MYKE® Pro Turf G (hereafter, MYKE® Pro) by Premier Tech, an inoculant containing arbuscular mycorrhizal fungus (AMF) *Glomus intraradices*. Premier Tech also produces an agricultural-grade inoculant of *G. intraradices* called AGTIV®. AMFs enhance plant nutrient uptake while reducing nutrient losses, thereby demonstrating the potential of AMF inoculants to improve

nutrient efficiency in agricultural crops with the proper pairings of plant and AMF species (Bender et al., 2016). Inoculation with *G. intraradices* has increased productivity and yield for a variety of food crops, including numerous fruits and vegetables, menthol mint, and maize (Bharti et al., 2013; Castellanos-Morales et al., 2012; Colla et al., 2014; Ll et al., 2012; Rouphael et al., 2010). Inoculation with *G. intraradices* has also improved crop tolerance to suboptimal soil conditions, such as drought, salinity, and alkalinity (Bharti et al., 2013; Colla et al., 2014; Evelin et al., 2012; Rouphael et al., 2010). *G. intraradices* can colonize both switchgrass and miscanthus roots and had a significant growth-promoting effect when tested with switchgrass grown in soil with a pH of 5 (An et al., 2008; Clark, 2007). This effect was not significant when tested in a soil with a pH of 4 (Clark, 2007). This suggests that *G. intraradices* may have significant growth promoting effects in more neutral soils but may lose this effect when soils become excessively acidic. It appears that *G. intraradices* has not otherwise been tested as a biofertilizer for either switchgrass or miscanthus and thus merits further study.

Finally, it is important to investigate inoculation of beneficial microbial consortia (or mixed-species inoculants) as biofertilizers for switchgrass and miscanthus production. Research indicates that increasing the diversity of soil microbial communities can enhance ecosystem functioning and plant productivity, particularly when the functional properties of the microbial species are complementary or synergistic (Bender et al., 2016; Tailor and Joshi, 2014). Studies investigating the use of various plant-beneficial bacterial and fungal consortia have demonstrated synergies that promote nutrient uptake, productivity, and yield for a variety of food crops more effectively than single-species inoculants (Colla et al., 2014; Dal Cortivo et al., 2018; Simarmata et al., 2016; Singh et al., 2018). There was limited or no available literature outlining studies investigating the use of microbial consortia as a biofertilizer for biomass crops. However,

Schmidt et al. (2017) investigated the growth-promoting effect of plant-beneficial bacterial and fungal consortia isolated from miscanthus plants. This study examined the individual growth promoting effects of numerous endophytic bacteria and fungi isolated from miscanthus roots and leaves when reapplied as inoculants to miscanthus grown in sterile soil. They used the results from this trial to select several isolates to produce mixed-species bacterial and fungal inoculants which were then tested alongside a mixed-species bacterial inoculant comprised of species isolated from poplar for their growth promoting effects greenhouse conditions with unsterilized soil, as well as polluted versus non-polluted field conditions (Schmidt et al., 2017). While there were no significant effects of any treatments in the non-sterile greenhouse experiment, the bacterial and fungal inoculants had significant growth promoting effects when applied separately at both polluted and non-polluted field sites (Schmidt et al., 2017). The benefits were observed to be more pronounced on the polluted site (Schmidt et al., 2017). When applied together, the bacterial and fungal inoculants did not significantly affect miscanthus plant growth, and the bacterial inoculant containing isolates from poplar trees consistently negatively affected miscanthus growth (Schmidt et al., 2017). This study demonstrates how mixed-species inoculants can have positive or negative effects on plant growth, depending on the complex interactions among the plant and microbial species and their environment. Additional research is required to investigate the success of the more accessible commercial mixed-species inoculants with these two biomass crops.

2.2.2 Biosolids

Another alternative to synthetic fertilizer is organic fertilizer, which can be derived from livestock manure, plant matter, or municipal sewage (Amundson et al., 2015). Organic fertilizers can enhance plant growth while increasing SOM and contributing to nutrient recycling and

recovery goals (Amundson et al., 2015). Municipal sewage (or biosolids) fertilizers are of particular interest for biomass crop production because any toxic trace elements that may be present in the biosolids and taken up by the plants will not pose a health concern since the crops are not for human consumption (Kołodziej et al., 2016). Studies that have investigated this avenue of fertilization for biomass crops have determined that biosolids are an alternative to synthetic fertilizers for both miscanthus and switchgrass based on their ability to increase yield while providing environmental benefits.

To investigate the viability of biosolids as a replacement for synthetic N fertilizer in switchgrass production for biofuels, as well as comparing one- and two-cut harvesting regimes, Liu et al. (2013) and Liu et al. (2014) conducted a small-plot and a field-scale trial in Virginia (USA). In both studies, fertilizer treatments were applied only one time in the spring of the first year of the trial. In their small-plot trial, Liu et al. (2013) have reported significantly higher switchgrass yields in plots receiving biosolids compared to the unfertilized control. Their evaluation of biomass quality revealed that biosolids fertilizers had minor negative impacts on theoretical ethanol potential (TEP; predicted ethanol yield per unit mass of biomass), but this was outweighed by the positive yield effects resulting in a significant increase in theoretical ethanol yield (TEY; predicted ethanol yield per unit land area under production). In their larger field-scale study, Liu et al. (2014) report weaker responses to fertilization than in their small-plot study, however the biosolids were also applied at lower rates to adhere to fertilizer regulations in commercial production systems. Overall, this field-scale study also concluded that biosolids were a viable replacement for synthetic N fertilizers due to significant increases in biomass yield and TEY compared to the control, sometimes even outperforming the synthetic N fertilizer (Liu et al., 2014). It is worth noting, however, that this response was averaged across the one-cut and

two-cut harvesting regimes but was largely driven by the positive yield effects observed in two-cut regimes (Liu et al., 2014). Finally, a more recent study by Brown et al. (2020) found that despite similar levels of N_2O emissions in switchgrass fertilized with synthetic N and biosolids fertilizers in Washington (USA), biosolids reduced the net greenhouse effect of switchgrass production by avoiding the energy costs of synthetic fertilizer production and long-haul shipping. Brown et al. (2020) concluded that biosolids represent a viable replacement to synthetic N fertilizers due to this reduced greenhouse effect while producing equivalent biomass and ethanol yields.

The literature suggests that research investigating the use of biosolids to fertilize miscanthus has taken place exclusively in Europe. A field trial in Poland by Kołodziej et al. (2016) tilled several rates of biosolids into the topsoil before planting miscanthus and observed its effects on biomass production and quality over the next six years. The study reported the highest overwinter survival, yield, and biomass quality at the two lowest biosolids application rates (10 and 20 Mg dry matter ha^{-1}), concluding that miscanthus benefits from low rates of biosolids amendments (Kołodziej et al., 2016). Furthermore, a life-cycle assessment study aiming to determine the environmental impact of natural gas derived from miscanthus biomass in the United Kingdom under varying fertilizer regimes reports a lower global warming potential for miscanthus fertilized with biosolids compared to synthetic N fertilizer (Gilbert et al., 2011). While this study did not have adequate detailed data to assess the actual agronomic effects of the different fertilizers, their model indicates that, in order for yield benefits to offset the climate impact of biosolids application, each 25 kg N ha^{-1} increase in biosolids application rate must produce a minimum yield increase of only 0.2 Mg ha^{-1} (Gilbert et al., 2011). However, a set of two greenhouse trials and one field trial in Wales found that miscanthus only responded to

extremely high rates of biosolids fertilization which far exceeded the Water Framework Directive's regulations for organic N application (Smith and Slater, 2010). The opposing results of the Kołodziej et al. (2016) and Gilbet et al. (2011) studies indicate that miscanthus responds differently to biosolids fertilizers depending on environmental conditions (specifically initial soil fertility), much like its variable response to synthetic N fertilizers. This demonstrates that there is potential for biosolids to reduce reliance on synthetic fertilizers, but more research under variable environmental conditions is required to understand when and where it will be effective.

2.3 Hypotheses and Predictions

As the negative impacts of over-applying synthetic fertilizers become fully realized, alternative methods to fertilize a variety of crops is of importance, as outlined above. The overall goal of this study is therefore to continue exploring the use of various biofertilizer alternatives for biomass crops grown in Ontario biomass. The long-term objective of this project is to establish sustainable yield-enhancing practices for the herbaceous biomass crops, switchgrass and miscanthus, in Ontario. This research will contribute to this objective by exploring the viability of four biofertilizers (JumpStart® by Novozymes BioAg Ltd., MYKE® Pro or AGTIV® by Premier Tech, Optimyc and MooR by VisscherHolland, and LysteGro® by Lystek) as sustainable yield-enhancing strategies for switchgrass and miscanthus. Three of these biofertilizers are microbial inoculants (JumpStart®, MYKE® Pro or AGTIV®, and Optimyc and MooR) and one is a biosolid fertilizer (LysteGro®). To do this, three short-term objectives were investigated over the course of two field seasons at three southern Ontario field sites, two of which are grower-owned and operated properties in Burlington (Ontario), and the third being the University of Guelph's research site in Guelph (Ontario). The research objectives are:

(1) to assess how each fertilizer treatment affects switchgrass and miscanthus physiological development and yield compared to the control and to conventional synthetic N fertilizer;
(2) to quantify the effects of synthetic N and biosolids fertilizers on soil nutrient levels and plant uptake compared to the control and other biofertilizers that are tested in this study;
(3) to quantify all fertilizer treatment and control treatment effects on soil biological communities; and
(4) to assess how fertilizer treatment affects GHG emissions from the soil under switchgrass compared to the control.

Based on the above objectives, the following hypotheses were formed:

(1) H_o: Fertilizer treatments will not positively influence physiological development parameters, crop yield, soil nutrients and plant uptake of nutrients, soil biological communities (soil health) and GHG emissions for either switchgrass or miscanthus compared to the control.
(2) H_1: All fertilizer treatments will enhance plant physiological development and yield compared to the unfertilized control for both switchgrass and miscanthus.
(3) H_2: Synthetic N and biosolids fertilizers will increase soil nutrient levels compared to the control, but microbial inoculant biofertilizers will have the strongest impact on plant nutrient uptake, for both switchgrass and miscanthus.
(4) H_3: Microbial inoculants would have the strongest positive effect on soil biological communities compared to the control, and that synthetic N fertilizers would negatively impact soil biology, for both switchgrass and miscanthus.

(5) H_4: N fertilizers will enhance N_2O emissions and biofertilizers (microbial inoculants and biosolids) will enhance CO_2 emissions, with synthetic fertilizers having the strongest overall greenhouse impact.

Chapter 3: Materials and Methods

3.1 Study Description

The goal of this study was to evaluate the agronomic (plant growth and yield) and environmental (soil health and GHG emissions) potential of several commercially available biofertilizer products for switchgrass (*Panicum virgatum* var. Cave-in-Rock) and miscanthus (*Miscanthus sacchariflorus*) biomass production in southern Ontario. The biofertilizers used were (1) JumpStart® inoculant of *P. bilaiae* [Novozymes BioAg], (2) MYKE® Pro / AGTIV® inoculant of *G. intraradices* [Premier Tech], (3) Optimyc and MooR inoculants of fungal and bacterial consortia, respectively [VisscherHolland], and (4) LysteGro® biosolids fertilizer [Lystek]. In addition to the above biofertilizer treatments, a zero-input control and a typical synthetic N fertilizer treatment were included. Please see **Table 3.1.1** for a detailed description of each of these treatments, noting that some adjustments were made between the 2019 and 2020 seasons. The study was conducted at three different field sites in southern Ontario: (1) Guelph Switchgrass, GS; (2) Burlington Switchgrass, BS; and (3) Burlington Miscanthus, BM. The GS field site is a University of Guelph research facility. The BS and BM field sites are properties owned and operated by members of the OBPC, Mr. James Fisher and Mr. Norman Richardson, respectively. Descriptions for each of these sites are provided below, including the key physical conditions, land management history, and layout of the experimental plots.

Table 3.1.1: Summary of treatments applied in the 2019 and 2020 field seasons.

Treatment	*Description*
Control	No inputs of any kind.
Synthetic N	Food-grade urea applied at 60 kg N ha^{-1} (switchgrass) and 55 kg N ha^{-1} (miscanthus) according to OBPC recommended rates (Samson et al., 2018; Withers et al., 2016).
JumpStart®	Dissolved in water to a concentration of 2.05×10^5 cfu *Penicillium bilaiae* L^{-1} (based on manufacturer recommendations for wheat seed treatment; Novozymes BioAg, 2019) and applied at 1 L m^{-2} and food-grade urea applied at 30 kg N ha^{-1}. 2020: triple 2019 application rate (6.15×10^5 cfu *P. bilaiae* L^{-1}); no urea
MYKE® Pro / AGTIV	Surface-applied at a rate of 3.00×10^3 *Glomus intraradices* spores m^{-2} according to manufacturer recommendations (Premier Tech, 2020; Premier Tech 2021) and food-grade urea at 30 kg N ha^{-1}. 2020: no urea
LysteGro (2019 only)	Surface-applied at a rate of 60 kg N ha^{-1} corrected for a 50% N volatilization rate according to manufacturer recommendations (Lystek, 2019; M. Dougherty, personal communication, July 8, 2019).
Optimyc + MooR (2020 only)	Optimyc applied at 750 g ha^{-1} in combination with MooR applied at 25 L ha^{-1} according to manufacturer recommendations (M. Boersma, personal communication, May 13, 2020) by mixing both products in water to a concentration of 0.15 g Optimyc L^{-1} and 5 mL MooR L^{-1}. Final solution was applied at 0.5 L m^{-2}. MooR contains *Bacillus licheniforms* (2106 cfu mL^{-1}), *Bacillus methylotrophicus* (4106 cfu mL^{-1}), and *Bacillus subtilis* (4106 cfu mL^{-1}). Optimyc contains *Entrophospora columbiana*, *Glomus clarum*, *Glomus etunicatum*, and *Rhizophagus irregularis*, each at 139 spores g^{-1}.

3.1.1 Guelph Switchgrass (GS) Field Site

The GS field site is located at the Guelph Turfgrass Institute (Guelph, ON), having four 200 m^2 (10 m by 20 m) blocks of switchgrass var. Cave-in-Rock planted in 2014. These blocks were initially established for a long-term biomass crop research project which concluded in 2017, the details of which can be found in Ashiq et al. (2018). For the present study, each of the four replicate blocks were divided into five treatment plots (four measuring 3 m by 10 m, one measuring 4 m by 10 m, each separated by a 1 m guard row (gap). The five treatments in 2019 were then assigned in a randomized complete block design (RCBD). In 2020, LysteGro® was removed from the study due to challenges associated with coordinating its application within the COVID-19 contact restrictions. Plots that had received LysteGro® in 2019 received Optimyc and MooR (VisscherHolland) in 2020. See **Figure 3.1.1** for the experimental map depicting the GS field layout.

Figure 3.1.1: Experimental layout of the Guelph Switchgrass site. Letter labels on the treatment plots correspond to the following treatments: C = control, F = synthetic N, J = JumpStart®, A = MYKE® Pro (2019) or AGTIV® (2020) and L = LysteGro (2019) or Optimyc + MooR (2020)

According to the 1981-2010 Canadian Climate Normals for this area, average daily temperature was 7.0˚C and average annual precipitation was 916.5 mm (ECCC, 2021b). During the growing season, defined as May to October for the purposes of this study, average daily temperature was 15.3˚C and total precipitation was 500.6 mm (ECCC, 2021b). In 2019, this area was cooler and drier than normal with a year-round average daily temperature of 6.6˚C and annual precipitation of 704.1 mm (ECCC, 2021c). However, in the 2019 growing season (May-October 2019), the average daily temperature was 15.3˚C and total precipitation was 404.9 mm (ECCC, 2021c), indicating a drier growing season with normal temperatures. In 2020, the second year of the study, the year-round average daily temperature was 8.0˚C and annual precipitation was 689.2 mm (ECCC, 2021e) which is warmer and drier than normal. In May-October of 2020,

average daily temperature is 15.5˚C and total precipitation is 392.2 mm (ECC, 2021e), also following a warmer and drier trend. For a monthly breakdown of temperature and precipitation data, please refer to **Appendix A**.

Soil samples collected at this site in 2019 (0-30 cm) were sent to SGS Laboratories (Guelph, ON; hereafter SGS Labs) for a texture analysis using the hydrometer method for particle size analysis as described by Gavlak et al. (2005). This texture analysis indicated that the soil texture ranged from a sandy loam to a loam (**Appendix A**). According to AAFC (1963), this field site occurs between an area of poorly drained Granby sandy loam and an area of gravelly and rapidly draining Donnybrook sandy loam (Hoffman et al., 1963). Neither of these soils are commonly cultivated due to their respective challenges and are classified as poor and fair to poor, respectively, in terms of their cropland quality (Hoffman et al., 1963). Observations at this site suggest that it belongs to the Donnybrook series because of the number of stones and gravel present. Donnybrook sandy loams are generally assigned as Class 4 or Class 6 agricultural lands according to the CLI soil capability classes due to poor fertility, topographical challenges, or adverse inherent soil characteristics (Gillespie et al., 1971).

3.1.2 Burlington Switchgrass (BS) Field Site

The BS field site is located at Holten Farm (Burlington, ON). This crop of switchgrass var. Cave-in-Rock was planted by seed in 2012 following one year of soybean production (J. Fischer, personal communication, May 13, 2021). Four blocks each containing five treatment plots (6 m by 6 m, each separated by a 1 m guard row) were established in June of 2019. Each block was oriented perpendicular to the dominant slope and in the most level locations within the field to reduce the possibility of the treatments leaching into adjacent blocks once applied. Furthermore, each of the blocks were at least 5 m from each other to mitigate this risk. The five treatments

from 2019 were then assigned in an RCBD. This site was not revisited for the 2020 field season due to difficulties coordinating sufficient transportation and labour within the COVID-19 contact restrictions. See **Figure 3.1.2** for an experimental map depicting the BS field layout.

Figure 3.1.2: Experimental layout of the Burlington Switchgrass site. Letters labels on the treatment plots correspond to the following treatments: C = control, F = synthetic N, J = JumpStart®, A = MYKE® Pro, and L = LysteGro.

The 1981-2010 Canadian Climate Normals for this area indicate an average daily temperature of 9.1˚C and average annual precipitation of 863.1 mm (ECCC, 2021a). During the growing season (May-October), Climate Normals indicate an average daily temperature of 17.4˚C and total annual precipitation of 462.4 mm (ECCC, 2021a). In 2019, this area was cooler and wetter than normal with a year-round average daily temperature of 8.5˚C and annual precipitation of 969.7 mm (ECCC, 2021d). In May-October 2019, the average daily temperature was 17.4˚C and total precipitation was 526.0 mm (ECCC, 2021d), indicating higher than average

precipitation during the growing season, but average growing season temperatures. In 2020, there was a year-round average daily temperature of 10.3˚C and annual precipitation of 855.4 mm (ECCC, 2021f) which was warmer and slightly drier than average. May-October of 2020 had an average daily temperature of 18.2˚C and total precipitation equalling 458.7 mm (ECC, 2021f), also warmer and slightly drier than the norm for these months. For a full breakdown of climatic variables, please see **Appendix A**.

Soil samples collected at this site in 2019 (0-30 cm) were sent to SGS Labs for textural analysis as previously described. The results of this analysis indicated that the soil has a silt loam texture (**Appendix A**). According to AAFC (1971), this field site occurs on an area of Oneida loam soil, however our texture analysis suggests it may be an Oneida silt loam. Oneida loams and silt loams are moderately well-drained and are considered Class 1 to Class 2 agricultural lands based on the CLI soil capability classes, depending on the severity of the slope which influences the risk of erosion (Gillespie et al., 1971). AAFC (1971) indicates slopes of 5-9% in this area, so it would be assigned to Class 2.

3.1.3 Burlington Miscanthus (BM) Field Site

The BM field site is located at Mabel May Farms (Burlington, ON). The strip of miscanthus (*Miscanthus sacchariflorus*) used for this study was planted from rhizomes in late spring of 2015 (N. Richardson, personal communication, May 16, 2021). Prior to the miscanthus planting, this land area was planted to mixed hay (N. Richardson, personal communication, May 16, 2021). Three blocks, each containing five treatment plots (6 m by 6 m, each separated by a 1 m guard row) were established in June 2019 and the five treatments were assigned to their respective plots in an RCBD. In 2020, four out of these five treatments were reapplied to the same blocks they were assigned to in 2019. The new Optimyc + MooR treatment, which replaced LysteGro at

the GS site, was not applied at this location in 2020 due to difficulties coordinating sufficient transportation and labour to bring the water required for its application during the COVID-19 restrictions. See **Figure 3.1.3** for an experimental map depicting the BM field layout for the 2019 and 2020 field seasons.

Figure 3.1.3: Experimental layout of the Burlington Miscanthus site. Letter labels on the treatment plots correspond to the following treatments: C = control, F = synthetic N, J = JumpStart®, A = MYKE® Pro (2019) or AGTIV® (2020), and L = LysteGro (2019). L plots were excluded in 2020.

The BM field site occurs within the same city as the BS field site, so the climate data is the same for both locations. As described above, 2019 was cooler and cooler and wetter than the 1981-2010 Climate Normals although the May-October 2019 growing season maintained average temperatures. In 2020, this area was warmer and slightly drier than the norm both when looking at the data for the full year and when only examining data from growing season months (May-October). The full monthly breakdown of climate data is presented in **Appendix A**.

Soil samples collected at this site in 2019 (0-30 cm) were sent to SGS Labs for textural analysis and it was determined that the soils have a loam texture (**Appendix A**). According to AAFC (1971), this field site occurs on a Chinguacousy clay loam. Soils in the Chinguacousy series are imperfectly drained and generally considered to be Class 1 agricultural land according to CLI soil capability classes, ideal for growing a wide a variety of crops (Gillespie et al., 1971).

3.2 Plant Morphology and Yield

Every two to three weeks from mid July to the end of October 2019, five randomly selected tillers were harvested from each treatment plot at each of the three field sites to measure several plant morphological metrics. A full list of sampling dates is provided for reference in **Appendix B**. Those five tillers were stored in plastic bags with a wet paper towel in a cooler to preserve them until we completed sampling and returned to the lab. At the lab, each tiller was laid out and measured from its base to the tip of the tallest point. Then, leaves were removed, counted, and their area was measured using a LI-3100 Area Meter. Stems and leaves were weighed separately, then dried at 65˚C for 2 weeks and weighed again. For each sampling period, the mean plant height, mean leaf number per tiller, mean leaf area per tiller, mean stem dry weight per tiller, mean leaf dry weight per tiller, and mean total dry weight per tiller were calculated for each plot. This data set was narrowed down for analysis by selecting only the peak season value for each metric at each site. The peak season value for each morphological metric was identified by taking the overall mean value for each metric (regardless of treatment) for each sampling day at each field site and choosing the day with the highest value for each metric. Only values from those days were analyzed.

Due to wide variation and lack of significance associated with the morphological data collected in 2019, and a reduced research program due to the COVID-19 pandemic restrictions,

the methodology used to track plant growth over the growing season was adjusted for the 2020 season. Morphological measures were reduced to monthly tiller height and tiller counts. At both sites, tiller height was measured by marking out one random 0.5-by-0.5 m (0.25 m^2) area within each treatment plot at each of the two field sites and the five tallest tillers in each 0.25 m^2 area were marked with flagging tape. Each month, from June to September at the Guelph Switchgrass site and from July to September at the Milton Miscanthus site (see **Appendix B** for exact dates), the height of each of the marked tillers was measured and the number of tillers within each of the 0.25 m^2 areas were counted. The average height of the five tillers was calculated for each plot on each sampling day. Then the date with the peak plant height (per the selection method outlined for the 2019 data) was selected for analysis. The tiller counts were converted from tillers per 0.25m^2 to tillers ha^{-1} and only the last sampling date in autumn was chosen for analysis to match the available data from 2019.

Finally, yield was measured in both 2019 and 2020 by harvesting all tillers in a randomly selected 0.5-by-0.5 m (0.25 m^2) area in each treatment plot at each site at the end of the growing season (see **Appendix B** for exact dates). Unfortunately, a miscommunication with the landowner led to the crop being harvested before the 2019 yield data was collected, and this site was not revisited in 2020 due to COVID-19 restrictions. Therefore, no final yield data is available for this site. All yield data for switchgrass presented in this thesis was collected at the Guelph Switchgrass site only. After being harvested from the 0.25m^2 area, the plants were dried at 65˚C until they reached a constant weight. Once constant weight was achieved, the weight was recorded (g 0.25 m^{-2}). The values were then converted to tonnes ha^{-1} for the statistical analysis. The tiller density data (tillers 0.25 m^{-2}) was collected in the same area before harvest and converted to tillers ha^{-1} for statistical analysis.

3.3 Soil Fertility and Nutrient Uptake

3.3.1 Field Data

Soil samples were collected to analyze soil nutrient availability (soil fertility) at all three field sites (GS, BS, BM) at two key points during the 2019 season: baseline (before treatment application) and end-of-season (during plant senescence). In 2020, soil fertility data was collected at the mid-season (peak of plant growth) sampling periods. See **Appendix B** for a full list of sampling days. On each sampling day, soil samples (0-30 cm) were collected from two randomly selected locations in each plot. The two samples were then homogenized, and one composite sample taken from the mixture sis. Once all sampling was completed, the single composite sample from each plot was sent to SGS Labs to analyze the availability of key plant nutrients N, P, K, magnesium (Mg) and calcium (Ca). SGS Labs uses the following Ontario-accredited methods for the estimation of these nutrients:

(1) Nitrate-nitrogen and ammonium-nitrogen (ppm): Soil is mixed with potassium chloride (at ratio of 1:5) which is shaken for half an hour and then filtered. Extract is then analyzed using autoanalyzer which measures the color intensity produced after treating extract with chemicals.

(2) Available Phosphorus (ppm): Olsen method which uses sodium bicarbonate was used for analyzing P. One part of soil is mixed with 20 parts of 0.5 M sodium bicarbonate solution (pH 8.5), which is shaken for 30 minutes. After adding chemicals (molybdate and stannous chloride solution) to the extract, a blue color is formed which is read on photoelectric colourimeter. See Olsen and Sommers (1983) for further details.

(3) Potassium (ppm): Ammonium acetate was used to extract potassium from the soil and the extracted mineral is then quantified measured using a flame photometer.

(4) Magnesium (ppm): Ammonium acetate was used to extract magnesium the soil and the extracted mineral is then quantified using a flame photometer.

(5) Calcium (ppm): Ammonium acetate was used to extract calcium from the soil and the extracted mineral is then quantified using a flame photometer.

Baseline values for each of these nutrients at each of the sites is provided in **Table 3.3.1**.

Table 3.3.1: Baseline soil availability (ppm) of nitrate (NO_3^-), ammonium (NH_4^+) phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) at the Guelph Switchgrass, Burlington Switchgrass, and Burlington Miscanthus sites. Baseline data was collected once per block. All nutrient contents are expressed in parts-per-million (ppm).

Guelph Switchgrass						
Block	*NO_3^-*	*NH_4^+*	*P*	*K*	*Mg*	*Ca*
1	6.3	4.7	32	76	192	3272
2	8.6	5.7	24	68	279	2190
3	4.8	3.5	23	67	258	1875
4	3.9	3.0	14	66	286	1822
Burlington Switchgrass						
Block	*NO_3^-*	*NH_4^+*	*P*	*K*	*Mg*	*Ca*
1	5.3	4.1	4	47	151	1644
2	5.4	4.6	6	51	127	1594
3	5.0	3.9	5	50	140	1668
4	5.4	3.6	5	49	169	1607
Burlington Switchgrass						
Block	*NO_3^-*	*NH_4^+*	*P*	*K*	*Mg*	*Ca*
1	17.9	4.5	12	79	243	1962
2	19.0	4.7	11	132	269	2616
3	17.2	2.6	9	78	220	2990

For the end-of-season 2019, nutrient availability data was taken from tests included in the VitTellus® Soil Health Index package conducted by A&L Canada Laboratories (London, ON; hereafter A&L Canada Labs), the details of which will be discussed in the next section which outlines the methods used to evaluate soil biological health. Hence, soil samples were not sent for analysis at SGS Labs. A&L Canada Labs uses the following methods for the estimation of these nutrients:

(1) Nitrate-nitrogen: Potassium sulphate extraction followed by colourimetric analysis using the cadmium reduction method, per the protocol provided by Standard Methods Committee (2018).

(2) Available Phosphorus: Extracted and analysed per both the Bray-P and Olsen-P methods, as described in Olsen and Sommers (1983). For the purposes of this study, the Olsen-P values were selected due to the increased suitability of this measure for neutral to alkaline soils (Olsen and Sommers, 1983), and to maintain consistency with the methodology used by SGS Labs as previously described.

(3) Potassium: Ammonium acetate extraction followed by inductively coupled plasma - optical emission spectrometry (ICP-OES) analysis, per the protocol provided by Soil and Plant Analysis Council (2000).

(4) Magnesium: Ammonium acetate extraction followed by ICP-OES analysis, per the protocol provided by Soil and Plant Analysis Council (2000).

(5) Calcium: Ammonium acetate extraction followed by ICP-OES analysis, per the protocol provided by Soil and Plant Analysis Council (2000).

To complement the soil fertility data, plant tissue nutrient content was also assessed at the peak of the growing season in both 2019 and 2020. Please refer to **Appendix B** for a full outline of the sampling days. This data was collected to determine if the applied treatments influenced plant uptake of nutrients, in comparison with the nutrients available in the soil. This was important to collect because some of the biofertilizer treatments work by improving plant access to existing soil nutrients rather than directly increasing the amount of nutrients in the soil. In 2019, five tillers were randomly selected and harvested from each plot at the GS, BS, and BM sites, respectively. These five plants were initially used to collect plant morphological data as

previously described. Once the samples were dried and the dry weights were recorded, the leaves and tillers of all five plants were ground together to form one composite plant tissue sample per plot. The ground plant tissues were then sent to SGS Labs in Guelph, ON for analysis of the total N, P, K, Mg, and Ca contents of the plant tissues. In 2020, five randomly selected plants were harvested from each plot at the GS and BM field sites, dried at 60˚C, ground together to form one composite sample per plot, and sent to SGS Labs for analysis of the N, P, K, Mg, and Ca content, the same as in 2019. SGS Labs uses the following methods for the estimation of these nutrients in the plant tissues:

(1) Nitrogen (%): Combustion of dried plant tissue in LECO N analyser, per the AOAC International (2006) protocol.
(2) Phosphorus (%): Tissue sample is dry ashed, acid digested using hydrochloric acid, and then diluted for analysis of phosphorus content via ICP-OES per the AOAC International (1996) protocol.
(3) Potassium (%): Tissue sample is dry ashed, acid digested using hydrochloric acid, and then diluted for analysis of potassium content via ICP-OES per the AOAC International (1996) protocol.
(4) Magnesium (%): Tissue sample is dry ashed, acid digested using hydrochloric acid, and then diluted for analysis of magnesium content via ICP-OES per the AOAC International (1996) protocol.
(5) Calcium (%): Tissue sample is dry ashed, acid digested using hydrochloric acid, and then diluted for analysis of calcium content via ICP-OES per the AOAC International (1996) protocol.

3.3.2 Incubation Study

To assess treatment effects on soil nutrient availability under controlled conditions, an incubation study was conducted with support from a University of Guelph Master of Environmental Science (MES) student, Ramanjit Kaur Bhatti. This study was used to assess each treatments' effect on the accumulation of these key nutrients through microbial processes without the presence of live plants which would be removing nutrients for growth as they become available. On November 7, 2019, four soil sub-samples (0-15 cm) were collected from each treatment plot across three replicates at the GS field site. The four sub-samples were then combined to form one composite sample per plot. These composite soil samples were then air-dried, ground, and passed through a 2 mm mesh sieve in preparation for the incubation study.

A subsample of air-dried sieved soil (700 g) was collected from each of the larger samples and stored in large jars. The moisture content was then adjusted to 22% by adding the required amount of distilled water. An additional 100 g of air-dried sieved soil was collected for baseline nutrient analysis (week 0) and stored at -20˚C until the end of the study. The jars were then sealed and stored in the incubator at 20˚C. The incubation began on January 14, 2020, and sub-samples were collected on weeks 1, 3, 5 and 7 of the incubation. At each sampling week, a 100 g soil sample was taken from the bulk 700 g soil for each treatment and stored at -20˚C. Later, these soils were analyzed at SGS Labs for nitrate-nitrogen, ammonium-nitrogen, available phosphorus, and potassium using the same methods as previously described. The total average availability of each nutrient over all eight samples (weeks 0-7) was determined and statistically analyzed. The analysis of nutrient release over time during this study is outlined in **Appendix C**.

3.4 Soil Biological Health

Bacterial and fungal community sizes in the top ten centimeters of soil were analyzed through quantitative polymerase chain reaction (qPCR) analysis of the highly conserved 16S and 18S ribosomal ribonucleic acid (rRNA) regions, respectively. This process involved five steps: (1) collecting soil samples, (2) extracting the deoxyribonucleic acid (DNA) from the soils, (3) confirming DNA extraction quality, (4) conducting an inhibition test, and (5) running the 16S and 18S qPCR assays. Soil samples were collected at three key times in the 2019 season: baseline (before treatment application), mid-season (peak plant growth), and end-of-season (plant senescence). End-of-season samples were not collected from the BS field site due to the premature harvest of the crop. In 2020, samples were collected only at the mid-season and end-of-season sampling times. On each sampling day, eight subsamples (0-10 cm) were collected from each plot in an X-shaped pattern (four samples on each diagonal), to capture variability across the plot. Samples were typically collected using soil probes. On days when the soils were too hard to use the probes, planting shovels or soil augers were used instead. Nitrile gloves were worn while sampling and all sampling equipment (including hands) was sterilized between each plot using 70% ethanol. The eight subsamples from each plot were homogenized in polypropylene bag and stored in a cooler until sampling was complete. Samples were then stored at 4˚C for one to a maximum of seven days until DNA extraction could be completed. See **Appendix B** for a list of dates that samples were collected, and the dates that associated DNA extractions occurred. All DNA extractions were conducted using the DNeasy PowerSoil Kit (Qiagen) according to manufacturer instructions. The isolated DNA extractions (volume of 100 µL) were stored at -20˚C until further analysis could be completed. Soil moisture for each soil

sample was also calculated by weighing a subsample of wet soil (approximately 10 g), drying the soil at 65˚C, recording the dry weight of the sample, and dividing it by its initial wet weight.

DNA was quantified and checked for purity using the NanoDrop™ 8000 Spectrophotometer (Thermo Fisher Scientific Corp.). Sample purity was assessed using $A_{260/280}$ ratios, with pure samples of 1.8 and a spectral profile with the trough at the 230 nm wavelength and the peak at the 260 nm wavelength (Matlock, 2015).

An inhibition test was conducted by spiking a known concentration of an M13 plasmid into a dilution series of sample DNA and using qPCR to identify inhibition. All plasmid standards used for the qPCR assays in this study (M13, 16S, and 18S) were constructed by cloning genes from environmental samples or pure culture DNA into TOPO TA plasmids (Life Technologies Corp.), and target gene identities were verified by sequencing at the University of Guelph's Laboratory Services (Guelph, ON). The inhibition test was run using a representative set of eight samples from the 2019 DNA extractions. From each undiluted DNA sample, 25-, 50-, and 100-times dilutions were prepared.

In total, the qPCR assay for the inhibition test included four dilutions of eight DNA samples (raw elution, 25-, 50-, and 100-times dilutions), two positive controls, and two negative controls. A MasterMix stock solution for the qPCR reactions was prepared according to the following proportions (per reaction): 10 µL SsoFast™ EvaGreen® Supermix (Bio-Rad Laboratories Inc.), 1 µL each of M13-F and M13-R primers (10 pmol μL^{-1}), 1 µL M13 plasmid (excluded for negative controls), and 3 µL PCR-grade deoxyribonuclease (DNase)- and ribonuclease (RNase)-free water (hereafter, nuclease-free water). MasterMix (16 µL) was pipetted into each of the required wells in a sterile 96-well plate. For each of the DNA samples, 4 µL of each dilution and the initial undiluted DNA sample was added to the appropriate wells with the 16 µL of

MasterMix (16 µL) for a reaction volume of 20 µL. For the positive controls, M13 plasmid (10^4 pmol μL^{-1}) (4 µL) was added to the MasterMix solution (16 µL). For the negative controls, nuclease-free water (4 µL) was added. The plate was then sealed, and the qPCR assay was run using the CFX96™ Real-Time PCR Detection System (Bio-Rad Laboratories Inc.) (hereafter, CFX96™). The reaction cycle was as follows: (1) initial denaturation at 98.0˚C for 2 minutes; (2) 40 cycles of denaturation at 98.0˚C for 10 seconds followed by annealing at 55.0˚C for 20 seconds with a plate reading at each repetition; and (3) a melt curve starting at 65.0˚C and increasing to 95.0˚C in increments of 1˚C every 10 seconds, with a plate reading at each temperature. The results of this test indicated that the 50-times dilution was most efficient. Therefore, all qPCR assays were run using 50x dilutions of the DNA extractions.

In preparation for the 16S qPCR analyses, a standard dilution series of the 16S plasmid ranging from 10^1 pmol μL^{-1} to 10^8 pmol μL^{-1} (increasing by a factor of ten) was created and its efficiency tested in a qPCR analysis (minimum efficiency of 95%). MasterMix stock solution was prepared according to the following proportions (per reaction): 10 µL SsoFast™ EvaGreen® Supermix (Bio-Rad Laboratories Inc.), 1 µL each of 338F and 518R primers (10 pmol μL^{-1}) (Muyzer et al., 1993), 4 µL nuclease-free water. MasterMix (16 µL) and 4 µL of each concentration in the standard dilution series were added to the appropriate wells of a 96-well plate (20 µL reaction volume). Two negative controls were also included on the plate, consisting of MasterMix (16 µL) and nuclease-free water (4 µL). The plate was sealed and transferred into the CFX96™ to run the following reaction cycle: (1) initial denaturation at 98.0˚C for 2 minutes; (2) 35 cycles of denaturation at 98.0˚C for 5 seconds followed by annealing at 55.0˚C for 5 seconds with a plate reading at each repetition; and (3) a melt curve starting at 65.0˚C and

increasing to 95.0˚C in increments of 1˚C every 5 seconds, with a plate reading at each temperature.

16S gene abundances were calculated using standard curves from 10-fold serial dilutions of cloned plasmids between 10^8 – 10^1 copies per µL, run in duplicate or triplicate. All 16S qPCR assays were optimized to reaction efficiencies of 101-122% with R^2 values ranging from 0.93-1.00. Two negative controls (16 µL MasterMix plus 4 µL nuclease-free water) were included on each plate. DNA dilutions (50x) (4 µL) were used for each reaction, with an appropriate volume of MasterMix (16 µL) as described above and quantified using a CFX96™ via the reaction cycle above. The amplification specificity of each qPCR reaction was tested via the melt curve created at the third stage of the reaction cycle. Results were expressed as gene copies per gram of dry soil (copies g dry soil^{-1}) based on the calculation of soil moisture for that sample.

Finally, in preparation for the 18S qPCR analyses, a standard dilution series of the 18S plasmid ranging from 10^1 pmol µL^{-1} to 10^8 pmol µL^{-1} (increasing by a factor of ten) was created and its efficiency tested in a qPCR analysis (minimum efficiency of 95%). A MasterMix stock solution was prepared according to the following proportions (per reaction): 10 µL SsoFast™ EvaGreen® Supermix (Bio-Rad Laboratories Inc.), 1 µL each of FF1 and FFR390 primers (10 pmol µL^{-1}) (Prévost-Bouré et al., 2011), 4 µL nuclease-free water. MasterMix (16 µL) and 4 µL of each concentration in the standard dilution series were added to the appropriate wells of a 96-well plate (20 µL reaction volume). Two negative controls were also included on the plate (16 µL of MasterMix, 4 µL of nuclease-free water). The plate was then sealed and transferred into the CFX96™ where the following reaction cycle was run: (1) initial denaturation at 95.0˚C for 3 minutes; (2) 40 cycles of denaturation at 95.0˚C for 15 seconds, annealing at 50.0˚C for 30 seconds, and extension at 70.0˚C for 45 seconds with a plate reading at every cycle; and (3) a

melt curve starting at 65.0˚C and increasing to 95.0˚C in increments of 0.5˚C every 5 seconds, with a plate reading at each temperature.

Genes were calculated using standard curves from 10-fold dilutions of cloned plasmids between 10^8 – 10^1 copies per µL, run in duplicate or triplicate. All 18S qPCR assays were optimized to reaction efficiencies of 90 – 104% with R^2 values ranging from 0.86 – 0.91. Two negative controls (16 µL MasterMix, 4 µL nuclease-free water) were included on each plate. DNA dilutions (50x) (4 µL) were used for each reaction, with an appropriate volume of MasterMix (16 µL) as described above and quantified using a CFX96™ via the reaction cycle described above. The amplification specificity of each qPCR reaction was tested through the melt curve created at stage 3 of the reaction cycle. Results were expressed as gene copies per gram of dry soil (copies g dry soil^{-1}).

In addition to the measurements of bacterial and fungal abundance, soil biological health was assessed by evaluating respiration rates, C substrate availability, and earthworm abundance. At the end of the 2019 season, two soil samples (0-30 cm) were collected from randomly selected locations within each plot at the GS and BM field sites (see **Appendix B** for exact dates). At the Guelph Switchgrass site, samples were only taken from three out of four replicates (blocks) due to the costs associated with the test. Samples were not collected from the BS field site due to the pre-emptive harvest of the crop and rapidly declining weather conditions. The samples were stored at 4˚C until being delivered to A&L Canada Labs to be evaluated by the VitTellus® Soil Health Index package. This package includes analysis of soil nutrient availability, pH, cation exchange capacity, percent base saturation, electrical conductivity, Solvita CO_2 test, and reactive carbon, as well as the final VitTellus® Soil Health Index. From this data, the Solvita CO_2 Burst test results were used to assess treatment effects on basal soil respiration and the reactive C

measurements were evaluated as an indicator of treatment effects on the availability of C substrate which is vital for supporting microbial communities. The VitTellus® Soil Health Index itself was not included in the analysis for this study because no explanation of how the index is calculated could be found, therefore its validity as an indicator of soil health could not be independently assessed. Samples were not collected for this test in 2020 due to a lack of significant differences observed in the 2019 data, as well as the cost of the test.

Earthworm abundance was measured at the GS site in spring of 2020, approximately two weeks after treatment application (see **Appendix B** for exact date). Earthworm abundance was measured according to a protocol adapted from Price and Gordon (1999). First, a 900 cm^2 metal quadrat was hammered into the soil to a depth of 10 cm at a randomly selected location in the control, synthetic N, and JumpStart® (representative biofertilizer treatment) plots of three of the four replicate blocks. Then, a solution of 5% formaldehyde was poured slowly into the quadrat, allowing the solution to seep into the soil and cause the worms to come to the surface seeking oxygen due to the irritation of their skin. The worms are not killed using the 5% formaldehyde solution. All worms in the quadrant were counted for about 20 minutes and they were placed outside the quadrant so that they could return to the soil. These earthworm counts were then converted from number of earthworms per 900 cm^2 to number of earthworms per m^2.

3.5 Greenhouse Gases

Soil GHG flux was measured and compared among treatment plots at the GS field site in the 2020 season. In June 2020, one static chamber was installed at a randomly selected location in the plots the received the following treatments in blocks two to four: control, synthetic N, JumpStart®, and Optimyc + MooR. Each chamber was driven into the soil deep enough to produce a 15 cm headspace. Block one and the AGTIV® treatment were excluded due to

resource constraints and concerns about completing the sampling within the limited time frame. The treatments chosen are representative of the three types of fertilizers used in this field season (no fertilizer, synthetic fertilizer, and biofertilizer). Of the two biofertilizers chosen, JumpStart® represents a biofertilizer that works by inoculating the soil with a microbe that solubilizes and increases accessibility of phosphorus in the soil (namely, phosphorus), whereas Optimyc + MooR represents a biofertilizer that works by inoculating the soil with consortia of AMF and rhizobacteria to promote the beneficial relationships among these microbes and plant roots that can improve plant uptake of soil nutrients.

One week after the chambers were installed, monthly sampling began. See **Appendix B** for the full list of sampling dates. Each sampling day began between 10:00 AM and 11:00 AM, according to the Collier et al. (2014) recommendation which states that gas samples meant to be representative of a full day should be taken in mid to late morning while the temperatures are moderate. Sampling rounds were separated by block to eliminate the bias associated with sampling the same treatment at the same time each day (Collier et al., 2014). Samples were collected from each chamber in a block using an air-tight syringe at T_0 (immediately after sealing chamber lid), T_1 (15 minutes after sealing chamber lid) and T_2 (30 minutes after sealing chamber lid). Before extracting the sample from the chamber, the syringe was filled and emptied back into the chamber to circulate the air in the chamber before extracting the final sample which was then injected into a labeled 12 mL Exetainer® (Labco Ltd.). Before each sampling round, ancillary measures of soil moisture and soil temperature were also collected to assess the influence of these factors on the variation of flux rates between sampling dates. Soil moisture was measured using a HydroSense II (Campbell Scientific) equipped with the 12 cm sensors. At the same time, soil temperature was measured at a 10 cm depth using a TFX-430 Thermometer (Ebro). This

sampling procedure is adapted from the procedure laid out in Collier et al. (2014). All samples were stored at room temperature until the end of the season when they could be sent to the Agriculture and AgriFood Canada laboratory in Saskatoon, Saskatchewan for analyses of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) concentrations via gas chromatography. When the gas chromatography results were received, flux rates were calculated in grams (or kilograms for CO_2) of gas flux per hectare per day (g or kg ha^{-1} day^{-1}) using the protocol outlined by Kahmark et al. (2020). Total global warming potential (GWP) was estimated by summing the CO_2-equivalents (CO_2e) for all three gases and expressed in kg CO_2e ha^{-1} day^{-1}. The CO_2e for carbon dioxide is 1-times the CO_2 flux rate (since this is the reference point), the CO_2e for methane was estimated at 28-times the CH_4 flux rate and the CO_2e for nitrous oxide was estimated at 265-times the N_2O flux rate, per the 100-year GWP estimates for these gases reported by IPCC (2014).

3.6 Statistical Analysis

The generalized linear mixed model procedure (PROC GLIMMIX) in SAS® OnDemand for Academics (SAS Institute Inc.) was used to run an analysis of variance (ANOVA) assessing treatment effects on each metric at each site. The fertilizer treatment sum of square was partitioned using an orthogonal contrast approach to evaluate comparisons between the biofertilizers and synthetic fertilizer treatments, and to compare biofertilizers among each other. Orthogonal contrasts were conducted in order to specifically compare these groups of interest in order to inform evaluations of biofertilizer performance in the absence of significant differences in the ANOVA. Dunnett's Correction was used as the post hoc test for least-square means comparisons between each fertilizer treatment and the control plots in order to determine which fertilizer treatments produced significant differences compared to the growth of these crops in

the absence of fertilizer for the duration of this study. Significant differences were defined according to an alpha (α) less than or equal to 0.05. The assumptions for the ANOVAs were confirmed using scatterplots of studentized residuals against the various independent variables and their predicted values. A Shapiro–Wilk's test ($\alpha \leq 0.05$) was used to confirm that residuals followed a normal distribution. Statistical analysis of treatment effects on the autumn-harvested yield, autumn tiller densities, field-sampled soil nutrient availability (N, P, K, Mg, Ca), field-sampled plant tissue nutrient contents (N, P, K, Mg, Ca), 16S bacterial and 18S fungal gene abundance, Solvita CO_2 Burst test, soil reactive C, earthworm abundances, and GHG flux rates (CO_2, CH_4, N_2O, and GWP as CO_2e) for each site with available data were conducted in the same manner.

In addition to ANOVAs and orthogonal contrasts assessing treatment effects on the GHG flux rates as described above, statistical analyses were used to assess seasonal variations in the flux of each gas. The relationships between flux rates of each gas and the collected soil data (temperature and volumetric soil moisture) were also evaluated. First, PROC GLIMMIX (SAS® OnDemand for Academics) was used to run ANOVAs testing the effect of the sampling date on the flux rate of each gas, regardless of treatment. Orthogonal contrast analysis was also employed to compare the flux of each gas in the summer (July and August dates) versus the autumn (September and October dates). Significant differences were defined according to an alpha of 0.05 and the assumptions for the ANOVAs were confirmed in the same manner as described above. The relationships between the flux rate for each gas and the soil temperature and moisture data that were collected on the same day were tested in SAS® OnDemand for Academics by conducting linear regression analyses using PROC GLIMMIX. Significant differences were once again defined according to an alpha less than or equal to 0.05.

Chapter 4: Results

4.1 Plant Morphology and Biomass Yield

4.1.1 Switchgrass

The peak season mean value for each treatment for the six plant morphological metrics measured throughout 2019 (tiller height, leaf number, leaf area, stem dry mass, leaf dry mass, and total dry mass) are presented in **Table 4.1.1**. Mixed model analysis of variance (ANOVA) for these values demonstrated no significant treatment effects on tiller height (cm), leaf area (cm^2), leaf dry mass (g), and total dry mass (g) at either the Guelph Switchgrass (GS) or Burlington Switchgrass (BS) field sites (**Table 4.1.2**). Least-square means comparison adjusted according to Dunnett's Correction also revealed no significant differences between the control and any of the fertilizer treatments for any physiological metric at either the GS or BS field site (data not presented). Partitioning of variance using orthogonal contrast also showed that there were no significant differences between the following treatment groups at the BS field sites: (1) Synthetic N fertilizer versus combined average of all three biofertilizers, (2) LysteGro biosolids fertilizer versus JumpStart® and MYKE® Pro inoculants of plant growth-promoting microbes, (3) JumpStart® versus LysteGro and MYKE® Pro, and (4) MYKE® Pro versus LysteGro and JumpStart® (**Table 4.1.2**).

Table 4.1.1: Mean peak season measure (± standard error) for six metrics of plant morphology (tiller height [cm], leaf number tiller^{-1}, leaf area [cm^2 tiller^{-1}], stem dry mass [g tiller^{-1}], leaf dry mass [g tiller^{-1}], and total dry mass [g tiller^{-1}] at the Guelph Switchgrass and Burlington Switchgrass sites in 2019.

Guelph Switchgrass						
Treatment	*Tiller height*	*Leaf number*	*Leaf area*	*Stem dry mass*	*Leaf dry mass*	*Total dry mass*
Control	148.8 ± 14.1	6.5 ± 0.3	150.6 ± 27.4	3.2 ± 0.2	1.3 ± 0.1	4.2 ± 0.6
Synthetic N	161.8 ± 6.2	6.3 ± 0.3	139.6 ± 3.3	3.1 ± 0.4	1.3 ± 0.2	4.8 ± 0.6
JumpStart®	147.6 ± 7.6	6.8 ± 0.3	164.3 ± 18.9	3.8 ± 0.4	1.3 ± 0.1	4.4 ± 0.1
MYKE® Pro	153.0 ± 7.9	7.0 ± 0.0	155.6 ± 15.9	2.6 ± 0.3	1.2 ± 0.0	3.8 ± 0.1
LysteGro	148.8 ± 8.5	7.0 ± 0.0	124.4 ± 12.7	2.8 ± 0.1	1.2 ± 0.1	4.0 ± 0.6
Burlington Switchgrass						
Treatment	*Tiller height*	*Leaf number*	*Leaf area*	*Stem dry mass*	*Leaf dry mass*	*Total dry mass*
Control	137.3 ± 9.9	5.8 ± 0.3	124.9 ± 10.2	3.0 ± 0.4	1.0 ± 0.1	3.8 ± 0.4
Synthetic N	136.7 ± 11.4	6.8 ± 0.5	128.4 ± 5.9	3.7 ± 0.5	0.9 ± 0.2	4.2 ± 0.6
JumpStart®	139.6 ± 9.4	6.8 ± 0.5	138.8 ± 20.8	2.8 ± 0.5	1.1 ± 0.2	3.6 ± 0.5
MYKE® Pro	142.2 ± 9.9	6.0 ± 0.4	119.3 ± 18.2	2.8 ± 0.4	0.9 ± 0.1	3.5 ± 0.5
LysteGro	141.3 ± 12.0	6.0 ± 0.0	126.6 ± 18.2	3.0 ± 0.5	1.2 ± 0.1	3.8 ± 0.6

Table 4.1.2: Mixed model analysis of variance assessing the effects of five fertilizer treatments (Control, Synthetic N, JumpStart®, MYKE® Pro, and LysteGro) on the peak value of six metrics of plant morphology (tiller height [cm], leaf number tiller^{-1}, leaf area [cm^2 tiller^{-1}], stem dry mass [g tiller^{-1}], leaf dry mass [g tiller^{-1}], and total dry mass [g tiller^{-1}] at the Guelph Switchgrass and Burlington Switchgrass sites in 2019.

Guelph Switchgrass							
Source of Variation	*df*	Tiller height *p-value*	Leaf number *p-value*	Leaf area *p-value*	Stem dry mass *p-value*	Leaf dry mass *p-value*	Total dry mass *p-value*
Fertilizer[1]	4	0.4174	**0.0444***	0.6303	**0.0188***	0.9270	0.7081
SYN vs BIO	1	0.0919	**0.0068****	0.7061	0.9222	0.6801	0.2639
LG vs JS & MP	1	0.8338	0.5744	0.1515	0.1190	0.8164	0.9373
JS vs LG & MP	1	0.6441	0.2707	0.3161	**0.0012****	0.4426	0.4376
MP vs LG & JS	1	0.5044	0.5744	0.6360	**0.0266***	0.5880	0.4837
Block	1	**0.0023****	0.1207	0.1953	**0.0300***	0.1222	**0.0194***
Burlington Switchgrass							
Source of Variation	*df*	Tiller height *p-value*	Leaf number *p-value*	Leaf area *p-value*	Stem dry mass *p-value*	Leaf dry mass *p-value*	Total dry mass *p-value*
Fertilizer[1]	4	0.8240	0.0928	0.8698	0.8039	0.5842	0.5903
SYN vs BIO	1	0.3602	0.1642	0.9926	0.3207	0.3549	0.1627
LG vs JS & MP	1	0.9332	0.3154	0.8832	0.5008	0.2918	0.4807
JS vs LG & MP	1	0.6600	0.0580	0.3373	0.8074	0.8363	0.9599
MP vs LG & JS	1	0.7211	0.3154	0.4123	0.6643	0.2134	0.5115
Block	1	**<0.0001*****	**0.0430***	0.0698	**0.0016****	0.1205	**0.0022****

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; MP = MYKE® Pro inoculant of *Glomus intraradices*; LG = LysteGro biosolids fertilizer; BIO = JumpStart®, MYKE® Pro, and LysteGro)

Two of the 2019 physiological metrics (leaf number tiller^{-1}, and stem dry mass in g tiller^{-1}) demonstrated significant differences at the GS site. First, orthogonal contrast demonstrated that plots at this site treated with synthetic N fertilizer had a significantly ($p < 0.05$) lower peak leaf number than those treated with biofertilizers (**Figure 4.1.1**). The ANOVA indicated a significant treatment effect on leaf number at this site (**Table 4.1.2**), but least-square means comparison adjusted according to the Tukey test indicated no significant differences among treatments. Second, there was a significant treatment effect ($p < 0.05$) on stem dry mass at the GS field site whereby JumpStart® produced a significantly higher peak stem dry mass than either MYKE® Pro or LysteGro which were statistically similar in a least-square means comparison adjusted according to the Tukey test (**Figure 4.1.2**). No significant differences were observed for either of these metrics at the BS field site. Per **Table 4.1.2**, several metrics demonstrate significant block effects, simply indicating that the RCBD approach was justified as this source of variation resulting from the blocks was separated from the treatment effects.

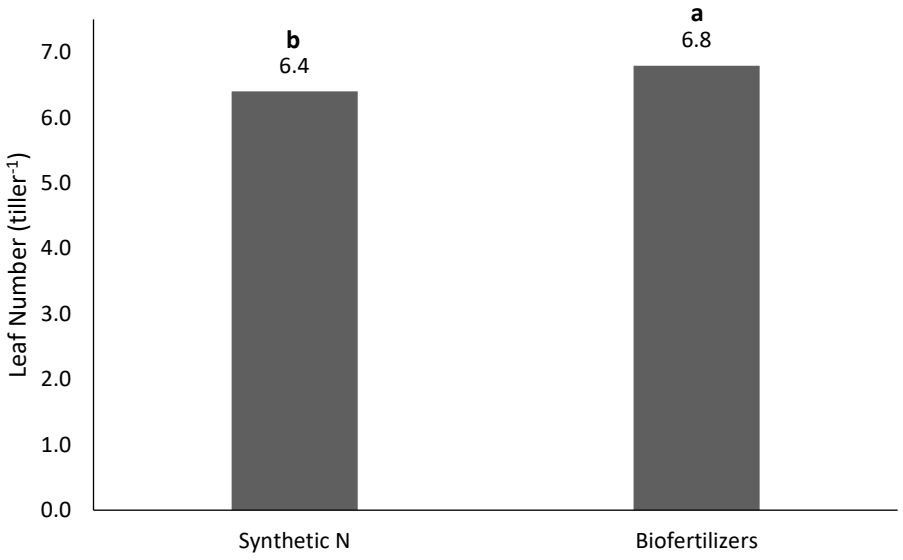

Figure 4.1.1: Peak leaf number per tiller for switchgrass as influenced by synthetic N fertilizer versus the combined average of three biofertilizers (JumpStart®, MYKE® Pro, and LysteGro) at the Guelph Switchgrass site in 2019. Different letters indicate significantly different means according to orthogonal contrast ($p \leq 0.05$).

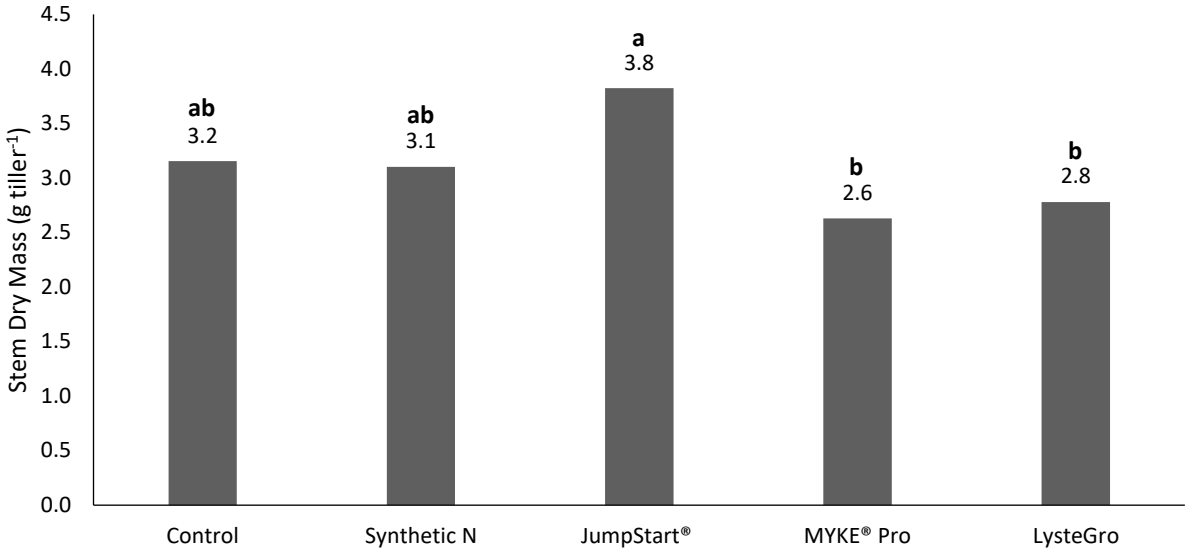

Figure 4.1.2: Peak stem dry mass (g tiller^{-1}) for switchgrass as influenced by fertilizer treatment at the Guelph Switchgrass site in 2019. Different letters indicate significantly different means according to the Tukey test ($p \leq 0.05$).

The mean peak season values for tiller height at the GS field site in 2020 are presented in **Table 4.1.3**. In 2020, synthetic N fertilizer significantly increased peak tiller height (cm) compared to the combined average for the three biofertilizers (JumpStart®, AGTIV® and Optimyc + MooR) (**Figure 4.1.3A**). Synthetic N fertilizer also resulted in significantly taller tillers compared to the control at the GS field site when analyzed via least-square means comparison adjusted according to Dunnett's Correction (**Figure 4.1.3B**).

Table 4.1.3: Mean peak season measure for tiller height (cm; ± standard error) at the Guelph Switchgrass site in 2020.

Treatment	*Tiller height*
Control	155.1 ± 6.3
Synthetic N	180.2 ± 3.9
JumpStart®	166.7 ± 8.8
AGTIV®	167.7 ± 9.2
Optimyc + MooR	156.2 ± 10.9

Table 4.1.4: Mixed model analysis of variance assessing the effects of five fertilizer treatments (Control, Synthetic N, JumpStart®, AGTIV®, and Optimyc + MooR) on peak tiller height (cm) at the Guelph Switchgrass site in 2020.

Source of Variation	*df*	*p-value*
Fertilizer[1]	4	0.0575
SYN vs BIO	1	**0.0287***
AG vs JS & OM	1	0.3941
JS vs AG & OM	1	0.5166
OM vs AG & JS	1	0.1466
Block	1	**0.0210***

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; AG = AGTIV® inoculant of *Glomus intraradices*; JS = JumpStart® inoculant of *Penicillium bilaiae*; OM = Optimyc + MooR inoculants of beneficial fungal and bacterial consortia; BIO = AGTIV®, JumpStart®, and Optimyc + MooR).

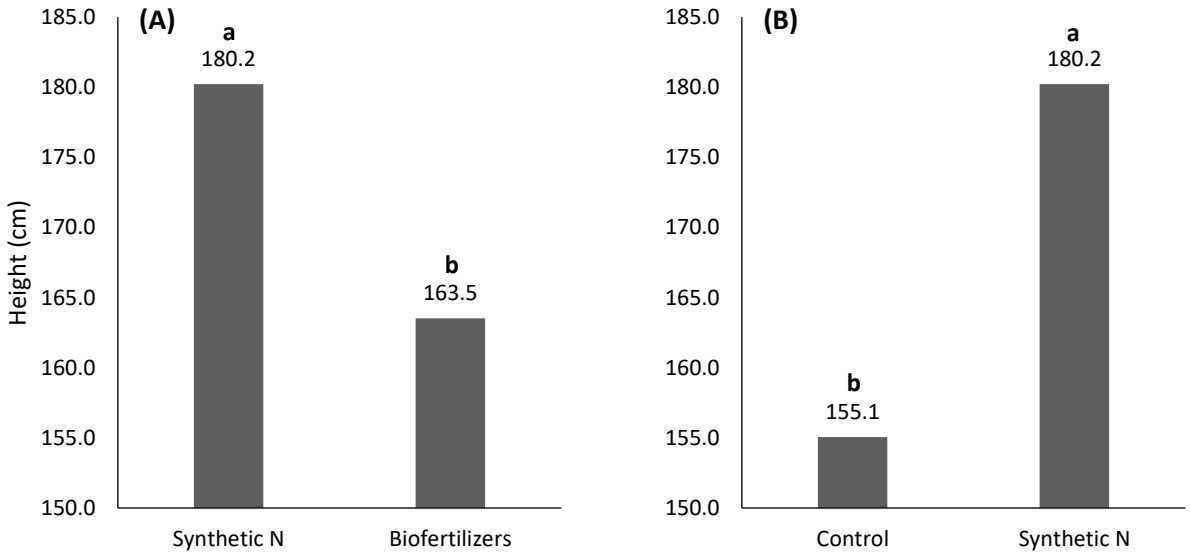

Figure 4.1.3: Peak tiller height (cm) for switchgrass as influenced by (A) synthetic nitrogen (N) fertilizer versus the combined average of three biofertilizers (JumpStart®, AGTIV®, and Optimyc + MooR) and (B) control versus synthetic N fertilizer at the Guelph Switchgrass site in 2020. Different letters indicate significantly different means according to (A) orthogonal contrast and (B) Dunnett's Correction ($p \leq 0.05$).

Mean values for autumn tiller density (tillers ha^{-1}) and autumn-harvested yield for each treatment at the GS site in both 2019 and 2020 are presented in **Table 4.1.5**. Mixed model analysis of variance indicated that autumn tiller density (tillers ha^{-1}) was not affected by treatment at GS field site in 2019 or 2020 (**Table 4.1.6**). There were also no significant differences in the least-square means comparison between each fertilizer treatment and the

control, adjusted according to Dunnett's Correction, or in any of the orthogonal contrast analyses (**Table 4.1.6**).

Table 4.1.5: Autumn tiller density (tillers ha^{-1}; ± standard error) and autumn-harvested yield (tonnes ha^{-1}; ± standard error) at the Guelph Switchgrass site in 2019 and 2020.

2019		
Treatment	*Tiller Density*	*Yield*
Control	$4.9 \times 10^6 \pm 0.5 \times 10^6$	9.4 ± 1.2
Synthetic N	$5.1 \times 10^6 \pm 0.2 \times 10^6$	10.5 ± 0.8
JumpStart®	$5.0 \times 10^6 \pm 0.4 \times 10^6$	9.5 ± 0.7
MYKE® Pro	$4.3 \times 10^6 \pm 0.3 \times 10^6$	8.8 ± 1.1
LysteGro	$3.9 \times 10^6 \pm 0.3 \times 10^6$	10.7 ± 2.2
2020		
Treatment	*Tiller Density*	*Yield*
Control	$5.0 \times 10^6 \pm 0.6 \times 10^6$	8.2 ± 2.0
Synthetic N	$4.3 \times 10^6 \pm 0.2 \times 10^6$	12.2 ± 1.3
JumpStart®	$4.3 \times 10^6 \pm 0.4 \times 10^6$	7.9 ± 0.7
AGTIV®	$4.8 \times 10^6 \pm 0.3 \times 10^6$	8.4 ± 0.7
Optimyc + MooR	$4.5 \times 10^6 \pm 0.5 \times 10^6$	8.3 ± 2.2

Table 4.1.6: Mixed model analysis of variance assessing the effects of fertilizer treatments (Control, Synthetic N, JumpStart®, MYKE® Pro/AGTIV® [2019/2020], and LysteGro [2019] or Optimyc + MooR [2020]) on autumn tiller density (tillers ha^{-1}) at the Guelph Switchgrass site in 2019 and 2020.

2019		
Source of Variation	*df*	*p-value*
Fertilizer[1]	4	0.2095
SYN vs BIO	1	0.1767
LG vs JS & MP	1	0.8369
JS vs LG & MP	1	0.0858
MP vs LG & JS	1	0.1224
Block	1	0.7457
2020		
Source of Variation	*df*	*p-value*
Fertilizer[2]	4	0.5764
SYN vs BIO	1	0.6737
AG vs JS & OM	1	0.3690
JS vs AG & OM	1	0.4450
OM vs AG & JS	1	0.8882
Block	1	**0.0361***

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; MP = MYKE® Pro inoculant of *Glomus intraradices*; LG = LysteGro biosolids fertilizer; BIO = JumpStart®, MYKE® Pro, and LysteGro)

[2] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; AG = AGTIV® inoculant of *Glomus intraradices*; OM = Optimyc + MooR inoculants of beneficial fungal and bacterial consortia; BIO = JumpStart®, AGTIV®, and Optimyc + MooR).

Biomass yield (tonnes ha^{-1}) was measured in autumn of both 2019 and 2020. No significant effect of treatment on autumn-harvested biomass yield was observed in the ANOVA for the GS field site in either year (**Table 4.1.7**). There was also no significant difference in the 2019 or 2020 autumn-harvested biomass yield between any of the fertilizer treatments and the control when analysed in a least-square means comparison adjusted according to Dunnett's Correction. However, orthogonal contrast analysis indicated that synthetic N produces significantly higher autumn-harvested biomass yield in 2020 than the combined average of the three biofertilizers (JumpStart®, AGTIV®, Optimyc + MooR) (**Figure 4.1.4**).

Table 4.1.7: Mixed model analysis of variance assessing the effects of fertilizer treatments (Control, Synthetic N, JumpStart®, MYKE® Pro/AGTIV® [2019/2020], and LysteGro [2019] or Optimyc + MooR [2020]) on yield (tonnes ha^{-1}) at the Guelph Switchgrass site in 2019 and 2020.

2019		
Source of Variation	*df*	*p-value*
Fertilizer[1]	4	0.8243
SYN vs BIO	1	0.5928
LG vs JS & MP	1	0.3568
JS vs LG & MP	1	0.9026
MP vs LG & JS	1	0.4209
Block	1	0.3339
2020		
Source of Variation	*df*	*p-value*
Fertilizer[2]	4	0.1636
SYN vs BIO	1	**0.0201***
AG vs JS & OM	1	0.9014
JS vs AG & OM	1	0.6941
OM vs AG & JS	1	0.7869
Block	1	0.0851

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; MP = MYKE® Pro inoculant of *Glomus intraradices*; LG = LysteGro biosolids fertilizer; BIO = JumpStart®, MYKE® Pro, and LysteGro).
[2] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; AG = AGTIV® inoculant of *Glomus intraradices*; OM = Optimyc + MooR inoculants of beneficial fungal and bacterial consortia; BIO = JumpStart®, AGTIV®, and Optimyc + MooR).

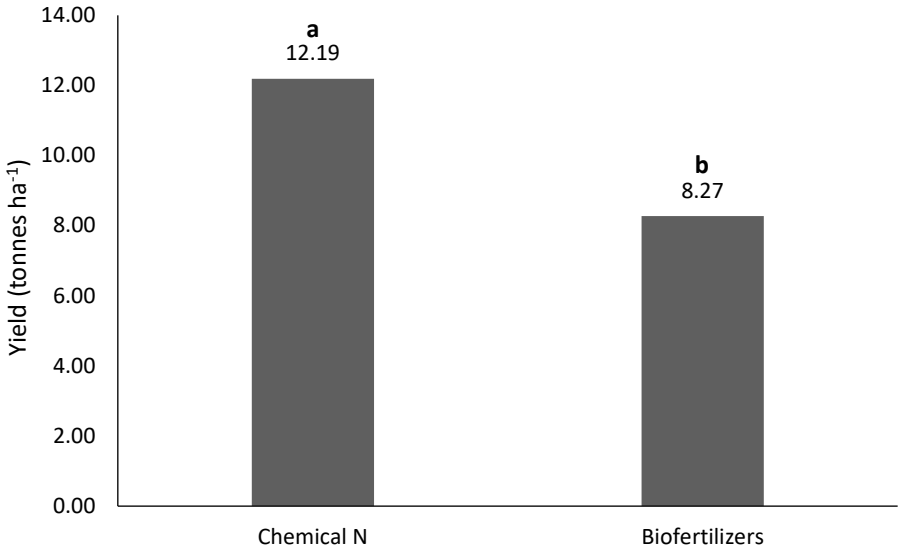

Figure 4.1.4: Influence of synthetic nitrogen (N) fertilizer versus the combined average of three biofertilizers (JumpStart®, AGTIV®, Optimyc + MooR) on switchgrass biomass yield (tonnes ha^{-1}) at the Guelph Switchgrass site in 2020. Different letters indicate significantly different means according to orthogonal contrast ($p \leq 0.05$).

4.1.2 Miscanthus

Table 4.1.8 summarizes the peak values for each of the six plant morphological metrics measured in 2019 at the BM field site – tiller height (cm), leaf number (# $tiller^{-1}$), leaf area (cm^2 $tiller^{-1}$), stem dry mass (g $tiller^{-1}$), leaf dry mass (g $tiller^{-1}$), and total dry mass (g $tiller^{-1}$). Mixed model analysis of variance (ANOVA) for the peak values of each of these metrics revealed no significant treatment effects on any of the metrics at the BM field site (**Table 4.1.9**). Least-square means comparison adjusted according to Dunnett's Correction also revealed no significant differences were observed between the control and any of the fertilizer treatments (data not presented). Finally, partitioning of variance using orthogonal contrast also showed that there were no significant differences between the following treatment groups for any of the six morphological metrics at the BM field site: (1) Synthetic N fertilizer versus combined average of the three biofertilizers, (2) LysteGro biosolids fertilizer versus JumpStart® and MYKE® Pro

inoculants of plant growth-promoting microbes, (3) JumpStart® versus LysteGro and MYKE® Pro, and (4) MYKE® Pro versus LysteGro and JumpStart® (**Table 4.1.9**). Per **Table 4.1.9**, several metrics demonstrate significant block effects, simply indicating that the RCBD approach was justified as this source of variation resulting from the blocks was separated from the treatment effects. The peak values for tiller height at the BM field site in 2020 are summarized in **Table 4.1.10**. There were no significant treatment effects on peak tiller height (cm) measured in at the BM field site 2020 (**Table 4.1.11**).

Table 4.1.8: Mean peak season measure (± standard error) for six metrics of plant morphology (tiller height [cm], leaf number tiller^{-1}, leaf area [cm^2 tiller^{-1}], stem dry mass [g tiller^{-1}], leaf dry mass [g tiller^{-1}], and total dry mass [g tiller^{-1}] at the Burlington Miscanthus site in 2019.

Treatment	*Tiller height*	*Leaf number*	*Leaf area*	*Stem dry mass*	*Leaf dry mass*	*Total dry mass*
Control	192.7 ± 12.1	9.9 ± 0.6	622.8 ± 71.3	8.3 ± 0.6	4.0 ± 0.1	12.4 ± 0.5
Synthetic N	184.8 ± 8.3	9.7 ± 0.2	765.5 ± 57.5	7.5 ± 0.6	3.8 ± 0.3	11.3 ± 0.9
JumpStart®	197.3 ± 20.1	11.1 ± 1.1	607.1 ± 113.3	9.8 ± 0.8	4.6 ± 0.2	14.4 ± 0.9
MYKE® Pro	191.0 ± 14.7	9.9 ± 0.3	654.3 ± 28.2	9.2 ± 1.7	4.2 ± 0.5	13.4 ± 2.1
LysteGro	195.8 ± 6.1	9.9 ± 0.1	694.4 ± 54.5	9.0 ± 0.7	4.4 ± 0.5	13.9 ± 1.2

Table 4.1.9: Mixed model analysis of variance assessing the effects of five fertilizer treatments (Control, Synthetic N, JumpStart®, MYKE® Pro, and LysteGro) on the peak value of six metrics of plant physiology (tiller height [cm], leaf number tiller^{-1}, leaf area [cm^2 tiller^{-1}], stem dry mass [g tiller^{-1}], leaf dry mass [g tiller^{-1}], and total dry mass [g tiller^{-1}] at the Burlington Miscanthus site in 2019.

		Height (cm)	Leaf Number	Leaf area (cm^2)	Stem dry mass (g)	Leaf dry mass (g)	Total dry mass (g)
Source of Variation	*df*	*p-value*	*p-value*	*p-value*	*p-value*	*p-value*	*p-value*
Fertilizer[1]	4	0.8771	0.3243	0.5617	0.5249	0.6766	0.5389
SYN vs BIO	1	0.3733	0.3353	0.2054	0.1354	0.2209	0.1423
LG vs JS & MP	1	0.8889	0.3709	0.4864	0.6881	0.9456	0.7511
JS vs LG & MP	1	0.7311	0.0730	0.4634	0.5493	0.5816	0.5435
MP vs LG & JS	1	0.6305	0.2971	0.9689	0.8398	0.6280	0.7674
Block	1	**0.0263***	0.1214	0.9356	0.4086	**0.0105***	0.8067

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; MP = MYKE® Pro inoculant of *Glomus intraradices*; LG = LysteGro biosolids fertilizer; BIO = JumpStart®, MYKE® Pro, and LysteGro).

Table 4.1.10: Mean peak season measure for tiller height (cm; ± standard error) at the Burlington Miscanthus site in 2020.

Treatment	*Tiller height*
Control	256.9 ± 7.2
Synthetic N	229.5 ± 9.4
JumpStart®	236.8 ± 9.1
AGTIV®	239.5 ± 5.3

Table 4.1.11: Mixed model analysis of variance assessing the effects of four fertilizer treatments (Control, Synthetic N, JumpStart®, and AGTIV®) on peak tiller height (cm) at the Burlington Miscanthus site in 2020.

Source of Variation	*df*	*p-value*
Fertilizer[1]	3	0.1609
SYN vs BIO	1	0.3771
AG vs JS	1	0.8032
Block	1	0.3439

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; AG = AGTIV® inoculant of *Glomus intraradices*; JS = JumpStart® inoculant of *Penicillium bilaiae*; BIO = AGTIV® and JumpStart®).

Autumn tiller density (tillers ha^{-1}) and autumn-harvested yield (tonnes ha^{-1}) for 2019 and 2020 at the BM field site are summarized in **Table 4.1.12**. In the mixed model ANOVA, there were no significant treatment effects on autumn tiller density (tillers ha^{-1}) at the BM field site in either 2019 or 2020 (**Table 4.1.13**). Least-square means comparison adjusted according to Dunnett's Correction also revealed that there were no significant treatment effects on tiller density compared to the control in either 2019 or 2020. In 2020, however, AGTIV® produced a significantly tiller density than JumpStart® at the BM field site in 2020 when assessed using orthogonal contrast (**Figure 4.1.5**).

Table 4.1.12: Autumn tiller density (tillers ha^{-1}; ± standard error) and autumn-harvested yield (tonnes ha^{-1}; ± standard error) at the Burlington Miscanthus site in 2019 and 2020.

2019		
Treatment	*Tiller Density*	*Yield*
Control	$1.6 \times 10^6 \pm 0.16 \times 10^6$	16.2 ± 2.1
Synthetic N	$1.4 \times 10^6 \pm 0.12 \times 10^6$	10.5 ± 1.0
JumpStart®	$1.4 \times 10^6 \pm 0.10 \times 10^6$	13.5 ± 3.1
MYKE® Pro	$1.4 \times 10^6 \pm 0.03 \times 10^6$	11.9 ± 1.8
LysteGro	$1.2 \times 10^6 \pm 0.10 \times 10^6$	11.7 ± 0.5
2020		
Treatment	*Tiller Density*	*Yield*
Control	$8.7 \times 10^5 \pm 0.01 \times 10^6$	15.7 ± 1.7
Synthetic N	$1.1 \times 10^6 \pm 0.10 \times 10^6$	15.7 ± 1.1
JumpStart®	$8.4 \times 10^5 \pm 0.07 \times 10^6$	10.9 ± 3.8
AGTIV®	$1.4 \times 10^6 \pm 0.2 \times 10^6$	20.5 ± 1.1

Table 4.1.13: Mixed model analysis of variance assessing the effects of fertilizer treatments (Control, Synthetic N, JumpStart®, MYKE® Pro/AGTIV® [2019/2020], and LysteGro [2019]) on autumn tiller density (tillers ha^{-1}) at the Burlington Miscanthus site in 2019 and 2020.

2019		
Source of Variation	*df*	*p-value*
Fertilizer[1]	4	0.1775
SYN vs BIO	1	0.5071
LG vs JS & MP	1	0.3043
JS vs LG & MP	1	0.5429
MP vs LG & JS	1	0.6563
Block	1	0.1426
2020		
Source of Variation	*df*	*p-value*
Fertilizer[2]	3	0.0821
SYN vs BIO	1	0.8575
AG vs JS	1	**0.0266***
Block	1	0.5549

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; BIO = JumpStart®, MYKE® Pro, and LysteGro; JS = JumpStart® inoculant of *Penicillium bilaiae*; MP = MYKE® Pro inoculant of *Glomus intraradices*; LG = LysteGro biosolids fertilizer)

[2] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (CT = control; SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; AG = AGTIV® inoculant of *Glomus intraradices*; BIO = JumpStart® and AGTIV®).

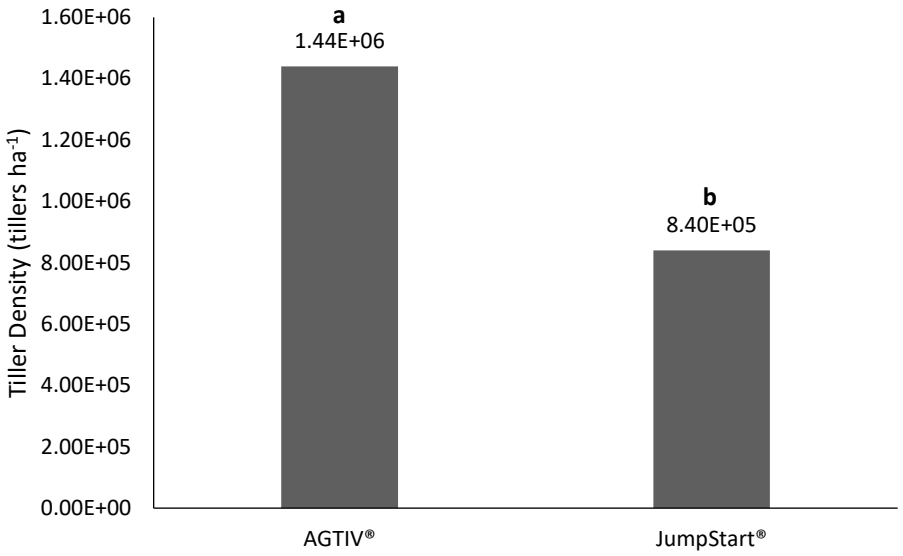

Figure 4.1.5: Autumn tiller density (tillers ha^{-1}) for miscanthus as influenced by AGTIV® versus JumpStart® at the Burlington Miscanthus site in 2020. Different letters indicate significantly different means according to orthogonal contrast ($p \leq 0.05$).

Finally, biomass yield (tonnes ha^{-1}) was measured in the fall of both study years. No significant effect of treatment on biomass yield was observed in the ANOVA models for the BM field site in either of the field seasons (**Table 4.1.14**). However, least-square means comparison adjusted according to Dunnett's Correction between each treatment and the control at each field site indicated that synthetic N fertilizer significantly reduced yield compared to the control at this site in 2019 (**Figure 4.1.6**). Additionally, at the BM field site in 2020 plots receiving AGTIV® had significantly higher biomass yield than plots receiving JumpStart® ($p < 0.05$) when analysed using orthogonal contrast (**Figure 4.1.7**).

Table 4.1.14: Mixed model analysis of variance assessing the effects of fertilizer treatments (Control, Synthetic N, JumpStart®, MYKE® Pro/AGTIV® [2019/2020], and LysteGro [2019]) on autumn-harvested biomass yield (tonnes ha^{-1}) at the Burlington Miscanthus site in 2019 and 2020.

2019		
Source of Variation	*df*	*p-value*
Fertilizer[1]	4	0.1019
SYN vs BIO	1	0.2437
LG vs JS & MP	1	0.5416
JS vs LG & MP	1	0.3278
MP vs LG & JS	1	0.6964
Block	1	**0.0241***
2020		
Source of Variation	*df*	*p-value*
Fertilizer[2]	3	0.0779
SYN vs BIO	1	0.9864
AG vs JS & OM	1	**0.0151***
Block	1	0.3204

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (CT = control; SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; MP = MYKE® Pro inoculant of *Glomus intraradices*; LG = LysteGro biosolids fertilizer; BIO = JumpStart®, MYKE® Pro, and LysteGro).

[2] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (CT = control; SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; AG = AGTIV® inoculant of *Glomus intraradices*; BIO = JumpStart® and AGTIV®).

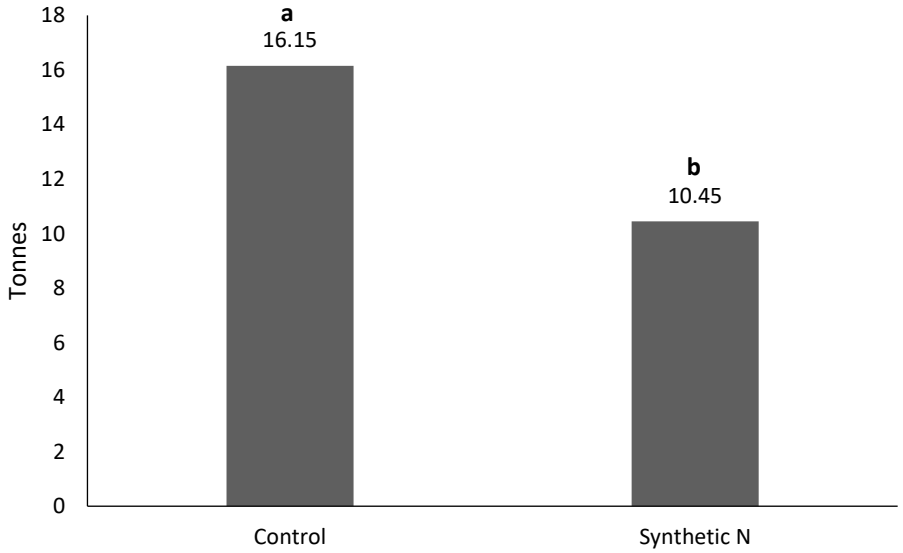

Figure 4.1.6: Biomass yield (tonnes ha^{-1}) for miscanthus as influenced by synthetic N versus the control at the Burlington Miscanthus site in 2019. Different letters indicate significantly different means according to Dunnett's Correction ($p \leq 0.05$).

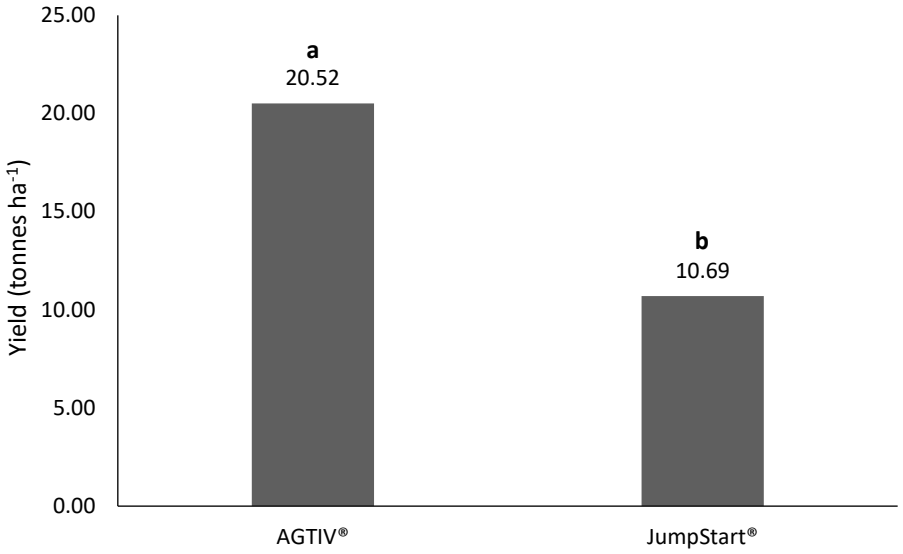

Figure 4.1.7: Biomass yield (tonnes ha^{-1}) for miscanthus as influenced by AGTIV® versus JumpStart® at the Burlington Miscanthus site in 2020. Different letters indicate significantly different means according to orthogonal analysis ($p \leq 0.05$).

4.2 Soil Fertility and Nutrient Uptake

4.2.1 Switchgrass

Soil samples were collected from 0 to 30 cm depth at the GS field site to assess treatment effects on the end-of-season soil fertility in 2019, and peak season soil fertility in 2020. Soil fertility was evaluated by quantifying the availability of the following key nutrients in these soil samples: nitrate (NO_3^-), ammonium (NH_4^+; 2020 only), phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca). End-of-season soil fertility data was not collected the BS field site due to the premature harvest of the crop at that location, and this site was not included in the 2020 study season. The mean value for each nutrient by treatment is presented in **Table 4.2.1**.

Table 4.2.1: End-of-season soil availability (ppm; ± standard error) of nitrate (NO_3^-), phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) at the Guelph Switchgrass site in autumn 2019, and the peak season soil availability (ppm; ± standard error) of NO_3^-, ammonium (NH_4^+), P, K, Mg, and Ca at the Guelph Switchgrass site in 2020. Soil samples were collected from 0 to 30 cm depth.

2019					
Treatment	*NO_3^-*	*P*	*K*	*Mg*	*Ca*
Control	3.3 ± 0.6	20.3 ± 1.5	80.7 ± 11.6	288.7 ± 30.7	2366.7 ± 400.8
Synthetic N	2.7 ± 0.6	23.0 ± 2.0	67.3 ± 6.6	268.3 ± 33.8	2030.0 ± 303.3
JumpStart®	2.3 ± 0.3	23.0 ± 3.6	61.7 ± 5.0	267.3 ± 21.9	2090.0 ± 277.2
MYKE® Pro	3.0 ± 0.5	16.3 ± 1.3	81.3 ± 1.4	272.0 ± 27.3	2283.3 ± 279.0
LysteGro	4.0 ± 0.9	21.7 ± 3.8	88.3 ± 10.8	253.0 ± 29.5	2263.3 ± 297.3

2020						
Treatment	*NO_3^-*	*NH_4^+*	*P*	*K*	*Mg*	*Ca*
Control	1.7 ± 0.8	2.4 ± 0.2	13.9 ± 2.4	75.2 ± 3.7	237.6 ± 25.6	2148.7 ± 209.0
Synthetic N	3.0 ± 1.8	2.5 ± 0.2	13.0 ± 2.8	77.5 ± 8.9	232.8 ± 25.2	2004.6 ± 211.1
JumpStart®	2.4 ± 1.2	2.3 ± 0.2	13.4 ± 1.6	70.6 ± 5.2	226.3 ± 20.5	2025.9 ± 233.8
AGTIV®	3.0 ± 1.1	2.7 ± 0.1	11.8 ± 1.7	71.7 ± 3.5	222.1 ± 23.4	2032.5 ± 273.6
Optimyc + MooR	2.2 ± 0.7	2.3 ± 0.1	17.8 ± 3.0	95.8 ± 7.8	232.1 ± 28.8	2108.5 ± 186.6

In 2019, orthogonal contrast analyses indicate that LysteGro significantly increased end-of-season soil NO_3^- availability compared to the combined average of JumpStart® and MYKE® Pro (**Figure 4.2.1A**), and that JumpStart® significantly reduced NO_3^- availability compared to the combined average of LysteGro and MYKE® Pro (**Figure 4.2.1B**). Furthermore, MYKE® Pro significantly reduced end-of-season soil P availability compared to the combined average of LysteGro and JumpStart® (**Figure 4.2.2**). No other treatment had any significant effects on the availability of these nutrients, nor were there any significant treatment effects on the availability of K, Mg, or Ca in the soil at the end of the 2019 season (**Table 4.2.2**). None of the treatments significantly affected the availability of any of the five nutrients compared to the control in 2019 according to the results of least-square means analysis adjusted according to Dunnett's Correction (data not presented).

Unlike end-of-season 2019, peak season soil NO_3^- was not significantly affected by treatment in 2020 (**Table 4.2.2**). However, peak season soil NH_4^+ was significantly higher in plots receiving AGTIV® compared to the combined average of JumpStart® and Optimyc + MooR

when variance was partitioned using orthogonal contrast analysis (**Figure 4.2.3**). Orthogonal contrast also indicated that P availability was significantly lower in AGTIV® plots compared to the combined average of JumpStart® and Optimyc + Moor (**Figure 4.2.4A**) and was significantly higher in Optimyc + MooR plots compared to the combined average of AGTIV® and JumpStart® (**Figure 4.2.4B**). Finally, least-square means analysis adjusted according to the Tukey test indicated that peak season soil K availability was significantly increased by Optimyc + MooR compared to JumpStart® in 2020 (**Figure 4.2.5**). No significant treatment effects were observed for Mg or Ca in 2020 (**Table 4.2.2**), nor did any of the treatments significantly alter the availability of any of the six nutrients compared to the control when assessed in a least-square means analysis adjusted according to Dunnett's correction (data not presented).

Table 4.2.2: Mixed model analysis of variance assessing the effects of fertilizer treatment (Control, Synthetic N, JumpStart®, MYKE® Pro/AGTIV® [2019/2020], and LysteGro [2019] or Optimyc + MooR [2020]) on the soil availability (ppm) of nitrate (NO_3^-), phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) at the Guelph Switchgrass site in autumn 2019, and the peak season soil availability (ppm) of NO_3^-, ammonium (NH_4^+), P, K, Mg, and Ca at the Guelph Switchgrass site in 2020. Soil samples were collected from 0 to 30 cm depth.

2019

		NO_3^-	P	K	Mg	Ca
Source of Variation	*df*	*p-value*	*p-value*	*p-value*	*p-value*	*p-value*
Fertilizer[1]	4	0.0659	0.0686	0.2660	0.5289	0.0681
SYN vs BIO	1	0.2615	0.1080	0.3647	0.8619	0.0501
LG vs JS & MP	1	**0.0180***	0.3421	0.1817	0.2964	0.4523
JS vs LG & MP	1	**0.0227***	0.0684	0.0551	0.6882	0.0781
MP vs LG & JS	1	0.8838	**0.0143***	0.4570	0.5033	0.2538
Block	1	**0.0045****	**0.0017****	0.6207	**0.0007*****	**<0.0001******

2020

		NO_3^-	NH_4^+	P	K	Mg	Ca
Source of Variation	*df*	*p-value*	*p-value*	*p-value*	*p-value*	*p-value*	*p-value*
Fertilizer[2]	4	0.7435	0.1787	0.0907	**0.0354***	0.9142	0.2010
SYN vs BIO	1	0.3850	0.8607	0.7201	0.5842	0.4461	0.1119
AG vs JS & OM	1	0.4220	**0.0184***	**0.0359***	0.1138	0.6522	0.3976
JS vs AG & OM	1	0.3603	0.1763	0.6203	0.0600	0.9162	0.4788
OM vs AG & JS	1	0.9065	0.2214	**0.0141***	**0.0026****	0.7289	0.1337
Block	1	**0.0389***	**0.0116***	**0.0006*****	0.0899	**<0.0001*****	**<0.0001*****

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; MP = MYKE® Pro inoculant of *Glomus intraradices*; LG = LysteGro biosolids fertilizer; BIO = JumpStart®, MYKE® Pro, and LysteGro).

[2] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; AG = AGTIV® inoculant of *Glomus intraradices*; OM = Optimyc + MooR inoculants of beneficial fungal and bacterial consortia; BIO = JumpStart®, AGTIV®, and Optimyc + MooR).

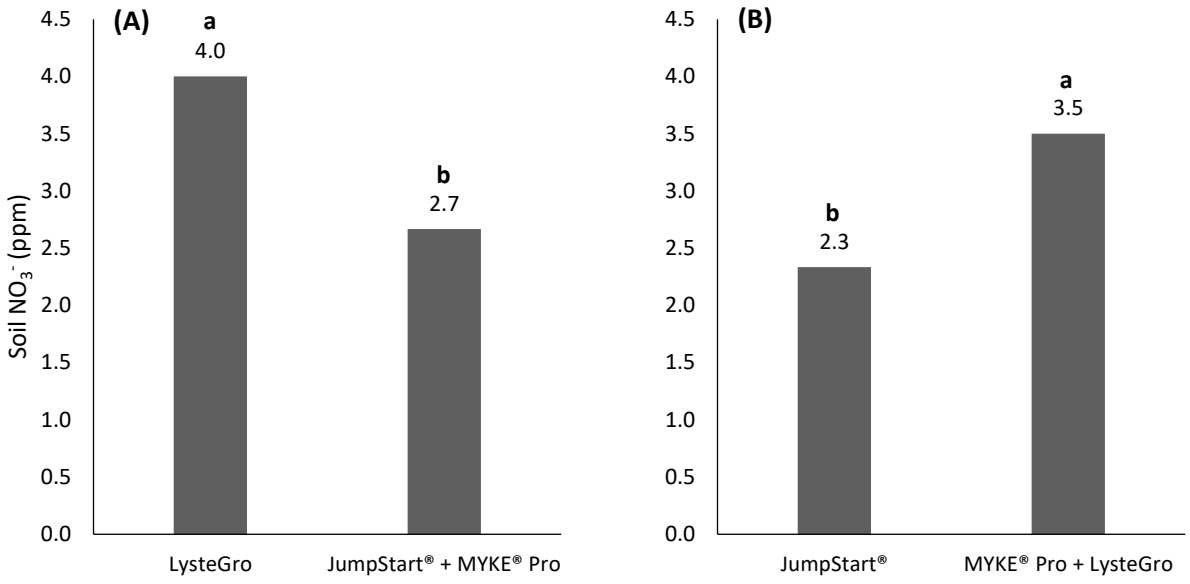

Figure 4.2.1: End-of-season soil NO_3^- availability (ppm) in the top 30 cm of soil as influenced by (A) LysteGro versus the combined average of JumpStart® and MYKE® Pro and (B) JumpStart® versus the combined average of MYKE® Pro and LysteGro at the Guelph Switchgrass site in 2019. Different letters indicate significantly different means according to orthogonal contrast ($p \leq 0.05$).

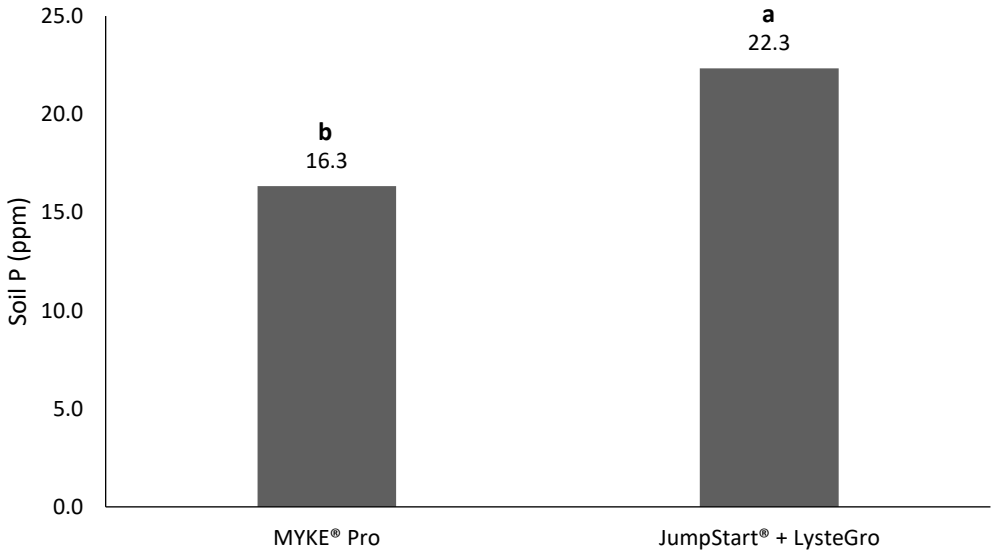

Figure 4.2.2: End-of-season soil P availability (ppm) in the top 30 cm of soil as influenced by MYKE® Pro versus the combined average of JumpStart® and LysteGro at the Guelph Switchgrass site in 2019. Different letters indicate significantly different means according to orthogonal contrast ($p \leq 0.05$).

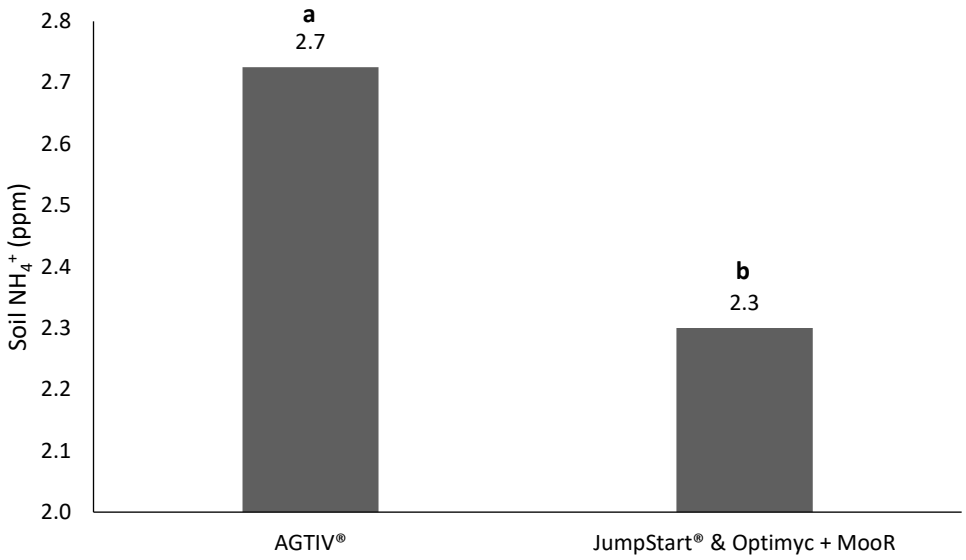

Figure 4.2.3: Peak season soil NH_4^+ availability (ppm) in the top 30 cm of soil as influenced by AGTIV® versus the combined average of JumpStart® and Optimyc + MooR at the Guelph Switchgrass site in 2020. Different letters indicate significantly different means according to orthogonal contrast ($p \leq 0.05$).

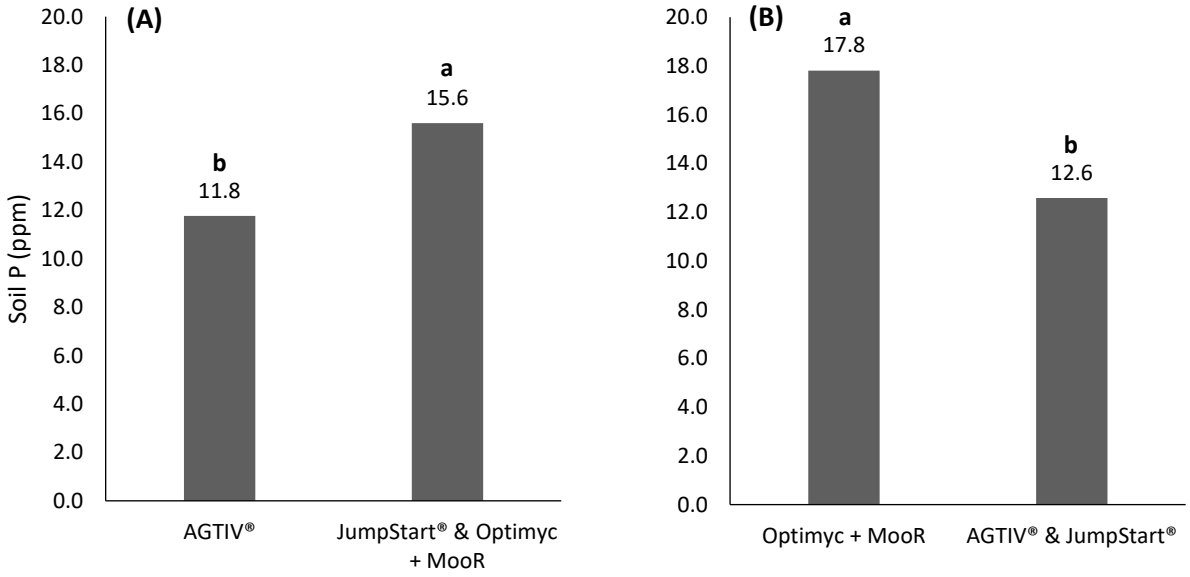

Figure 4.2.4: Peak season soil P availability (ppm) in the top 30 cm of soil as influenced by (A) AGTIV® versus the combined average of JumpStart® and Optimyc + MooR and (B) Optimyc + MooR versus the combined average of AGTIV® and JumpStart® at the Guelph Switchgrass site in 2020. Different letters indicate significantly different means according to orthogonal contrast ($p \leq 0.05$).

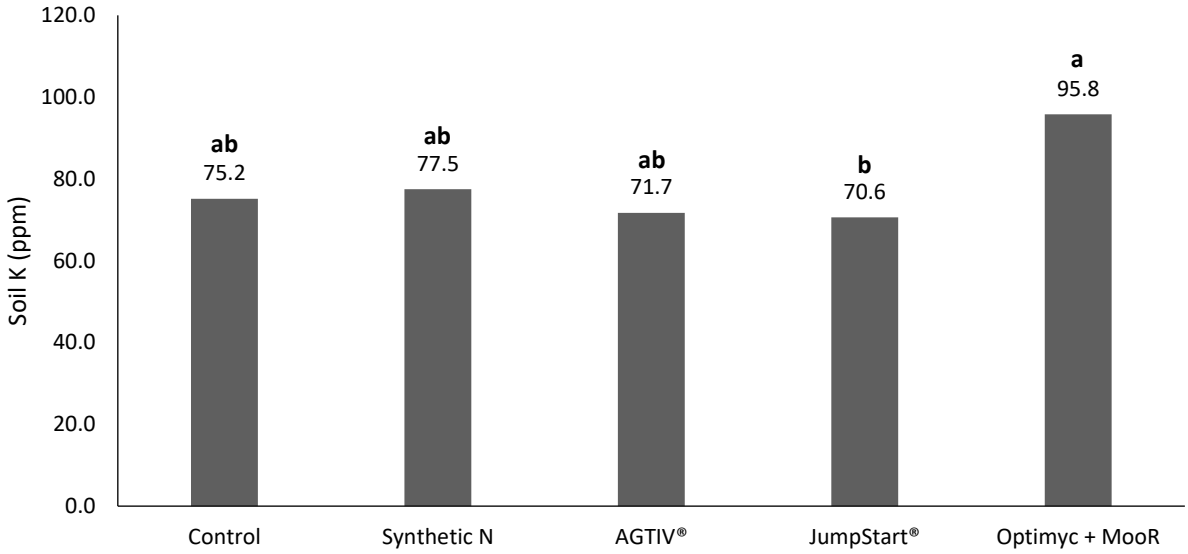

Figure 4.2.5: Peak season soil K availability (ppm) in the top 30 cm of soil as influenced by fertilizer treatment at the Guelph Switchgrass site in 2020. Different letters indicate significantly different means according to the Tukey test ($p \leq 0.05$).

Plant tissue samples were collected at the peak of the growing season in both 2019 and 2020 to evaluate the concentration of five nutrients (N, P, K, Mg, and Ca) in switchgrass plant tissues under the various fertilizer treatments. These data begin to elucidate how treatment effects on soil fertility translate into plant uptake of these key nutrients. The mean value for each nutrient by treatment is presented in **Table 4.2.3**. for the GS site, and **Table 4.2.4** for the BS site.

Table 4.2.3: Peak season switchgrass tissue concentration (%; ± standard error) of nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) at the Guelph Switchgrass site in 2019 and 2020.

2019					
Treatment	*N*	*P*	*K*	*Mg*	*Ca*
Control	0.43 ± 0.01	0.14 ± 0.01	0.99 ± 0.07	0.10 ± 0.01	0.17 ± 0.03
Synthetic N	0.67 ± 0.05	0.15 ± 0.01	1.18 ± 0.04	0.12 ± 0.01	0.12 ± 0.02
JumpStart®	0.46 ± 0.04	0.15 ± 0.01	1.00 ± 0.06	0.10 ± 0.01	0.10 ± 0.02
MYKE® Pro	0.48 ± 0.03	0.17 ± 0.01	1.19 ± 0.05	0.13 ± 0.01	0.13 ± 0.01
LysteGro	0.46 ± 0.05	0.16 ± 0.01	1.07 ± 0.04	0.10 ± 0.01	0.10 ± 0.02
2020					
Treatment	*N*	*P*	*K*	*Mg*	*Ca*
Control	0.58 ± 0.05	0.15 ± 0.01	0.95 ± 0.10	0.10 ± 0.01	0.18 ± 0.01
Synthetic N	0.76 ± 0.08	0.17 ± 0.02	0.95 ± 0.05	0.13 ± 0.02	0.20 ± 0.03
JumpStart®	0.62 ± 0.04	0.15 ± 0.01	0.93 ± 0.03	0.09 ± 0.01	0.18 ± 0.02
AGTIV®	0.63 ± 0.01	0.15 ± 0.01	0.92 ± 0.01	0.10 ± 0.01	0.18 ± 0.03
Optimyc + MooR	0.68 ± 0.04	0.15 ± 0.01	1.07 ± 0.04	0.10 ± 0.01	0.17 ± 0.01

Table 4.2.4: Peak season switchgrass tissue concentration (%; ± standard error) of nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) at the Burlington Switchgrass site in 2019.

Treatment	*N*	*P*	*K*	*Mg*	*Ca*
Control	0.77 ± 0.16	0.15 ± 0.01	1.03 ± 0.06	0.10 ± 0.01	0.19 ± 0.02
Synthetic N	0.95 ± 0.05	0.15 ± 0.01	1.04 ± 0.05	0.11 ± 0.01	0.22 ± 0.02
JumpStart®	0.98 ± 0.09	0.16 ± 0.02	1.02 ± 0.03	0.11 ± 0.01	0.19 ± 0.02
MYKE® Pro	1.04 ± 0.08	0.15 ± 0.01	1.16 ± 0.07	0.11 ± 0.01	0.21 ± 0.02
LysteGro	1.04 ± 0.05	0.18 ± 0.01	1.14 ± 0.05	0.11 ± 0.01	0.21 ± 0.02

At the GS field site in 2019, there were significant treatment effects on plant tissue N, K, and Mg, but no significant effects on P or Ca (**Table 4.2.5**). The synthetic N treatment significantly increased peak season 2019 switchgrass tissue N concentration (%) compared to all other treatments in a least-square means comparison adjusted according to the Tukey test (**Figure 4.2.1**). Switchgrass tissue K concentration (%) at the GS site in 2019 was significantly increased by MYKE® Pro compared to the control when analysed in least-square means comparisons adjusted according to the Tukey test (**Figure 4.2.7**). Finally, orthogonal contrast analysis indicated that MYKE® Pro application resulted in significantly lower switchgrass tissue Mg concentration (%) compared to the combined average of JumpStart® and LysteGro at the GS site in 2019 (**Figure 4.2.8**). Peak season plant samples for tissue nutrient analysis were also collected from the BS field site in 2019, as this sampling occurred before the site was prematurely harvested, however there were no significant treatment effects on switchgrass tissue concentrations of any of the five measured nutrients (**Table 4.2.6**).

In 2020, significant treatment effects on switchgrass tissue N and K concentrations at the GS field site, but not on tissue P, Mg, or Ca (**Table 4.2.5**). As observed at the GS field site in 2019, synthetic N significantly increased switchgrass tissue N concentration (%) compared to the control when analysed in least-square means comparisons adjusted according to the Tukey test (**Figure 4.2.9**) and Dunnett's Correction (data not presented). Unlike 2019, however, synthetic N

did not significantly increase switchgrass tissue N compared to any of the biofertilizer treatments. Switchgrass tissue K concentration (%) was significantly higher for plants receiving Optimyc + MooR compared to the combined average of AGTIV® and JumpStart® when assessed via orthogonal contrast (**Figure 4.2.10**). The BS field site was not included in the trial in 2020.

Table 4.2.5: Mixed model analysis of variance assessing the effects of fertilizer treatment (Control, Synthetic N, JumpStart®, MYKE® Pro/AGTIV® [2019/2020], and LysteGro [2019] or Optimyc + MooR [2020]) on the peak season switchgrass (Panicum virgatum) tissue concentration (%) of nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) at the Guelph Switchgrass site in 2019 and 2020.

2019						
		N	P	K	Mg	Ca
Source of Variation	*df*	*p-value*	*p-value*	*p-value*	*p-value*	*p-value*
Fertilizer[1]	4	**0.0010****	0.5523	**0.0155***	0.0762	0.3557
SYN vs BIO	1	**0.0001*****	0.6261	0.0841	0.2982	0.6541
LG vs JS & MP	1	0.8153	0.2290	**0.0366***	0.1890	0.3128
JS vs LG & MP	1	0.7372	0.7390	0.7103	0.3313	0.8521
MP vs LG & JS	1	0.5669	0.1672	**0.0149***	**0.0258***	0.2192
Block	1	0.4819	0.0692	0.0553	**0.0123***	0.1505
2020						
		N	P	K	Mg	Ca
Source of Variation	*df*	*p-value*	*p-value*	*p-value*	*p-value*	*p-value*
Fertilizer[2]	4	**0.0445***	0.5463	0.2198	0.3192	0.4836
SYN vs BIO	1	0.1615	0.7576	0.7062	0.5677	0.6993
AG vs JS & OM	1	0.7216	0.6614	0.1851	0.4248	0.5874
JS vs AG & OM	1	0.4047	0.6623	0.3334	0.2462	0.7809
OM vs AG & JS	1	0.2326	0.9980	**0.0278***	0.6762	0.4239
Block	1	0.1220	0.0101*	0.2049	**0.0160***	**0.0004*****

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; MP = MYKE® Pro inoculant of *Glomus intraradices*; LG = LysteGro biosolids fertilizer; BIO = JumpStart®, MYKE® Pro, and LysteGro).
[2] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; AG = AGTIV® inoculant of *Glomus intraradices*; OM = Optimyc + MooR inoculants of beneficial fungal and bacterial consortia; BIO = JumpStart®, AGTIV®, and Optimyc + MooR).

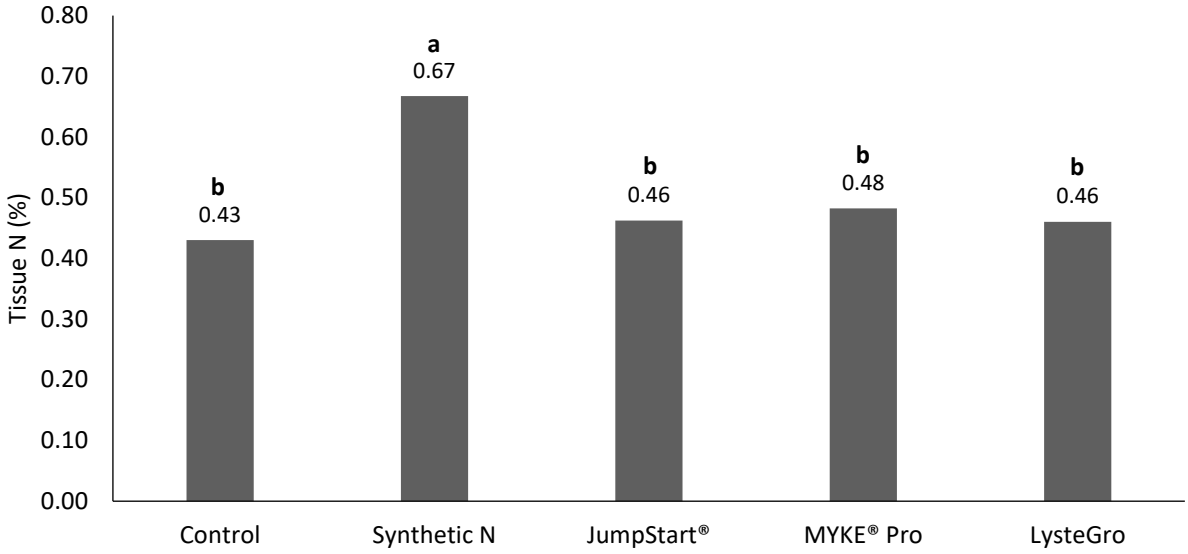

Figure 4.2.6: Peak season switchgrass tissue N concentration (%) as influenced by fertilizer treatment at the Guelph Switchgrass site in 2019. Different letters indicate significantly different means according to the Tukey test ($p \leq 0.05$).

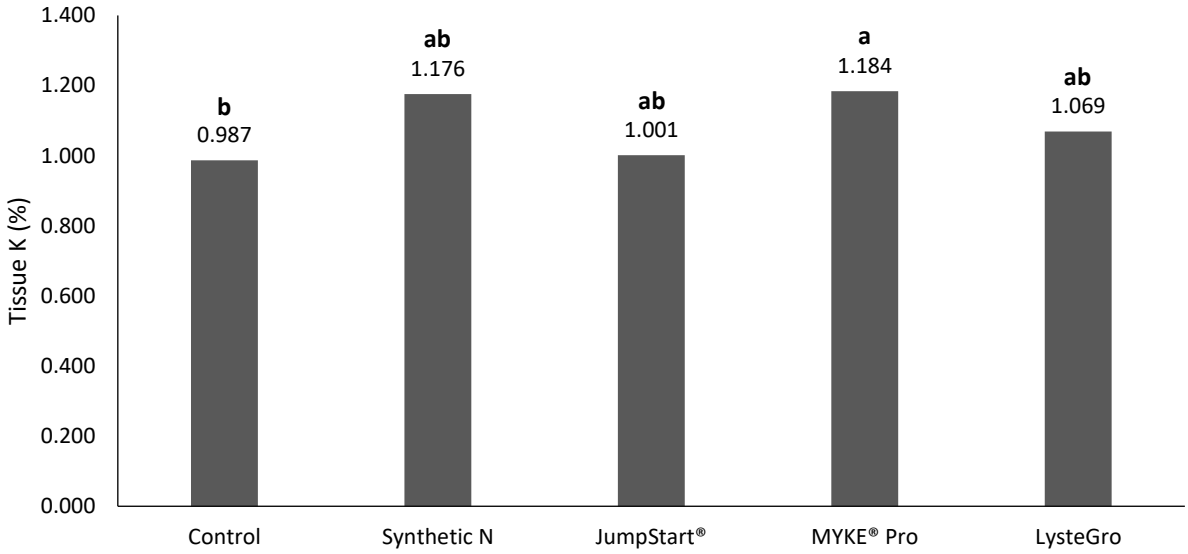

Figure 4.2.7: Peak season switchgrass tissue K concentration (%) as influenced by fertilizer treatment at the Guelph Switchgrass site in 2019. Different letters indicate significantly different means according to the Tukey test ($p \leq 0.05$).

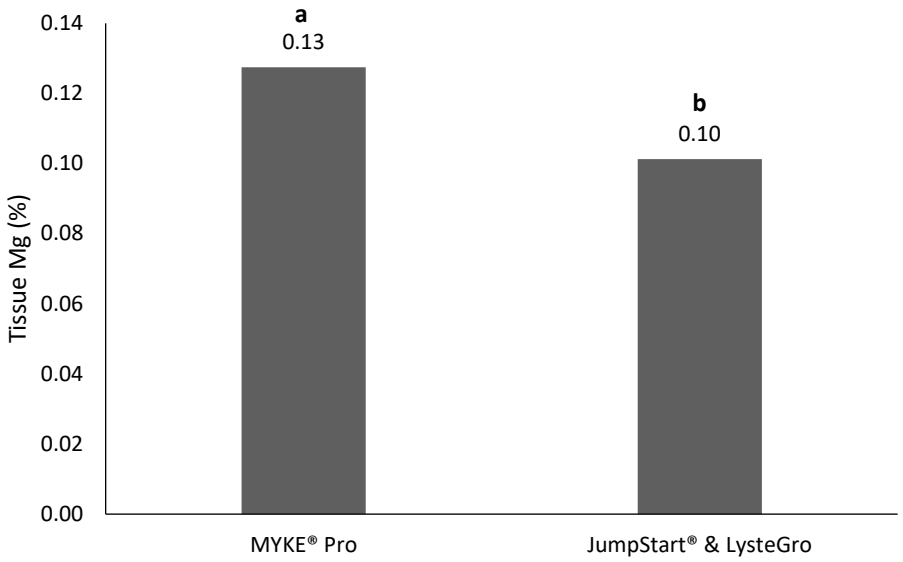

Figure 4.2.8: Peak season switchgrass tissue Mg concentration (%) as influenced by MYKE® Pro versus the combined average of JumpStart® and LysteGro at the Guelph Switchgrass site in 2019. Different letters indicate significantly different means according to orthogonal contrast ($p \leq 0.05$).

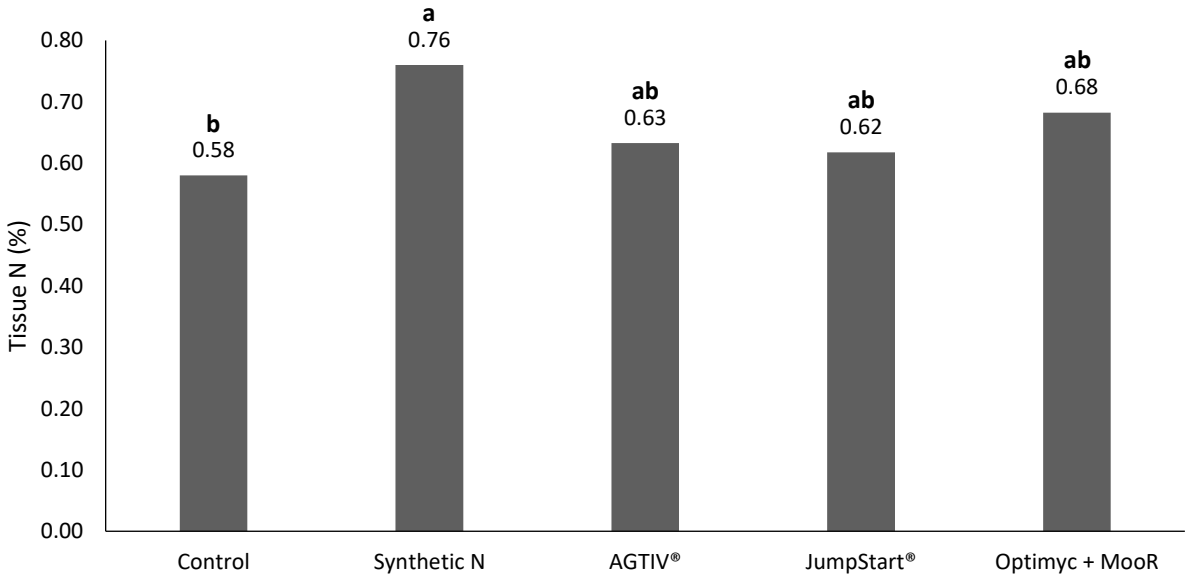

Figure 4.2.9: Peak season switchgrass tissue N concentration (%) as influenced by fertilizer treatment at the Guelph Switchgrass site in 2020. Different letters indicate significantly different means according to the Tukey test ($p \leq 0.05$).

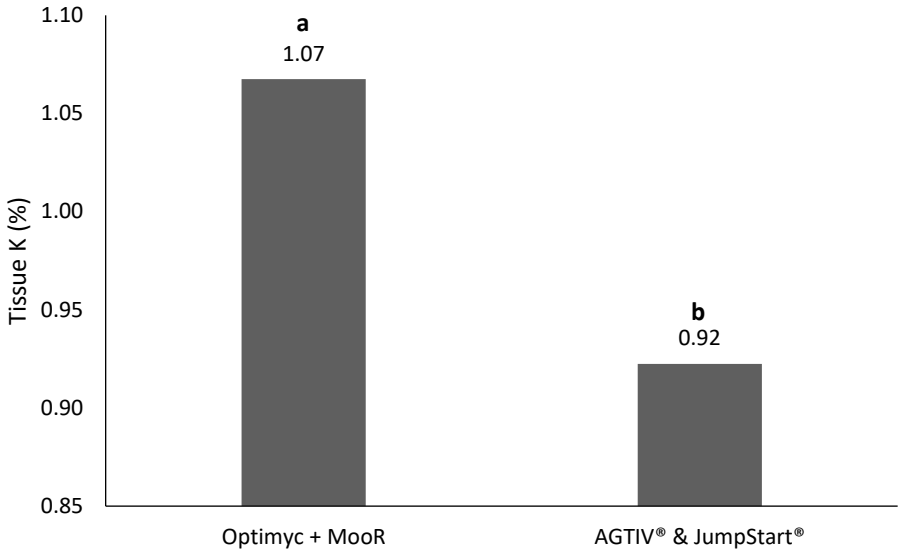

Figure 4.2.10: Peak season switchgrass tissue K concentration (%) as influenced by Optimyc + MooR versus the combined average of AGTIV® and JumpStart® at the Guelph Switchgrass site in 2020. Different letters indicate significantly different means according to orthogonal contrast ($p \leq 0.05$).

Table 4.2.6: Mixed model analysis of variance assessing the effects of fertilizer treatment (Control, Synthetic N, JumpStart®, MYKE® Pro, and LysteGro) on the peak season plant tissue concentration (%) of nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) at the Burlington Switchgrass site in 2019.

		N	P	K	Mg	Ca
Source of Variation	*df*	*p-value*	*p-value*	*p-value*	*p-value*	*p-value*
Fertilizer[1]	4	0.3044	0.4676	0.2269	0.9744	0.5870
SYN vs BIO	1	0.5351	0.4624	0.3078	0.8897	0.3564
LG vs JS & MP	1	0.6287	0.9108	0.0618	0.8449	0.2444
JS vs LG & MP	1	0.8056	0.1570	0.4216	0.6995	0.4339
MP vs LG & JS	1	0.8056	0.2084	0.2277	0.8449	0.6607
Block	1	1.0000	0.4208	1.0000	1.0000	1.0000

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; MP = MYKE® Pro inoculant of *Glomus intraradices*; LG = LysteGro biosolids fertilizer; BIO = JumpStart®, MYKE® Pro, and LysteGro).

4.2.2 Miscanthus

Soil samples were collected from 0 to 30 cm depth at the BM field site to assess treatment effects on the end-of-season soil fertility in 2019, and peak season soil fertility in 2020. Soil fertility was evaluated by quantifying the availability of the following key nutrients in these soil

samples: NO_3^-, NH_4^+ (2020 only), P, K, Mg, and Ca. The mean value for each nutrient by treatment is presented in **Table 4.2.7**.

Table 4.2.7: End-of-season soil availability (ppm; ± standard error) of nitrate (NO_3^-), phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) at the Burlington Miscanthus site in autumn 2019, and the peak season soil availability (ppm; ± standard error) of NO_3^-, ammonium (NH_4^+), P, K, Mg, and Ca at the Burlington Miscanthus site in 2020. Soil samples were collected from 0 to 30 cm depth.

2019					
Treatment	*NO_3^-*	*P*	*K*	*Mg*	*Ca*
Control	8.3 ± 1.7	10.0 ± 1.5	79.0 ± 13.6	232.3 ± 13.8	1820.0 ± 215.2
Synthetic N	8.7 ± 0.7	9.3 ± 0.7	66.3 ± 3.7	221.0 ± 12.9	2040.0 ± 180.4
JumpStart®	8.3 ± 0.9	7.0 ± 0.6	65.0 ± 5.8	230.0 ± 17.9	2053.3 ± 265.6
MYKE® Pro	8.3 ± 0.7	12.3 ± 2.4	78.7 ± 9.2	233.7 ± 15.9	2300.0 ± 392.7
LysteGro	6.7 ± 0.9	10.3 ± 0.9	88.3 ± 7.9	233.0 ± 14.0	2006.7 ± 83.5

2020						
Treatment	*NO_3^-*	*NH_4^+*	*P*	*K*	*Mg*	*Ca*
Control	8.8 ± 4.3	2.9 ± 0.2	6.7 ± 0.4	90.9 ± 7.2	234.0 ± 32.4	2622.9 ± 363.2
Synthetic N	7.3 ± 1.0	2.7 ± 0.2	6.7 ± 0.9	97.8 ± 7.9	234.9 ± 14.2	2511.2 ± 391.7
JumpStart®	4.6 ± 0.6	2.9 ± 0.0	8.2 ± 1.0	100.2 ± 0.5	231.5 ± 18.3	2557.5 ± 361.7
AGTIV®	4.5 ± 1.3	3.0 ± 0.3	6.6 ± 0.6	93.7 ± 4.4	248.1 ± 15.2	2994.3 ± 539.4

There were no significant treatment effects on the soil availability of any of these nutrients in either year, except for end-of-season soil P availability in 2019 (**Table 4.2.8**). Orthogonal contrast analyses indicated that JumpStart® significantly reduced soil P availability compared to the combined average of MYKE® Pro and LysteGro (**Figure 4.2.11A**), and that MYKE® Pro significantly increased soil P availability compared to the combined average of JumpStart® and LysteGro (**Figure 4.2.11B**).

Table 4.2.8: Mixed model analysis of variance assessing the effects of fertilizer treatment (Control, Synthetic N, JumpStart®, MYKE® Pro/AGTIV® [2019/2020], and LysteGro [2019]) on the soil availability (ppm) of nitrate (NO_3^-), phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) at the Burlington Miscanthus site in autumn 2019, and the peak season soil availability (ppm) of NO_3^-, ammonium (NH_4^+), P, K, Mg, and Ca at the Burlington Miscanthus site in 2020.

2019						
		NO_3^-	P	K	Mg	Ca
Source of Variation	*df*	*p-value*	*p-value*	*p-value*	*p-value*	*p-value*
Fertilizer[1]	4	0.6116	0.1122	0.3816	0.9723	0.2781
SYN vs BIO	1	0.4447	0.8920	0.3264	0.5475	0.7036
LG vs JS & MP	1	0.1797	0.4392	0.1687	0.9334	0.4625
JS vs LG & MP	1	0.4946	**0.0141***	0.1191	0.8428	0.5693
MP vs LG & JS	1	0.4720	**0.0497***	0.8222	0.9084	0.2094
Block	1	0.5987	0.4356	0.8350	0.9184	**0.0032****

2020							
		NO_3^-	NH_4^+	P	K	Mg	Ca
Source of Variation	*df*	*p-value*	*p-value*	*p-value*	*p-value*	*p-value*	*p-value*
Fertilizer[2]	3	0.4503	0.8001	0.4921	0.4002	0.7908	0.6793
SYN vs BIO	1	0.1838	0.3785	0.4685	0.9443	0.8104	0.4814
AG vs JS & OM	1	0.8599	0.7615	0.2116	0.2872	0.4233	0.3542
Block	1	0.2489	0.1514	0.4948	**0.0441***	**0.0271***	**0.0402***

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; MP = MYKE® Pro inoculant of *Glomus intraradices*; LG = LysteGro biosolids fertilizer; BIO = JumpStart®, MYKE® Pro, and LysteGro).
[2] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; AG = AGTIV® inoculant of *Glomus intraradices*; BIO = JumpStart® and AGTIV®).

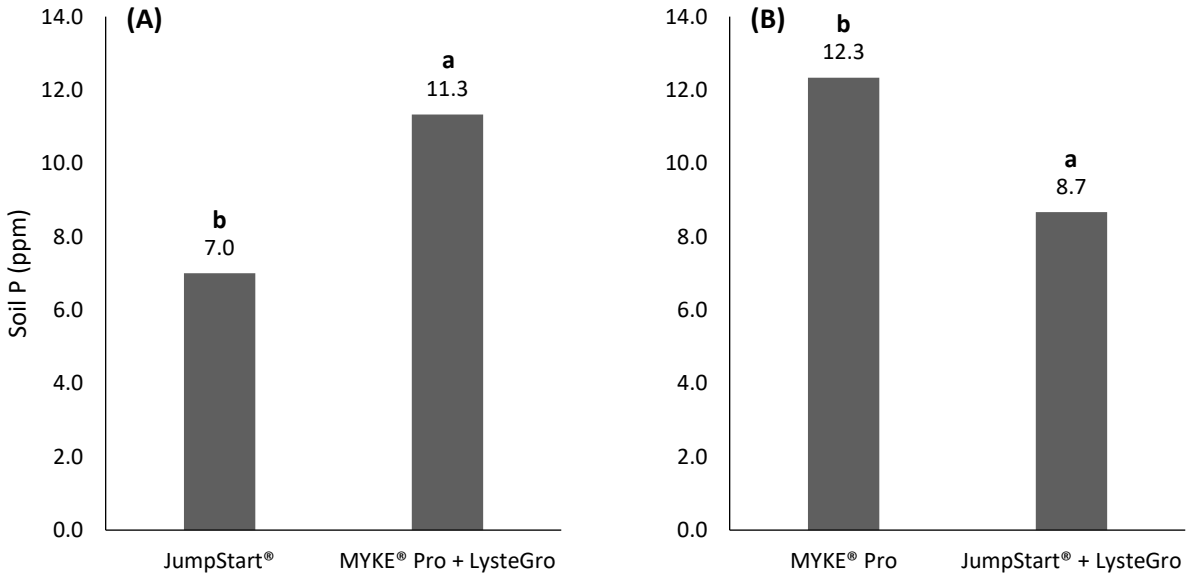

Figure 4.2.11: Autumn soil P availability (ppm) as influenced by (A) JumpStart® versus the combined average of MYKE® Pro and LysteGro and (B) MYKE® Pro versus the combined average of JumpStart® and LysteGro at the Burlington Miscanthus site in 2019. Different letters indicate significantly different means according to orthogonal contrast ($p \leq 0.05$).

In addition to the soil nutrient availability data, plant samples were collected from the BM field site at the peak of the growing season in both 2019 and 2020 to determine how the treatments affected plant tissue concentrations (%) of N, P, K, Mg, and Ca (**Table 4.2.9**). In 2019, there were significant differences in miscanthus tissue concentrations of N, K, Mg, and Ca, but not P (**Table 4.2.10**). According to orthogonal contrast analyses, miscanthus tissue N concentration (%) was significantly higher in plants receiving LysteGro compared to the combined average of JumpStart® and MYKE® Pro (**Figure 4.2.12**), and miscanthus tissue K concentration (%) was significantly higher in plants receiving synthetic N fertilizer versus the combined average of JumpStart®, MYKE® Pro, and LysteGro (**Figure 4.2.13**). Miscanthus tissue Mg concentration (%) was significantly lower in plants receiving JumpStart® compared to the combined average of MYKE® Pro and LysteGro (**Figure 4.2.14A**), and significantly lower in plants receiving MYKE® Pro compared to the combined average of JumpStart® and LysteGro (**Figure 4.2.14B**) according to orthogonal contrast analyses. An orthogonal contrast analysis indicated that miscanthus tissue Ca concentration (%) was significantly lower in plants receiving JumpStart® compared to the combined average of MYKE® Pro and LysteGro (**Figure 4.2.15**).

Table 4.2.9: Peak season miscanthus tissue concentration (%; ± standard error) of nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) at the Burlington Miscanthus site in 2019 and 2020.

2019					
Treatment	*N*	*P*	*K*	*Mg*	*Ca*
Control	1.19 ± 0.01	0.19 ± 0.01	1.38 ± 0.01	0.13 ± 0.003	0.21 ± 0.02
Synthetic N	1.27 ± 0.02	0.18 ± 0.01	1.30 ± 0.08	0.13 ± 0.003	0.24 ± 0.04
JumpStart®	1.19 ± 0.06	0.20 ± 0.01	1.43 ± 0.14	0.15 ± 0.007	0.22 ± 0.03
MYKE® Pro	1.34 ± 0.05	0.20 ± 0.01	1.52 ± 0.11	0.13 ± 0.009	0.23 ± 0.03
LysteGro	1.34 ± 0.09	0.22 ± 0.02	1.62 ± 0.08	0.17 ± 0.012	0.28 ± 0.02
2020					
Treatment	*N*	*P*	*K*	*Mg*	*Ca*
Control	0.70 ± 0.01	0.13 ± 0.009	0.87 ± 0.06	0.10 ± 0.015	0.16 ± 0.02
Synthetic N	0.77 ± 0.05	0.15 ± 0.006	0.83 ± 0.09	0.12 ± 0.022	0.23 ± 0.01
JumpStart®	0.86 ± 0.02	0.14 ± 0.007	0.79 ± 0.06	0.11 ± 0.000	0.21 ± 0.02
AGTIV®	0.83 ± 0.11	0.15 ± 0.000	0.89 ± 0.03	0.12 ± 0.012	0.20 ± 0.01

In 2020, Ca was the only plant tissue nutrient concentration (%) significantly affected by treatment (**Table 4.2.5**). In a least-square means comparison adjusted according to Dunnett's Correction, miscanthus plants receiving synthetic N had significantly higher tissue Ca concentration (%) than plants in control plots (data not presented). Furthermore, miscanthus tissue Ca concentration (%) was significantly higher in plants receiving synthetic N compared to the combined average of AGTIV® and JumpStart® biofertilizers (**Figure 4.2.16A**) and was significantly lower in plants receiving AGTIV® compared to plants receiving JumpStart® (**Figure 4.2.16B**) according to orthogonal contrast analyses. No significant differences were observed among treatments for miscanthus tissue N, P, K, or Mg concentrations (%) at the BM field site in 2020 (**Table 4.2.10**).

Table 4.2.10: Mixed model analysis of variance assessing the effects of fertilizer treatment (Control, Synthetic N, JumpStart®, MYKE® Pro/AGTIV® [2019/2020], and LysteGro [2019]) on the peak season plant tissue concentration (%) of nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) at the Burlington Miscanthus site in 2019 and 2020.

2019						
		N	P	K	Mg	Ca
Source of Variation	*df*	*p-value*	*p-value*	*p-value*	*p-value*	*p-value*
Fertilizer[1]	4	0.1285	0.4377	0.0936	0.3267	0.3186
SYN vs BIO	1	0.7306	0.2669	**0.0291***	0.0812	0.8708
LG vs JS & MP	1	**0.0353***	0.7457	0.1777	0.7772	0.0917
JS vs LG & MP	1	0.2534	0.3217	0.1693	**0.0089****	**0.0053****
MP vs LG & JS	1	0.2253	0.4083	0.9912	**0.0196***	0.1622
Block	1	1.0000	1.0000	0.4915	0.2301	**0.0004*****
2020						
		N	P	K	Mg	Ca
Source of Variation	*df*	*p-value*	*p-value*	*p-value*	*p-value*	*p-value*
Fertilizer[2]	3	0.1846	0.4486	0.4608	0.7711	0.0569
SYN vs BIO	1	0.8764	0.1038	0.9390	0.4368	**0.0314***
AG vs JS	1	0.0523	0.2077	0.2453	0.5573	**0.0461***
Block	1	0.4480	0.1621	0.1716	0.1554	1.000

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; MP = MYKE® Pro inoculant of *Glomus intraradices*; LG = LysteGro biosolids fertilizer; BIO = JumpStart®, MYKE® Pro, and LysteGro).
[2] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; AG = AGTIV® inoculant of *Glomus intraradices*; BIO = JumpStart® and AGTIV®).

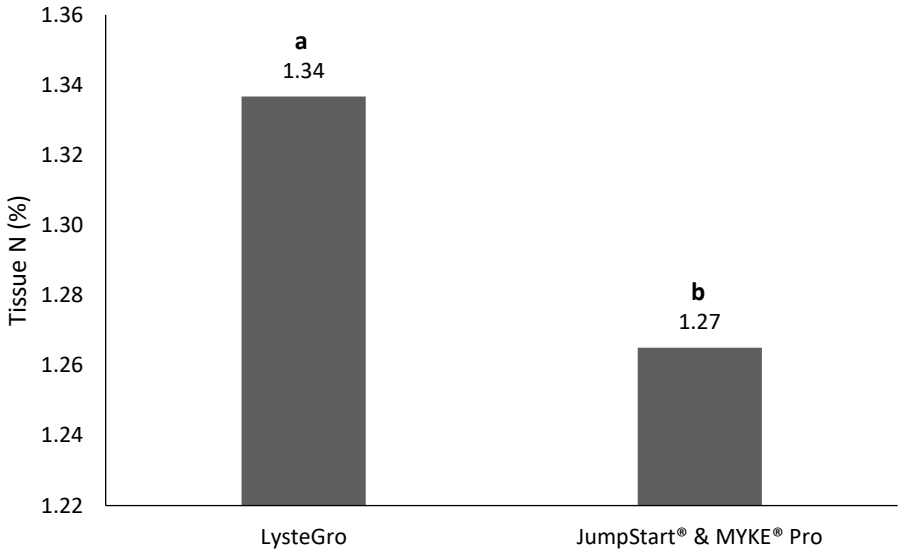

Figure 4.2.12: Peak season miscanthus tissue N concentration (%) as influenced by LysteGro versus the combined average of JumpStart® and MYKE® Pro at the Burlington Miscanthus site in 2019. Different letters indicate significantly different means according to orthogonal contrast ($p \leq 0.05$).

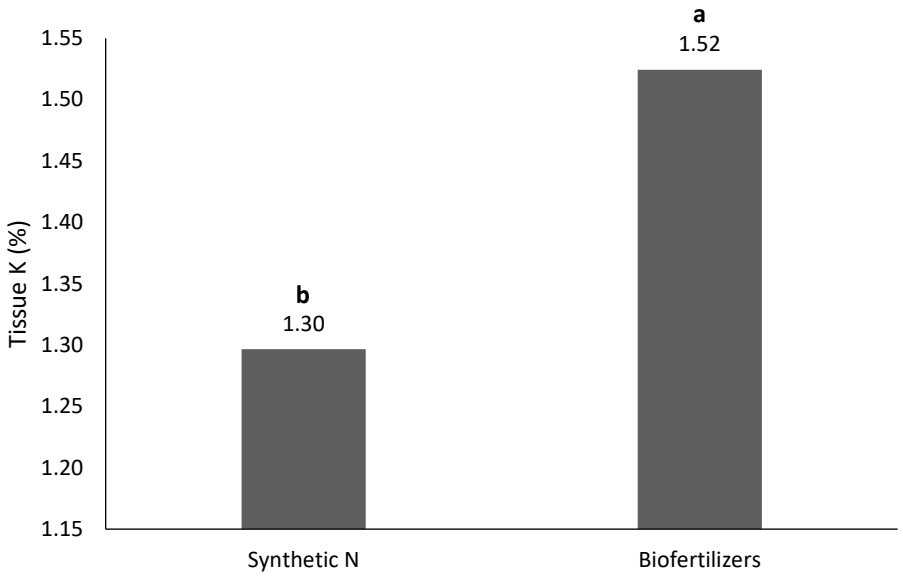

Figure 4.2.13: Peak season miscanthus tissue K concentration (%) as influenced by synthetic N fertilizer versus the combined average of three biofertilizers (JumpStart®, MYKE® Pro, and LysteGro) at the Burlington Miscanthus site in 2019. Different letters indicate significantly different means according to orthogonal contrast ($p \leq 0.05$).

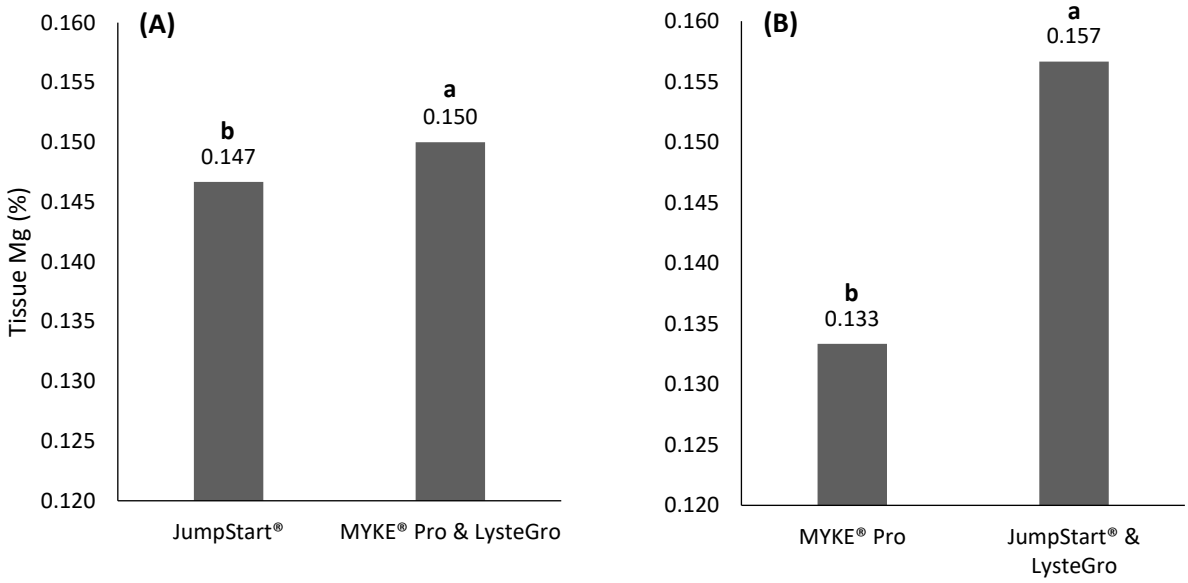

Figure 4.2.14: Peak season miscanthus tissue Mg concentration (%) as influenced by (A) JumpStart® versus the combined average of MYKE® Pro and LysteGro and (B) MYKE® Pro versus the combined averaged of JumpStart® and LysteGro at the Burlington Miscanthus site in 2019. Different letters indicate significantly different means according to orthogonal contrast ($p \leq 0.05$).

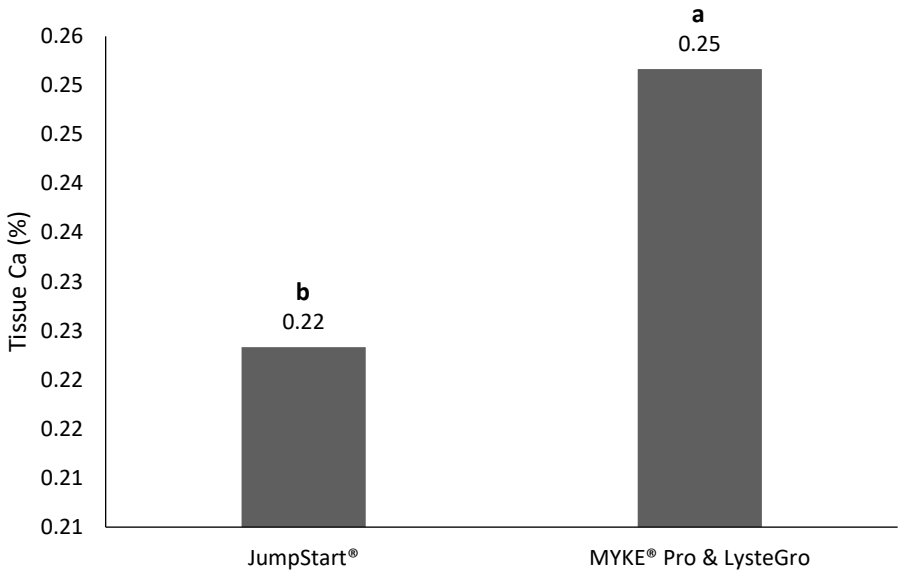

Figure 4.2.15: Peak season miscanthus tissue Ca concentration (%) as influenced by JumpStart® versus the combined average of MYKE® Pro and LysteGro at the Burlington Miscanthus site in 2019. Different letters indicate significantly different means according to orthogonal contrast ($p \leq 0.05$).

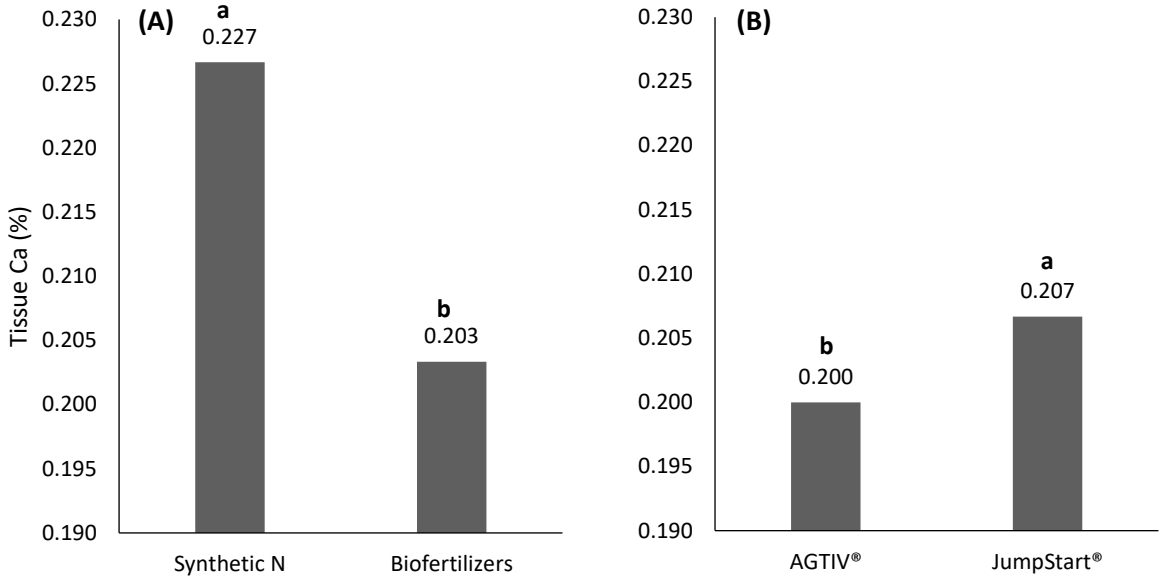

Figure 4.2.16: Peak season miscanthus tissue Ca concentration (%) as influenced by (A) synthetic N fertilizer versus the combined average of AGTIV® and JumpStart® and (B) AGTIV versus JumpStart® at the Burlington Miscanthus site in 2020. Different letters indicate significantly different means according to orthogonal contrast ($p \leq 0.05$).

4.2.3 Incubation Study

The final component of the soil fertility assessment is the ancillary incubation study conducted in collaboration with Master of Environmental Sciences (MES) student, Ramanjit Kaur Bhatti, which evaluated how each fertilizer treatment affected the bioavailability of N, P, and K in bare soils collected from the GS field site the end of the 2019 season. Significant effects of the fertilizer treatments were observed for nitrate-N, ammonium-N, total mineral N, P and K (**Table 4.2.11**). There were also significant effects of time (weeks in incubation) for most of these nutrients, as well as a significant treatment-time (fertilizer-week) interaction for both NH_4^+ and P (**Table 4.2.11**), however the time in incubation is not the focus of this study so the treatment (fertilizer) effects are what will be discussed in this section. Please see Appendix C for a discussion of the trends in nutrient release over time from this study.

Synthetic N fertilizer produced the highest mean NH_4^+ content, followed closely by LysteGro, with all treatments significantly ($p < 0.05$) increasing NH_4^+ compared to the control (**Figure 4.2.17**). Mean soil NO_3^- content increased significantly from 10.3 ppm in the control to 16.5 and 15.8 ppm in LysteGro and synthetic N treatments, respectively (**Figure 4.2.17**). Total mineral-N content was significantly ($p < 0.05$) lower in the control (11.9 ppm) compared to each of the fertilizer treatments which measured in at 19.0, 18.4, 14.6 and 14.0 ppm in LysteGro, synthetic N, JumpStart® and MYKE® Pro, respectively (**Figure 4.2.17**). The highest available P content was recorded in MYKE® Pro, followed by JumpStart®. Mean P availability was lowest in the control (16.8 ppm), increasing significantly ($p < 0.05$) to 22.8, 21.8, 20.9, and 20.6 ppm in MYKE® Pro, JumpStart®, LysteGro and synthetic N treatments, respectively (**Figure 4.2.18**). Finally, mean K availability was 58.5 ppm in the control, then 73.2, 64.4, 63.5, and 57.4 ppm in soils receiving LysteGro, synthetic N, MYKE® Pro, and JumpStart®, respectively (**Figure**

4.2.19). The highest available K was recorded in soil that received LysteGro, which was significantly ($p < 0.05$) higher than control soils, with all other treatments being statistically similar to the control (**Figure 4.2.19**).

Table 4.2.11: Mixed model analysis of variance assessing the effects of fertilizer treatment (Control, Synthetic N, JumpStart®, MYKE® Pro, and LysteGro) on the bioavailability (ppm) of nitrate (NO_3^-), ammonium (NH_4^+), total mineral nitrogen (Total N), phosphorus (P), and potassium (K), in bare soils collected from the Guelph Switchgrass site in the fall of 2019 and incubated under controlled conditions over seven weeks.

		NO_3^-	NH_4^+	Total N	P	K
Source of Variation	*df*	*p-value*	*p-value*	*p-value*	*p-value*	*p-value*
Fertilizer	4	< **0.0001******	< **0.0001******	< **0.0001******	**0.0005*****	< **0.0001******
Week	4	< **0.0001******	< **0.0001******	< **0.0001******	< **0.0001******	0.0746
Fertilizer*Week	16	0.4928	**0.0004*****	0.2970	**0.0010****	0.9990
Block	1	0.8659	0.4910	0.9444	< **0.0001******	< **0.0001******

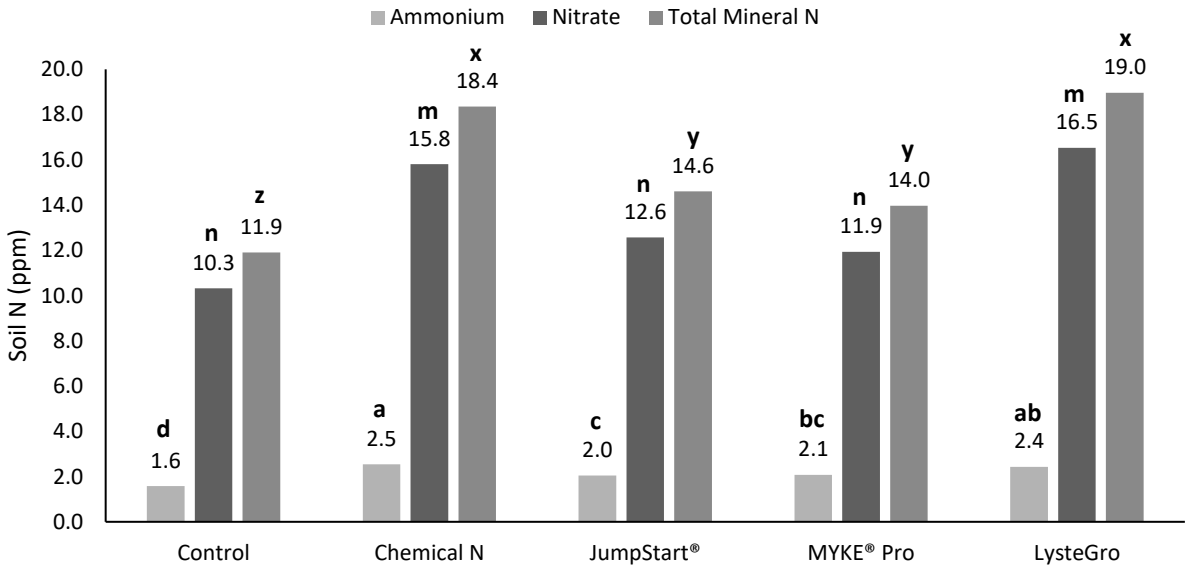

Figure 4.2.17: Average availability of NH_4^+, NO_3^-, and total mineral N in the soil over a seven-week incubation period as affected by fertilizer treatment. Different letters indicate significantly different means according to least-square means comparison adjusted according to the Tukey test ($p \leq 0.05$).

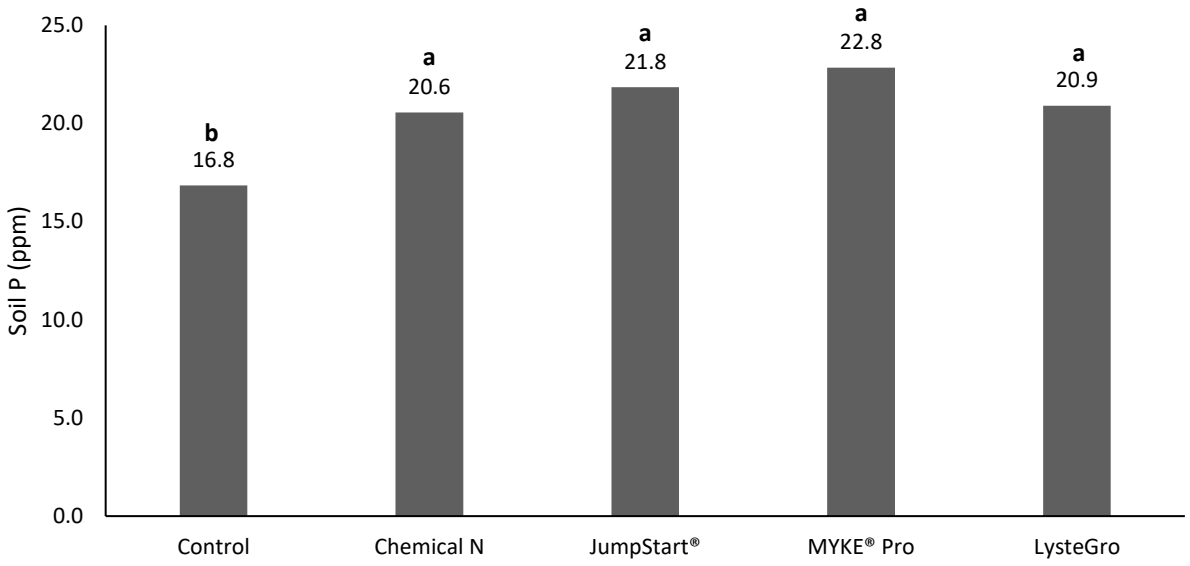

Figure 4.2.18: Average availability of P in the soil over a seven-week incubation period as affected by fertilizer treatment. Different letters indicate significantly different means according to least-square means comparison adjusted according to the Tukey test ($p \leq 0.05$).

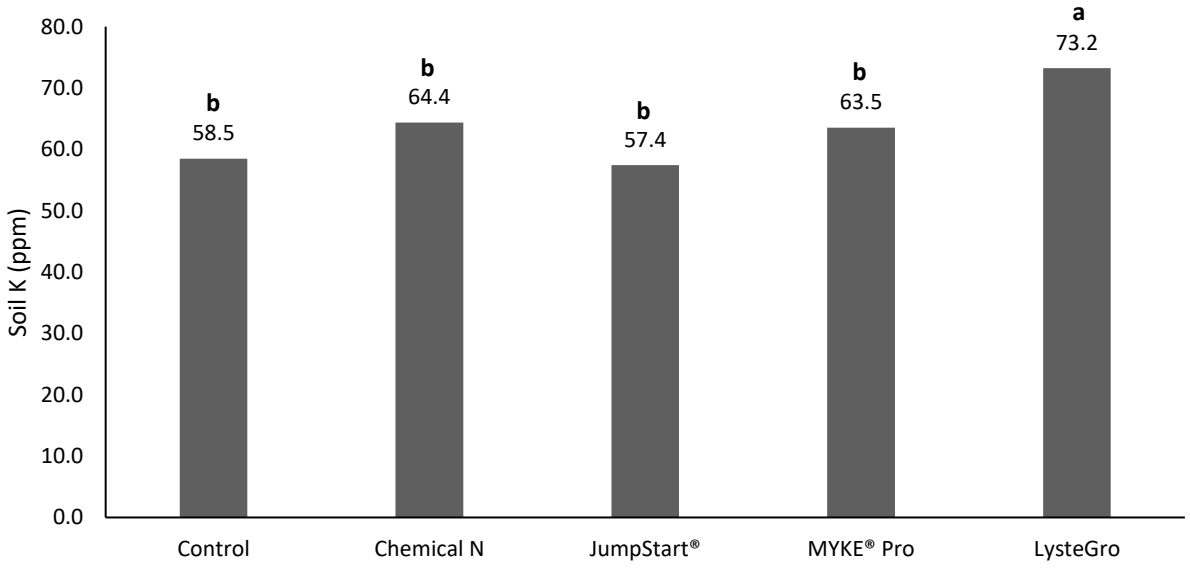

Figure 4.2.19: Average availability of K in the soil over a seven-week incubation period as affected by fertilizer treatment. Different letters indicate significantly different means according to least-square means comparison adjusted according to the Tukey test ($p \leq 0.05$).

4.3 Soil Biological Health

4.3.1 Switchgrass

At the GS field site in 2019, soil biological health was measured by quantifying the abundance of 16S bacterial genes and 18S fungal genes (gene copies per g dry soil or copies g dry soil^{-1}) in the top 10 cm of the soil profile at the peak and the end of the growing season. At the end of the 2019 growing season, soil respiration in the top 30 cm was estimated using the Solvita CO_2 Burst and the amount of carbon substrate (required to fuel the metabolism of heterotrophic microbes) in the top 30 cm to was measured using the reactive C test. At the BS field site, the only data collected was the peak season 16S and 18S abundance due to the early harvest of the crop. Baseline abundance of 16S and 18S gene copies was also measured at both the BS and GS field site to account for any differences that may have existed among plots before treatments were applied. In 2020, peak and end-of-season 16S and 18S gene abundance in the top 10 cm were measured again at the GS field site, along with early season earthworm abundance in control, synthetic N, and JumpStart® plots. Solvita CO_2 Burst and reactive C analyses were omitted, and the BS site was not revisited in 2020.

At the GS field site, there were no significant differences observed in the abundance of either the 16S bacterial or 18S fungal genes among any of the plots, indicating that all plots were statistically similar at the beginning of the trial ($p > 0.05$). Baseline, peak, and end-of-season abundances of each gene are summarized in **Table 4.3.1**. At the peak of the 2019 growing season, there was no significant treatment effect on the abundance of 16S or 18S genes (**Table 4.3.2**). However, orthogonal contrast analyses indicated that the 2019 end-of-season 16S bacterial gene abundance was significantly higher ($p < 0.05$) in plots receiving MYKE® Pro compared to the combined average of plots receiving JumpStart® and LysteGro (**Figure 4.3.1**)

and 18S fungal gene abundance was significantly lower ($p < 0.05$) in plots receiving JumpStart® compared to the combined average of plots receiving MYKE® Pro and LysteGro (**Figure 4.3.2**). No significant differences in the abundance of either gene were observed between the biofertilizer treatments and the synthetic N fertilizer treatment (**Table 4.3.2**), nor were there any significant differences observed between any of the fertilizer treatments and the control when analyzed in a least-square means comparison adjusted according to Dunnett's Correction ($p > 0.05$, data not presented). The Solvita CO_2 Burst and the reactive C test results are summarized in **Table 4.3.3**. Mixed model ANOVA for this data also indicated no significant treatment effects when analysed using least-square means comparisons adjusted according to either the Tukey test or Dunnett's Correction, nor were there any significant differences among treatments analysed using orthogonal contrast (**Table 4.3.4**).

Table 4.3.1: 16S bacterial and 18S fungal gene abundances (copies g dry soil^{-1}; ± standard error) at the baseline, peak, and end-of-season sampling dates at the Guelph Switchgrass site in 2019.

16S Bacterial Gene			
Treatment	*Baseline*	*Peak Season*	*End-of-season*
Control	$2.1 \times 10^9 \pm 0.7 \times 10^9$	$2.4 \times 10^9 \pm 0.9 \times 10^9$	$5.0 \times 10^9 \pm 0.8 \times 10^9$
Synthetic N	$1.9 \times 10^9 \pm 0.1 \times 10^9$	$2.6 \times 10^9 \pm 1.0 \times 10^9$	$3.6 \times 10^9 \pm 0.5 \times 10^9$
JumpStart®	$2.0 \times 10^9 \pm 0.4 \times 10^9$	$2.6 \times 10^9 \pm 0.8 \times 10^9$	$3.1 \times 10^9 \pm 1.0 \times 10^9$
MYKE® Pro	$2.3 \times 10^9 \pm 0.4 \times 10^9$	$2.2 \times 10^9 \pm 0.3 \times 10^9$	$5.3 \times 10^9 \pm 1.3 \times 10^9$
LysteGro	$1.8 \times 10^9 \pm 0.4 \times 10^9$	$2.7 \times 10^9 \pm 0.6 \times 10^9$	$3.6 \times 10^9 \pm 0.7 \times 10^9$
18S Fungal Gene			
Treatment	*Baseline*	*Peak Season*	*End-of-season*
Control	$1.3 \times 10^6 \pm 0.3 \times 10^6$	$1.3 \times 10^6 \pm 0.2 \times 10^6$	$3.7 \times 10^6 \pm 0.9 \times 10^6$
Synthetic N	$9.3 \times 10^5 \pm 0.1 \times 10^6$	$1.7 \times 10^6 \pm 0.1 \times 10^6$	$3.1 \times 10^6 \pm 0.7 \times 10^6$
JumpStart®	$1.2 \times 10^6 \pm 0.2 \times 10^6$	$2.3 \times 10^6 \pm 0.7 \times 10^6$	$1.4 \times 10^6 \pm 0.5 \times 10^6$
MYKE® Pro	$1.8 \times 10^6 \pm 0.7 \times 10^6$	$1.8 \times 10^6 \pm 0.7 \times 10^6$	$3.9 \times 10^6 \pm 1.5 \times 10^6$
LysteGro	$1.1 \times 10^6 \pm 0.3 \times 10^6$	$2.6 \times 10^6 \pm 0.7 \times 10^6$	$2.4 \times 10^6 \pm 0.8 \times 10^6$

Table 4.3.2: Mixed model analysis of variance assessing the effects of fertilizer treatment (Control, Synthetic N, JumpStart®, MYKE® Pro, and LysteGro) on 16S bacterial gene and 18S fungal gene abundances (copies g dry soil^{-1}; ± standard error) in the top 10 cm of the soil profile at the Guelph Switchgrass site in 2019.

16S Bacterial Gene			
		Peak Season	End-of-season
Source of Variation	*df*	*p-value*	*p-value*
Fertilizer[1]	4	0.8625	0.0917
SYN vs BIO	1	0.3725	0.8389
LG vs JS & MP	1	0.6851	0.6908
JS vs LG & MP	1	0.9617	0.0587
MP vs LG & JS	1	0.6506	**0.0281***
Block	1	**0.0029****	**0.0259***
18S Fungal Gene			
		Peak Season	End-of-season
Source of Variation	*df*	*p-value*	*p-value*
Fertilizer[1]	4	0.7215	0.1288
SYN vs BIO	1	0.8677	0.2591
LG vs JS & MP	1	0.5234	0.8039
JS vs LG & MP	1	0.8115	**0.0498***
MP vs LG & JS	1	0.3853	0.0780
Block	1	**0.0434***	0.2556

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; MP = MYKE® Pro inoculant of *Glomus intraradices*; LG = LysteGro biosolids fertilizer; BIO = JumpStart®, MYKE® Pro, and LysteGro).

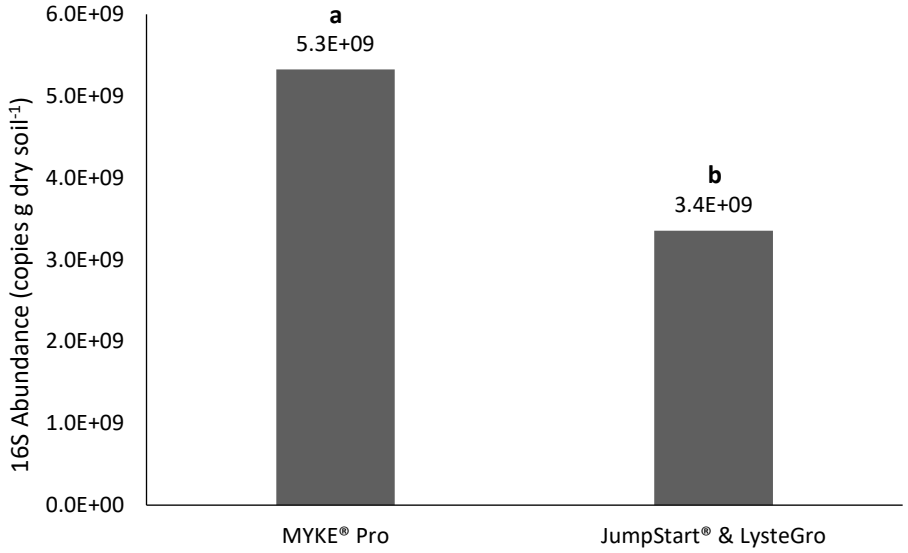

Figure 4.3.1: End-of-season 16S bacterial gene abundance (copies g dry soil^{-1}) in the top 10 cm of soil as influenced by MYKE® Pro versus the combined average of JumpStart® and LysteGro at the Guelph Switchgrass site in 2019. Different letters indicate significantly different means according to orthogonal contrast ($p \leq 0.05$).

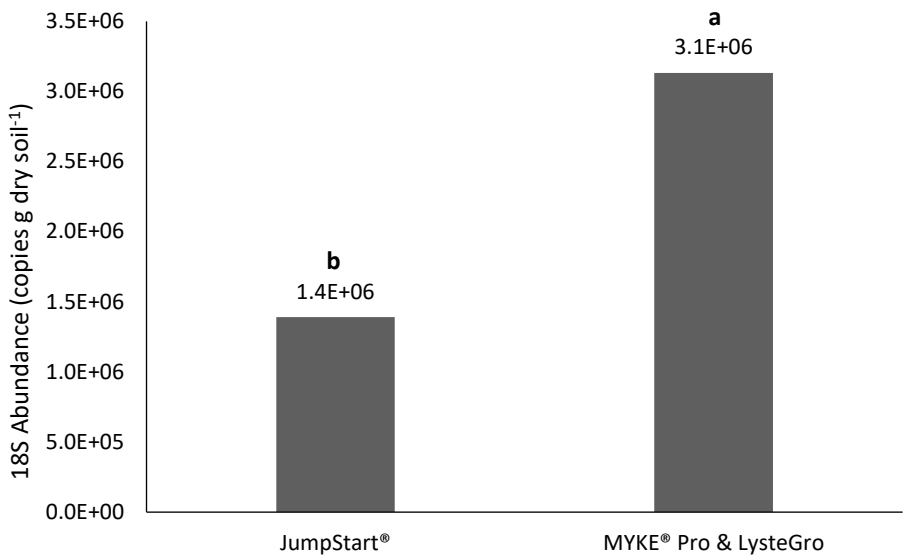

Figure 4.3.2: End-of-season 18S fungal gene abundance (copies g dry soil^{-1}) in the top 10 cm of soil as influenced by JumpStart® versus the combined average of MYKE® Pro and LysteGro at the Guelph Switchgrass site in 2019. Different letters indicate significantly different means according to orthogonal contrast ($p \leq 0.05$).

Table 4.3.3: Autumn Solvita CO_2 Burst (ppm; ± standard error) and soil reactive C (ppm± standard error) test results from the Guelph Switchgrass site in 2019.

Treatment	*Solvita CO_2 Burst*	*Soil Reactive C*
Control	62.5 ± 17.8	769.5 ± 38.3
Synthetic N	38.9 ± 16.9	810.4 ± 15.2
JumpStart®	31.1 ± 8.5	715.3 ± 52.8
MYKE® Pro	58.2 ± 8.5	753.0 ± 15.9
LysteGro	44.5 ± 7.0	756.2 ± 49.1

Table 4.3.4: Mixed model analysis of variance assessing the effects of fertilizer treatment (Control, Synthetic N, JumpStart®, MYKE® Pro, and LysteGro) on the Solvita CO_2 Burst (ppm) and reactive carbon (ppm) for the top 30 cm of the soil profile at the Guelph Switchgrass site in 2019.

		Solvita CO_2 *Burst*	Soil Reactive C
Source of Variation	*df*	*p-value*	*p-value*
Fertilizer[1]	4	0.3761	0.4045
SYN vs BIO	1	0.7388	0.1138
LG vs JS & MP	1	0.8908	0.5948
JS vs LG & MP	1	0.1620	0.3526
MP vs LG & JS	1	0.1994	0.7122
Block	1	0.2522	0.0906

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; MP = MYKE® Pro inoculant of *Glomus intraradices*; LG = LysteGro biosolids fertilizer; BIO = JumpStart®, MYKE® Pro, and LysteGro).

16S bacterial and 18S fungal gene abundances at the peak sampling date at the Burlington Switchgrass (BS) field site are summarized in **Table 4.3.5**. Mixed model ANOVA of baseline measurements of 16S bacterial gene abundance at the BS site in 2019 indicated no significant differences among any of the plots ($p > 0.05$), meaning that all plots had a statistically similar abundance of this gene before treatments were applied. Baseline 18S abundance was significantly higher ($p = 0.0081$) in plots assigned to receive synthetic N fertilizer compared to the combined average of plots assigned to receive the three biofertilizer treatments – JumpStart®, MYKE® Pro, and LysteGro (data not presented). At the peak season sampling date, 16S gene abundance was significantly ($p < 0.05$) lower in plots receiving MYKE® Pro compared to all other treatments in a least-square means comparison adjusted according to the Tukey test (**Table 4.3.6**; **Figure 4.3.3**). Peak season 18S gene abundance at the BS field site in 2019 was significantly ($p < 0.05$) lower in MYKE® Pro plots compared to the control and to JumpStart® plots in least-square means comparison adjusted according to the Tukey test (**Figure 4.3.4**).

Table 4.3.5: 16S bacterial and 18S fungal gene abundances (copies g dry soil^{-1}; ± standard error) at the baseline and peak season sampling dates at the Burlington Switchgrass site in 2019.

16S Bacterial Gene		
Treatment	*Baseline*	*Peak Season*
Control	$2.2 \times 10^9 \pm 0.5 \times 10^9$	$3.5 \times 10^9 \pm 0.3 \times 10^9$
Synthetic N	$2.4 \times 10^9 \pm 0.4 \times 10^9$	$2.2 \times 10^9 \pm 0.6 \times 10^9$
JumpStart®	$1.8 \times 10^9 \pm 0.4 \times 10^9$	$3.4 \times 10^9 \pm 0.4 \times 10^9$
MYKE® Pro	$1.9 \times 10^9 \pm 0.5 \times 10^9$	$1.1 \times 10^9 \pm 0.3 \times 10^9$
LysteGro	$1.5 \times 10^9 \pm 0.4 \times 10^9$	$2.2 \times 10^9 \pm 0.5 \times 10^9$
18S Fungal Gene		
Treatment	*Baseline*	*Peak Season*
Control	$1.6 \times 10^6 \pm 0.3 \times 10^6$	$3.6 \times 10^6 \pm 0.8 \times 10^6$
Synthetic N	$2.1 \times 10^6 \pm 0.2 \times 10^6$	$2.1 \times 10^6 \pm 0.3 \times 10^6$
JumpStart®	$1.1 \times 10^6 \pm 0.3 \times 10^6$	$3.3 \times 10^6 \pm 0.4 \times 10^6$
MYKE® Pro	$1.4 \times 10^6 \pm 0.4 \times 10^6$	$1.1 \times 10^6 \pm 0.5 \times 10^6$
LysteGro	$1.2 \times 10^6 \pm 0.2 \times 10^6$	$1.7 \times 10^6 \pm 0.4 \times 10^6$

Table 4.3.6: Mixed model analysis of variance assessing the effects of fertilizer treatment (Control, Synthetic N, JumpStart®, MYKE® Pro, and LysteGro) on 16S bacterial gene and 18S fungal gene abundances (copies g dry soil^{-1}) in the top 10 cm of the soil profile at the Burlington Switchgrass site in 2019.

16S Bacterial Gene		
		Peak Season
Source of Variation	*df*	*p-value*
Fertilizer[1]	4	**0.0008*** **
SYN vs BIO	1	0.7697
LG vs JS & MP	1	0.4607
JS vs LG & MP	1	**0.0010** **
MP vs LG & JS	1	**0.0003*** **
Block	1	**0.0237***
18S Fungal Gene		
		Peak Season
Source of Variation	*df*	*p-value*
Fertilizer[1]	4	**0.0021** **
SYN vs BIO	1	0.3050
LG vs JS & MP	1	0.7605
JS vs LG & MP	1	**0.0013** **
MP vs LG & JS	1	**0.0022** **
Block	1	**0.0164***

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; MP = MYKE® Pro inoculant of *Glomus intraradices*; LG = LysteGro biosolids fertilizer; BIO = JumpStart®, MYKE® Pro, and LysteGro).

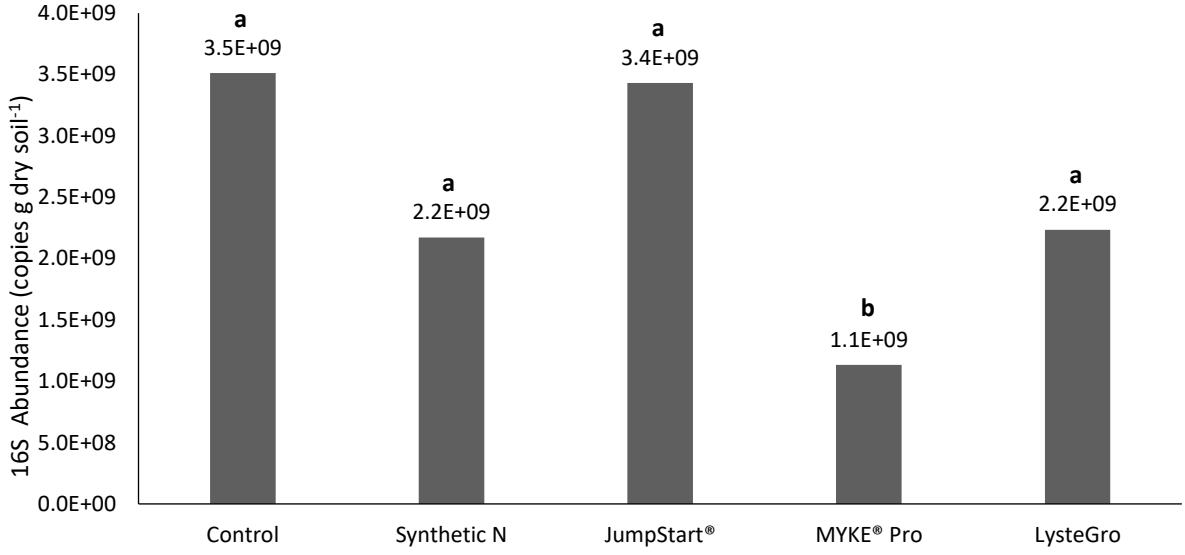

Figure 4.3.3: Peak season 16S bacterial gene abundance (copies g dry soil^{-1}) in the top 10 cm of soil as influenced by fertilizer treatment at the Burlington Switchgrass site in 2019. Different letters indicate significantly different means according to least-square means comparison adjusted per the Tukey test ($p \leq 0.05$).

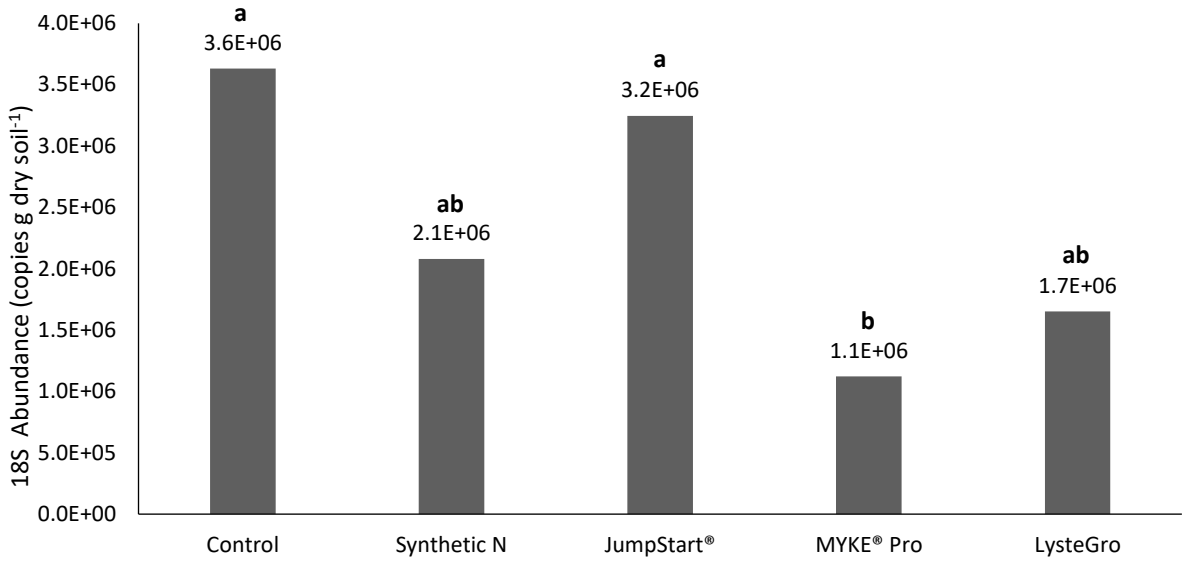

Figure 4.3.4: Peak season 18S fungal gene abundance (copies g dry soil^{-1}) in the top 10 cm of soil as influenced by fertilizer treatment at the Burlington Switchgrass site in 2019. Different letters indicate significantly different means according to least-square means comparison adjusted per the Tukey test ($p \leq 0.05$).

Significant treatment effects on 16S bacterial gene and 18S fungal gene abundances at the GS field site were only observed at the peak season sampling period in this year, with no significant differences among treatments in the end-of-season data (**Table 4.3.8**). Least-square means comparison adjusted according to the Tukey test indicated that peak season 16S abundance at the GS field site in 2020 was significantly ($p < 0.05$) higher in plots receiving Optimyc + MooR than in synthetic N and control plots (**Figure 4.3.5**). Furthermore, least-square means comparison adjusted according to Dunnett's Correction indicated that both AGTIV® and Optimyc + MooR significantly ($p < 0.05$) increased 16S gene abundance compared to the control (data not presented). Peak season 18S fungal gene abundance was significantly ($p < 0.05$) lower in plots receiving synthetic N fertilizer versus plots that received Optimyc + MooR, according to least-square means comparisons adjusted according to the Tukey test (**Figure 4.3.6**). Finally,

earthworm densities from spring 2020 at the GS field site are summarized in **Table 4.3.9**. No significant differences were observed in the density of earthworms (**Table 4.3.10**).

Table 4.3.7: 16S bacterial and 18S fungal gene abundances (copies g dry soil^{-1}; ± standard error) at the peak and end-of-season sampling dates at the Guelph Switchgrass site in 2020.

16S Bacterial Gene		
Treatment	*Peak Season*	*End-of-season*
Control	$7.2 \times 10^9 \pm 1.0 \times 10^9$	$1.3 \times 10^{10} \pm 0.3 \times 10^{10}$
Synthetic N	$7.7 \times 10^9 \pm 0.6 \times 10^9$	$1.5 \times 10^{10} \pm 0.4 \times 10^{10}$
JumpStart®	$9.7 \times 10^9 \pm 2.8 \times 10^9$	$1.5 \times 10^{10} \pm 0.2 \times 10^{10}$
AGTIV®	$1.6 \times 10^{10} \pm 0.4 \times 10^{10}$	$1.1 \times 10^{10} \pm 0.2 \times 10^{10}$
Optimyc + MooR	$1.8 \times 10^{10} \pm 0.08 \times 10^{10}$	$9.0 \times 10^9 \pm 1.7 \times 10^9$
18S Fungal Gene		
Treatment	*Peak Season*	*End-of-season*
Control	$3.1 \times 10^5 \pm 0.9 \times 10^5$	$5.1 \times 10^5 \pm 0.9 \times 10^5$
Synthetic N	$2.4 \times 10^5 \pm 0.3 \times 10^5$	$5.5 \times 10^5 \pm 1.1 \times 10^5$
JumpStart®	$5.1 \times 10^5 \pm 2.1 \times 10^5$	$4.7 \times 10^5 \pm 0.7 \times 10^5$
AGTIV®	$9.6 \times 10^5 \pm 2.6 \times 10^5$	$5.6 \times 10^5 \pm 1.4 \times 10^5$
Optimyc + MooR	$8.7 \times 10^5 \pm 0.6 \times 10^5$	$5.0 \times 10^5 \pm 0.4 \times 10^5$

Table 4.3.8: Mixed model analysis of variance assessing the effects of fertilizer treatment (Control, Synthetic N, JumpStart®, AGTIV®, and Optimyc + MooR) on 16S bacterial gene and 18S fungal gene abundances (copies g dry soil^{-1}) in the top 10 cm of the soil profile at the Guelph Switchgrass site in 2020.

16S Bacterial Gene Abundance			
		Peak Season	End-of-season
Source of Variation	*df*	*p-value*	*p-value*
Fertilizer[1]	4	**0.0091****	0.3212
SYN vs BIO	1	**0.0113***	0.2627
AG vs JS & OM	1	0.4282	0.7313
JS vs AG & OM	1	**0.0304***	0.0961
OM vs AG & JS	1	0.1283	0.1716
Block	1	0.2212	0.3539
18S Fungal Gene Abundance			
		Peak Season	End-of-season
Source of Variation	*df*	*p-value*	*p-value*
Fertilizer[1]	4	**0.0183***	0.9934
SYN vs BIO	1	**0.0114***	0.8266
AG vs JS & OM	1	0.2768	0.7611
JS vs AG & OM	1	**0.0297***	0.7097
OM vs AG & JS	1	0.2093	0.9452
Block	1	0.3053	0.7923

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; AG = AGTIV® inoculant of *Glomus intraradices*; OM = Optimyc + MooR inoculants of beneficial fungal and bacterial consortia; BIO = JumpStart®, AGTIV®, and Optimyc + MooR).

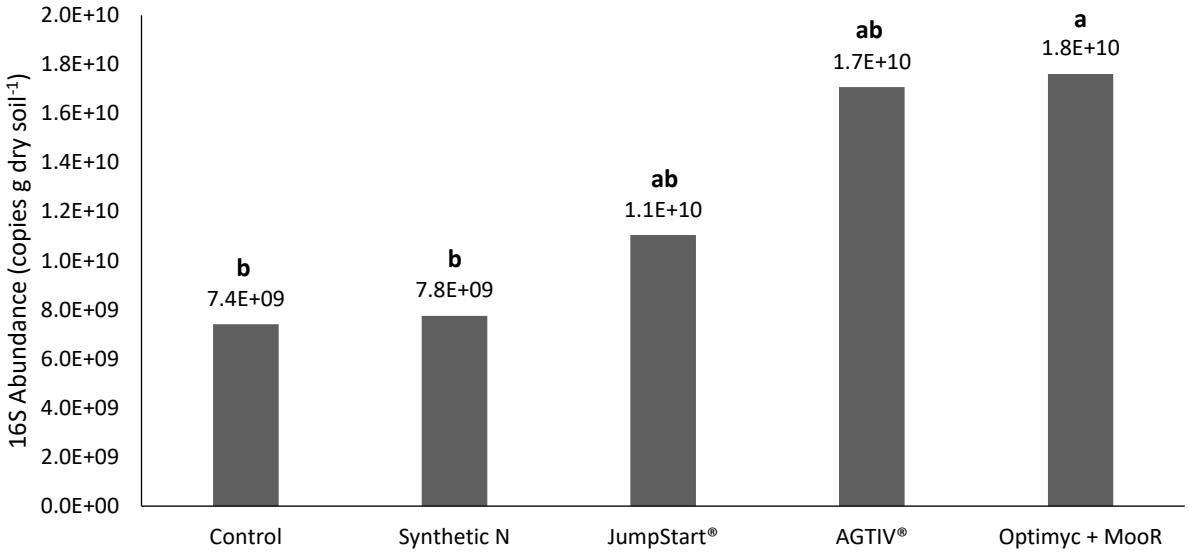

Figure 4.3.5: Peak season 16S bacterial gene abundance (copies g dry soil^{-1}) in the top 10 cm of soil as influenced by fertilizer treatment at the Guelph Switchgrass site in 2020. Different letters indicate significantly different means according to least-square means comparison adjusted per the Tukey test ($p \leq 0.05$).

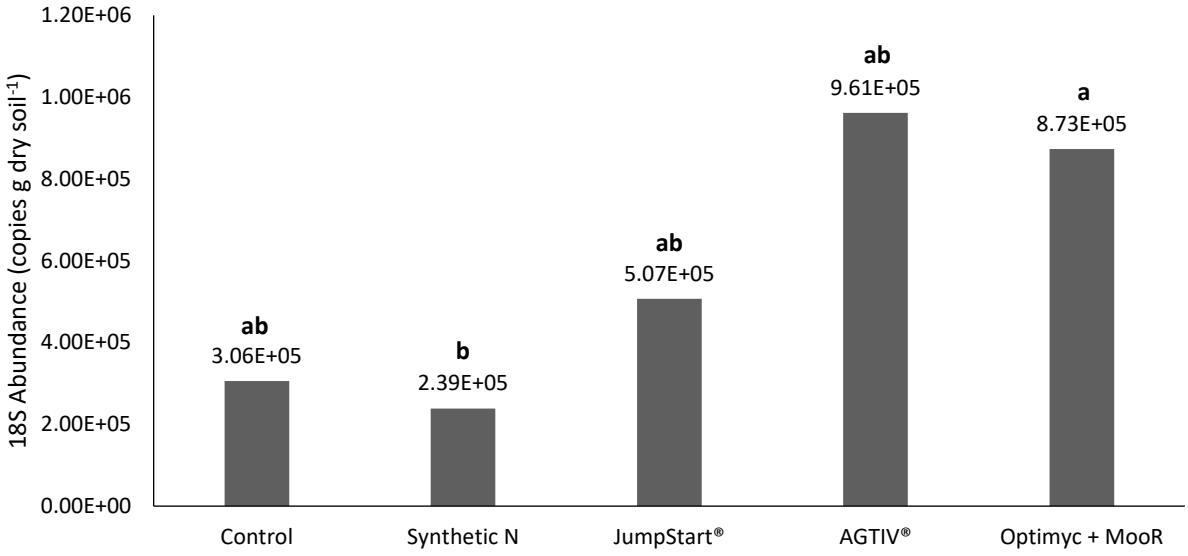

Figure 4.3.6: Peak 18S fungal gene abundance (copies g dry soil^{-1}) in the top 10 cm of soil as influenced by fertilizer treatment at the Guelph Switchgrass site in 2020. Different letters indicate significantly different means according to least-square means comparison adjusted per the Tukey test ($p \leq 0.05$).

Table 4.3.9: Earthworm density (worms m^{-2}; ± standard error) at the Guelph Switchgrass site in the spring of 2020.

Treatment	*Earthworm Density*
Control	89 ± 53
Synthetic N	52 ± 41
JumpStart®[1]	33 ± 6

[1] JumpStart® plots were chosen as a representative biofertilizer treatment

Table 4.3.10: Mixed model analysis of variance assessing the effects of fertilizer treatment (Control, Synthetic N, and JumpStart®) on earthworm abundance (worms m^{-2}) at the Guelph Switchgrass site in 2020.

Source of Variation	*df*	*p-value*
Fertilizer	4	0.1116
Block	1	**<0.0001******

4.3.2 Miscanthus

At the BM field site in 2019, soil biological health in Miscanthus plots was measured by quantifying the abundance of 16S bacterial genes and 18S fungal genes (copies g dry $soil^{-1}$) in the top 10 cm of the soil profile at the peak and the end of the growing season. At the end of the 2019 growing season, soil respiration and available carbon substrate in the top 30 cm was measured using the Solvita CO_2 Burst and the reactive C tests, respectively. Baseline abundance of 16S and 18S gene copies was also measured to account for any differences that may have existed among plots before treatments were applied. In 2020, peak and end-of-season 16S and 18S gene abundance in the top 10 cm were measured again at the BM field site. Solvita CO_2 Burst and reactive C analyses were omitted in this year.

The 2019 baseline, peak, and end-of-season abundances of each gene are summarized in **Table 4.3.11**. Baseline 16S bacterial gene and 18S fungal gene abundances demonstrated no significant differences ($p > 0.05$), indicating that all plots had a statistically similar abundance of soil bacteria and fungi at the start of the trial. At the peak of the growing season, plots receiving synthetic N fertilizer had a significantly ($p < 0.05$) higher abundance of 16S bacterial gene copies versus the combined average of the three biofertilizer treatments – JumpStart®, MYKE® Pro,

and LysteGro – according to orthogonal contrast analysis (**Figure 4.3.7**). However, at the end-of-season sampling there were no significant differences in 16S gene abundance among any of the treatments (**Table 4.3.12**). There were no significant differences in 18S fungal gene abundance at either the peak or end-of-season sampling at the BM site in 2019 (**Table 4.3.12**).

Table 4.3.11: 16S bacterial and 18S fungal gene abundances (copies g dry soil^{-1}; ± standard error) at the baseline, peak, and end-of-season sampling dates at the Burlington Miscanthus site in 2019.

16S Bacterial Gene			
Treatment	*Baseline*	*Peak Season*	*End-of-season*
Control	$4.0 \times 10^9 \pm 0.8 \times 10^9$	$4.5 \times 10^9 \pm 0.4 \times 10^9$	$4.8 \times 10^9 \pm 1.0 \times 10^9$
Synthetic N	$4.9 \times 10^9 \pm 0.6 \times 10^9$	$2.5 \times 10^{10} \pm 1.9 \times 10^{10}$	$6.2 \times 10^9 \pm 0.8 \times 10^9$
JumpStart®	$3.8 \times 10^9 \pm 0.4 \times 10^9$	$4.6 \times 10^9 \pm 1.0 \times 10^9$	$5.4 \times 10^9 \pm 0.9 \times 10^9$
MYKE® Pro	$4.0 \times 10^9 \pm 0.6 \times 10^9$	$5.2 \times 10^9 \pm 0.6 \times 10^9$	$5.0 \times 10^9 \pm 1.5 \times 10^9$
LysteGro	$3.4 \times 10^9 \pm 0.4 \times 10^9$	$5.2 \times 10^9 \pm 1.2 \times 10^9$	$5.2 \times 10^9 \pm 0.8 \times 10^9$
18S Fungal Gene			
Treatment	*Baseline*	*Peak Season*	*End-of-season*
Control	$2.2 \times 10^6 \pm 0.2 \times 10^6$	$2.9 \times 10^6 \pm 0.6 \times 10^6$	$2.5 \times 10^6 \pm 0.1 \times 10^6$
Synthetic N	$1.8 \times 10^6 \pm 0.1 \times 10^6$	$3.4 \times 10^6 \pm 1.2 \times 10^6$	$3.4 \times 10^6 \pm 0.5 \times 10^6$
JumpStart®	$2.3 \times 10^6 \pm 0.6 \times 10^6$	$2.9 \times 10^6 \pm 1.1 \times 10^6$	$3.3 \times 10^6 \pm 1.1 \times 10^6$
MYKE® Pro	$2.4 \times 10^6 \pm 0.8 \times 10^6$	$2.8 \times 10^6 \pm 0.6 \times 10^6$	$2.7 \times 10^6 \pm 0.6 \times 10^6$
LysteGro	$1.9 \times 10^6 \pm 0.5 \times 10^6$	$4.4 \times 10^6 \pm 1.9 \times 10^6$	$3.2 \times 10^6 \pm 1.2 \times 10^6$

Table 4.3.12: Mixed model analysis of variance assessing the effects of fertilizer treatment (Control, Synthetic N, JumpStart®, MYKE® Pro, and LysteGro) on 16S bacterial gene and 18S fungal gene abundances (copies g dry soil^{-1}) in the top 10 cm of the soil profile at the Burlington Miscanthus site in 2019.

16S Bacterial Gene			
		Peak Season	End-of-season
Source of Variation	*df*	*p-value*	*p-value*
Fertilizer[1]	4	0.2891	0.8192
SYN vs BIO	1	**0.0497***	0.3590
LG vs JS & MP	1	0.9832	0.8642
JS vs LG & MP	1	0.8175	0.7632
MP vs LG & JS	1	0.8339	0.6385
Block	1	0.3321	0.3608
18S Fungal Gene			
		Peak Season	End-of-season
Source of Variation	*df*	*p-value*	*p-value*
Fertilizer[1]	4	0.8518	0.9034
SYN vs BIO	1	0.7824	0.4707
LG vs JS & MP	1	0.3074	0.9297
JS vs LG & MP	1	0.5163	0.6879
MP vs LG & JS	1	0.6918	0.7531
Block	1	**0.0090****	0.1997

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; MP = MYKE® Pro inoculant of *Glomus intraradices*; LG = LysteGro biosolids fertilizer; BIO = JumpStart®, MYKE® Pro, and LysteGro).

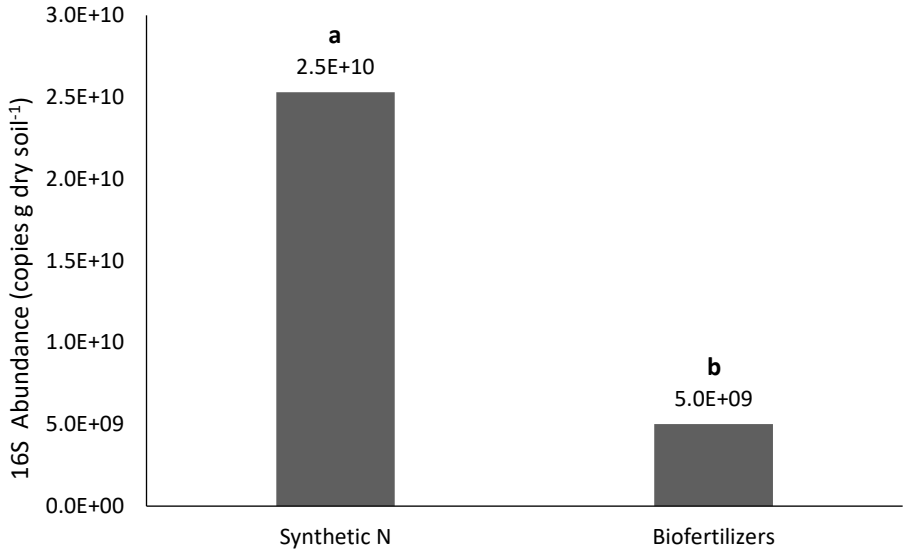

Figure 4.3.7: Peak season 16S bacterial gene abundance (copies g dry soil^{-1}) in the top 10 cm of soil as influenced by (A) synthetic N versus the combined average of three biofertilizers (JumpStart®, MYKE® Pro and LysteGro) at the Burlington Miscanthus site in 2019. Different letters indicate significantly different means according to orthogonal contrast ($p \leq 0.05$).

The Solvita CO_2 Burst conducted at the end of the 2019 growing season indicated that soils from JumpStart® plots released significantly ($p < 0.05$) less CO_2 through microbial respiration than the combined average of MYKE® Pro and LysteGro plots (**Figure 4.3.8**). The Solvita CO_2 Burst and soil reactive C test results for all treatments are summarized in **Table 4.3.13**. No significant differences in soil reactive C were observed among any of the treatments (**Table 4.3.14**).

Table 4.3.13: Autumn Solvita CO_2 Burst (ppm; ± standard error) and soil reactive C (ppm; ± standard error) test results from the Burlington Miscanthus site in 2019.

Treatment	*Solvita CO_2 Burst*	*Soil Reactive C*
Control	49.2 ± 12.3	939.5 ± 11.9
Synthetic N	31.8 ± 5.7	941.6 ± 19.4
JumpStart®	30.5 ± 2.3	907.3 ± 30.9
MYKE® Pro	51.8 ± 10.7	927.5 ± 11.9
LysteGro	48.0 ± 5.9	945.5 ± 11.9

Table 4.3.14: Mixed model analysis of variance assessing the effects of fertilizer treatment (Control, Synthetic N, JumpStart®, MYKE® Pro, and LysteGro) on the Solvita CO_2 Burst (ppm) and reactive carbon (ppm) for the top 30 cm of the soil profile at the Burlington Miscanthus site in 2019.

		Solvita CO_2	Reactive C
Source of Variation	*df*	*p-value*	*p-value*
Fertilizer[1]	4	0.0884	0.3029
SYN vs BIO	1	0.1347	0.3482
LG vs JS & MP	1	0.3313	0.1138
JS vs LG & MP	1	**0.0275***	0.1001
MP vs LG & JS	1	0.1361	0.9355
Block	1	0.2211	**0.0242***

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; MP = MYKE® Pro inoculant of *Glomus intraradices*; LG = LysteGro biosolids fertilizer; BIO = JumpStart®, MYKE® Pro, and LysteGro).

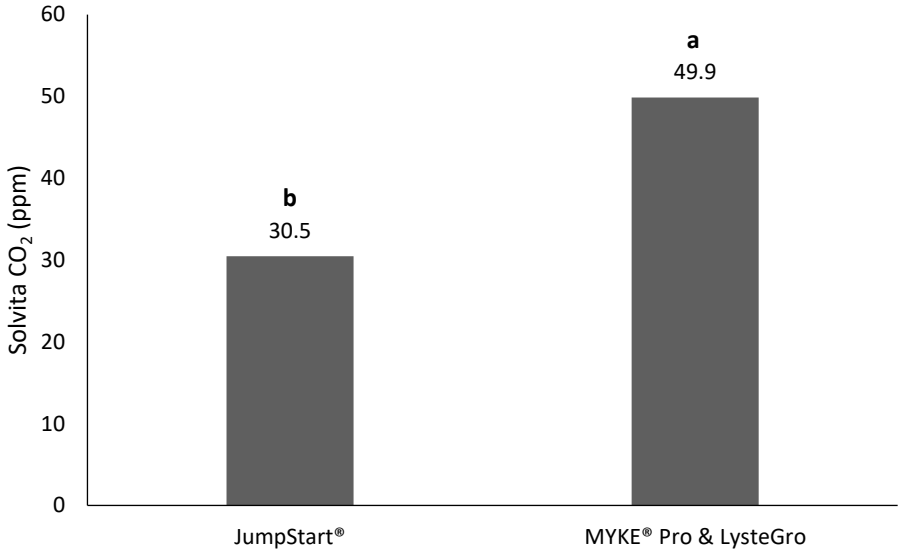

Figure 4.3.8: End-of-season Solvita-CO_2 Burst results (ppm) as influenced by JumpStart® versus the combined average of MYKE® Pro and LysteGro at the Burlington Miscanthus site in 2019. Different letters indicate significantly different means according to orthogonal contrast ($p \leq 0.05$).

Peak and end-of-season 16S and 18S gene abundances for 2020 are presented in **Table 4.3.15**. No significant differences in the abundance of either the 16S bacterial or 18S fungal genes were observed at the peak of the 2020 growing season (**Table 4.3.16**). At the end-of-season sampling, plots receiving AGTIV® had a significantly ($p < 0.05$) higher abundance of 16S bacterial gene copies than the control in a least-square means comparison adjusted according

to Dunnett's Correction (data not presented), but this difference was not significant in least-square means comparison adjusted according to the Tukey test (**Table 4.3.16**). As in 2019, no significant differences in the abundance of 18S fungal gene copies at either the peak or end-of-season sampling were observed at the BM field site in 2020 (**Table 4.3.16**).

Table 4.3.15: 16S bacterial and 18S fungal gene abundances (copies g dry soil^{-1}; ± standard error) at the peak and end-of-season sampling dates at the Burlington Miscanthus site in 2020.

16S Bacterial Gene		
Treatment	*Peak Season*	*End-of-season*
Control	$8.9 \times 10^9 \pm 0.8 \times 10^9$	$1.1 \times 10^{10} \pm 0.3 \times 10^{10}$
Synthetic N	$1.7 \times 10^{10} \pm 0.3 \times 10^{10}$	$2.0 \times 10^{10} \pm 0.4 \times 10^{10}$
JumpStart®	$1.7 \times 10^{10} \pm 0.7 \times 10^{10}$	$2.8 \times 10^{10} \pm 0.4 \times 10^{10}$
AGTIV®	$3.0 \times 10^{10} \pm 0.9 \times 10^{10}$	$3.1 \times 10^{10} \pm 0.4 \times 10^{10}$
18S Fungal Gene		
Treatment	*Peak Season*	*End-of-season*
Control	$5.8 \times 10^5 \pm 2.5 \times 10^5$	$6.6 \times 10^5 \pm 0.9 \times 10^5$
Synthetic N	$7.1 \times 10^5 \pm 2.4 \times 10^5$	$5.8 \times 10^5 \pm 1.5 \times 10^5$
JumpStart®	$8.1 \times 10^5 \pm 3.4 \times 10^5$	$1.4 \times 10^6 \pm 0.5 \times 10^6$
AGTIV®	$5.8 \times 10^5 \pm 1.4 \times 10^5$	$2.2 \times 10^6 \pm 1.1 \times 10^6$

Table 4.3.16: Mixed model analysis of variance assessing the effects of fertilizer treatment (Control, Synthetic N, JumpStart®, and AGTIV®) on 16S bacterial gene and 18S fungal gene abundances (copies g dry soil^{-1}) in the top 10 cm of the soil profile at the Burlington Miscanthus site in 2020.

16S Bacterial Gene Abundance			
		Peak Season	End-of-season
Source of Variation	*df*	*p-value*	*p-value*
Fertilizer[1]	3	0.2295	0.0663
SYN vs BIO	1	0.9129	0.2054
AG vs JS	1	0.6580	0.7313
Block	1	0.1595	0.0586
18S Fungal Gene Abundance			
		Peak Season	End-of-season
Source of Variation	*df*	*p-value*	*p-value*
Fertilizer[1]	3	0.8963	0.1866
SYN vs BIO	1	0.9067	0.0715
AG vs JS	1	0.6521	0.4414
Block	1	0.4953	0.5528

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; AG = AGTIV® inoculant of *Glomus intraradices*; BIO = JumpStart® and AGTIV®).

4.4 Greenhouse Gases

The final portion of this research project was a preliminary evaluation of the climate impact of switchgrass biomass crop production related to the effects of different fertilizer treatments on the soil flux of three key greenhouse gases (GHGs) from the soil (carbon dioxide, CO_2; methane, CH_4; and nitrous oxide, N_2O) and flux rate patterns of these gases throughout the growing season.

Flux rates for CO_2, CH_4, and N_2O separated by treatment for each sampling date are presented in **Table 4.4.1**. Based on data collected one day of each month from July to October of 2020, there were no significant effects of treatment on the flux of CO_2 (kg CO_2 ha^{-1} day^{-1}) or N_2O (g N_2O ha^{-1} day^{-1}) from soils under switchgrass crops at the GS field site (**Table 4.4.2**). On the August 2020 sampling day, however, least-square means analysis adjusted according to the Tukey test indicated that plots receiving synthetic N fertilizer absorbed significantly ($p < 0.05$) more CH_4 than plots receiving JumpStart® on August 19, 2020 (**Figure 4.4.1**). CH_4 flux was not significantly affected by any of the treatments in July, September, or October (**Table 4.4.2**).

Table 4.4.1: Flux rate of carbon dioxide, CO_2 (kg CO_2 ha^{-1} day^{-1}; ± standard error), methane, CH_4 (g CH_4 ha^{-1} day^{-1}; ± standard error) and nitrous oxide, N_2O (g N_2O ha^{-1} day^{-1}; ± standard error) from the soil, and global warming potential (GWP; kg CO_2e ha^{-1} day^{-1}; ± standard error), separated by treatment, over the course of the growing season at the Guelph Switchgrass site in 2020.

Sampling Day	*Treatment*	*CO_2 Flux*	*CH_4 Flux*	*N_2O Flux*	*GWP[1]*
July 15	Control	131.5 ± 7.7	-4.4 ± 3.5	0.0 ± 1.6	131.3 ± 7.7
	Synthetic N	120.8 ± 18.6	-2.0 ± 0.7	2.5 ± 1.8	121.4 ± 18.4
	JumpStart®	107.6 ± 11.0	-2.5 ± 0.5	2.4 ± 1.7	108.1 ± 10.6
	Optimyc + MooR	117.6 ± 8.6	-2.5 ± 1.2	2.6 ± 1.3	118.2 ± 8.3
August 19	Control	77.0 ± 4.6	-5.6 ± 1.4	-0.1 ± 0.9	76.8 ± 4.9
	Synthetic N	60.1 ± 3.1	-5.9 ± 1.0	-0.3 ± 0.7	59.8 ± 3.2
	JumpStart®	64.7 ± 12.4	-3.0 ± 0.9	0.2 ± 0.1	64.7 ± 12.4
	Optimyc + MooR	65.1 ± 4.1	-3.5 ± 0.8	-0.2 ± 0.8	64.9 ± 3.9
September 15	Control	48.7 ± 5.6	-3.1 ± 2.3	1.1 ± 0.5	48.0 ± 5.7
	Synthetic N	29.7 ± 14.1	-5.6 ± 2.9	-0.4 ± 0.6	29.4 ± 14.1
	JumpStart®	44.7 ± 4.7	-3.7 ± 0.3	-0.1 ± 0.7	44.6 ± 4.7
	Optimyc + MooR	32.3 ± 12.8	-8.2 ± 2.7	-0.2 ± 0.5	32.0 ± 12.8
October 14	Control	30.9 ± 5.0	-4.0 ± 0.8	-0.2 ± 0.7	30.7 ± 5.1
	Synthetic N	27.5 ± 3.1	-1.2 ± 1.5	1.5 ± 0.4	27.8 ± 3.2
	JumpStart®	24.7 ± 3.5	-3.6 ± 2.6	0.4 ± 0.4	24.7 ± 3.6
	Optimyc + MooR	24.1 ± 5.9	-4.8 ± 1.0	0.2 ± 0.3	24.0 ± 5.9

[1] Does not take into consideration carbon sequestered in plant biomass via photosynthesis.

Table 4.4.2: Mixed model analysis of variance assessing the effects of fertilizer treatment (Control, Synthetic N, JumpStart®, and Optimyc + MooR) on the flux rate of three greenhouse gases (carbon dioxide, CO_2; methane, CH_4; and nitrous oxide, N_2O) from the soil, and on the global warming potential (GWP), at the Guelph Switchgrass site in 2020.

Carbon Dioxide (CO_2) Flux; kg CO_2 ha^{-1} day^{-1}					
		July	August	September	October
Source of Variation	*df*	*p-value*	*p-value*	*p-value*	*p-value*
Fertilizer[1]	3	0.4718	0.4876	0.3337	0.7785
SYN vs BIO	1	0.5308	0.6198	0.3958	0.6388
JS vs OM	1	0.5087	0.9719	0.3082	0.9418
Block	1	0.1599	0.5855	0.1101	0.1496
Methane (CH_4) Flux; g CH_4 ha^{-1} day^{-1}					
		July	August	September	October
Source of Variation	*df*	*p-value*	*p-value*	*p-value*	*p-value*
Fertilizer[1]	3	0.7501	**0.0023****	0.3798	0.4936
SYN vs BIO	1	0.7865	**0.0084****	0.8977	0.1842
JS vs OM	1	1.0000	0.5655	0.1779	0.6146
Block	1	0.2046	**0.0119***	0.3001	0.9420
Nitrous Oxide (N_2O) Flux; g N_2O ha^{-1} day^{-1}					
		July	August	September	October
Source of Variation	*df*	*p-value*	*p-value*	*p-value*	*p-value*
Fertilizer[1]	3	0.4065	0.9494	0.3563	0.2386
SYN vs BIO	1	0.9897	0.7519	0.7780	0.1210
JS vs OM	1	0.9100	0.6502	0.8939	0.7907
Block	1	0.0821	0.1521	0.7838	0.4322
Global Warming Potential (GWP); kg CO_2e ha^{-1} day^{-1}					
		July	August	September	October
Source of Variation	*df*	*p-value*	*p-value*	*p-value*	*p-value*
Fertilizer[1]	3	0.4949	0.4834	0.3256	0.7872
SYN vs BIO	1	0.5314	0.6080	0.3981	0.6029
JS vs OM	1	0.5073	0.9803	0.3072	0.9336
Block	1	0.1785	0.6407	0.1147	0.1604

[1] The partitioning of treatment (fertilizer) sum of squares was done using an orthogonal contrast approach (SYN = synthetic N fertilizer; JS = JumpStart® inoculant of *Penicillium bilaiae*; OM = Optimyc + MooR inoculants of beneficial fungal and bacterial consortia; BIO = JumpStart® and Optimyc + MooR).

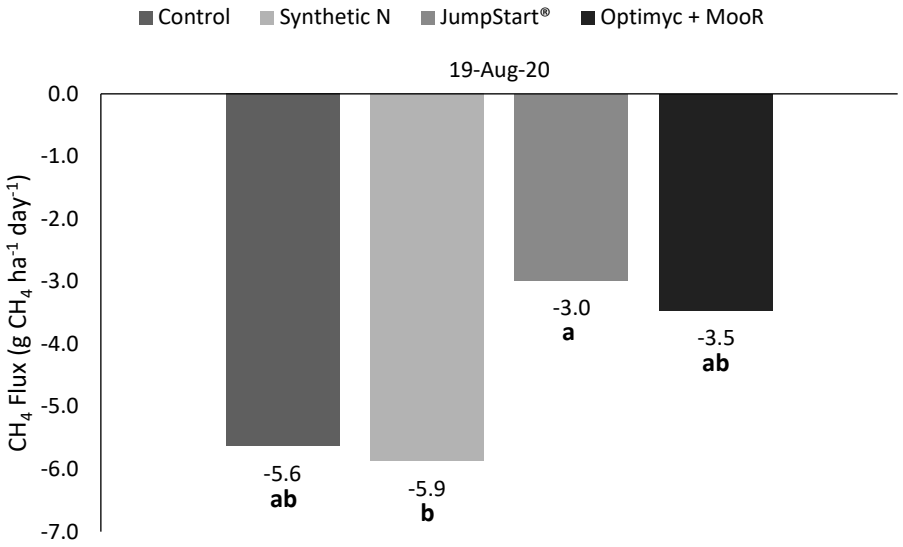

Figure 4.4.1: CH_4 flux from the soil (g CH_4 ha^{-1} day^{-1}) as influenced by fertilizer treatment at the Guelph Switchgrass site on August 19, 2020. Different letters indicate significantly different means according to least-square means analysis adjusted per the Tukey test ($p \leq 0.05$).

In addition to investigating treatment effects on the flux rate for each of these three gases individually, the effect of the fertilizers on the total global warming potential (GWP) of switchgrass plots (kg CO_2e ha^{-1} day^{-1}) was evaluated. The GWP associated with each treatment on each sampling day is presented in **Table 4.4.1**. Analysis by least-square means comparison adjusted according to the Tukey test and Dunnett's Correction, as well as defined orthogonal contrasts, indicated that there were no significant treatment effects on GWP (**Table 4.4.2**).

It was also important to investigate how the flux for each of the three greenhouse gases changed over the course of the growing season. The flux rate for each gas on each sampling date are presented in **Table 4.4.3**. CO_2 flux rates show a significant ($p < 0.05$) declining trend over the course of the growing season (**Table 4.4.4**, **Figure 4.4.2**). There is also significantly more ($p < 0.05$) CO_2 emissions during the two summer sampling days (July and August) compared to the two autumn sampling days (**Table 4.4.4**). CH_4 flux rates decline to their minimum value in

September, increasing again at the end of the growing season (**Figure 4.4.3**), however there are no significant differences among the sampling dates or seasons (**Table 4.4.4**). N_2O flux rates follow a similar trend of declining to their minimum values in August and September before rising slightly again (**Figure 4.4.4**). While there were significant differences among sampling dates – with the August 19 and September 15, 2020, N_2O flux rates being significantly lower than the July 15, 2020 flux rate – there were no significant differences observed between the summer and fall months when grouped (**Table 4.4.4**).

Table 4.4.3: Flux rate of carbon dioxide, CO_2 (kg CO_2 ha^{-1} day^{-1}; ± standard error), methane, CH_4 (g CH_4 ha^{-1} day^{-1}; ± standard error) and nitrous oxide, N_2O (g N_2O ha^{-1} day^{-1}; ± standard error) from the soil over the course of the growing season at the Guelph Switchgrass site in 2020.

Sampling Day	*CO_2 Flux*	*CH_4 Flux*	*N_2O Flux*
July 15	119.3 ± 5.8	-2.8 ± 0.9	1.9 ± 0.7
August 19	66.7 ± 3.6	-4.5 ± 0.6	-0.1 ± 0.3
September 15	38.9 ± 5.0	-5.2 ± 1.1	0.1 ± 0.3
October 14	26.8 ± 2.1	-3.4 ± 0.8	0.5 ± 0.3

Table 4.4.4: Mixed model analysis of variance assessing the effects of sampling day on the flux rate of carbon dioxide, CO_2 (kg CO_2 ha^{-1} day^{-1}), methane, CH_4 (g CH_4 ha^{-1} day^{-1}) and nitrous oxide, N_2O (g N_2O ha^{-1} day^{-1}) from the soil at the Guelph Switchgrass site in 2020.

		CO_2 Flux	CH_4 Flux	N_2O Flux
Variable	*df*	*p-value*	*p-value*	*p-value*
Month[1]	46	**< 0.0001******	0.1737	**0.0062****
Summer vs Fall		**< 0.0001******	0.4401	0.1639
Block	1	0.1941	**0.0191***	**0.0082****

[1]The partitioning of treatment (month) sum of squares was done using an orthogonal contrast approach (Summer = July 15 and August 19, 2020; Fall = September 15 and October 14, 2020)

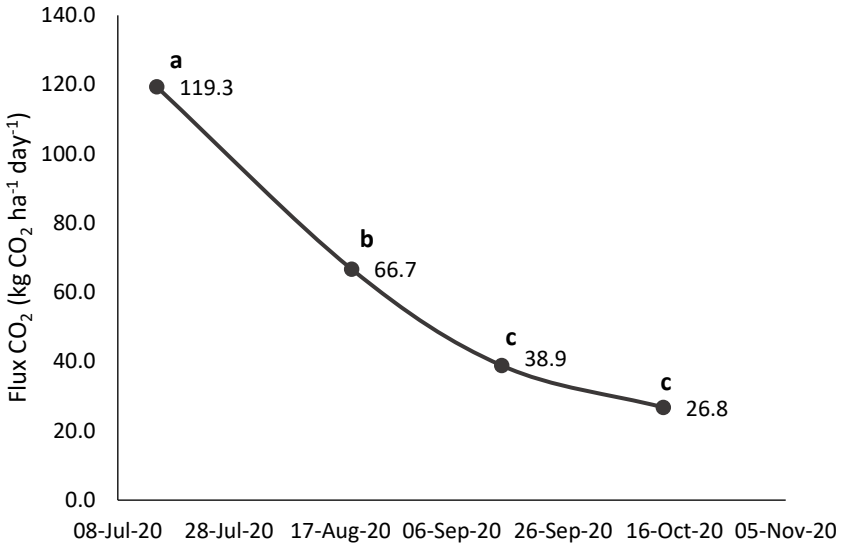

Figure 4.4.2: CO_2 flux (kg CO_2 ha^{-1} day^{-1}) at the Guelph Switchgrass site over the course of the 2020 field season. Different letters indicate significantly different means according to least-square means analysis adjusted per the Tukey test ($p \leq 0.05$).

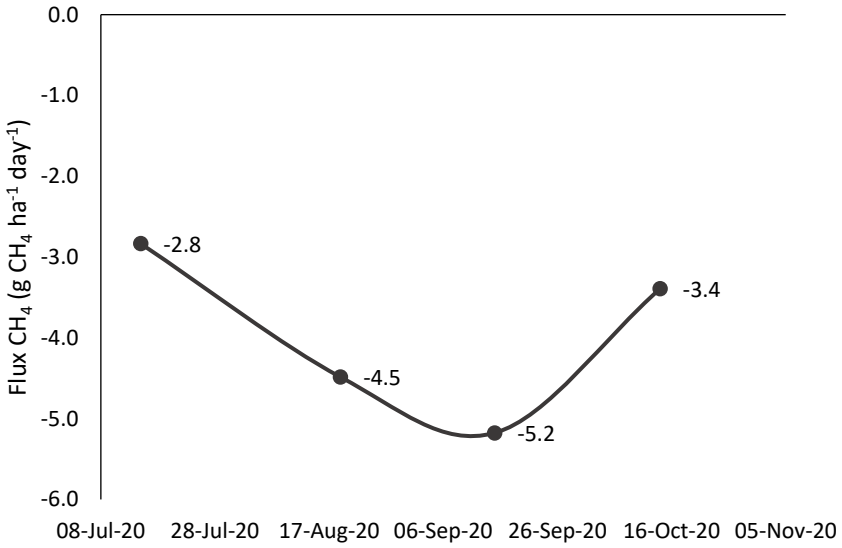

Figure 4.4.3: CH_4 flux (g CH_4 ha^{-1} day^{-1}) over time at the Guelph Switchgrass site over the 2020 field season. There are no statistically significant differences among data points ($p > 0.05$).

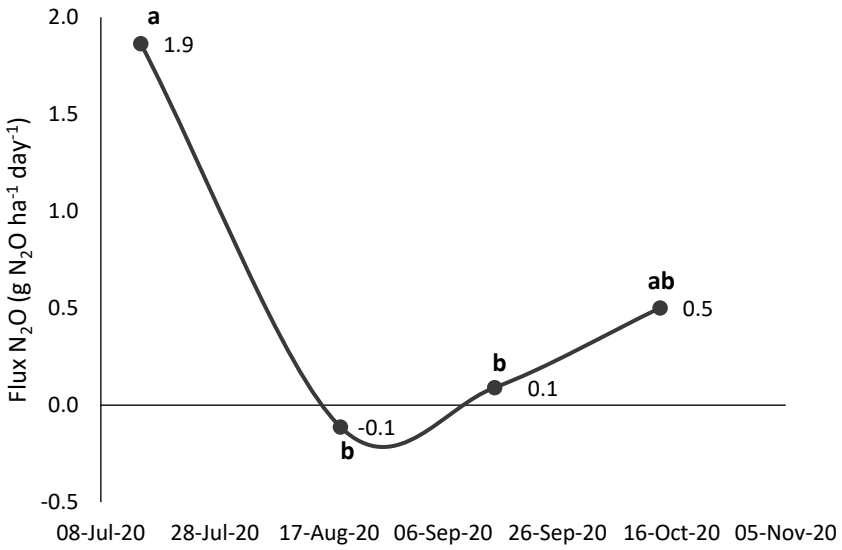

Figure 4.4.4: N_2O flux (g N_2O ha^{-1} day^{-1}) at the Guelph Switchgrass site over the 2020 field season. Different letters indicate significantly different means according to least-square means analysis adjusted per the Tukey test ($p \leq 0.05$).

Finally, linear regression analyses were conducted to determine the influence of soil temperature (˚C) and volumetric water content (%) at 10 cm depth, which can be used to explain the trends in flux rate for these gases over the course of the growing season. The regressions analyses revealed a significant ($p < 0.05$) well-fitting ($R^2 = 0.8110$) positive relationship between soil temperature and CO_2 flux (**Figure 4.4.5**). There is also a significant ($p < 0.05$) but more loosely fitting ($R^2 = 0.179$) negative relationship between soil volumetric water content and the CO_2 flux (**Figure 4.4.6**) at the GS field site. There was no significant relationship between either of these soil conditions and CH_4 or N_2O flux rates (g CH_4 ha^{-1} day^{-1} and g N_2O ha^{-1} day^{-1}, respectively) at the GS site (**Table 4.4.5**).

Table 4.4.5: Linear regression analysis of the relationship between soil temperature at 10 cm depth (˚C) and soil volumetric water content (%) on the flux rate of carbon dioxide, CO_2 (kg CO_2 ha^{-1} day^{-1}), methane, CH_4 (g CH_4 ha^{-1} day^{-1}) and nitrous oxide, N_2O (g N_2O ha^{-1} day^{-1}) from the soil at the Guelph Switchgrass site in 2020.

Soil Temperature (˚C)							
		CO_2 Flux		CH_4 Flux		N_2O Flux	
Variable	*df*	*Estimate*	*p-value*	*Estimate*	*p-value*	*Estimate*	*p-value*
Intercept	46	-78.51	< **0.0001******	-5.30	**0.0068*****	-1.37	0.1822
Slope	46	9.27	< **0.0001******	0.12	0.4677	0.13	0.0524
Soil Volumetric Water Content (%)							
		CO_2 Flux		CH_4 Flux		N_2O Flux	
Variable	*Df*	*Estimate*	*p-value*	*Estimate*	*p-value*	*Estimate*	*p-value*
Intercept	46	117.40	< **0.0001******	-5.06	**0.0022*****	0.90	0.3084
Slope	46	-259.24	**0.0028*****	5.16	0.4710	-1.49	0.7097

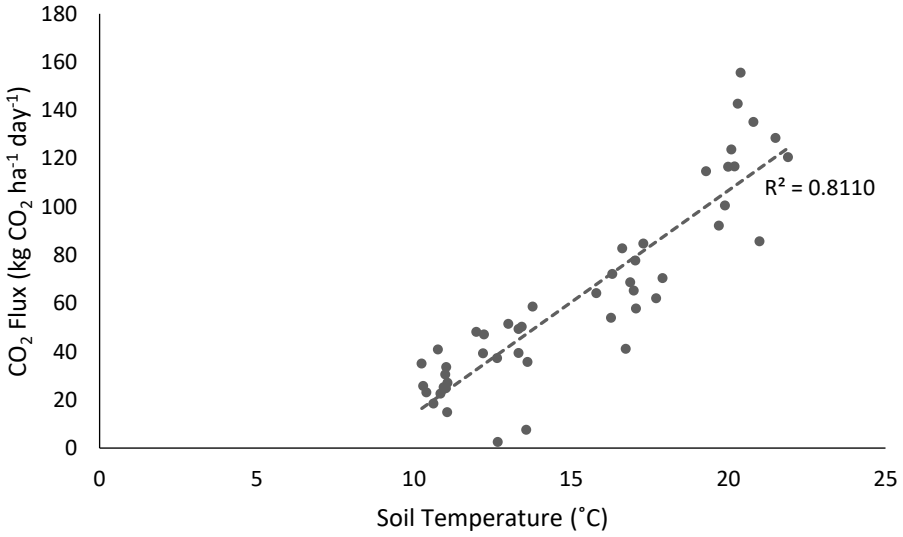

Figure 4.4.5: Linear regression model demonstrating the significant positive relationship between soil temperature (˚C) at 10 cm depth and CO_2 flux rate (kg CO_2 ha^{-1} day^{-1}) at the Guelph Switchgrass site in 2020 ($p < 0.05$, $R^2 = 0.811$).

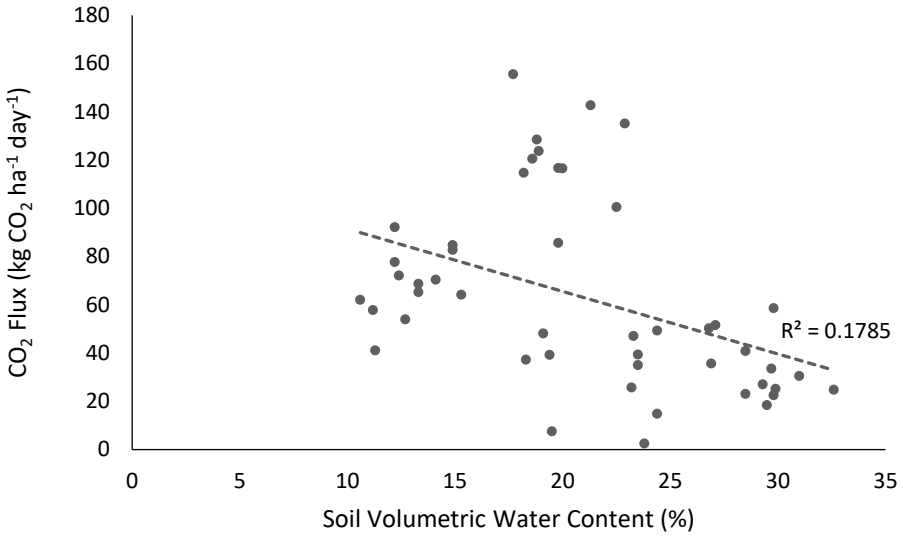

Figure 4.4.6: Linear regression model demonstrating the significant negative relationship between soil volumetric water content (%) at 10 cm depth and CO_2 flux rate (kg CO_2 ha^{-1} day^{-1}) at the Guelph Switchgrass site in 2020 ($p < 0.05$, $R^2 = 0.179$).

Chapter 5: Discussion

5.1 Plant Morphology and Biomass Yield

The most prominent agronomic input for switchgrass and miscanthus production is fertilizer, primarily synthetic N (Hall et al., 2011; Samson et al., 2018; Withers et al., 2016). However, due to the known negative environmental impacts associated with synthetic fertilizers (Ashworth et al., 2015; Bender et al., 2016; Oates et al., 2016; Steffen et al. 2015) and some inconsistency in the yield response of both of these biomass crops to synthetic N (i.e. Arundale et al., 2014; Cadoux et al., 2012; Fike et al., 2017; Finnan and Burke, 2016; Marsal et al., 2016; Owens et al., 2013; Parrish & Fike, 2005), there is growing interest in biofertilizer options like those which have been tested in the present study. Biomass crop producers must see a sufficient positive effect on plant growth and ultimately crop yield for each dollar spent on agronomic inputs to justify the investment (Zering, 2014). Therefore, a major component of this study is the data collected to determine how each fertilizer treatment affected final yield, as well as several other plant morphological characteristics, for switchgrass and miscanthus in southern Ontario. Basic data regarding the costs associated with each fertilizer treatment will also be briefly discussed.

5.1.1 Switchgrass

Autumn-harvested yield was not significantly affected by any of the five treatments at the Guelph Switchgrass (GS) field site in 2019 (**Table 4.1.7**). Yield data was not collected for the Burlington Switchgrass (BS) field site due to miscommunication that caused workers to harvest the crop before the late season data was collected. The results from the GS site indicate that the three biofertilizers applied that year (JumpStart®, MYKE® Pro, and LysteGro) produced yields that are comparable to synthetic N, and that all four fertilizers produced yields that are comparable to the control plots. Therefore, there was no yield response to the treatments as

applied that year. In 2020, however, synthetic N significantly increased fall-harvested yield compared to the combined average of the three biofertilizers applied that year (JumpStart®, AGTIV®, and Optimyc + MooR; **Figure 4.1.4**). As observed in 2019, there were no significant yield differences among any of the three biofertilizers or between any of the fertilizer treatments and the control in 2020. The change in significance of the synthetic N treatment effect from 2019 to 2020 aligns with Lemus et al. (2008b) where authors found that switchgrass crops responded to synthetic N in the two years following its application, but not in the year it was applied. Although Lemus et al. (2008b) did not apply additional N fertilizer beyond the first year of their study, and their study also did not include biofertilizer treatments, it does demonstrate that switchgrass may not be responsive to synthetic N in the first year of application as observed in the present study.

Most existing studies in the literature which investigate the use of synthetic N fertilizers in established switchgrass fields report a consistent yield response to synthetic N over multiple years at the same study site, whether the response is significantly positive (Fike et al., 2017; Guretzky et al., 2011; Lemus et al., 2008a; Vogel et al., 2002) or not significant (Fike et al., 2017; Jung and Lal, 2011; Owens et al., 2013; Palmer et al. 2014). The response observed in the present study may have changed from 2019 to 2020 because all treatments, including synthetic N, were applied much earlier in 2020. Logistical considerations and cooler spring weather led to delays in the 2019 season, meaning that treatments were not applied until July. Samson et al. (2018) recommends that growers apply the recommended synthetic fertilizers when switchgrass stems are about 15-25 cm tall, however switchgrass at the GS site averaged greater than 95 cm tall when 2019 treatments were applied (data not presented). In 2020, treatments were applied at the GS field site in late May which allowed the plants to make better use of the fertilizers earlier

in the season and for a larger portion of their active growth period. Therefore, it appears that increasing residence time for fertilizer treatments could enhance the benefits to autumn-harvested biomass yield.

It is also interesting that none of the biofertilizer treatments produced significant yield response compared among each other or to the control at the GS field site in either year. The lack of significant yield response from JumpStart® contradicts findings from the Simpson (2018) small-plot field trial comparing the yield response of switchgrass to synthetic N, JumpStart® and a control at the GS field site. In the Simpson (2018) study, JumpStart® significantly increased yield compared to the control, and had a numerically higher but not statistically different yield than plots treated with synthetic N. It is unclear why the present study observed such an opposite response compared to Simpson (2018) given that both studies occurred at the same site using the same products. The lack of significant yield response from MYKE® Pro (2019) and AGTIV® (2020) contradicts findings by Clark (2007), who found that inoculation with *G. intraradices* (the active ingredient in MYKE® Pro / AGTIV® biofertilizer) significantly increased switchgrass yield on the more neutral of the two soils they tested. The lack of significant difference between LysteGro and the control in 2019 contradicts findings from studies suggesting that biosolids fertilizers can significantly increase switchgrass yields (Liu et al., 2013; Liu et al., 2014), and there is little literature with which to contextualize the lack of significant differences in the Optimyc + MooR treatment. While this lack of significant response of switchgrass biomass yield to biofertilizers does contradict existing literature, it is worth noting that there are very few studies that have tested commercial biofertilizer products, particularly microbial inoculants, with perennial grasses. Therefore, the results from this study may simply add to the growing body of literature beginning to investigate the viability of these products for commercial biomass

production. Furthermore, this study only covers two years of application for these fertilizers, which may not have captured any potential long-term impacts of these products on switchgrass biomass yield. Therefore, it is recommended that future biofertilizer field studies should be conducted over more than two years to better account for these potential long-term effects.

Most importantly, it should be stated that the GS switchgrass plots were established in 2014 and, in the years prior to the commencement of this project, all plots received a fertilizer rate of 60 kg N ha^{-1}. This long-term application of inorganic N fertilizers (2014 to 2018) could also have contributed to the lack of significant differences reported in this thesis. Switchgrass is an excellent nutrient recycler and would be able to reuse the nutrients from these previous fertilizer applications very efficiently (Arundale et al., 2014; Guretzky et al., 2011; Parrish and Fike, 2005; Vogel et al., 2002). It is therefore recommended that any fertilizer treatment study on switchgrass should ensure that the sites being used have not received external fertilizers for at least two years prior to the experiment.

The plant morphological data from the GS field site somewhat contradicts findings from the biomass yield data derived from this site. Significantly lower peak leaf number recorded in synthetic N plots compared to the combined average of the three biofertilizer treatments (**Figure 4.1.1**), and significantly higher peak stem dry mass recorded in JumpStart® plots compared to LysteGro and MYKE® Pro (**Figure 4.1.2**) do not correspond with similarly significant differences in yield (**Table 4.1.7**). It is unclear why these significant treatment effects on plant morphology did not translate into significant yield effects in 2019. Furthermore, there were no observed treatment effects on any plant morphological metrics for switchgrass at the BS field site (**Table 4.1.2**). These discrepancies between the sites makes it more difficult to explain treatment effects on plant morphological parameters. Possible explanations include geographical

differences in soil texture (sandy loam to loam at GS site, silt loam at BS site), baseline nutrient availability, and weather. We did not collect any weather data at either (GS and BS) site, but data from the nearest climate stations indicate that the BS site had warmer temperatures and increased precipitation compared to the GS site. Despite some of these discrepancies, it can be speculated that significant differences observed for stem weight and leaf number at the GS field site may not have translated to significant differences in yield because these differences were observed at the peak values for the measured morphological traits, not at the end of the season when the yield data was collected.

In 2020, synthetic N fertilizer significantly increased peak tiller height (cm) compared to the control and the combined mean tiller height for the three biofertilizer treatments (**Figure 4.1.3**). This result corresponds with significant increases to autumn biomass yield (tonnes ha^{-1}) observed in synthetic N plots compared to the combined average of the three biofertilizer treatments during 2020 (**Figure 4.1.4**). The lack of significant treatment effects on autumn tiller density (tillers ha^{-1}) at the GS field site in either 2019 or 2020 (**Table 4.1.6**) suggests that fertilization does not influence yield through effects on tiller density, but rather through its effects on the biomass produced by each tiller. There is little to no existing research investigating the link between morphological characteristics and yield in switchgrass biomass crops, however, so these results merit further investigation.

Finally, it is important to consider the cost effectiveness of each of the treatments based on the yield achieved per dollar spent. While a complete economic evaluation is beyond the scope of this thesis, some preliminary insights can be gained by comparing the cost of each treatment to the biomass yield it produced for switchgrass in this study. **Table 5.1.1** provides a breakdown of the cost associated with each treatment at the rate applied in this study, along with the mean

yield that the treatment produced over all available years of study for both switchgrass and miscanthus (to be discussed in the next section). It is clear from this data that synthetic N applied at 60 kg N ha^{-1} is the least expensive of all fertilizer treatments. For switchgrass, synthetic N fertilizer also produced the highest yield over the two study years, making this treatment the most logical choice for growers. LysteGro biofertilizer produced the second highest mean biomass yield for switchgrass based on the single year it was applied, demonstrating that this treatment also represents a viable option for increasing switchgrass yield. However, LysteGro is also the most expensive biofertilizer option which may become prohibitive to growers who would otherwise be interested in choosing this biofertilizer as an alternative to synthetic fertilizers. Furthermore, it is important to note that all biofertilizer treatments from this study are more expensive for growers to apply than the traditional synthetic fertilizer. Therefore, if biofertilizers are established as a viable method of increasing switchgrass yield while reducing the environmental impact of its production compared to synthetic N fertilizers, government policies should be established to provide financial support to growers choosing to apply effective biofertilizers in place of synthetic fertilizers.

Table 5.1.1: Product pricing ($ CAD) and biomass yield (tonnes ha^{-1}) for fertilizer treatments options applied to mature switchgrass at the Guelph Switchgrass site and mature miscanthus at the Burlington Miscanthus site.

		Yield (tonnes ha^{-1}) [1]	
Treatment	*Price ($ ha^{-1} year^{-1})*	Switchgrass (GS)	Miscanthus (BM)
Synthetic N (60 kg N ha^{-1})	28.97	11.33	13.05
JumpStart®	88.65 - 100.79[2]	8.70	12.09
MYKE® Pro (2019) / AGTIV® (2020)	34.45-35.70[3]	8.61	16.22
Optimyc + MooR (2020 only)	98.84[4]	8.56	N/A
LysteGro (2019 only)	100.58	10.66	11.68

[1] Averaged over all available years of study.
[2] Price changes depending on the amount purchased and is based on the application rate used in 2020, which was triple the rate applied in 2019.
[3] Price changes depending on the amount purchased and is based on cost of AGTIV® (agricultural-grade version of the product), not MYKE® Pro (retail-grade version of the product).
[4] Average annual cost, based on the following 7-year cycle: Optimyc and MooR both applied in year 1, just MooR applied in years 2-4, and no applications in years 5-7.

5.1.2 Miscanthus

At the Burlington Miscanthus (BM) field site, autumn-harvested miscanthus biomass yield was not significantly affected by any of the treatments in 2019 (**Table 4.1.14**). This indicates that, in the first year of application to mature miscanthus stands, all three biofertilizers (JumpStart®, MYKE® Pro, and LysteGro) produced statistically similar yields to the synthetic N fertilizer, and that all four fertilizer treatments produce statistically similar yields to the control. As observed with switchgrass, however, yield response to some of the treatments became significant in the second study year.

In 2020, synthetic N significantly lowered autumn-harvested miscanthus biomass yield at the BM site compared to the control (**Figure 4.1.6**). This result was unexpected and does not fit with most of the literature which reports either a significantly positive or no significant yield response of miscanthus to N fertilizer (Arundale et al., 2014; Cadoux et al., 2012; Christian et al., 2008; Davis et al., 2014; Finnan and Burke, 2016; Lee et al., 2017; Shield et al., 2014). The only study that indicates a potential negative response of miscanthus yield to N fertilizer is Lewandowski

and Schmidt (2006) which reports a negative response to total soil N exceeding 114 kg N ha^{-1} according to their boundary line model developed using data collected in southwest Germany. End-of-season season soil fertility data collected in 2019 indicates that control plots had a slightly lower availability of soil NO_3^- than plots receiving synthetic N (**Table 4.2.7**). This small is unlikely to explain the observed differences in yield. Future research should continue to examine the relationship between miscanthus biomass yield and total soil N availability to determine under which conditions miscanthus is most likely to benefit from N fertilization.

Orthogonal contrast statistical analyses also found that miscanthus at the BM field site was significantly higher yielding in plots receiving AGTIV® compared to plots receiving JumpStart® in 2020 (**Figure 4.1.7**). AGTIV® plots had the highest autumn-harvested biomass yield of all treatments, but this difference only achieved statistical significance in this direct contrast comparison with JumpStart® which has the lowest yield of all the treatments. There does not seem do be any other literature that has investigated inoculants of *G. intraradices* (the active ingredient in AGTIV®) as a biofertilizer for miscanthus. Therefore, this study represents novel results suggesting that commercial inoculants of *G. intraradices*, like MYKE® Pro and AGTIV® by Premier Tech, should be further investigated on commercial miscanthus field sites.

It was important to measure treatment effects on plant morphological metrics to begin to understand which aspects of plant growth are affected to produce any observed treatment effects on yield. There were no significant treatment effects on the peak values of the measured plant morphological metrics for miscanthus at the BM field site in 2019 (**Table 4.1.9**). There were also no significant treatment effects on peak miscanthus tiller height in 2020 (**Table 4.1.11**). Therefore, it can be concluded that treatments produced comparable patterns in plant morphological development in the first two years of application to mature miscanthus. While the

lack of significant differences observed here correspond with the lack of significant treatment effects on autumn-harvested biomass yield at this site in 2019, significant differences in yield were observed in 2020 despite the lack of significant treatment effects on peak tiller height. This indicates that peak tiller height is not likely to be a key factor driving yield differences at the BM field site. Autumn tiller density, however, may be a stronger determining factor of miscanthus yield. The results of this study found no significant treatment effects on autumn tiller density in 2019 (**Table 4.1.13**), but AGTIV® significantly increased autumn tiller density compared to JumpStart® in 2020 (**Figure 4.1.5**). These results follow the same trend as were observed for autumn-harvested miscanthus biomass yield at the BM site in their respective years. This suggests that any inputs that act to increase miscanthus tiller production may be more likely to increase final biomass yield at the end of the season, however further research is required to confirm this thought.

Finally, it is important to assess the cost effectiveness of each of the treatments based on the yield achieved per dollar spent. As stated above, a complete economic evaluation is beyond the scope of this thesis. However, some preliminary insights can be gained by comparing the cost of each treatment to the biomass yield it produced for miscanthus in this study for each of the treatments. For a breakdown of the cost associated with each treatment at the rate applied in this study, along with the mean yield that the treatment produced over all available years of study for both switchgrass and miscanthus, please refer back to **Table 5.1.1**.

This data demonstrates that synthetic N applied at 60 kg N ha^{-1} is the least expensive of all fertilizer treatments. Unlike switchgrass, however, synthetic N fertilizer did not result in the highest miscanthus biomass yield over the two study years. The highest miscanthus biomass yields were achieved by MYKE® Pro / AGTIV® (2019/2020), which also represents the least

expensive biofertilizer treatment in this study. Furthermore, the MYKE® Pro / AGTIV® biofertilizer was more cost effective than the synthetic N fertilizer based on the data from the present study. MYKE® Pro / AGTIV® produced an average biomass yield of 0.45-0.47 tonnes ha^{-1} yr^{-1} for each dollar spent on the biofertilizer (dependant on the amount purchased). Conversely synthetic N fertilizer produced an average biomass yield of 0.45 oven dried tonnes ha^{-1} yr^{-1} per dollar spent on the fertilizer. These estimations were made by dividing the mean annual biomass yield of each treatment by its annual cost per hectare. This demonstrates how MYKE® Pro / AGTIV® may represent both an economically and environmentally beneficial option for miscanthus producers, as long as the yield benefits associated with this biofertilizer are consistent over numerous years. It remains important to emphasize, however, that all biofertilizer treatments from this study are more expensive than the traditional synthetic fertilizer. If biofertilizers are established as a viable method of increasing miscanthus yields while reducing the environmental impact of its production compared to synthetic N fertilizers, government policies should be established to provide financial support to growers choosing to apply effective biofertilizers in place of synthetic fertilizers.

5.2 Soil Fertility and Nutrient Uptake

5.2.1 Switchgrass

When farmers apply a fertilizer, the expectation is that it will enhance availability of nutrients in the soil and facilitate nutrient uptake by crops to fuel their growth and yield. ANOVA analysis, as indicated in **Table 4.2.2**, resulted in treatments having no significant influence on any of the tested soil nutrients. Given this outcome, to address the overall goal of this study (evaluating the potential use of biofertilizers as an environmentally friendly alternative for biomass growers), orthogonal contrast analyses assessing the effects of fertilizer treatments

on soil nutrient availability were used. Interestingly, soil mineral N availability (NO_3^- or NH_4^+) was never significantly higher in synthetic N plots compared to the control at the GS site (**Table 4.2.2**). However, switchgrass receiving synthetic N had significantly higher tissue N concentrations at the peak of the growing season compared to other treatments (**Figure 4.2.6**, **Figure 4.2.9**) which then resulted in significantly higher biomass yield in 2020. The plant uptake of N from soil could have resulted in low residue soil N levels, as observed in Owens et al. (2015) where fertilizer N recovery was high with low initial soil N levels, and thereby showing lack of significance among treatments. Furthermore, switchgrass can effectively translocate nutrients from aboveground tissue to the root system for storage in belowground structures during the winter months (Lemus et al., 2008b; Wayman et al., 2014). This translocation of N taken up by the plant during active growth stages and storage in belowground structures after senescence may explain the lack of response to fertilizer treatments on biomass yields. However, the fact that in general there were no significant differences in biomass yields (2019) as influenced by fertilizer treatments is promising in relation to the use of biofertilizers in switchgrass cultivation.

Orthogonal contrast analysis indicated that LysteGro significantly increased soil NO_3^- compared to the combined average of JumpStart® and MYKE® Pro in autumn 2019, and JumpStart® plots had significantly lower autumn soil NO_3^--N availability compared to the combined average of LysteGro and MYKE® Pro. These differences driven by relatively large increases in soil N with LysteGro application agrees with existing literature which consistently reports an increase in soil fertility, and specifically increased soil N availability, with biosolids fertilizer application (Asemaninejad et al., 2021; Athemenh et al., 2015; Li et al., 2013; Price et al., 2015).

Looking at the effects of the biofertilizer treatments on nutrients other than N, field data indicates that there may be some improvements to soil fertility although the results vary among years. First, soil P in autumn 2019 was significantly higher in the combined average of JumpStart® and LysteGro plots compared to plots receiving MYKE® Pro (**Figure 4.2.2**), with JumpStart® producing the highest soil P availability (on par with synthetic N plots) among all the treatments in that year (**Table 4.2.1**). This agrees with existing literature that clearly demonstrates the ability of *P. bilaiae*, the active fungus in JumpStart®, to solubilize soil P (Asea et al., 1988; Leggett et al., 2015; Takeda and Knight, 2006; Wakelin et al., 2004). However, the performance of inoculants of P-solubilizing microbes as reported in the literature can be more variable under field conditions than controlled lab and greenhouse experiments due to differences in soil chemistry, physical structure, and climatic conditions (Alori et al., 2017; Sharma et al., 2013). The performance of P-solubilizing microbes can also be influenced by interactions with other soil microbes (Alori et al., 2017). Therefore, no firm conclusions can be made from this study, unless similar research is undertaken in field conditions for at least 3 to 4 years. In this context, annual variations in these conditions over the course of this study may explain the difference in soil P availability associated with JumpStart® from 2019 to 2020. Furthermore, the 2020 soil samples were collected at the peak of the growing season when plants actively take up nutrients from the soil, so if JumpStart® is contributing to enhanced soil P availability as well as enhanced P uptake by plants, this may also explain the reduced soil P availability observed at this time.

Peak season soil P under Optimyc + MooR in 2020 was higher than any of the other treatments, being significantly higher than the combined average of AGTIV® and JumpStart® (**Figure 4.2.4**). AGTIV® had the lowest soil P level and was significantly lower than the

combined average of JumpStart® and Optimyc + MooR (**Figure 4.2.4**). The lower soil P level in AGTIV® plots agrees with Ll et al. (2012) who found that soil P decreased under inoculation with *G. intraradices* (the active fungus in AGTIV®), while plant P content improved. Understanding the mechanism behind improved soil P availability under Optimyc + MooR is more complex due to the multiple fungi and bacteria contained in the two products. Furthermore, the plots that were treated with Optimyc + MooR in 2020 had received LysteGro in 2019 which may be contributing to the improved soil P availability observed for the Optimyc + MooR treatment in 2020. There is also a lack of literature documenting the effects of the various fungi and bacteria in the Optimyc + MooR products on soil nutrient availability, let alone their potential interactions, making it difficult to predict how much these inoculants may be contributing to the observed results. Optimyc + MooR also produced the highest peak season soil K in 2020, being significantly higher than JumpStart® (**Figure 4.2.5**). Again, the potential contributions from LysteGro applied on these plots in the previous year and lack of literature documenting the exact activities and interactions of the bacteria and fungi in these biofertilizer treatments makes it difficult to fully explore these results.

The next component of this study was to evaluate the peak season switchgrass tissue nutrient concentrations. This data can indicate the treatments' contributions to plant uptake of soil nutrients. All values for peak season switchgrass tissue nutrient concentrations observed in the present study (**Table 4.2.3**, **Table 4.2.4**) fall within or only slightly below values previously reported in the literature for switchgrass grown across the United States, which range from 2.0 to 35 g N kg^{-1} (0.20-3.5% N) (de Koff and Allison, 2015; Makaju et al., 2013; Mulkey et al., 2006; Sadeghpour et al., 2014; Waramit et al., 2011), 0.2 to 3.3 g P kg^{-1} (0.02-0.33% P), 0.1 to 21.6 g K kg^{-1} (0.01-2.16% K), 0.6 to 2.6 g Mg kg^{-1} (0.06-0.26% Mg), and 1.4 to 3.0 g Ca kg^{-1} (0.14-

0.30% Ca) (de Koff and Allison, 2015; Makaju et al., 2013; Sadeghpour et al., 2014). Therefore, switchgrass in this study was able to uptake nutrients and incorporate them into biomass within the range that is typical for the species regardless of fertilizer treatment. This is promising in the effort of promoting biofertilizers as environmentally friendly alternatives to inorganic fertilizer use by Ontario switchgrass growers.

The present study found that 60 kg N ha^{-1} of synthetic N fertilizer significantly increased peak season switchgrass tissue N concentration compared to the control at the GS field site in both study years (**Figure 4.2.6**, **Figure 4.2.9**). Increases in switchgrass tissue N with synthetic N fertilizer were also observed at the BS field site from 2019 (**Table 4.2.4**) but were not statistically significant (**Table 4.2.6**). Waramit et al. (2011) reported significant differences in switchgrass tissue N among N rates earlier in the growing season during the vegetative growth stage, but these differences lessened as the season progressed into the autumn months, especially for their lower N rate (65 kg N ha^{-1}) which is closer to the application rate used in the present study. Lemus et al. (2008a) reported similar switchgrass tissue N concentrations among 0, 56, and 100 kg N ha^{-1} fertilization rates, but a significant increase in switchgrass tissue N at 200 kg N ha^{-1}. Sadeghpour et al. (2014) observed a significant increase in switchgrass tissue N with synthetic N fertilizer application at 134 kg N ha^{-1}, but the lower N rate (67 kg N ha^{-1}) slightly reduced tissue N compared to the control. Therefore, the significant increase in switchgrass tissue N with synthetic N fertilizer observed in this study seem to fit with the general trend of increasing tissue N with increasing N fertilizer rates observed in the literature, although the results from the present study indicate increasing tissue N at a lower N rate than what has previously been reported.

Sadeghpour et al. (2014) have also reported significant reductions to switchgrass tissue P content with increasing N rate, and like with tissue N, tissue K slightly declined in the 67 kg N ha^{-1} treatment compared to the control but increased significantly in the 134 kg N ha^{-1} treatment. Although there were no significant effects of synthetic N on switchgrass tissue nutrient concentrations except N in the present study, there were small numerical increases in tissue P at the GS site (**Table 4.2.3**) that follow similar trends as those reported by Sadeghpour et al. (2014). The present study also found numerical increases in switchgrass tissue K concentration at 60 kg N ha^{-1} compared to the control, which agrees with the general trend of increasing tissue K with increasing synthetic N application observed by Sadeghpour et al. (2014). Sadeghpour et al. (2014) have also reported no significant effect of N fertilizer treatment on switchgrass tissue Mg or Ca content which agrees with the results from the present study.

Results from this research regarding the significant increase in switchgrass tissue concentrations of key nutrients associated with biofertilizers is a novel addition to the literature. Few trials have investigated the use of biofertilizers (especially microbial inoculants) for switchgrass, and those that do exist focus primarily on biomass yield responses, as well as some insight into biomass quality (i.e., An *et al.*, 2008; Clark, 2007; Liu et al., 2013; Liu et al., 2014; Simpson, 2018). Therefore, the results showing a significant increase in switchgrass tissue K with Optimyc + MooR compared to the combined average of AGTIV® and JumpStart® at the GS site in 2020 (**Figure 4.2.10**) is one of the first indications that this type of biofertilizer may be able to significantly improve uptake of these nutrients by switchgrass. As with the soil data, however, it is important to note that the significant effect of Optimcy + MooR on plant tissue nutrient concentrations observed in 2020 may be partially connected to the application of LysteGro on the same plots in the previous season (2019). For this reason, additional research

should be conducted to test these products separately and together to determine which treatment is responsible for the enhanced tissue nutrient concentrations, and to see if there may be any additional benefits to applying Optimyc + MooR in the year following the application of LysteGro.

5.2.2 Miscanthus

In 2019, soils at the BM field site demonstrated significant treatment effects on soil P availability, whereby JumpStart® produced significantly lower soil P levels than the combined average of MYKE® Pro and LysteGro, and MYKE® Pro produced significantly higher soil P levels than the combined average of JumpStart® and LysteGro (**Figure 4.2.11**). Furthermore, JumpStart® produced the lowest soil P levels out of all treatments at the end of the season in 2019 (**Table 4.2.7**). This contradicts existing literature which has repeatedly reported improved soil P availability when soils were inoculated with *P. bilaiae* (Asea et al., 1988; Leggett et al., 2015; Takeda and Knight, 2006; Wakelin et al., 2004). As mentioned in the previous section, however, the performance of P-solubilizing microbes can vary depending on several factors, including soil physical conditions, soil chemistry, interactions with other soil microbes, and climatic conditions (Alori et al., 2017; Sharma et al., 2013). Because most of these variables were not measured during this study, the reason that JumpStart® inoculant of *P. bilaiae* failed at this site in 2019 cannot be determined.

Regarding the heightened soil P under MYKE® Pro, there is uncertainty in the literature regarding the ability of *G. intraradices* (the active ingredient in MYKE® Pro) to directly contribute to the solubilization of soil P bound to soil particles. Antunes et al. (2007) reports no evidence of altered pH or improved P availability by *G. intraradices* beyond enhancing plant access to existing sources of available P by exploiting a larger volume of the growth medium.

However, studies have generally found improved soil P availability and plant uptake when soils are inoculated with *G. intraradices*, which may be due to direct contributions by the mycorrhizal fungus itself or symbioses between *G. intraradices* and other P-solubilizing microbes in the soil (Antunes et al., 2007; Villegas and Fortin, 2001; Villegas and Fortin, 2002). Therefore, it is possible that *G. intraradices* improved P availability in the soil due to direct contributions to P solubilization or through symbioses with existing soil microbial communities, explaining the increased soil P observed in the present study. There remains, however, a poor understanding of how mycorrhizal fungi may contribute to mediating soil P availability which needs to be further explored to support more concrete explanations for this observation.

In 2020, there were no significant treatment effects on soil nutrient availability. This may be because soil samples in this year were collected at the peak of the growing season when plants are actively taking up nutrients from the soil to incorporate into their biomass thus masking treatment effects on the availability of those nutrients in the soil. Therefore, it was important to also investigate treatment effects on miscanthus tissue nutrient concentrations at the peak of the growing season, which will be discussed next.

When searching the literature to determine whether the plant tissue nutrient concentrations from this study align with previously reported values, no studies could be found reporting miscanthus plant tissue Ca concentrations. Furthermore, only one study reported miscanthus tissue Mg concentrations, and exact values were not reported, so the range was estimated from figures presented in the paper which was by Himken et al. (1997). With these limitations in consideration, all values for peak season miscanthus tissue nutrient concentrations (**Table 4.2.9**) fall within ranges previously reported for various miscanthus genotypes (including cultivars of *Miscanthus sinesis* and *Miscanthus* × *giganteus*), which range from 1.7 to 59.2 g N kg^{-1} (0.17-

5.9% N) (Beale and Long, 1997; Cadoux et al., 2012; Dohleman et al. 2012; Himken et al., 1997; Yu et al., 2013), 0.4 to 7.5 g P kg^{-1} (0.04-0.75% P) (Beale and Long, 1997; Cadoux et al., 2012; Himken et al., 1997; Yu et al., 2013), 2.5 to 62.6 g K kg^{-1} (0.25-6.3% K) (Beale and Long, 1997; Cadoux et al., 2012; Himken et al., 1997; Yu et al., 2013), and approximately 1.0 to 2.5 g Mg kg^{-1} (0.1-0.25% Mg) (Himken et al., 1997). Therefore, the miscanthus in this study seems to have been able to uptake nutrients and incorporate them into their biomass within the range that is typical for the species regardless of fertilizer treatment. It is important to note, however, that Yu et al. (2013) found that genotype was a significant factor influencing miscanthus tissue nutrient dynamics and no studies could be found reporting tissue nutrient concentrations for *M. saccariflorus* which is the species used in the present study. This creates difficulty in determining whether the plant tissue nutrient data from the present study is typical of *M. sacchariflorus* specifically. However, this also means that the present study represents a novel contribution to the literature which may begin to build a precedent for normal plant tissue nutrient concentrations for *M. sacchariflorus*.

The treatment effects on peak season miscanthus tissue nutrient concentrations differed among study years, with far more significant effects occurring in 2019 compared to 2020. In 2019, LysteGro significantly increased plant tissue N concentrations compared to the combined average of JumpStart® and MYKE® Pro (**Figure 4.2.12**). JumpStart® had significantly lower tissue Mg than the combined average of MYKE® Pro and LysteGro (**Figure 4.2.14A**) and MYKE® Pro also produced significantly lower tissue Mg than the combined average of JumpStart® and LysteGro (**Figure 4.2.14B**). LysteGro had numerically higher mean tissue concentrations for P, K, and Ca in 2019 compared to all other treatments (**Table 4.2.9**), contributing to significant differences whereby synthetic N produced significantly lower

miscanthus tissue K than the combined average of all three biofertilizers (**Figure 4.2.13**) and JumpStart® produced significantly lower miscanthus tissue Ca than the combined average of MYKE® Pro and LysteGro (**Figure 4.2.15**). The contributions of LysteGro to significant increases in miscanthus tissue nutrient concentrations in 2019 is unsurprising because the product itself adds organic forms of a wide variety of nutrients to the soil, meaning more of these nutrients would be available for plant uptake. This agrees with observations from Kołodziej et al. (2016) which reported higher plant tissue concentrations of all nutrients measured in their study with the application of biosolids, even at the end of the growing season. Smith and Slater (2010) also report significant increases in end-of-season miscanthus tissue N with application of a biosolids biofertilizer (like LysteGro), however these authors do not report significant differences from the control for any other nutrient.

The only significant treatment effects observed for miscanthus tissue nutrients in 2020 was that synthetic N significantly increased tissue Ca concentrations compared to the combined average of the two biofertilizers applied that year (**Figure 4.2.16A**), and that AGTIV® produced a significantly lower tissue Ca concentration compared to JumpStart® (**Figure 4.2.16B**). Increased peak season miscanthus tissue Ca concentration disagrees with findings from Gołąb-Bogacz et al. (2021), which reports no significant effect of 60 kg N ha^{-1} fertilizer on miscanthus tissue Ca content compared to the control. No other literature has reported the effects of synthetic N fertilizer on Ca uptake by miscanthus but increasing tissue Ca content with increasing NH_4^+-N fertilizer has been observed for *Eucalyptus urograndis* seedlings (Santos et al., 2020).

5.2.3 Incubation Study

The best evidence of how each fertilizer treatment affected soil fertility in this project comes from the incubation experiment, which demonstrated significant increases in N, P, and K

availability associated with the various treatments. The significant increase in soil NO_3^--N, NH_4^+-N, and total mineral N by LysteGro (**Figure 4.2.17**) can likely be attributed to the supply of readily mineralizable organic N in the biosolids material. This agrees with existing literature, as increased availability of NO_3^--N and NH_4^+-N with the application of municipal biosolids has been observed in numerous instances (Asemaninejad et al., 2021; Athemenh et al., 2015; Hartl et al., 2003; Horrocks et al., 2016; Li et al., 2013; Oladeji et al., 2019; Price et al., 2015; Ramadass and Palaniyandi, 2007; Ranjbar et al., 2016; Rigby et al., 2016). One study contradicting these results is Majeed et al. (2021), which reports numerically higher soil mineral N in their control plots compared to any of their biosolids treatments, however none of the differences are statistically significant. The authors explain that this is likely due to enhanced vegetative growth in biosolids-treated plots; because the plants had access to more nitrogen during their growth, an increased amount of nitrogen was taken up from the soil compared to control plots where plants had to grow under lower N availability (Majeed et al., 2021). Therefore, this study indicates support for increasing soil mineral N under biosolids applications, like LysteGro. The significant increases in all three forms of soil N associated with synthetic N fertilizer (**Figure 4.2.17**) is also likely due to the direct addition of labile N as urea and increased N mineralization in the soils and therefore these results also agree with existing literature (Malhi et al 2006; Nascente et al., 2017).

No previous studies reporting increased soil N availability associated with inoculation of either *P. bilaiae* (JumpStart®) or *G. intraradices* (MYKE® Pro) could be found, therefore these observations represent novel additions to the literature. Although 30 kg N ha^{-1} synthetic N fertilizer was applied with the inoculants, this occurred several months before samples were collected for the incubation study and it is therefore expected that this small dose of fertilizer

would not explain the significant differences observed in this study. Veresoglou et al. (2012) summarizes existing evidence that AMF, like *G. intraradices*, may contribute to increased soil N availability by altering the surrounding soil microbial community in ways that promote N fixation, nitrification, and the release of complex forms of organic N through accelerated decomposition, as well as reducing soil N losses through leaching and denitrification; these mechanisms may explain how MYKE® Pro may have created the significant increase in soil N observed in the present study. Direct evidence of the influence that *G. intraradices* may have had on the soil N cycle cannot be observed from the data collected in the present study, however, so contributions to increased soil N availability through these effects cannot be commented on. These initial observations of commercial biofertilizers promoting increased soil N availability are promising, but further studies should be conducted to confirm these observations and to clarify the mechanisms driving this response.

Soil P availability was significantly higher in all fertilizer treatments compared to the control and statistically similar among each other (**Figure 4.2.18**). Increased soil P availability in soils treated with synthetic N fertilizer has been previously reported in the literature (Zhang et al., 2021), however most studies report a decline in soil available P with synthetic N fertilizer applications (Chen et al., 2018; Jing et al., 2021; Ll et al., 2012 Singh et al, 2001). Jing et al. (2021) and Ll et al. (2012) explain that soil available P likely declined in their study due to increased plant uptake of the most readily available forms of P. However, Jing et al. (2021) also reports higher rates of P mobilization from stable to bioavailable forms with higher N application rates. Because the soils in the incubation experiment were isolated from plants, soil P may have increased due to this increased P mobilization in the absence of plant uptake. Zhang et al. (2021) also report that increased P bioavailability in soils receiving synthetic N fertilizers may be

explained by increased soil acidity (lower soil pH) which mobilizes calcium-phosphate compounds. This effect is also likely to have occurred in the soils from the present study as these soils are calcareous, with a pH of 7.5 at the time the incubation study samples were collected, and therefore contains P bound in calcium-phosphate compounds. It should be noted, however, that pH was not measured over the course of the incubation experiment.

Increased soil P has been previously reported for biosolids fertilizers like LysteGro (Davis et al., 2018; Horrocks et al., 2016; Maguire et al., 2001; Ramadass and Palaniyandi, 2007; Ranjbar et al., 2016; Warman et al., 2004) and *P. bilaiae* inoculants like JumpStart® (Asea et al., 1988; Leggett et al., 2015; Takeda and Knight, 2006; Wakelin et al., 2004). Increased soil P observed in soils treated with municipal biosolids has been attributed to the presence of plant-available P in the biosolids material, as well as stabilization of existing soil P and enhanced soil microbial activity resulting in increased soil P (Horrocks et al., 2016; Ramadass and Palaniyandi, 2007; Ranjbar et al., 2016). While one study by Hartl et al. (2003) did not observe significant increases to soil P with biosolids application, the authors report that crop uptake and removal of P was approximately equal to the amount of P applied with their biosolids treatments. This effect of plant growth and uptake on soil P accumulation would not be observed in the incubation study because bare soils were used for the experiment, therefore there was no influence active plant growth. *P. bilaiae* is a P-solubilizing fungus which acts by secreting oxalic and citric acids which act as chelating agents and acidifying the soil (Cunningham and Kuiack 1992; Takeda and Knight, 2006). Soil P availability has been shown to increase with inoculation of *G. intraradices* due to localized changes in pH induced by the AMF causing increased solubility of inorganic P compounds and/or through symbioses between *G. intraradices* and other soil microbes (Villegas and Fortin, 2001; Villegas and Fortin, 2002). However, there is a generally poor understanding in

the literature regarding the direct contributions of AMF like *G. intraradices* to improved soil P solubility (Antunes et al, 2007). Overall, the increased soil P availability observed for the three biofertilizer treatments in the present study fits with existing patterns in the literature, despite the poor understanding of the direct mechanisms driving observed improvements to soil P availability and plant uptake with AMF inoculation.

Soil K availability was only significantly higher than the control in soils receiving LysteGro (**Figure 4.2.19**). This finding agrees with existing literature where there are numerous studies reporting increased soil K availability after biosolids applications due to direct additions of labile forms of K (Castro et al., 2009; Hartl et al., 2003; Ramadass and Palaniyandi, 2007; Ranjbar et al., 2016; Warman et al., 2004). Therefore, the present study adds to the existing body of research indicating that biosolids fertilizers like LysteGro can serve as an effective means of enhancing soil K. While there were no other significant treatment effects on soil K, it may be worth continuing to investigate the ability of AMF microbial inoculants to increase soil K availability as MYKE® Pro resulted in numerically higher soil K levels than the control which could become statistically significant under different environmental conditions.

Overall, the incubation study has provided clear evidence that JumpStart®, MYKE® Pro, and LysteGro biofertilizers can contribute to improved soil fertility by significantly increasing the availability of nitrogen and phosphorus, as well as enhanced potassium availability under LysteGro. The lack of existing literature documenting these effects, especially for the microbial inoculants JumpStart® (*P. bilaiae*) and MYKE® Pro (*G. intraradices*) make it difficult to contextualize and explain the results observed in the present study, however the results are promising and should justify further research into the mechanisms driving these observations.

Further studies should also be conducted to confirm that these soil fertility enhancements by biofertilizers also occur under field conditions.

5.3 Soil Biological Health

5.3.1 Switchgrass

Treatment effects on the abundance of 16S bacterial and 18S fungal gene abundance varied by site and by year, likely due to differences in soil and climatic conditions. At the GS field site in 2019, only MYKE® Pro significantly increased autumn 16S gene abundance compared to the combined average of JumpStart® and LysteGro (**Figure 4.3.1**). Conversely, peak season data from the BS field site in 2019 indicates significant negative effects of MYKE® Pro on both 16S bacterial and 18S fungal gene abundance (**Figure 4.3.3**, **Figure 4.3.4**). Furthermore, when the agricultural-grade inoculant of *G. intraradices* (AGTIV®) was applied at the GS field site in 2020, no significant differences in 16S bacterial or 18S fungal gene abundance were observed for this treatment on its own (**Table 4.3.8**, **Figure 4.3.5**, **Figure 4.3.6**).

Research into how AMF may affect the surrounding microbial community is relatively scarce and there are reports of AMF, and specifically *G. intraradices* (the active ingredient in MYKE® Pro) having either positive (Albertsen et al., 2006; Mechri et al., 2014; Trabelsi and Mhamdi, 2013) or negative (Gui et al., 2017; Mechri et al., 2014; Welc et al., 2010) effects on the surrounding soil microbial communities. Authors documenting negative impacts of AMF, including *G. intraradices*, on surrounding soil microbial communities suggest this may be caused by competition between the AMF and surrounding microbes for access to soil nutrients, as well as AMF regulation of the surrounding soil microbial community driven by mycelium exudates (Gui et al., 2017; Mechri et al., 2014; Welc et al., 2010). Studies that have observed positive effects of *G. intraradices* on soil microbial communities indicate that mycelium exudates and

other regulatory pathways driven by the AMF to suppress certain soil microbes (i.e., pathogenic bacteria and fungi) and promote other microbial species that enhance the symbiosis between *G. intraradices* and its host plant (Trabelsi and Mhamdi, 2013). AMF inoculation may also support strong soil microbial communities through their effects on the soil environment, such as improved soil structure and stability and regulation of soil decomposition and nutrient cycles, however, no direct connections between soil environmental effects and soil microbial communities have been reported yet (Johnson and Gehring, 2007). Overall, it is accepted that AMF do influence the surrounding soil microbial community structure, but the nature of this influence is heavily dependant on the specific plant-AMF species pairings and the conditions of the surrounding soil environment (Johnson and Gehring, 2007).

The results of the present study demonstrating significant positive and negative effects of *G. intraradices* (MYKE® Pro) on 16S bacterial gene abundance and significant negative effects of the AMF on 18S fungal gene abundance in the soil at the GS and BS field sites, respectively, are an addition to the literature documenting the range of effects AMF may have on surrounding microbial communities. The lack of significant effect observed for the AGTIV® at the GS site in 2020 provides evidence that annual variations in climatic conditions may also be a key factor determining what effect *G. intraradices* has on the microbial community (**Appendix A**). It is also important to keep in mind, however, that this project only collected data on the total abundance of 16S bacterial and 18S fungal genes in the soil and therefore did not evaluate potential effects of the treatment on soil microbial community structure or the activity of specific microbial functions. Overall, more research is needed to fully understand the conditions determining whether the effect of *G. intraradices* inoculation on surrounding microbial

communities will be positive or negative, as well as more detailed data regarding how this AMF inoculant may alter microbial community structure and function.

The other significant treatment effect observed at the GS site in 2019 was for JumpStart®, the inoculant of P-solubilizing fungus *P. bilaiae*. JumpStart® produced significantly lower autumn 18S fungal gene abundance compared to the combined average of MYKE® Pro and LysteGro treatments (**Figure 4.3.2**). In contrast, at the BS site in 2019, JumpStart® produced significantly higher season 18S abundance than MYKE® Pro in the least-square means comparison (**Figure 4.3.4**). No studies investigating the effect of *P. bilaiae* inoculation on surrounding soil microbial communities could be found in the existing literature. Therefore, the results from the present study represent a novel addition to the literature indicating that the overall effect of *P. bilaiae* (JumpStart®) on the abundance of bacterial and fungal genes in the soil may be positive or negative, depending on soil conditions. Because the effect was consistent among the two years JumpStart® was applied at the GS field site, these results also indicate that more stable soil environmental factors may be more significant than annual variability (i.e., in soil temperature and moisture) in determining whether *P. bilaiae* will have a positive or negative influence on the abundance of bacterial and fungal genes in the surrounding soils. The results from this study are preliminary, however, so additional studies should be conducted to verify these findings and to further investigate the effects *P. bilaiae* may have in promoting healthy soil microbial community structure and function.

In 2020, data from the GS field site indicated a negative effect of synthetic N fertilizer on peak season 16S bacterial and 18S fungal gene abundance compared to biofertilizer treatments (**Figure 4.3.5**, **Figure 4.3.6**). There is an abundance of research investigating the effect of synthetic N fertilizers on soil microbial communities which also documents negative effects of N

fertilizer on soil microbial communities related to ammonia toxicity and soil acidification (Miura et al., 2016; Zhou et al., 2015) and positive effects of N fertilizer on soil microbial abundance related to enhanced root exudates and soil fertility status (Lupwayi et al., 2012; Zhu et al., 2016). Negative effects of N fertilizers on soil microbial communities seem to be more apparent under more intensive conventional management practices (i.e., conventional tillage, residue removal) and when N is applied in excess of plant needs (Lupwayi et al., 2012; Miura et al., 2016; Zhou et al., 2015). While the present study documents no significant differences in the abundance of 16S bacterial or 18S fungal genes between control plots and plots receiving synthetic N, the reduction in the abundance of both genes compared to biofertilizer treatments indicate that 60 kg N ha^{-1} of synthetic N fertilizer at this site results in negative influences on the soil biological communities. Additional studies should be conducted to evaluate how synthetic N fertilizers applied to switchgrass crops affect soil microbial community structure and function which will provide more information about the soil health effects.

The last point of discussion within the soil microbial community data is that Optimyc + MooR resulted in a significantly higher peak season abundance of 16S bacterial genes in the soil compared to the synthetic N and control treatments, and a significantly higher peak season abundance of 18S fungal genes compared to the synthetic N treatment at the GS field site in 2020 (**Figure 4.3.5**, **Figure 4.3.6**). As has been stated in previous sections, it becomes very difficult to contextualize the results from the Optimyc + MooR treatment due to the 2019 application of LysteGro on the same plots which may have had effects lasting into the 2020 season. This difficulty is further complicated by the lack of literature documenting the exact individual and interactive effects of the multiple bacterial and fungal species in the Optimyc + MooR products. Logically, however, it makes sense that inoculating soils with several species of

bacteria and fungi would lead to significant increases in the presence of 16S bacterial and 18S fungal genes, as has been observed in the present study. Future investigations will be required to determine the mechanism for this enhancement. Specifically, a study investigating treatment effects on soil microbial community composition would indicate whether the enhanced 16S and 18S gene abundance is driven primarily by the growth of the species present in the biofertilizer inoculants, or if the products are enhancing the population size of existing soil microbial communities.

Three additional metrics of soil biological health were collected at the GS field site over the two years: the Solvita CO_2 Burst test and soil reactive C test at the end of the 2019 season, and an earthworm density count in the spring after treatment application in 2020. None of these metrics were significantly affected by any of the applied treatments. This is the first time that a study has compared the effects of commercial biofertilizers, synthetic N fertilizer, and a control on these three metrics in a switchgrass biomass crop field. Therefore, these results are a novel addition to the literature.

The lack of significant differences indicates that the treatments did not significantly affect soil microbial respiration rate, available C substrate, or earthworm abundance in the switchgrass field within the time period of this study but does not rule out that significant differences might develop with repeated applications. For example, Morrow et al. (2016) did not observe significant differences in soil reactive C between conventional tillage and no-till treatments at the site which had only began implementing the assigned treatments two years prior to sample collection. These authors did, however, observe significant differences at field sites where various tillage and crop rotation treatments had been established for longer periods of time (Morrow et al., 2016) indicating that this soil health metric may not be affected by changes in

agricultural management in the first several years. Similarly, Saini et al. (2021) observed no significant effects of N fertilizer on soil reactive C in switchgrass fields until the third year of treatment application where the highest N rate produced the highest reactive C level. Furthermore, while Li et al. (2018) have reported increased cumulative respiration in switchgrass biomass crop soils receiving N fertilization, these differences did not appear until 120 days into the authors' incubation study. This differs from the present study which measures soil microbial respiration with the 24-hour Solvita CO_2 Burst test, and no studies could be found that used the Solvita CO_2 Burst test to assess soils from switchgrass biomass crop fields. Finally, earthworm abundance was seen to respond positively to increasing rates of single superphosphate fertilizer on sheep grazing lands after 35 years of consistent treatment application (Schon et al., 2021), demonstrating that earthworm abundance can be affected by long-term fertilizer application. Earthworm abundance has also been significantly affected by to long-term establishment of various other farm management practices (Bai et al., 2018). However, no studies have been found reporting a significant response of earthworm abundance to short-term changes in agricultural management. Longer term trials of biofertilizer products should be established to determine if significant differences in soil health metrics appear after several years of applying the products.

It can be concluded from this section that there is promising potential for biofertilizers to improve soil biological health under switchgrass biomass crops, particularly by enhancing 16S bacterial and 18S gene abundance (which are indicative of bacterial and fungal population sizes). Further study will be required, however, to determine the long-term effects of these treatments that may emerge with consistent application. Additional studies should also investigate treatment

effects on soil microbial community composition and activity to create a clearer picture of how the biofertilizers affect soil ecosystem services.

5.3.2 Miscanthus

In contrast to the several significant treatment effects on soil microbial communities observed at the switchgrass field sites over the two years of this research project, very few significant results were observed at the miscanthus (BM) field site. The only significant difference observed among treatments for 16S bacterial and 18S fungal gene abundance (copies g dry soil^{-1}) in 2019 was that the 16S gene was significantly more abundant in plots receiving synthetic N fertilizer than the combined average of plots receiving biofertilizer treatments at the peak of the 2019 growing season at the BM site (**Figure 4.3.7**). At the end-of-season sampling date in 2020, plots treated with AGTIV® biofertilizer had significantly higher 16S bacterial gene abundance than control plots when least-square means comparisons were made using Dunnett's Correction ($p < 0.05$), but treatment effects were not significant when the least-square means comparisons were corrected according to the Tukey test ($p = 0.0663$). No other significant differences in gene abundance were observed for any treatment, at any sampling date in either 2019 or 2020 at this site (**Table 4.3.12**, **Table 4.3.16**).

Considering the significant increase in 16S gene abundance under synthetic N fertilizer, it seems that this does agree with some of the existing literature and can be explained by synthetic N fertilizer enhancing plant root exudates which support soil bacterial communities and removing N limitation on bacterial community growth (Lupwayi et al., 2012; Zhu et al., 2016). There are also studies, however, that indicate a lack of conclusive results regarding the effect of fertilizer type on soil microbial abundance within the first year of treatment application (Coelho et al., 2019) and significant negative effects of synthetic N on 16S gene abundance related to

ammonia toxicity and altered soil pH (Miura et al., 2016; Zhou et al., 2015). Due to the diversity of responses reported in the literature, and the lack of consistent response among sampling dates and years in the present study, it is difficult to confidently determine the effect that synthetic N had on the abundance of 16S bacterial genes under miscanthus crops at this site. Furthermore, several studies in the literature have reported significant negative effects of synthetic N fertilizers on soil microbial community diversity and stability, as well as increased denitrification potential, which create negative effects on overall soil health regardless of 16S gene abundance (Ren et al., 2020; Wang et al., 2018; Zhou et al., 2015). Additional years of study under long-term applications of synthetic N fertilizers and biofertilizers on miscanthus crops should be conducted to confirm the net effect of different fertilizer types on soil biological communities in terms of bacterial and fungal abundance, as was measured in the present study, as well as how the various fertilizers influence community structure and function which is more detailed indicator of soil health.

The significant increase in 16S bacterial gene abundance attributed to AGTIV® (inoculant of the AMF *G. intraradices*) is in agreement with studies in the literature that have reported similar positive effects on soil bacterial abundance which some authors attribute to mycelium exudates from the fungus which may promote the abundance of bacteria that enhance the symbiosis between *G. intraradices* and its plant host (Albertsen et al., 2006; Mechri et al., 2014; Trabelsi and Mhamdi, 2013). It must be noted the present study did not investigate the quantity or composition of mycelial exudates or mycorrhizosphere community composition, this represents a possible explanation and merits further study to determine the mechanism. Future studies into the effect that inoculants of *G. intraradices* (like MYKE® Pro and AGTIV® used in the present study) may have on soil health under miscanthus biomass crops is also required to determine

whether this positive effect on soil bacterial gene abundance is consistent across years and soil environmental conditions since there are also several studies reporting suppressive effects of *G. intraradices* on surrounding microbial communities (Gui et al., 2017; Mechri et al., 2014; Welc et al., 2010).

Soil samples were collected at the end of the 2019 growing season to assess soil biological health using the Solvita CO_2 Burst test and measuring soil reactive C. The only significant difference observed at the BM field site was that JumpStart® plots had a significantly lower Solvita CO_2 Burst than the combined average of MYKE® Pro and LysteGro (**Figure 4.3.8**). These results are a novel addition to the literature, in that there are no previously published studies that have reported Solvita CO_2 Burst results from miscanthus biomass crops, nor are there any published studies that have compared the effect of various biofertilizers on the Solvita CO_2 Burst results. The lack of existing literature makes it difficult to explain the reason that JumpStart® would have resulted in a significantly lower basal soil respiration rate than the other two biofertilizer treatments. It can, however, be speculated that the added nutrients and organic matter from the biosolids in LysteGro and the plant-AMF symbiosis that may have resulted in improved establishment of *G. intraradices* in MYKE® Pro may have promoted these two biofertilizers over the free-living P-solubilizing fungus, *P. bilaiae*, present in JumpStart®. If true, this may have allowed the former two products to have a stronger positive effect on soil microbial activity. It is important to note that this is purely speculation, however, as no data was collected regarding soil organic matter additions or colonization success of biofertilizer inoculants.

In conclusion, this study seems to indicate that inoculants of *G. intraradices*, like MYKE® Pro or AGTIV®, may produce significant soil health benefits when applied to miscanthus

biomass crops by enhancing the abundance of soil bacteria and improving basal soil respiration rate (an indicator of soil microbial activity). There may also be significant enhancements of soil bacterial communities by synthetic N fertilizers, but the results from this study are inconclusive and do not provide sufficient information about how synthetic N fertilizers or any of the other treatments affected soil microbial community composition and functions. As such, this portion of the research provides promising first results regarding the potential for biofertilizers to improve soil health under miscanthus biomass crops, however, further research is required to validate these results under a variety of field conditions and provide more insight into the effects these products may have on specific soil microbial species, functional groups, and gene expressions.

5.4 Greenhouse Gases

The final portion of this thesis research was to evaluate treatment effects on the flux rates of three key greenhouse gases– carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) – from the soil, as well as treatment effects on the global warming potential (GWP) based on the net flux of each of the three GHGs from the soil, expressed in kg CO_2e ha^{-1} day^{-1}. Seasonal patterns in the release of the three greenhouse gases were evaluated, using linear regression analyses to determine whether these patterns may be tied to seasonal changes in soil temperature and moisture content. Together, this data adds to the growing body of literature evaluating the climate impact of biomass crop production under various management strategies, which is critical to determining the viability of these crops as components of a climate-friendly economy.

The only significant treatment effect observed for any of the measured GHGs over the course of the season is that the synthetic N fertilizer had a significantly lower CH_4 flux (higher CH_4 consumption) in August compared to JumpStart® in the least-square means comparison (**Figure 4.4.1**). Existing literature that has evaluated the effect of synthetic N on GHG fluxes from

switchgrass field soils has reported no significant effect of the fertilizer on methane flux rates (Mbonimpa et al., 2015; Nikièma et al., 2011, Schmer et al., 2012), although Nikièma et al. (2012) report a non-significant trend of decreasing CH_4 consumption with increasing N fertilizer up to 112 kg N ha^{-1}. These findings are consistent with García-Marco et al. (2014) in which the authors explain that NO_3^- addition, one of the most significant regulatory factors determining CH_4 flux rate, can inhibit methanotrophic metabolism. However, the study by García-Marco et al. (2014) also explains that NO_3^- addition can reduce CH_4 release in anoxic soil conditions because methanogenesis is inhibited by intermediate products of denitrification. Overall, these authors conclude that the effect of added soil N on methane flux will depend on the balance of N cycling activities and O_2 availability in the soil. Therefore, although the present study's findings suggest that synthetic N increased CH_4 consumption compared to biofertilizer treatments (especially JumpStart®), a firm conclusion cannot be made because N addition has been observed to either increase or decrease CH_4 flux rates depending on other soil conditions.

There were no significant treatment effects on the CO_2 flux rates on any of the sampling dates (**Table 4.4.2**). Studies in the existing literature have consistently reported no significant effects of synthetic N fertilizer on the soil CO_2 flux in switchgrass fields (Mbonimpa et al., 2015; Nikièma et al., 2011; Schmer et al., 2012), although Mbonimpa et al. (2015) observed a numerical increase in soil CO_2 emissions with N fertilizer application. Therefore, the lack of significant differences observed for the synthetic N treatment in the present study agrees with existing literature. Furthermore, García-Marco et al. (2014) found that glucose addition and soil temperature were the two most significant factors affecting CO_2 flux from agricultural soils, accounting for 40 and 35% of the variation, respectively. Nitrate addition did account for 19% of the variation in CO_2 flux rates, however, the increase in CO_2 flux was much smaller between

soils receiving 25 versus 50 kg N ha^{-1} than soils receiving 50 versus 75 kg N ha^{-1} indicating that only higher levels of N fertilization will result in large increases to soil CO_2 emissions (García-Marco et al., 2014). There is a lack of research on the effect of biofertilizers on soil GHG flux rates switchgrass biomass crop fields, therefore this project represents a novel addition to the literature. Based on the findings from García-Marco et al. (2014), however, it makes sense that neither of the biofertilizers for which GHG flux rates were monitored significantly affected soil CO_2 fluxes compared to the control since neither of these products added labile C (like glucose) to the soil nor would they have affected soil temperature.

There were also no significant treatment effects on the N_2O flux rates on any of the sampling dates in the present study (**Table 4.4.2**). This is more unexpected than the lack of significant treatment effects on the CO_2 flux rates since García-Marco et al. (2014) found that nitrate addition is one of the most significant contributing factors determining N_2O flux rates from agricultural soils, explaining about 33% of the variance. Furthermore, Schmer et al. (2012) have reported significant increases in the soil N_2O flux under switchgrass receiving 67 kg N ha^{-1} compared to the control, and significant increases in N_2O flux with synthetic N fertilizers have repeatedly been observed in a variety of agricultural systems (McSwiney and Robertson, 2005; Millar et al., 2018; Shcherbak et al. 2014; Zhou et al., 2014). The lack of significant differences observed in the present study, however, are not completely unprecedented in the literature as Nikièma et al. (2011) also reports no significant affect of synthetic N fertilizer on the soil N_2O flux from switchgrass fields up to 112 kg N ha^{-1}. These authors speculate that this lack of significant response to N fertilizers may be due to rapid uptake of the added N by the crop, site conditions that did not favour denitrification (i.e., insufficient soil WFPS), or a combination of the two. This speculation agrees with findings from García-Marco et al. (2014) explaining that

nitrate addition resulted in much larger increases in N_2O flux when soil WFPS exceeded 80%. Therefore, it could be that the field site from the present study experienced soil moisture under 80% WFPS and rapid N uptake by switchgrass, resulting in the lack of significant increases in N_2O flux rates with synthetic N. No existing studies investigating the influence of biofertilizers on soil N_2O flux rates in switchgrass fields could be found. Therefore, as with the CO_2 and CH_4 flux rates, it seems that the present study represents another novel addition to the literature regarding biofertilizer effects on soil N_2O flux under switchgrass.

When the CO_2-equivalents of each of the gases were calculated and added together to estimate the soil GWP, there were also no significant effects of any of the treatments (**Table 4.4.2**). This is likely because the largest proportion of net GHG flux expressed as CO_2 equivalents came from the soil CO_2 emissions which was also not significantly affected by any of the treatments, as previously discussed. The mean daily GWP for each month ranges between 108.3 to 131.3 kg CO_2e ha^{-1} day^{-1}. What seems to be the only existing study investigating the soil GWP for switchgrass fields reports a cumulative GWP from direct GHG emissions from the soil ranging from 24,550 to 26,050 kg CO_2e ha^{-1} $year^{-1}$, which works out to an annual mean of about 67 to 71 kg CO_2e ha^{-1} day^{-1} (Nikièma et al., 2011). Although the range reported by Nikièma et al. (2011) is lower than the present study, it is also important to note that this range is based on an annual average whereas the present study only collected data for the months of July to October. Therefore, values from Nikièma et al. (2011) are expected to be lower since the study includes estimates from the winter months which have lower flux rates than warmer months, especially compared to the summer which makes up half of the total data collected in the present study. Nikièma et al. (2011) do report an increase in GWP resulting from direct GHG (CO_2, CH_4, N_2O) emissions from soils under switchgrass receiving 57 and 112 kg N ha^{-1} resulting in an

increase of 0.7 to 1.5 Mg CO_2e ha^{-1} $year^{-1}$, respectively, compared to the control. However, the authors first comment that N fertilizer had minimal effects on soil GHG fluxes, consistent with their findings of no significant treatment effects on the annual flux of any of the three gases. This is consistent with the lack of significant differences observed for the synthetic N treatment in the present study. No existing studies could be found reporting the effect of biofertilizers on the net GWP of soils under switchgrass biomass crops, so the present study represents the first reporting of this in the literature.

It is important to note that the present study did not investigate the potential of soil organic carbon (SOC) sequestration by switchgrass during this time, nor has this work measured C sequestration in above- or belowground switchgrass biomass. Several studies in southern Ontario (Bazrgar et al., 2020; Graham et al., 2019, Jarecki et al., 2020) have reported a potential for significant SOC gain in soil influenced by continuous cultivation of switchgrass. Furthermore, Nikièma et al. (2011) measured about 2260 to 5600 kg C ha^{-1} of C storage in aboveground biomass for switchgrass crops in their trial which acts to offset the overall GWP of the cropping system. Therefore, if these sources of C sequestration were taken into consideration for the present study, the total system GWP would be considerably lower and could even reach negative values. The GWP values reported here represent only the direct contribution of the soils to the climate impact of switchgrass production.

Significant seasonal differences were observed for soil CO_2 (**Figure 4.4.2**) and N_2O (**Figure 4.4.4**) fluxes, whereas soil CH_4 fluxes were statistically similar throughout the growing season (**Figure 4.4.3**). All recorded CO_2 flux rates fall between 2.4 to 155.4 kg CO_2 ha^{-1} day^{-1} with mean values for each month ranging from 26.78 kg CO_2 ha^{-1} day^{-1} in October to 119.34 kg CO_2 ha^{-1} day^{-1} in July. This range is similar to those that have been previously reported in the

literature (Bates et al., 2021; Mbonimpa et al., 2015; Nikièma et al., 2011; Schmer et al., 2012), indicating that the field site used in the present study is a fairly typical switchgrass biomass crop field in this aspect. The significant decline in CO_2 flux rate from July to August to September and October also agrees with patterns previously observed in the literature and has been explained as being the result of seasonal changes in soil temperature and moisture conditions (Bates et al., 2021; Mbonimpa et al.; 2015, Nikièma et al., 2011; Schmer et al., 2012). The literature consistently reports that CO_2 release increases with temperature (Bates et al., 2021; Mbonimpa et al., 2015; Nikièma et al., 2011; Schmer et al., 2012), and this has study also observed a significant positive relationship between CO_2 flux and soil temperature (**Figure 4.4.5**). However, the relationship between soil CO_2 flux and soil moisture is less clear. Bates et al. (2021) and Mbonimpa et al. (2015) report increasing CO_2 release with increasing soil moisture, however Schmer et al. (2012) found increasing CO_2 flux only with increasing soil moisture only up to a water-filled pore space (WFPS) of 15%. Linear regression analysis from the present study indicated a slight negative relationship between soil volumetric water content (VWC) and CO_2 flux, with the widest variation around the trendline occurring between 15-20% VMC (**Figure 4.4.6**) which seems to agree with Schmer et al. (2015). Decreasing CO_2 flux after a certain threshold of soil moisture is expected because excessive soil moisture will result in anoxic conditions which then inhibits microbial respiration that drives soil CO_2 release (McKnight et al., 2017; Wei et al., 2014). Overall, the regressions observed in the present study agree with the previously reported seasonal patterns described above, as periods of lower temperature and higher VMC were recorded for September and October (lowest CO_2 flux), and higher temperatures and lower VMC were reported for July and August (highest CO_2 fluxes).

The N_2O flux from the present study ranged between -3.12 and 5.47 g N_2O ha^{-1} day^{-1}, which is slightly lower than the ranges reported by most studies (Ferchaud et al., 2020; Nikièma et al., 2011; Schmer et al., 2012), but slightly higher than the range reported by Bates et al. (2021). However, the results from Bates et al. (2021) are unique in that their measured fluxes are predominantly negative with a 17-month mean daily flux of -30.41 g N_2O ha^{-1} day^{-1}. Overall, the present study falls within the typical range of values reported in the literature, again indicating that the GS field site has a similar N_2O flux to other switchgrass field sites. Looking at the seasonal patterns in the present study, mean daily N_2O fluxes were significantly lower in August and September than July (**Figure 4.4.4**). These significant differences do not seem to be explained by changes in soil temperature or moisture levels as the regression analyses did not indicate any significant relationships between N_2O flux rate and either of those environmental factors (**Table 4.4.5**). In Ferchaud et al. (2020), N_2O emissions only demonstrated a positive linear relationship with soil moisture when excluding flux rates recorded at a WFPS less than 60%. The authors explain that this is likely because denitrification, the microbial process responsible for most soil N_2O emissions, becomes dominant at WFPS ≥60%. Furthermore, García-Marco et al. (2014) report that WFPS is the primary factor driving high N_2O emissions from agricultural soils due to the importance of high soil moisture in driving denitrification. It is possible that no significant relationship between soil moisture and N_2O rates could be found in the present study because WFPS may not have exceeded 60% at any of the sampling dates. The lowest average soil VMC (13.0%) occurred in August, which was also the month with the lowest mean daily N_2O flux, which further suggests that soil moisture is at least partially influencing the seasonal patterns in N_2O fluxes that were observed, despite the lack of significant relationship. The September mean daily N_2O flux was statistically similar to August and both months were

significantly lower than the mean daily N_2O flux for July. García-Marco et al. (2014) explain that labile soil C availability is also a significant explanatory factor behind soil N_2O flux rates, therefore it can be speculated that more active plant growth in July may have resulted in the release of more root exudates and increased root turnover by the switchgrass crop compared to September, at which time the switchgrass is nearer to maturity and senescence, and that this could have promoted the increased N_2O flux in July. This cannot be confirmed, however, as root exudates, root turnover, and soil labile C were not monitored over the course of the study.

There were no significant patterns in CH_4 flux rates in the present study, however all but four recorded flux rates were negative, and the full dataset ranged between -13.54 and 1.85 g CH_4 ha^{-1} day^{-1}. Monthly means ranged between -5.18 and -2.84 g CH_4 ha^{-1} day^{-1} (**Figure 4.4.3**). There was no significant relationship between methane flux rate and soil temperature or soil moisture (**Table 4.4.5**) which may explain the lack of significant differences among sampling dates. García-Marco et al. (2014) found that the most significant factors affecting CH_4 flux rates in agricultural settings were, in order of magnitude: glucose addition, soil temperature, and nitrate addition. These authors also note, however, that methanotrophic microorganisms (consumers of CH_4) are typically favoured over methanogenic microorganisms (producers of CH_4) under conditions of lower soil WFPS. Overall, it seems that switchgrass fields tend to favour CH_4 consumption rather than CH_4 emission, which is a good indicator of how these biomass crops can contribute to climate-friendly economic development. Future research should continue to investigate seasonal patterns in CH_4 as well as CO_2 and N_2O fluxes at other field sites and with more regular sampling to confirm the results from the present study and better capture fluctuations under various soil and climatic conditions.

In conclusion, these findings are exciting new additions to the literature indicating that biofertilizers do not significantly increase the flux of three major GHGs from the soil under switchgrass biomass crops. Furthermore, this study adds to the body of literature examining how N fertilizer affects GHG flux rates in switchgrass biomass crop fields, which is largely under-studied compared to conventional crops. While the lack of significant increase in the flux of any of the three measured GHGs in plots receiving synthetic N fertilizer is a promising indication that synthetic N fertilizer may not always result in higher direct GWP for biomass crop production systems, it is important to remember that this response may change under differing environmental conditions (i.e., soil texture and moisture levels). Therefore, while low rates of N fertilizer may not always produce negative climate impacts, it is important to conduct further research to better understand the conditions under which the effect of synthetic N fertilizer on individual GHG fluxes and net GWP of switchgrass fields may be stronger.

Chapter 6: Conclusions

6.1 Switchgrass

Switchgrass is one of the most rapidly expanding biomass crops in southern Ontario, but biomass crops are still a relatively new agricultural industry. There is much to learn about how to produce this grass most efficiently and sustainably as a high-yielding crop, with fertilizer management being one of the most important considerations to optimize for high biomass yields with minimal environmental impacts. In this study, six fertilizer treatments including a control, a synthetic N fertilizer, and four commercially available biofertilizers (JumpStart®, MYKE® Pro / AGTIV®, Optimyc + MooR, LysteGro) were tested at two switchgrass field sites (Guelph and Burlington, ON) over the course of two growing seasons (2019 and 2020). The overall goal of this study was to investigate whether any of the above biofertilizers could be recommended to growers as more environmentally friendly alternatives to traditional N fertilizers while maintaining acceptable biomass yields.

The synthetic N treatment and LysteGro biofertilizer treatment produced the highest switchgrass yields, although synthetic N was the only treatment that resulted in a difference in biomass yield. Therefore, these two fertilizers seem to be the best candidates to be recommended to growers out of the fertilizers that were tested. Synthetic N produced the highest yield at the lowest cost per hectare, while also increasing soil fertility in the incubation study and indicating some potential to increase CH_4 consumption. However, the synthetic N treatment also led to a significant reduction in 16S bacterial and 18S fungal gene abundance in the soil, which could be indicative of reduced soil health and ecosystem function. Additional research into the synthetic N fertilizer's effect on soil microbial community structure and activity would be required to confirm potential negative effects on soil health. Furthermore, synthetic N fertilizers can result in

environmental damage due to the high energy cost of production, excess nutrient leaching into surface and groundwater systems, and increased N_2O emissions from the soil under conditions that favour denitrification. Although significantly increased N_2O emissions were not observed in the present study, this may have been due to the soil moisture being too low to favour denitrification on the four days that GHG fluxes were measured. Furthermore, a full life cycle assessment of the energy costs associated with each treatment was beyond the scope of this study, and nutrient leaching was also not measured. While these components were not included in the present study, they may be important considerations to growers who are trying to reduce the environmental impacts of their operations and should be considered in future research.

LysteGro produced the highest yield out of all treatments in 2019, the only year it was applied. This result could not be confirmed in the second study year because this treatment had to be replaced due to the logistical challenges of application compounding with COVID-19 restrictions. LysteGro also significantly increased soil N under field conditions and increased the availability of three plant macronutrients (N, P, K) in the soil incubation study, providing promising results that this treatment may help to build fertility in nutrient-depleted soils. Furthermore, this biofertilizer did not reduce 16S bacterial or 18S fungal gene abundance like the synthetic N treatment. Although it did not significantly increase the abundance of these genes in the soils, using LysteGro in place of synthetic N may prevent potential detrimental effects to the vital soil ecosystem services which are largely driven by soil microbial communities. Additional years of study should be conducted to test this fertilizer over longer periods of time and at other field sites to confirm these initial results, but this product seems to represent a promising alternative for growers. One major challenge associated with this product is that it is one of the

most expensive products tested and would therefore require government financial support to the growers for them to adopt this biofertilizer.

The three other biofertilizers, JumpStart®, MYKE® Pro / AGTIV®, and Optimyc + MooR did not provide results the supported the overall goal of the study. All three products produced yields that were similar to the control, meaning that there is no yield-associated economic benefit to applying them. Furthermore, these products are all more expensive to apply than the traditional synthetic N fertilizer. Although these three products did demonstrate some potential environmental benefits which have been discussed in their respective chapters, the magnitude and consistency of the effects do not justify recommending these products for use by southern Ontario switchgrass growers at this time.

In summary, this study indicates that the best fertilizer options for Ontario switchgrass producers are the traditional synthetic N fertilizer or LysteGro biofertilizer. Synthetic N may be applied conservatively coupled with careful management of soil N levels to increase yields while minimizing detrimental environmental impacts. However, government financial support for environmentally friendly agricultural management practices can promote the use of LysteGro to support switchgrass yields while rehabilitating depleted soils. Additional years of study should be conducted to confirm these results under a wider range of soil and climatic conditions, and long-term studies should be established to monitor the longevity and consistency of the effects of these treatments. Future research may also provide more detailed insight into the soil biological health effects of these products by collecting data on soil microbial diversity, activity, and community composition. Studies with more frequent GHG flux measurements and biomass C sequestration by switchgrass would also be beneficial.

6.2 Miscanthus

Similar to switchgrass, synthetic N fertilizer input is the dominant agricultural management requirement for miscanthus. Therefore, growers and researchers in southern Ontario are interested in reducing the environmental impacts associated with fertilizer management for miscanthus cultivation. The present study contributes to the above interest by measuring the agronomic and environmental impacts of five fertilizer options in a southern Ontario miscanthus grower's field (Burlington, ON), including a control, a synthetic N fertilizer, and three commercially available biofertilizers (JumpStart®, MYKE® Pro / AGTIV®, LysteGro) over the course of two growing seasons (2019 and 2020).

Among the five treatments, MYKE® Pro / AGTIV® biofertilizer provided the most interesting results. AGTIV® applied in 2020 produced much higher yields (around 20 tonnes per hectare) compared to biofertilizer MYKE® Pro applied in 2019. The cost of AGTIV® was also the lowest of all tested biofertilizers, although this product still costs more per hectare than 60 kg N ha^{-1} synthetic N which is the current standard N fertilizer rate. Despite the slight increase in cost compared to traditional inorganic fertilizer, AGTIV® (the agricultural grade version of MYKE® Pro) is the treatment of highest interest for future research from this study. Field data demonstrated some evidence that MYKE® Pro / AGTIV® may contribute to improved soil nutrient availability, however significant treatment effects were only observed in 2019 so further study will be required to confirm these results. AGTIV® applied at the BM site in 2020 also showed evidence of potential soil biological health benefits as this treatment significantly increased 16S bacterial gene abundance at the peak of the 2020 growing season. Unfortunately, resources were not available to collect greenhouse gas data at the BM field site, so a discussion of the climate impact of these treatments when applied to miscanthus fields is not

possible. That said, the high yields observed in 2020 and promising soil fertility and biological health data indicate that AGTIV® may be a viable and environmentally friendly alternative to synthetic N fertilizers for miscanthus producers in Ontario.

The other two biofertilizers (JumpStart®, LysteGro) and the synthetic N fertilizer did not perform as well as AGTIV® or the control. Synthetic N fertilizer performed surprisingly poorly with regards to yield, particularly in 2019 when it reduced yields compared to the control plots. By 2020, however, synthetic N and control plots had the same mean yield. Additional years of study would be required to confirm whether yields would continue to increase in the synthetic N plots compared to the control, which may then help determine appropriate synthetic N use at this site. In addition to poor results for yield, synthetic N did not significantly affect soil nutrient availability except by increasing plant tissue Ca content in 2020, suggesting minimal soil fertility benefits. One promising result from the synthetic N treatment was significant increases in 16S bacterial gene abundance in soils at the peak of the 2019 growing season. This effect was not observed in the fall of the same year or at any time in 2020, therefore the soil biological health benefits of this treatment seem to be inconsistent. JumpStart® did not significantly affect yield or nutrient availability in the soil samples collected from the field and resulted in a significant decrease in basal soil respiration (indicative of soil microbial activity levels) compared to the combined average of LysteGro and MYKE® Pro treatments in 2019. Therefore, it appears that there are no economic or environmental incentives to apply JumpStart® to miscanthus biomass crops. LysteGro also did not significantly affect miscanthus yield or soil biological health, although it did produce significantly higher miscanthus tissue N and Mg content at the peak of the 2019 growing season. Only one year of data was collected for the LysteGro treatment due to the logistical complications of its application compounding with COVID-19 restrictions in 2020.

Therefore, these findings do not encompass multiple years of application or the performance of this biofertilizer under varying climatic conditions. As such, the results from this study should not be used to draw firm conclusions about the efficacy of LysteGro as a biofertilizer for miscanthus biomass crops in southern Ontario. Future research will be required to better understand the potential agronomic and environmental impacts of LysteGro. This is especially true given the promising results that have been reported in previous studies for biosolids-based fertilizers applied to miscanthus biomass crops.

In summary, the findings from the present study indicated that AGTIV® seems to be the most promising biofertilizer option for miscanthus biomass producers in southern Ontario. This is because this product was able to promote increased biomass yields while imparting some environmental benefits over the course of this short field trial. Additional studies should be conducted, however, to confirm these results under a range of field and climatic conditions. Long-term studies should also be established to confirm the longevity and consistency of the effects observed in the present study. Furthermore, future studies may consider conducting more detailed analyses for soil biological health that incorporate microbial diversity, activity, and community composition, as well as assessments of GHG fluxes associated with this treatment compared to a control and synthetic N fertilizer. If AGTIV® continues to prove an effective and environmentally friendly alternative to synthetic fertilizers for miscanthus biomass production, government financial support programs may be required to help offset the additional cost associated with this biofertilizer compared to traditional synthetic N fertilizer.

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Appendix A: Additional Site Description Details

Table A1: Soil texture analysis [SGS Labs, Guelph, ON] for the soils at the Guelph Switchgrass, Burlington Switchgrass, and Burlington Miscanthus sites. Samples were collected from 0-30 cm depth.

Sample Location	*% Sand*	*% Silt*	*% Clay*	*Texture*
Guelph Switchgrass Block 1	57	33	10	Sandy Loam
Guelph Switchgrass Block 2	50	41	9	Loam
Guelph Switchgrass Block 4	51	39	10	Loam
Burlington Switchgrass Block 1	23	60	17	Silt Loam
Burlington Switchgrass Block 3	27	61	12	Silt Loam
Burlington Switchgrass Block 4	28	54	18	Silt Loam
Burlington Miscanthus Block 1	51	42	7	Loam
Burlington Miscanthus Block 2	43	47	10	Loam
Burlington Miscanthus Block 3	42	49	9	Loam

Table A2: Guelph, Ontario temperature and precipitation data for 2019 (ECCC, 2021c) and 2020 (ECCC, 2021e), and 1981-2010 climate normals (ECCC, 2021b) using data from the nearest Government of Canada climate stations with data available for the desired years.

2019	*Jan*	*Feb*	*Mar*	*Apr*	*May*	*Jun*	*Jul*	*Aug*	*Sep*	*Oct*	*Nov*	*Dec*
Daily Max. (˚C)	-3.3	-0.8	2.3	10.3	17.4	23.2	28.4	26.1	22.1	14.8	3.3	1.6
Daily Min. (˚C)	-12.4	-10.3	-7.3	-0.2	5.1	10.1	14.0	11.9	8.8	2.4	-4.4	-5.8
Precipitation (mm)	28.5	38.6	60.3	93.3	79.4	53.2	50.6	59.1	30.1	132.5	34.4	44.1

2020	*Jan*	*Feb*	*Mar*	*Apr*	*May*	*Jun*	*Jul*	*Aug*	*Sep*	*Oct*	*Nov*	*Dec*
Daily Max. (˚C)	0.9	-1.0	6.9	10.7	17.3	25.8	29.6	26.5	21.4	12.8	10.4	1.4
Daily Min. (˚C)	-5.8	-8.8	-3.2	-1.3	4.6	10.5	15.4	12.5	7.7	2.1	0.0	-5.1
Precipitation (mm)	78.8	22.7	47.3	39.6	45.0	36.4	81.0	95.0	70.6	64.2	58.6	50.0

1981-2010	*Jan*	*Feb*	*Mar*	*Apr*	*May*	*Jun*	*Jul*	*Aug*	*Sep*	*Oct*	*Nov*	*Dec*
Daily Max. (˚C)	-2.6	-1.2	3.6	11.5	18.5	23.6	26.0	24.8	20.4	13.5	6.3	0.2
Daily Min. (˚C)	-10.3	-9.7	-5.6	0.8	6.4	11.5	14.0	12.9	8.6	2.9	-1.4	-6.8
Precipitation (mm)	65.2	54.9	61.0	74.5	82.3	82.4	98.6	83.9	87.8	67.4	87.1	71.2

Table A3: Burlington, Ontario temperature and precipitation data for 2019 (ECCC, 2021d) and 2020 (ECCC, 2021f), and 1981-2010 climate normals (ECCC, 2021a) using the nearest Government of Canada climate stations with data available for the desired years.

2019	*Jan*	*Feb*	*Mar*	*Apr*	*May*	*Jun*	*Jul*	*Aug*	*Sep*	*Oct*	*Nov*	*Dec*
Daily Max. (˚C)	-1.7	0.8	4.2	10.9	17.0	23.6	29.5	27.0	23.4	15.4	5.0	3.5
Daily Min. (˚C)	-9.7	-8.1	-5.4	1.9	6.6	13.0	17.9	16.0	12.9	6.6	-2.6	-4.5
Precipitation (mm)	65.2	93.2	54.4	95.2	121.6	97.0	72.6	53.8	36.2	144.8	40.7	95.0

2020	*Jan*	*Feb*	*Mar*	*Apr*	*May*	*Jun*	*Jul*	*Aug*	*Sep*	*Oct*	*Nov*	*Dec*
Daily Max. (˚C)	2.7	1.8	7.9	11.0	17.9	27.1	31.3	28.4	22.6	14.5	12.2	3.5
Daily Min. (˚C)	-4.7	-6.9	-1.5	1.0	7.2	15.2	19.7	17.2	12.1	5.5	3.4	-2.6
Precipitation (mm)	114.5	49.2	74.6	47.7	47.8	91.0	43.0	129.0	60.8	87.1	51.9	58.8

1981-2010	*Jan*	*Feb*	*Mar*	*Apr*	*May*	*Jun*	*Jul*	*Aug*	*Sep*	*Oct*	*Nov*	*Dec*
Daily Max. (˚C)	-0.6	0.8	5.2	12.4	19.4	25.0	28.0	26.7	21.8	15.1	8.0	2.4
Daily Min. (˚C)	-8.1	-7.1	-3.3	2.6	8.2	13.8	16.9	16.1	11.9	5.7	0.7	-4.3
Precipitation (mm)	66.0	54.5	61.6	70.6	81.0	69.1	75.3	82.0	83.1	71.9	84.9	63.0

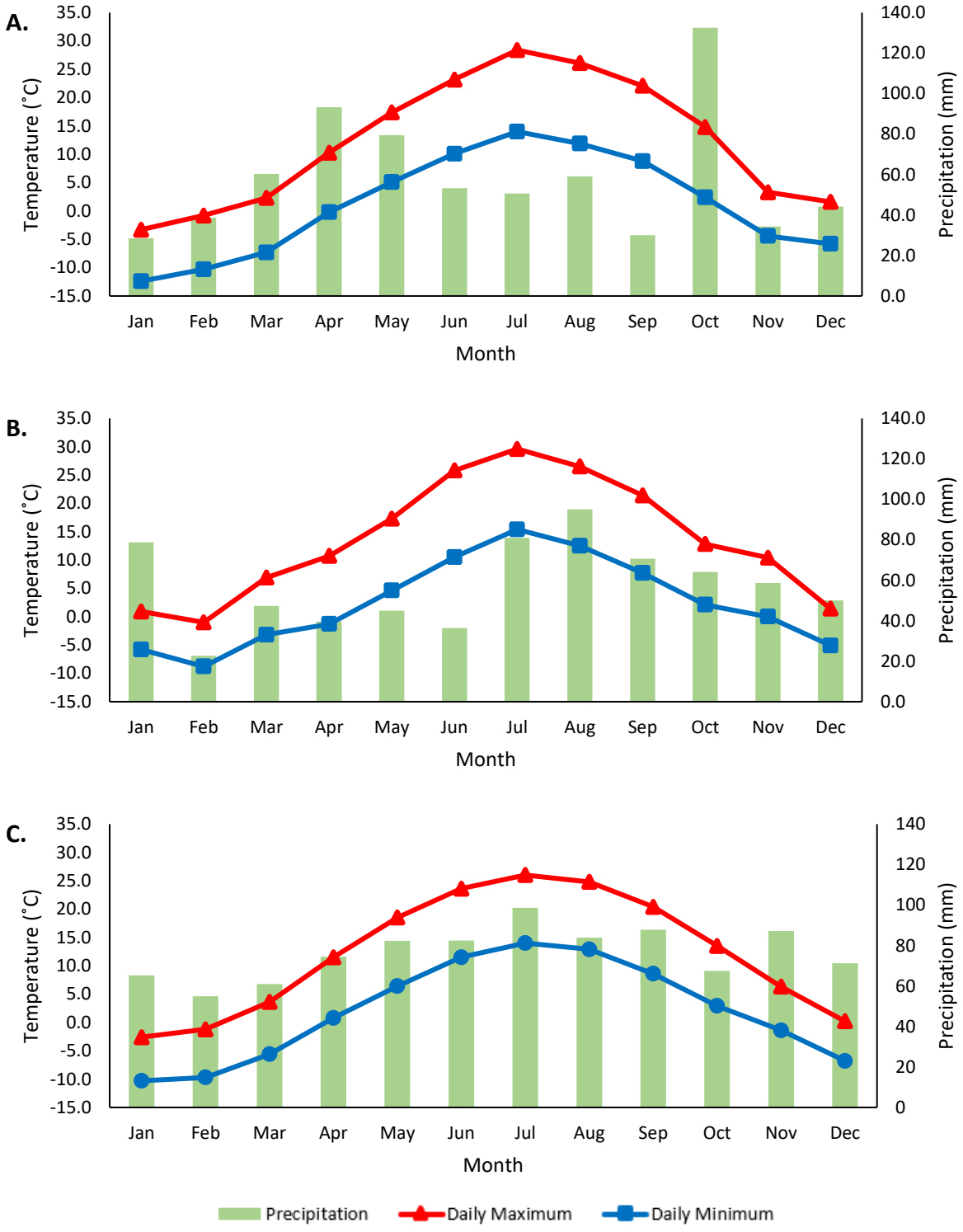

Figure A1: Guelph, Ontario temperature and precipitation data for 2019 (A.; ECCC, 2021c) and 2020 (B.; ECCC, 2021e), and 1981-2010 climate normals (C.; ECCC, 2021b) using data from the nearest Government of Canada climate stations with data available for the desired years.

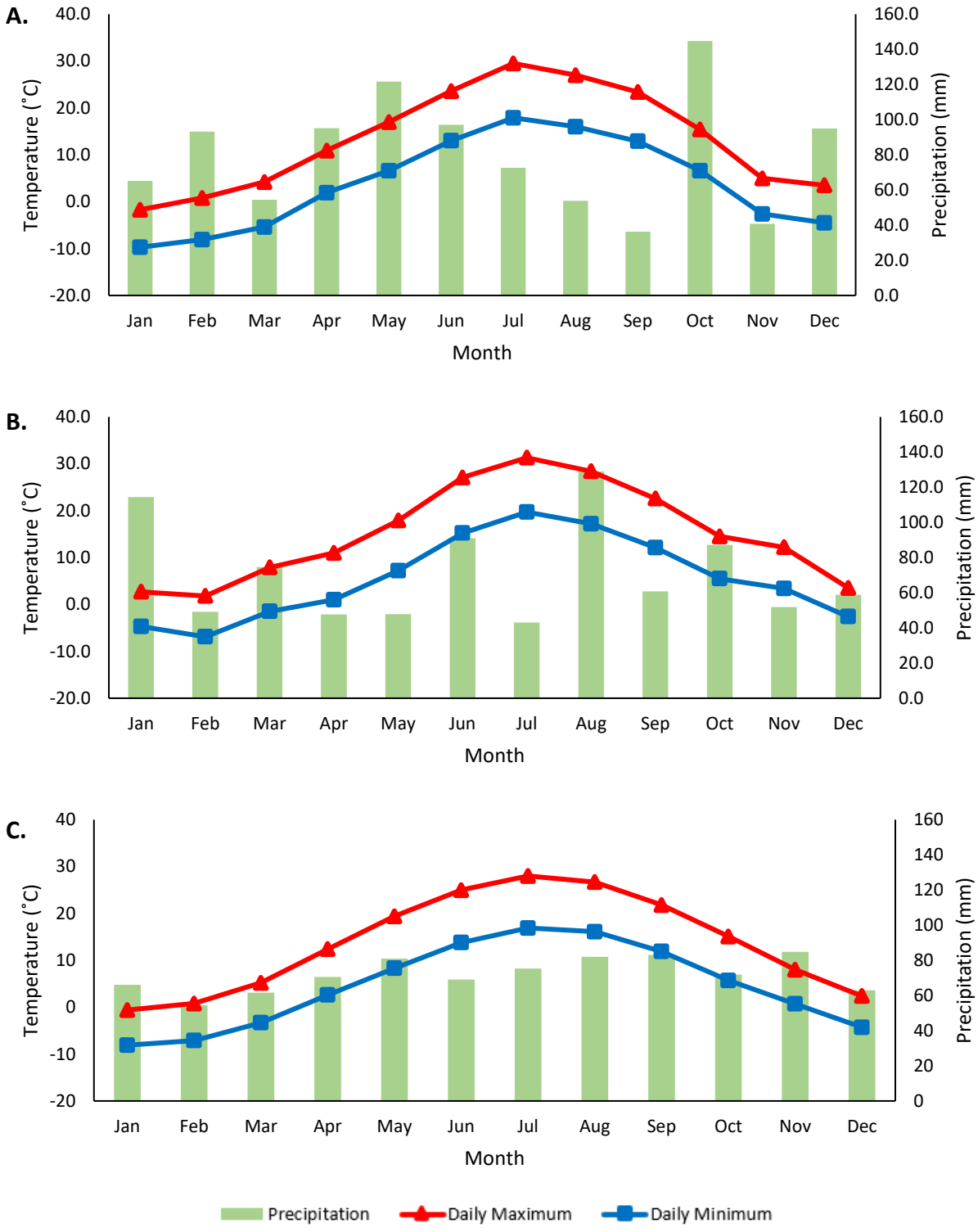

Figure A2: Burlington, Ontario temperature and precipitation data for 2019 (A.; ECCC, 2021d) and 2020 (B.; ECCC, 2021f), and 1981-2010 climate normals (C.; ECCC, 2021a), using data from the nearest Government of Canada climate stations with data available for the desired years.

Appendix B: Record of Sampling Dates

Table B1: Schedule of sampling dates for each metric at the Guelph Switchgrass (GS), Burlington Switchgrass (BS), and Burlington Miscanthus (BM) sites for the duration of the study.

Activity	Site	2019 Dates	2020 Dates
Plant Morphology Sampling	GS	Jul. 18, Jul. 31, Aug. 14, Aug. 28, Sep. 16, Oct. 3, Oct. 24	Jun. 12, Jul. 21, Aug. 31, Sep. 24
	BS	Jul. 18, Aug. 1, Aug. 15, Aug. 29, Sep. 18, Sep. 30	N/A
	BM	Jul. 18 (Jul. 22*), Aug. 6, Aug. 19, Sep. 3, Sep. 20, Oct. 2, Oct. 23	Jul. 23, Aug. 20, Sep. 25
Yield Sampling	GS	Nov. 7	Oct. 28
	BS	N/A	N/A
	BM	Nov. 6	Oct. 28
Soil Fertility Sampling	GS	Jun. 27, Nov. 7	Aug. 6
	BS	N/A	N/A
	BM	Jul. 3, Nov. 6	Aug. 14
Plant Tissue Nutrients Sampling	GS	Aug. 14	Aug. 6
	BS	Aug. 15	N/A
	BM	Aug. 19	Aug. 14
Soil Microbial Communities Sampling	GS	Jun. 27, Aug. 21, Nov. 7	Jul. 30, Oct. 8
	BS	Jul. 3, Aug. 29	N/A
	BM	Jul. 3, Sep. 9, Nov. 6	Jul. 28, Oct. 1
DNA Extraction on Soil Microbial Samples	GS	Jun. 28, Aug. 22, Nov. 14	Jul. 31, Oct. 9
	BS	Jul. 5, Sep. 4	N/A
	BM	Jul. 4, Sep. 11, Nov. 13	Jul. 29, Oct. 2
VitTellus® Soil Health Index Sampling	GS	Nov. 6	N/A
	BM	Nov. 7	N/A
Earthworm Abundance Sampling	GS	N/A	Jun. 10
Greenhouse Gas Flux Sampling	GS	N/A	Jul. 14, Aug. 19, Sep. 15, Oct. 14

* In 2019, plant morphology data for block 3 at the BM site was collected after blocks 1 and 2 due to an error in the initial treatment application for this block

Appendix C: Additional Analyses from Incubation Study

C 1 Nitrogen Mineralization

Nitrogen mineralization is a microbial process through which organic nitrogen is transformed to plant available inorganic nitrogen. In this process, NH_4^+ and NO_3^- ions are produced through ammonification and nitrification, respectively. The results pertaining to NH_4–N and, NO_3–N and total mineral nitrogen during the incubation study are presented below.

C 1.1 NH_4–N Content

The effect of the fertilizer treatments on NH_4–N content over the course of the incubation study is presented in **Figure C1**. Treatment differences were significant ($p \leq 0.0001$). The highest mean NH_4-N content throughout the incubation was recorded in urea (2.54 mg kg^{-1}) followed by LysteGro (2.43 mg kg^{-1}), compared to 1.59 mg kg^{-1} in the control. JumpStart® and MYKE® Pro treatments also produced higher NH_4-N content than the control.

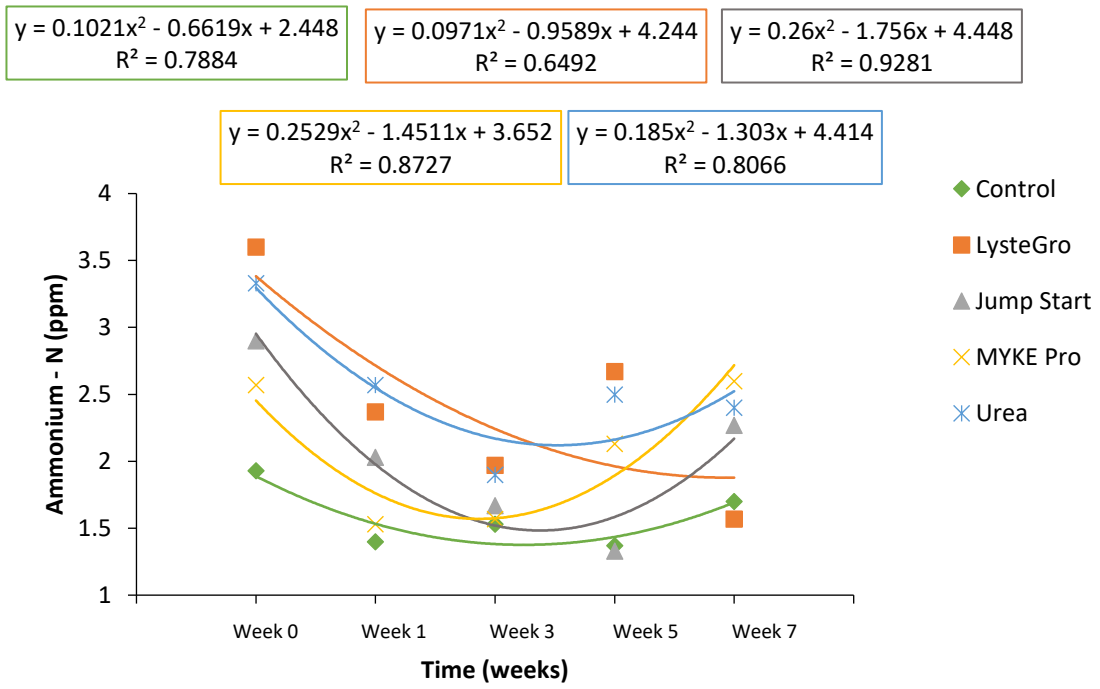

Figure C1: Effect of fertilizer treatment on NH_4-N content (mg kg^{-1}) in soil over time when incubated at 20˚C and 22% soil moisture.

The effect of time (week number) was also significant ($p \leq 0.0001$). In LysteGro treatment, a decreasing trend in NH_4-N was recorded over the incubation period. While in other treatments (urea, MYKE® Pro, JumpStart®) and the control, NH4-N initially decreased, but began increasing by weeks 3 to 5.

C 1.2 NO_3-N Content

The data on soil NO_3-N content as affected by different treatments is presented in **Figure C2**. Several treatments had significant ($p \leq 0.0001$) positive effects on NO_3-N content compared to control. The mean soil NO_3-N content increased significantly from 10.32 mg kg^{-1} in control to 16.53 mg kg^{-1} and 15.81 mg kg^{-1} in LysteGro and urea application, respectively. The combined effect of half dose of urea application along with JumpStart® and MYKE® Pro also improved

NO_3-N recovery over control, however the effect size was smaller than LysteGro and urea. NO_3-N content was 60.17%, 53.19%, 21.80% and 15.6% higher in LysteGro, urea, half dose of urea with JumpStart® and half dose of urea with MYKE® Pro, respectively, over control.

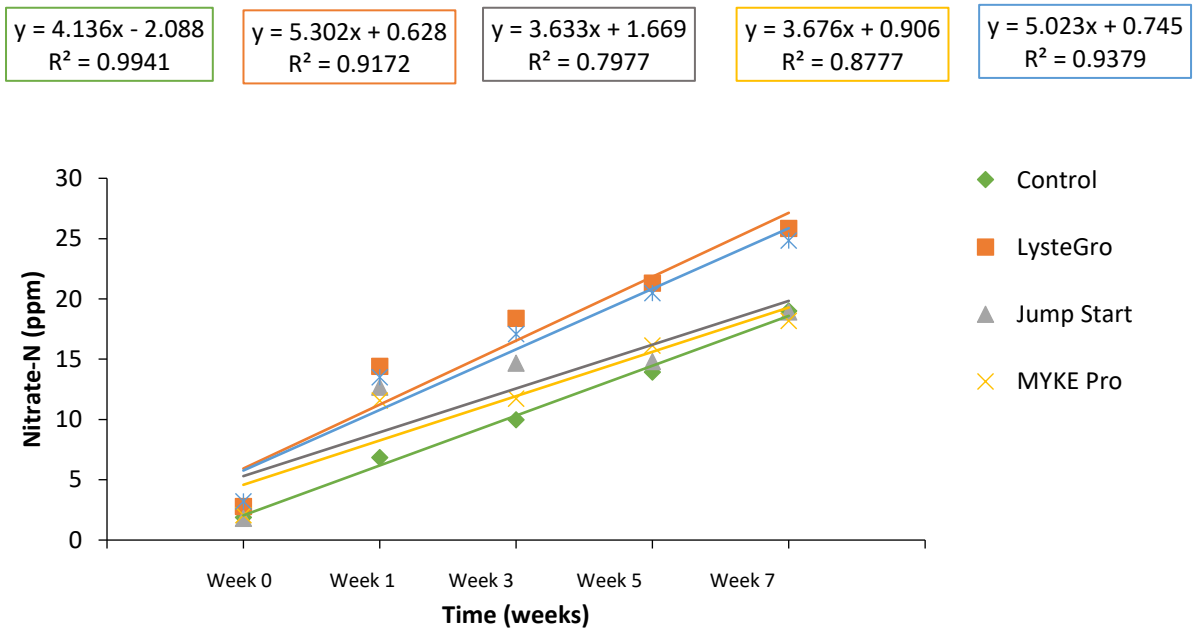

Figure C2: Effect of fertilizer treatment on NO_3-N content (mg kg^{-1}) in soil over time when incubated at 20˚C and 22% soil moisture.

Incubation period (in weeks) also significantly ($p \leq 0.0001$) affected soil NO_3-N content. For all treatments, the NO_3-N content increased over time during the incubation and a linear relationship ($R^2 \geq 0.8777$) was recorded for each treatments' relationship between incubation period and NO_3-N content (**Figure C2**). The highest soil NO_3-N content of 25.83 mg kg^{-1} followed by 24.83mg kg^{-1} was obtained in LysteGro and urea application, respectively, at the seventh week of the incubation. This is pertinent that LysteGro and urea treatments had similar increase in slope with time because it indicates that LysteGro biosolid-based biofertilizer produces similar increases in soil NO_3-N as traditional synthetic N fertilizers. These two

treatments also had the steepest slope among all the treatments indicating significant treatment effects.

C 1.3 Total Mineral Nitrogen

The data indicates that NO_3-N makes up major proportion of the mineral nitrogen pool as NH_4-N contributed only 2-3 mg kg^{-1} to it. The total mineral nitrogen content (NH_4-N + NO_3-N) differs significantly ($p \leq 0.0001$) with various treatments (**Figure C3**).

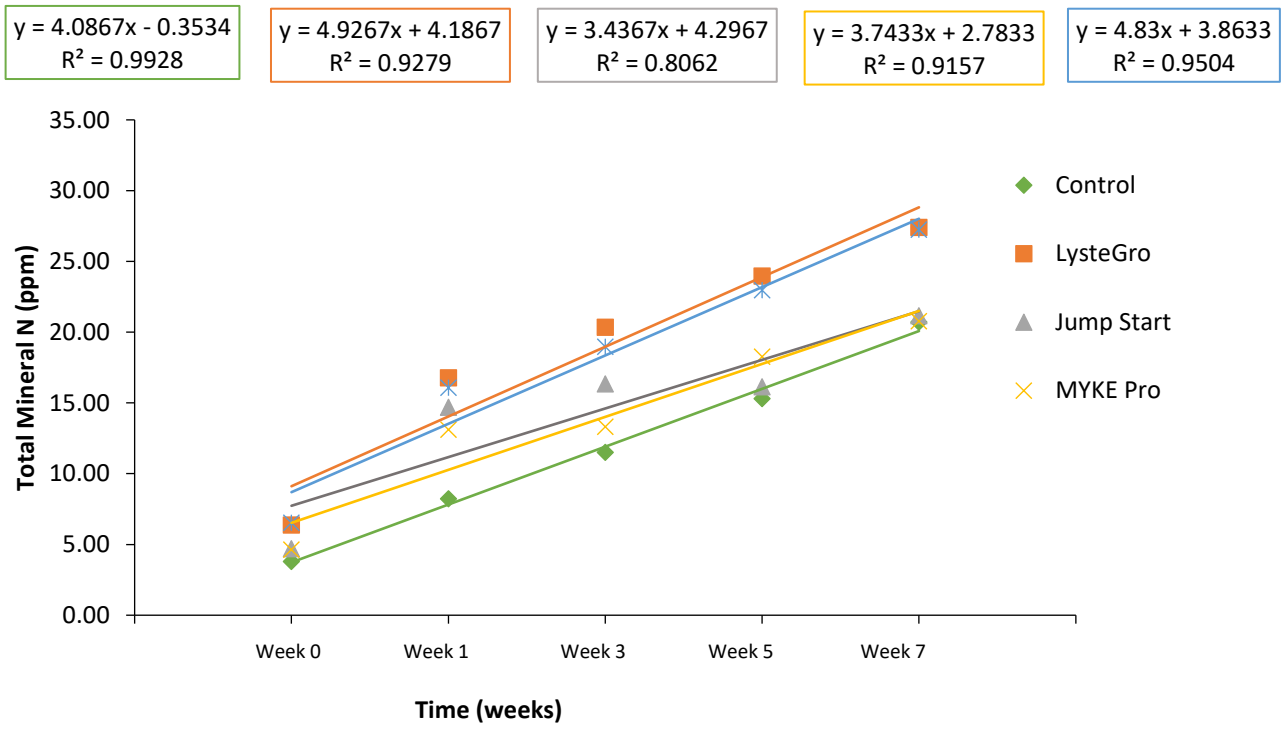

Figure C3: Effect of fertilizer treatment on total mineral N content (mg kg^{-1}) in soil over time when incubated at 20˚C and 22% soil moisture.

The mean mineral-N content increased from 11.91 mg kg^{-1} in control to 18.97, 18.35, 14.61 and 14.01 mg kg^{-1} in LysteGro, urea, JumpStart® and MYKE® Pro treatments, respectively. The corresponding increase values were 59.3% (LysteGro), 54.1% (urea), 22.7% (JumpStart®) and

17.6% (MYKE® Pro) higher total mineral N than that of control. The mineral N content in soil increased ($p \leq 0.0001$) with time during the incubation period. A linear relationship ($R^2 \geq 0.8062$) was observed for all treatments' relationships between incubation period and total mineral N content.

There was no significant interaction effect between fertilizer treatments and incubation period ($p > 0.05$). The highest total mineral-N was obtained in soils treated with LysteGro (27.40 mg kg^{-1}) followed by urea (27.23 mg kg^{-1}) at week 7 of incubation. At this stage of incubation, the total mineral N content was almost equal in control (20.70 mg kg^{-1}), JumpStart® (21.17 mg kg^{-1}) and MYKE® Pro (20.77 mg kg^{-1}) treatments.

The increase of NO_3-N, NH_4-N and mineral N content in soils receiving LysteGro was likely related to the supply of easily mineralizable N by this biosolids-based biofertilizer. It was highlighted by Iglesias-Jimenez and Alvarez (1993) that biosolids contain 16-21% of N as NH_4-N and NO_3-N, meaning these products can be used as source of inorganic N in agriculture. Increased availability of NO_3 and NH_4 in soil with biosolids application has been reported in various studies (Cuevas et al., 2000; Horrocks et al. 2016; Iglesias-Jimenez and Alvarez 1993; Ramadass and Palaniyandi, 2007; Singh et al., 1988).

Many reports have indicated the increased recovery of NH_4-N, NO_3-N and total mineral N in soil with urea application, which is related to increased mineralization in these soils (Malhi et al., 2006; Noguera et al., 2010). Prosser (1990) indicated that urea applied to soil undergoes hydrolysis to form ammonia which is further transformed to NO_3^- through the nitrification process. The low proportion of NH_4-N in total mineral N content of is mainly due to rapid oxidation process, which converts NH_4-N to NO_3-N (Fageria, 2014; Gupta 2015; Nascente et al., 2017).

C2 Available Phosphorus (P) Content

The data presented in **Figure C4**. indicates that the amount mineralized P (available P) in soil differs among various fertilizer treatments. Application of different fertilizers significantly ($p \leq 0.0001$) improved P availability over control irrespective of incubation period. The highest available P content was recorded in LysteGro followed by JumpStart® treatment. The mean P content throughout the incubation in soil ranged from 16.84 mg kg^{-1} in the control to 22.84 mg kg^{-1}, 21.85 mg kg^{-1}, 20.50 mg kg^{-1} and 20.57 mg kg^{-1} in LysteGro, JumpStart®, MYKE® Pro and urea treatments, respectively.

The available P content also differed significantly ($p \leq 0.0001$) with incubation period (**Figure C4**). Mean available P content increased throughout the incubation period for urea treatment. However, for LysteGro, JumpStart®, and MYKE® Pro treatments it was highest at week 3 and declined slightly afterwards. Singh et al. (1988) saw a similar trend of initial increase followed by a decrease in available P in an incubation study of soil with various organic matter. These authors attributed the decrease due to the absorption of mineralized P on clay minerals in soil (Singh et al., 1988).

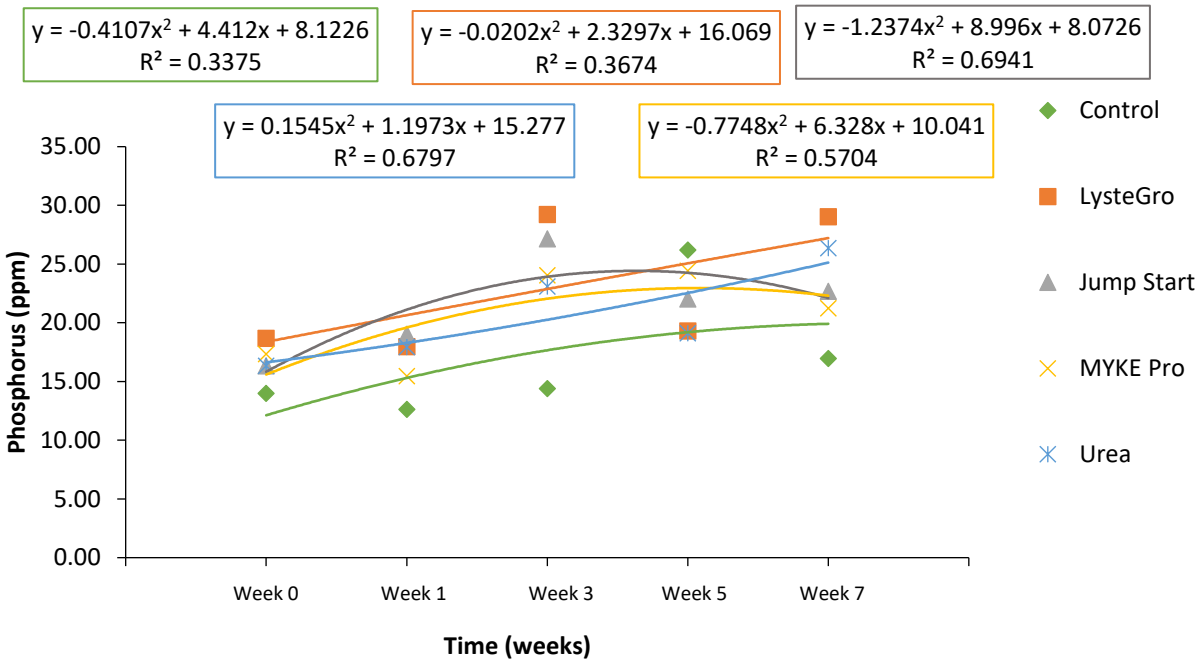

Figure C4: Effect of fertilizer treatment on P content (mg kg^{-1}) in soil over time when incubated at 20˚C and 22% soil moisture.

The initial increased P availability in LysteGro treatment was mainly due to the increased supply of P and improved microbial activities by the application of compost. Various reports are available in the literature demonstrating the improved P availability with the incorporation of biosolids to soils. (Ramadass and Palaniyandi, 2007; Zhang et al. 2006). Similarly, Horrocks et al. (2016) also reported that one year of biosolids application significantly improved the soil Olsen P. The increase in Olsen P with each tonne of biosolids applied was 0.15 mg kg^{-1}. It was further highlighted in an earlier report by Iglesias-Jimenez et al. (1993) that biosolids are as efficient as inorganic P fertilizers with respect P supply. It was suggested that biosolids may stimulate the transformation of organic P into its inorganic forms due to enhanced phosphatase enzyme activity (Stevenson, 1986; Peucci, 1990).

Previous studies have also demonstrated increased P availability with application of fungi of *Penicilium* spp. and *Glomus* spp. Cunningham and Kuiack (1992) reported that *P. bilai* produced oxalic and citric acid which caused acidification which solubilized the insoluble P complexes and enhanced the P availability. Similarly, mycorrhizal association also improved P uptake from the poorly soluble iron and aluminum phosphates and rock phosphate (Bolan, 1991; Miyasaka and Habte, 2001; Vassiev et al., 2001).

4.4. Available K:

The available K content in response to the various fertilizer treatments is depicted in **Figure C5**. Treatment effects on K availability were significant ($p \leq 0.0001$). The highest available K content was recorded in LysteGro treated soil, followed by JumpStart® and urea treatments. The mean K availability recorded was 58.47 mg kg^{-1} in the control, followed by 73.25 mg kg^{-1}, 57.44 mg kg^{-1}, 63.53 mg kg^{-1} and 64.38 mg kg^{-1} in soils receiving LysteGro, JumpStart®, MYKE® Pro and urea, respectively. The data indicated that the LysteGro application enhanced soil available K by 25.2% over the control.

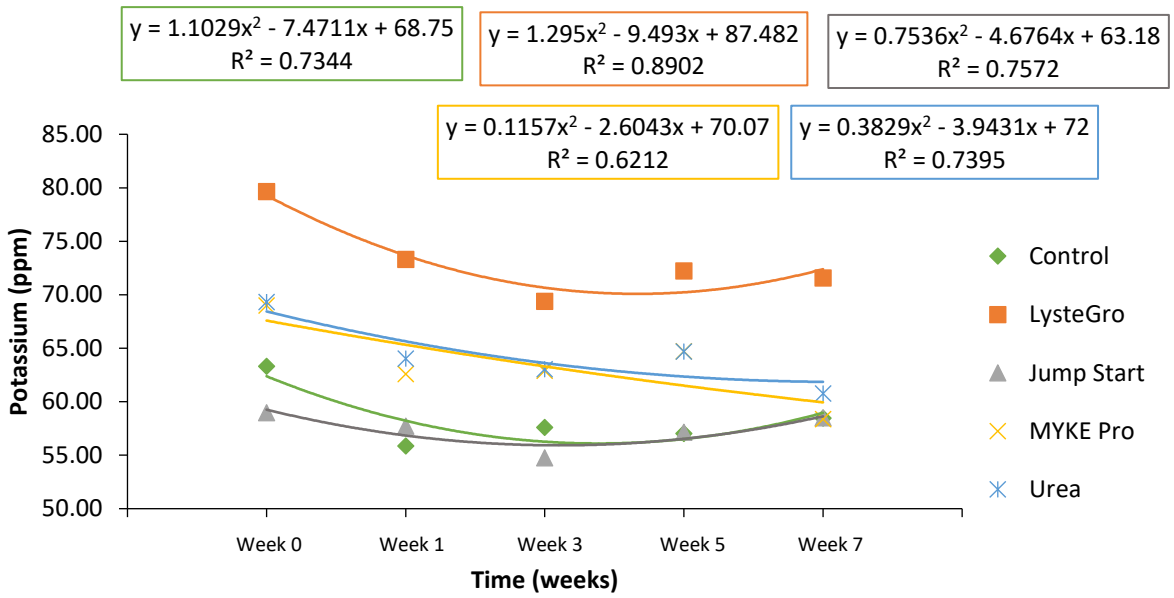

Figure C5. Effect of different treatments on K content (mg kg^{-1}) in soil over time when incubated at 20˚C and 22% soil moisture.

A long-term experiment deHaan (1981) demonstrated that the available K content in biosolids fertilizer was comparable to mineral K fertilizers. Application of biosolids for five consecutive years enhanced the soil K availability by 26 % over the control treatment (Hartl et al. 2003). A significant increase in soil available K with biosolids application was also reported by different researchers (Blanchet et al., 2016; Castro et al. 2009; Ramadass and Palaniyandi, 2007; Ranjbar et al., 2016; Warman et al., 2004). Therefore, the findings from the present study are consistent with existing literature.

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