Biochar and PGPR as Methods for Low-input Management of Bioenergy Grasses

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Abstract

Fast growing, highly productive, low N demanding, C_3 and C_4 perennial rhizomatous grasses (PRGs) have the potential to be excellent future lignocellulosic bioenergy feedstocks under temperate climatic conditions. Biochar, a black carbon sequestering material, and an active plant growth promoting rhizobacteria (PGPR) community have both been shown to enhance plant growth, although they have not been tested with relevant PRGs under Québec (high-latitude temperate zone) field conditions. Agronomic field studies were conducted on bioenergy crop production with two grass species (switchgrass - SG - C_4 species and reed canarygrass - RCG - C_3 species) using biochar (0 or 20 t ha^{-1}) and PGPR (able to mobilize soil phosphorus or supply nitrogen) as soil amendments. The SG research was conducted at three field sites in southern Québec. The sites represented three soil types in two climatic regions (sites 1 and 2 in southwestern Québec and associated with McGill University, and site 3 associated with Laval University in southeastern Québec). In these studies, interactions occurred between biochar and PGPR for many of the measured switchgrass growth variables (height, stand density, tiller DWs, dry biomass production, N export) that resulted in increasing growth and biomass yield at the comparatively warmer growing season sites 1 and 2. At site 3, with a cooler summer climate, both biochar and PGPR caused improved levels of at least some variables. The reed canarygrass work was conducted at one site, associated with McGill University, for 2 years. A positive biochar by N interaction ($P < 0.05$) occurred for both C_4 and C_3 grass productivity. Biochar amendment tended to increased nitrogen use efficiency (NUE) at 50 kg N ha^{-1} and 150 kg N ha^{-1} for C_4 and C_3 grasses, respectively. A similar pattern was observed for apparent N recovery for both PRGs and C content of reed canarygrass. Based on three years of data collection at three sites (nine site-years) for switchgrass, plus 2 additional site years for reed canarygrass, PGPR along with biochar generally, and particularly in the second and third years of the experiments promoted bioenergy plant growth and N use efficiencies, demonstrating their potential utility in development of low-input bioenergy feedstock production systems for high-latitude temperate zone areas, such as southern Québec.

Résumé

Ayant de la croissance rapide, hautement productive et une faible consommation de N, les herbes vivaces à rhizomes (GPR) C_3 et C_4 ont le potentiel d'être d'excellentes futures matières premières bioénergétiques lignocellulosiques dans des zones climatiques tempérées. Le biochar, un matériel noir séquestrant de carbone, est une communauté actif de PGPR dont tous les deux ont été demonstrés à améliorer la croissance des plantes, cependant elles n'ont pas été testées avec des PRG pertinentes dans les conditions Québecoises (zones tempérées de haute latitude). Des études agronomiques ont été menées sur la production agricole des cultures pour bioénergie avec deux espèces modèles (panic érigé-C_4, et alpiste roseau-C_3) en utilisant le biochar (0 ou 20 t ha^{-1}) et PGPR (mesure de mobiliser le phosphore du sol ou de l'azote) comme amendements du sol dans trois champs dans le sud du Québec. Les sites ont représenté les trois types de sol dans deux régions climatiques (sites 1 et 2 dans le sud-ouest du Québec et associé à l'Université McGill, et le troisième site associé à l'Université Laval dans le sud-est du Québec). Dans ces études, des interactions significatives ont eu lieu entre le biochar et PGPR pour la plupart des variables mesurées croissance (hauteur, densité, matière sèche de tiges, rendement de matière sèche, niveau de N) sur les sites avec une saison de croissance relativement chaude (1 et 2). Sur le site 3, avec un climat plus frais pendant l'été, le biochar et PGPR ont provoqué des niveaux améliorés d'au moins certaines des variables pendant les trois années. Une interaction entre le biochar et N ($P < 0.05$) a été observée pour la productivité des herbes de C_4 et C_3. Le biochar à augmenté le NUE aux niveaux de 50 kg N ha^{-1} et 150 kg N ha^{-1} pour les herbes C_4 et C_3, respectivement. Une tendance similaire a été observée pour N pour les GPR et teneur en C d'alpiste Roseau RCG. D'après les données sur trois sites, et collectionnées pour trois années (neuf années-sites), le PGPR ainsi que le biochar améliore la croissance des plantes et l'utilisation efficace de N, ce qui démontre leur potentielle dans le développement des systèmes bioénergetiques à faibles intrants de production des matières premières dans des zones tempérées d'haute latitudes comme le sud du Québec.

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Contributions of Authors to manuscript

This thesis format is manuscript-based and, as such, complies with the rules of 'Thesis Preparation and Submission Guidelines.' Chapter 3, chapter 4 and chapter 5 represent three separate manuscripts. The constant co-author on these manuscripts is Dr. Donald L. Smith. Of the other co-authors listed, Dr. Inna Teshler, a research associate with Dr. D. L. Smith, helped me in organizing and managing the field experiments. Dr. Xiaomin Zhou, financial officer, Reséau BiofuelNet Canada, provided guidance and support in arranging data and statistical analysis.

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LIST OF ABBREVIATIONS

%	Percentage
2,4 D	2,4 Dichlorophenoxyacetic acid
ANOVA	Analysis of Variance
ANR	Apparent nitrogen recovery
BacN	N-fixing PGPR
BacP	P-solubilizing PGPR
BNF	Biological nitrogen fixation
C	Carbon
C_3 species	Species that utilize the C_3 photosynthetic pathway
C_4 species	Species that utilize the C_4 photosynthetic pathway
CEC	Cation exchange capacity
cfu	Colony forming unit
Cl	Chlorine
cm	Centimeter
CO_2	Carbon dioxide
CRD	Completely randomized design
Cu	Copper
cv	Cultivar
DM	Dry matter
DOE	Department of Energy
DW	Dry weight
Fe	Iron
FW	Fresh weight
g	Gram
GHGs	Greenhouse gases
GJ	Gigajoule
h	Hour
ha	Hectare
HECP	Herbaceous Energy Crops Program
IPCC	International Panel for Climate Change
K	Potassium
kg	Kilogram
L	Liter
LB	Luria Bertani
M	Meter
Mg	Mega gram
mg	Milligram
min	Minute
mL	Milliliter
MPN	Most probable number
MRT	Mean residence time
N	Nitrogen
N_2O	Nitrous oxide
NH_4^+	Ammonium

Nif H	One of the nitrogenase genes
NO_3	Nitrate
NUE	Nitrogen use efficiency
^oC	Degree centigrade
P	Phosphorus
PGPR	Plant growth promoting rhizobacteria
PN	Combination of both N fixing and P solubilizing PGPR
PRGS	Perennial rhizomatous grasses
RCBD	Randomized complete block design
RCG	Reed canarygrass
REAP	Resource Efficient Agricultural Production
SAS	Statistical Analysis System
SE	Standard error
SG	Switchgrass
t	Ton
TCD	Thermal conductivity detector
WFPS	Water filled pore space
WUE	Water use efficiency
Zn	Zinc

CHAPTER 1

1 General Introduction

1.1 Introduction

Global climate is changing due to increases in the concentration of atmospheric greenhouse gases (GHGs), with the largest climate changes occurring at higher latitudes. This increase in atmospheric GHGs is anthropogenic and largely related to increases in carbon dioxide (CO_2) emissions, for the most part due to combustion of fossil fuels (IPCC, 2007), whereas increases in nitrous oxide (N_2O) (Smith and Almaraz, 2004) and methane are from agricultural sources (Smith and Almaraz, 2004). Climate change models predict warming and changes in precipitation; it is clear that this is already occurring in Quebec. In southern Canada, the average annual temperature increased 0.5 to 1.5^{o}C over the 20th century (Almaraz et al., 2008). Given these circumstances, the Government of Québec has strongly favored promotion of sustainable alternative energy sources as one mechanism to help meet Québec's GHG emission reduction target for 2020.

Herbaceous perennial crops with extensive root systems have long growing seasons, generally over-winter well once established, and have lower N (nitrogen) fertilizer demands since the N is internally recycled from the aboveground biomass to the rhizomes in autumn, where it is stored and translocated to new emerging shoots in the spring (Heaton et al., 2004). Both C_3 (plants that utilize the C_3 photosynthetic pathway) and C_4 (plants that utilize the C_4 photosynthetic pathway) perennial species have higher nutrient, water and solar radiation efficiencies than other plants; the former benefit most from the progressively higher atmospheric CO_2 levels, and the latter are better able to handle heat and drought stress, which are often associated with climate change. Perennial rhizomatous grasses are promising lignocellulosic feedstocks for biomass energy (a renewable form) production due to their better biofuel qualities, such as low ash, mineral, and moisture contents (Lewandowski and Schmidt, 2006). They also provide greater energy returns due to lesser energy inputs during cultivation and seeding, lower N fertilizer demands, and sequestration of carbon (C) from the atmosphere into the soil through extensive root systems.

Biochar is a charcoal like porous substance that produced from pyrolysis (thermal decomposition of biomass in low or oxygen free environment); it has gained attention with

the discoveries of long abandoned biochar treated sites in the Amazon basin that show substantially higher fertility than surrounding soils and still retain large amounts of pyrogenic carbon (Glaser et al., 2002). Biochar application to soil also improves soil moisture content and cation exchange capacity (CEC), the latter leading to greater nutrient retention (Liang et al., 2008). The binding of ammonium to the biochar surface is of particular interest as this can reduce N leaching. Biochar amended soil may require less N fertilizer to achieve target crop yields, leading to, for instance, less production of the GHG N_2O (Almaraz and Smith, 2009). Changes in soil properties that buffer developing crops against drier conditions are an important adaptation to climate change in Québec; biochar can contribute to this. Using biochar in agricultural systems also sequesters carbon from the atmosphere into soils, reducing the potential for future climate change. Moreover, biochar creates a C pool with high stability and, therefore, a longer soil-residence time (Glaser et al., 2001), which would result in some of the benefits of high soil organic matter, but with less frequent additions. These may have a role the in the cool temperate agricultural soils of Canada.

The success of bioethanol production from sugarcane in Brazil, has been attributed to lower inputs of N fertilizer since up to 80% of the plant N is derived from biological N fixation (BNF) by associated Plant Growth Promoting Rhizobia (PGPR) (Dobereiner, 1996; Pessoa-Jr et al., 2005). Numerous studies have reported investigation of diazotrophic associations with C_4 temperate grasses and their subsequent growth promotion (e.g. Bredja et al., 1998; Ker et al., 2012). Plant growth promotion by PGPR's, other than through BNF, have also been reported through the production of phytohormones, enhancement of enzymatic activities, increased nutrient uptake and other mechanisms (Dobbelaere et al., 2003). Some researchers have theorized that aliphatic carbon compounds found in the soil humus are breakdown products of extracellular polysaccharide, protein and chitin secreted or lysed from microbial cells, as well as hydrophobic long chain fatty acids synthesized by microbial cells. These substances can persist for several hundred years (Kleber et al., 2007). Thus, an active PGPR community could also contribute to long term C sequestration. Overall, biochar C persists in soils for millennia, whereas plant residues are generally degraded within months to decades. Thus, these two factors can contribute to retention of C in soils, although biochar for the longer term. In addition, if crops can be inoculated with PGPR that facilitate N uptake or acquisition of N from the atmosphere, the N fertilizer requirement of the crop will be

reduced, thereby reducing GHG emissions by new low-input production systems based on these PGPR and biochar.

The development of truly low-input sustainable systems for bioenergy crop production requires that we move beyond conventional management practices and explore innovative soil amendments and PGPRs that are as yet untested in Québec. Other results have suggested that biochar promotes seed germination, nutrient uptake and overall plant growth, but there are no field studies on the effect of biochar, along with specific PGPR inoculation, on PRG production as a bioenergy crop. The objective of this research is to optimize the production of biomass for biofuel production in Québec, allowing net reduction in GHG emissions, sequestration of C into soils through addition of biochar, PGPR inoculation and utilization of PRG root systems.

1.2 List of Hypotheses

1. Biochar, plus inoculation with N-fixing PGPR and P-solubilizing PGPR has positive effects on switchgrass (SG - a C_4 perennial warm-season grass) growth and productivity under temperate field conditions.
2. The positive effects of biochar, N-fixing and P-solubilizing PGPR inoculation on SG growth and biomass yields are reasonably constant across soil/climate conditions within southern Québec.
3. Biochar and N fertilizer increase SG growth, N use efficiency and apparent N recovery in southern Québec.
4. Biochar, N-fixing PGPR and P-solubilizing PGPR inoculation improve C_3 cool-season perennial reed canarygrass (RCG) growth under field conditions in southern Québec.
5. Biochar along with N fertilizer promote reed canarygrass (RCG) biomass yield, N export, N use efficiency and apparent N recovery under southern Québec field condition.

1.3 List of Objectives

1. To determine the effect of biochar (20 t ha^{-1}), N-fixing PGPR and P-solubilizing PGPR on the growth and productivity of a C_4 grass (SG) under high latitude field conditions.

2. To determine if the effects of biochar, N-fixing and P-solubilizing PGPR inoculation on SG growth are constant across sites within southern Québec.
3. Determine the agronomic growth performance and N use efficiency of a C_4 grass (SG) receiving biochar and N fertilizer treatment under field conditions in a high latitude region (southern Québec).
4. To investigate the effect of biochar, N-fixing PGPR and P-solubilizing PGPR on a C_3 grass (RCG) growth and biomass yield under southern Québec field conditions.
5. To determine the effects of biochar, along with N fertilizer, on a C_3 grass (RCG) growth, N export, N use efficiency and apparent N recovery, with a view toward development of a sustainable low-input system for energy grass production.

CHAPTER 2

2 Literature Review

2.1 Energy Crop (Bioenergy Grasses):

Throughout most of the period of human culture people have exploited forage crops, wood and peat as sources of renewable energy. Perennial rhizomatous grasses are herbaceous crops that have little or no woody tissue, and are mostly comprised of bunch-type grasses, generally harvested like hay at the end of growing season (Lemus, 2004). In comparison to annual biofuel crops (i.e. corn, wheat, soybean), perennial energy grasses have greater energy returns because of lower overall levels of intensive inputs, equipment use and reliance on fossil fuel energy during cultivation and seeding (Heaton et al., 2004; Lewandowski and Schmidt, 2006). These grasses regrow from their roots and do not require replanting for long periods of time (>5 years) (Lemus, 2004). They are highly productive, have a high capacity to sequester carbon from the atmosphere into soil, cause minimal soil disturbance throughout their growing period and accumulate soil organic carbon over the long term (Potter et al., 1999; Lemus, 2004). SG and RCG are both native species of eastern Canada and show promise as lignocellulosic biomass crops in near future. *Miscanthus* is an introduced grass that has also shown great promise in this area (Heaton et al., 2004).

2.1.1 *Panicum virgatum* L.

SG is a warm-season C_4 grass native to North America (ranging from Central America to the prairies of southern Canada); it has a northern adaptation limit of about 51° N (Parrish and Fike, 2005; Porter, 1966). Over 10 years the US Department of Energy (DOE) achieved yield increases of approximately 50% through selection of the best regionally adapted varieties, optimizing cutting frequency and timing, and reducing the level and timing of nitrogen fertilizer application (McLaughlin and Walsh, 1998; Lewandowski et al., 2003; McLaughlin et al., 2005). While the European Union chose *Miscanthus* x *giganteus* as a model perennial biomass crop, the DOE Herbaceous Energy Crops Program (HECP) concentrated on SG for ethanol and electricity production (Parrish and Fike, 2005; Sanderson et al., 2000), with yields in the 30 Mg DM ha^{-1} yr^{-1} range reported in parts of the US (Lewandowski et al., 2003). In Europe (UK) yields have ranged from 10.6-15.4 Mg DM ha^{-1}

(Lewandowski et al., 2003), and in Canada (southwestern Québec) 10.9-13.0 Mg DM ha^{-1} (Madakadze et al., 1998; Mehdi et al., 2000). The lower amount of ash in SG is important in its utility as a high-value feedstock energy crop, as is its high energy content of approximately 19.2 GJ t^{-1} for overwintered material and 18.5 GJ t^{-1} for fall harvested material, in eastern Canada (Sampson et al., 2000)

2.1.2 *Phalaris arundinacea* L.

RCG is a cool-season C_3 species that is invasive in temperate and boreal wetland communities in North America, as well as in Canada. It is grown as a forage crop (Lewandowski et al., 2003). RCG exhibits winter hardiness due to the storage of non-structural carbohydrates in its roots; these enable the crop to overwinter as rhizomes and to produce tillers early in the following year (Sampson et al., 2000). The development of RCG as an energy crop has been recent (last 15 years), with breeding programs in Finland and Sweden aimed at improving its biomass and lignin content, while lowering its mineral concentration (Lewandowski et al., 2003). RCG was found to produce greater biomass when nutrients were added at low and high levels becoming 35 and 195% more productive, respectively (Kercher and Zedler, 2004). In Finland and Sweden, RCG yields ranged from 5-12 Mg DM ha^{-1}, while its production in the UK yielded 6-12 Mg DM ha^{-1} (Lewandowski et al., 2003). Yields of 10.6 Mg DM ha^{-1} for RCG have been reported in Indiana, US (Cherney et al., 1986) and about 7.3 Mg DM ha^{-1} in southwestern Québec (Coulman, 1996).

2.1.3 Miscanthus

Miscanthus is a C_4 grass (Lewandowski et al., 2000) originating from East Asia. *M.* x *giganteus* is a very good candidate for bioenergy in temperate zones because of its high productivity, nutrient use efficiency, lodging resistance and disease and cold resistance. In Europe, the research and cultivation of *Miscanthus* as a biomass crop has been going on since 1983 (Lewandowski et al., 2000), with reported biomass yields of 13-24 Mg DM ha^{-1} when harvested in the early spring, to as much as 20-35 Mg DM ha^{-1} when harvested in late fall; spring harvests result in greater quality biomass as nutrient cycling to rhizomes has occurred (Lewandowski et al., 2000). Some recent trials of *M.* x *giganteus* in the US (mid-east) resulted in production of two to fourfold more biomass than upland switchgrass, 9 to 27 Mg ha^{-1} (Heaton et al., 2004). In eastern Canada (latitude ~45° N) small-scale research was

conducted in the late 1990s with yields of approximately 9 Mg DM ha^{-1} (Mehdi et al., 2000).

2.2 Biochar

2.2.1 Background

Biochar is a carbon-sequestering product that is produced through thermal decomposition of biomass under low or oxygen-free conditions along with relatively low temperature, such as <700^{o}C (Lehmann et al., 2003). Biochar characteristics are such that it tends to retain nutrients and has a high water holding capacity, which prevent leaching of nutrients through the soil profile and below the root zone (Novak et al., 2009; Major et al., 2009). Biochar is not a new concept for soil improvement and land application. Patches of black soil found in the Amazon Basin (so-called Amazonian Dark Earths or "terra preta") seem to have been covered with large amounts of residues from biomass burning (Liang et al., 2008). The scientific literature indicates that these soils were created by indigenous people, as far back as 10,000 years before present (BP – before 1950 AD), as determined by radiocarbon dating, with biochar present to varying depths (down to 1 meter). These applications were likely a result of both habitation activities and deliberate soil application by Amerindian populations before the arrival of Europeans (Lehmann et al., 2006). Man-made soils (Anthrosols) have been reported from a number of locations; soil enriched with organic material from peatland and heathland have been reported from 3000 years BP, as determined by radiocarbon dating, on the German island of Sylt (Blume and Leinueber, 2004). Others exist in the Netherlands, northern Belgium and north-western Germany, with material added to depths similar to their Amazonian counterparts (i.e. down to 1 meter). Similar soils have also been identified in Ecuador, Peru, West Africa (Benin, Liberia), and the savanna of South Africa (Lehmann et al., 2003). The terra preta (Anthrosols) contain high levels of nutrients such as nitrogen (N), phosphorus (P), potassium (K) and calcium (Ca) which is attributed, in part, to a high char content which made them much darker in colour than adjacent soils (Glaser et al., 2001). Large amounts of biochar-derived C stocks remain in these soils today, hundreds of years after they were abandoned. The total C storage is more than twice as high as Amazonian soils without biochar (Glaser et al., 2002). Such C storage in soils far exceeds the potential C sequestration in plant biomass even if bare soil were, theoretically, restocked to primary forest (Sombroek et al., 2003).

2.2.2 Production

When pyrolysis (thermo-chemical method) is used to produce biofuel biochar residue is a byproduct. Slow and fast pyrolysis technologies have been used to produce biochar; qualities depend on conditions during each step in the process and on the feedstock material. Biomass pyrolysis, for example, produces gaseous, liquid and solid products. The gases produced are often burned to generate energy for the pyrolysis process. Although the most valuable product is usually the liquid bio-oil, the solid biochar product is also valuable when used as a fuel, carbon sink, or, fertilizer (Briens at el., 2008; Glaser, 2007; Lehmann et al., 2006). During the production of biochar yields depend more on the type of biomass (feedstock) than temperature (Lehmann et al., 2006). A very promising technology for making organic slow-release nitrogenous fertilizers from biomass pyrolysis products has been patented (Radlein et al., 1997). Chan et al. (2008) have recently shown the potential of poultry litter biochar as soil amendments.

2.2.2.1 Physical properties

Physical characteristics of biochar depend mainly upon feedstock and pyrolysis conditions (Downie et al., 2009). Increasing pyrolysis temperature from 300 to 800 ^{o}C decreased the yield of biochar from 67 to 26% and increased the carbon content from 56 to 93%. The particles of char produced this way contain primarily carbon and inorganic matter (ash) and are highly porous (Downie et al., 2009). Micropores contribute most to surface area and are responsible for the high adsorptive capacity. Mesopores are important for liquid-solid adsorption processes; macropores are important for aeration, hydrology, movement of roots and bulk soil structure (Kolb, 2009).

2.2.2.2 Chemical properties

Biochar composition can be crudely divided into relatively recalcitrant carbon, labile or leachable carbon and ash (Lehmann, 2011). Charred biomass consists not only of recalcitrant aromatic ring structures, but also of more easily degradable aliphatic and oxidized carbon structure (Schmidt et al., 2000). The particulate form of biochar facilitates initiation of fairly rapid surface oxidation, within a few months (Cheng et al., 2006); but this is largely restricted to the outer areas of a particle, even after several hundred years in soils (Lehmann et al., 2005). The long-term improvement in soil fertility arises from the fact that the biomass thermal cracking process (pyrolysis) generates stable compounds consisting of

single and condensed ring aromatic carbon in particles with a high surface area per unit mass (Lehmann, 2007). This surface becomes oxidized and cation exchange capacity (CEC) develops (Liang et al., 2006) over time (Cheng et al., 2008; Cheng et al., 2006) and can lead to greater nutrient retention in "aged" rather than "fresh" biochar. The resulting high CEC captures positively charged plant nutrients like NH_4^+, K^+, Ca^{2+} and Mg^{2+}, which are retained on the biochar surface and not lost through volatilization ($NH_4^+ \rightarrow NH_3$) or leaching (K^+, Ca^{2+} and possibly Mg^{2+})(Glaser et al., 2001). Some biochars have shown fairly high concentrations of carbonates, which can be valuable as a liming material for overcoming soil acidity (Van Zwieten et al., 2010).

2.2.2.3 Biological properties

Biochar particles contain pores and large internal surface areas which are important for biological processes. Higher bacterial growth rates and physical protection of microorganisms within the pore structure were reported for biochar in soil (Pietikäinen et al., 2000). With regard to greater water holding capacity, biochar normally has water-containing pore spaces that allow continued hydration of microorganisms in a drying soil (Glaser et al., 2002). Greater microbial biomass has been suggested as a reason for greater decomposition of soil carbon in the presence of biochar (Wardle et al., 2008). The physical stability of biochar helps it act as a habitat for extraradical fungal hyphae that sporulate in the micropores, due to lower competition from saprophytes (Lehmann, 2011).

2.2.3 Effects of biochar

2.2.3.1 Effects on nitrogen cycle

Biochar application to soil can affect the N cycle, chiefly by stimulating N retention in biochar. Nitrogen is the macronutrient most sensitive to pyrolysis temperature (starts volatizing at 200 °C) therefore N-depleted biochar is less important as a direct source of nutrients, at least with regard to N than it is as a soil conditioner and driver of nutrient transformations. The conversion of NH_4^+ to NO_3^- is slowed by binding of NH_4^+ to biochar. This potentially diminishes the subsequent loss of N as N_2O and N_2 via denitrification. Thus, the biochar surface is of particular interest because it can slow the rate of nitrification (NH_4^+ amended soils may require less nitrogen fertilizer to achieve target crop yields, leading to, for instance, less contamination of ground water by nitrates (Almasri and Kaluarachchi 2004) and less production of the greenhouse gas N_2O (Almaraz et al., 2009). Biochar reduces N_2O

emission between 50 and 80% following its incorporation into the soil under soybean and grass systems, respectively, with the reductions explained as being due to increased aeration and possibly better stabilization of soil carbon (Rondon et al., 2005). It has been also assumed that biochar improved soil aeration in wet soils, thus reducing denitrification (Yanai et al., 2007). Following the addition of biochar to pine (*Pinus* sp.) forest soils nitrification rates were increased (Ball et al., 2010; Deluca and Sala, 2006). Biochar was also found to enhance biological nitrogen fixation (BNF) and beneficially influence the soil microbial community (Rondon et al., 2007). In addition to biochar decreased the leaching of inorganic nitrogen due to its adsorption capacity, high CEC and water holding capacity (Clough et al., 2010).

2.2.3.2 Effects on carbon cycle

Biochar application to soil was also able to reduce the emissions of some important greenhouse gases, such as a virtually complete suppression of methane emissions from soil amended with 20 g kg^{-1} in Colombia (Rondon et al., 2005). It has been assumed that improving aeration with the application of biochar to soil may decrease the number of methanogens and increase that of methanotrophs, absorbing more methane than is released (Lehmann, 2005). The chemical properties of biochar make it an important mechanism for this type of improved carbon sequestration. Organic materials such as crop litter, animal manure and composted wastes undergo decomposition in soil. The mean residence time (MRT) of microbially-processed soil organic carbon is as short as 30 years (Marschner et al., 2008). In contrast, biochar creates a carbon pool with high stability (Glaser et al., 2001). Liang et al. (2008) reported a MRT of 4,035 years. Modeling to long-term equilibrium yielded slightly longer MRTs of 1,300 and 2,600 years for black carbon from savanna fires in Australia at a mean annual temperature of 27°C (Lehmann et al., 2008).

2.2.3.3 Effect on nutrient transformation in soil

The cation exchange capacity (CEC) of biochar makes it a unique substance for retention of plant-available nutrients in the soil and offers the possibility of improving crop yields while decreasing environmental pollution. High rates of biochar addition in the tropical environment have been associated with increased plant uptake of P, K, Ca, Zn and Cu (Lehmann and Rondon, 2006). In particular, animal-derived biochars may directly supply nutrients in the soil which indirectly may increase the nutrient retention capacity of soil and

displace fertilizer use (Shinogi, 2003). Biochar mineral (ash) contents, which include several essential macro- and micro-nutrients, represent an important resource in the soil food web (Lehmann, 2011).

2.2.3.4 Effect on phosphorus and other nutrient cycles

Phosphorus (P) and other nutrients cause eutrophication when they leach or run off from agricultural land into water bodies. Several studies have demonstrated enhanced P uptake in the presence of biochar, but very little work has focused on the mechanisms through which biochar directly or indirectly influences the biotic and abiotic components of the P cycle. Low temperature biochars could facilitate phosphate adsorption to the surface as phosphorus is more resistant to heat than either nitrogen or sulphur (Lehmann, 2007). Alkaline phosphatase increased by 65%, and amino peptidease by 15%, with increasing rates of corn biochar application to an Alfisol (Jin, 2010).

2.2.3.5 Effect on tillage and irrigation requirements

As described above, biochar application to soil results in higher organic matter contents and water holding capacity of soils, which may facilitate reductions in tillage used in agricultural systems. Biochar application to a clay savanna soil in Colombia increased surface water infiltration (Major et al., 2009). Biochar enhanced soil moisture retention in drought-susceptible sandy soils in Ghana and increased yields of maize, yam and other crops (Lehmann et al., 2009).

2.2.3.6 Effect on soil microorganisms

Despite the importance of soil microorganisms to soil fertility and nutrient cycling, the impact of biochar on soil microbial communities is poorly understood. Application of biochar reduces nitrous oxide and methane emissions from acid savannah soils (Rondon et al., 2005), suggesting that the application of biochar may increase soil aeration or otherwise affect soil microbial communities. In addition to affecting measurable biogeochemical processes, biochar-enriched soils are associated with increased bacterial (Pietikäinen et al., 2000) and fungal (Warnock et al., 2007) growth rates, and greater overall cell biomass (Zackrisson et al., 1996). Warnock et al. (2007) proposed that biochar encourages the growth of microorganisms through increased nutrient availability (N, P and metal ions), induction of 'mycorrhizal helper bacteria' with beneficial metabolite production, and direct physical

protection of bacteria, from grazing predation, within biochar pores. About 16 of 20 microbial isolates from biochar-amended soils corresponded to plant growth promoting and/or biocontrol agents (Graber et al., 2010). The plant growth promoting organism *Trichoderma* was only isolated from the rhizosphere of pepper plants when biochar had been applied (Graber et al., 2010). The earthworm *Geopharous* may feed on microbes and microbial metabolites that are more abundant on biochar surfaces (Lavelle 1988). The tropical endogenic earthworm species *Pontscolex corethrurus* was found to prefer biochar amended soil, and ingests it for purposes other than obtaining nutrients (Topoliantz and Ponge, 2003, 2005). Biochar may be used as an inoculant carrier, substituting for the increasingly expensive and GHG releasing peat (Tilak and Subba Rao 1978, Ogawa 1989, Beck 1991).

2.2.3.7 Effect on plant growth

The beneficial properties of biochar (i.e. improved nutrient and/or water availability) will likely improve plant root growth. The number of storage roots of asparagus and the root length of rice have been found to increase following tropical soil treatment with coconut biochar (Matsubara et al., 2002). Increased radish dry matter due to N fertilizer addition varied from 95% in the control to 266% in 100 t ha^{-1} biochar-amended soil (Chan et al., 2007). A study on common bean (*Phaseolus vulgaris* L.) found that the proportion of fixed nitrogen increased from 50% without biochar additions to 72% with 90 g kg^{-1} biochar added (Rondon et al., 2007). Biochar addition to a Colombian savanna oxisol, over a 4 year study period, resulted in no treatment effects in the first year and significant yield increases in the last 3 years (Major et al., 2009). Some recent studies demonstrated positive effects of biochar on some crop yields, such as upland rice yield beginning on the first year after biochar application on a sandy soil in Brazil (Fabiano et al., 2012).

2.2.3.8 Effect on environment

Biochar as a byproduct of bioenergy manufacture helps not only sequestration of CO_2 but also may decrease production of greenhouse gases such as N_2O and methane. Reduced leaching has been demonstrated in greenhouse studies, which may be due to the adsorption behavior of biochar (Lehmann et al., 2003). Some studies have revealed that biochar sorbed more herbicide like atrazine, diuron, teobutylazine than un-charred manure, biosolids, etc. (Zheng 2010, Yu 2006, Wang 2010). A study regarding the degradation of the insecticides

chloropyrifos and carbofuran, found that degradation decreased with increasing amounts of biochar applied, while the uptake of the insecticides by onion plants also decreased with greater biochar application rates (Yu, 2009). A study showed that, compared to compost, biochar is much more efficient at reducing the bioavailability of cadmium and zinc, mostly because biochar raised soil pH more than compost did (Beesley et al., 2010). A dairy manure biochar (produced at 350 ^{o}C) sorbed several times more lead than activated charcoal(very low ash) due to their large surface area (Cao et al., 2000). Coconut charcoal was found to be most efficient in promoting oil biodegradation in laboratory work on crude oil contaminated desert soil (Cho et al., 1997).

2.3 PGPR

It is well known that a considerable number of bacterial species, mostly those associated with the plant rhizosphere, are able to exert beneficial effects on plant growth. Therefore, their use as inoculants or control agents for agricultural improvement has been a focus of numerous researchers for many years (Lucy et al. 2004; Bashan and de-Bashan 2005; Rodriguez et al. 2008). This group of bacteria has been termed "plant growth promoting rhizobacteria" (PGPR) (Bashan and Holguin 1998), and among them are strains from genera such as *Pseudomonas*, *Azospirillum*, *Burkholderia*, *Bacillus*, *Enterobacter*, *Rhizobium*, *Erwinia*, *Serratia*, *Alcalígenes*, *Arthrobacter*, *Acinetobacter*, *Flavobacterium,* etc. Bacteria that inhabit plant external surfaces or are internal to tissues are commonly named epiphytic and endophytic, respectively (Andrews and Harris 2000; Kuklinsky-Sobral et al., 2004). Both can contribute to the health, growth and development of plants. Although plant growth promoting bacteria occur in soil, usually their numbers are not sufficient to compete with other bacterial strains commonly established in the rhizosphere. Therefore, to improve their agronomic utility, inoculation of plants with target microorganisms, to increase the levels normally found in soil, is necessary to take advantage of their beneficial properties for plant yield enhancement (Igual et al., 2001). A prerequisite for introducing these beneficial bacteria into the environment is that, in addition to plant growth promotion, they should have the ability to compete with existing soil microflora.

2.3.1 Nitrogen-fixing PGPR

Microorganisms capable of biological nitrogen fixation are all bacteria and those beneficial to crops are generally soil bacteria and include rhizobia and free-living diazotrophs. These N-fixing bacteria are collectively considered to be plant growth promoting rhizobacteria (PGPR) and are often found near, on (*epiphytic*), or within (*endophytic*) the roots of plants (Glick, 1995; Gray and Smith, 2005; Vessey, 2003). The diazotrophs isolated from sugarcane include *Azospirillum* and *Acetobacter* or *Gluconacetobacter* species, as well as endophytic diazotrophs of the genera *Herbaspirillum* and *Burkholderia* (Boddey et al., 1987; Dobbelaere et al., 2003). Members of the diazotrophic genus *Azospirillum* are important sources of N fixation and N transfer to many plants (Dobbelaere et al., 2003). *G. diazotrophicus*, the predominant diazotroph from sugarcane, has also been shown to colonize rice, wheat, maize and *Arabidopsis thaliana* (Cocking et al., 2000). Inoculation of *Azospirillum lipoferum* and *A. brasilense*, isolated from kallar grass, onto rice provided nearly 70% of the plant's nitrogen (Malik et al., 1997). Yanni et al. (1997) reported increased grain yield of rice with the inoculation of two endophytic strains of *Rhizobium leguminosarum* var. *Trifoli* which was 10-45% over a wide range of N supply in a field experiment. Beside this, biochar has been reported as an effective inoculants carrier as other carrier material that ensured the survival of *Bradyrhizobium japonicum* for more than 6 months at an acceptance level (Khavazi et al., 2007). Kumutha et al. (2011) reported a significant increase of native mycorrhizal response to biochar with *Azospirillum* application in terms of root colonization of maize. Recently Ker et al., (2012) reported PGPR associated with SG (mixed PGPR) that, when inoculated onto SG plantations, caused positive growth and yield responses under field conditions. These mixed PGPR consist of strain of *Paenibacillus polymyxa*, two strains of *Pseudomonas* species, two strains of *Rahnella* species and three strains of *Serratia* species; where *Paenibacillus polymyxa* was confirmed to possess the Nif H gene, a marker for N fixation capabilities (Ker et al., 2012).

2.3.2 Phosphate-solubilizing PGPR

About 50% of the total organic forms of phosphorus (stable) are synthesized by plants and microorganisms in soil (Dalal, 1977; Anderson, 1980; Harley et al., 1983). Several P-solubilizing bacteria have been reported; some of these have been reported to promote growth of, for instance, lettuce and maize (Chabot et al., 1993; 1996). In addition to this,

combined inoculation of P-solubilizing bacteria, with other microorganisms (*Azospirillum*) resulted in improved grain and dry matter yields, with a increase in N and P uptake in barley, compared with separate inoculation with each strain (Alagawadi et al., 1992). Six phosphobacteria strains, including P-solubilizing bacteria and phosphate metabolizing bacteria, were isolated from the rhizosphere of perennial rye grass (*Lolium perenne*), white clover (*Trifolium repens*), wheat (*Triticum aestivum*), oat (*Avena sativa*) and yellow lupin (*Lupinus luteres*) from a volcanic soil in Chile and are now being screened under pot and field conditions (Jorquera et al., 2008). A strain of *Burkholderia cepacia* has been shown to have phosphatase activity and to improve the yield of tomato, onion, potato and banana in field tests, and is currently being used as a commercial biofertilizer in Cuba (Martinez et al., 2003).

Connecting text for Chapter 3

This chapter presents findings related to the SG growth and yield using biochar and specific PGPR (N-fixing, P-solubilizing or their combination) at three field sites located in two climatic regions (warmer associated with McGill University and cooler associated with Laval University) of southern Québec. It is important to determine the agronomic responses of SG inoculated with PGPR and grown in soil amended with biochar under high-latitude temperate zone field conditions. We investigated SG growth variables (i.e. height, stand count, fresh weight and dry weight) during 3 growing seasons, N export, NUE of aboveground dry biomass when grown in soil amended with biochar and/or inoculated with specific or combined PGPR. This chapter describes and disucsses the results of this work with a view toward development of low-input sustainable production systems for SG.

I have contributed to all the work contained in the following chapter, which includes reviewing the pertinent literature, conducting field experiments, analyzing the data and writing the following chapter. The chapter will be submitted, as a manuscript, to a selected journal for publication. The manuscript is co-authored by the me, Dr. Donald L. Smith, Dr. Inna Teshler and Dr. Xiaomin Zhou of the Department of Plant Science, Macdonald Campus of McGill University and Dr. Suzanne Allaire from Department of Soil and Agroenvironment Engineering and Horticulture Research Center and Dr. Anne Vanasse of Department of Plant Science, Laval University. Dr. Teshler assisted in managing the field experiments at the McGill Univesity field site. Dr. Zhou provided important input regarding methods of statistical analysis. Drs. Allaire and Vanasse managed the field site at Laval University. This research was supported by funds from Fonds de Recherche Nature et Technologies (FQRNT).

CHAPTER 3

3 Title: Effect of biochar and specific PGPR on switchgrass growth in southern Québec

3.1 Abstract

SG (*Panicum virgatum* L.) is fast growing, highly productive and a native C_4 perennial grass that has been identified as a potential lignocellulosic biomass crop for North America. Biochar, a carbon sequestering product has been shown to increase soil pH and nutrient availability, leading to crop yield improvements that persist for years. In addition to biochar, an active PGPR community can contribute to long-term carbon sequestration, as well as provided nitrogen through BNF or enhancing the availability of other nutrients (P, Zn, Fe, etc.) to plants. While biochar and PGPR have the potential to improve the efficiency of bioenergy grass production, they have not been tested under high-latitude temperate zone field conditions. Therefore, the objective of this study was to determine the effect of PGPR (able to mobilize soil phosphorus or supply nitrogen) and biochar (20 t ha^{-1}) on SG growth variables (i.e. height, stand count, dry biomass, aboveground N export) at three field sites in two climatic regions (warmer and cooler) of southern Québec. Here, we present findings regarding SG productivity for three consecutive years under field conditions. Overall, PGPR inoculation and biochar application resulted in an increase of up to 30% in dry biomass yield (range 9-30% with increases above 18% generally being statistically significant) and a 40% increase in nitrogen export (kg N ha^{-1}), compared to the control treatment. The data also indicated biochar by PGPR interactions for some measured variables. This positive effect of biochar and PGPR inoculation on SG growth across southern Québec indicates a possible sustainable low-input energy grass production system for temperate climatic conditions.

3.2 Introduction

Renewable energy resources have become a high priority as alternatives to fossil fuels, due to the need to find new renewable energy sources and due to their environmental benefits. There is clear evidence of climate change (increased frequencies of heat waves, drought, violent storms, heavy precipitation events) occurring in Québec (Almaraz et al., 2008), leading the Government of Québec to proactively implement innovative policies to reduce GHG emission. The development of a viable bioenergy sector is strongly favored in Québec; biomass, from promising alternative energy crops with low fertilizer requirements, long growing seasons, and sustained root systems (to store carbon in soil) continues to gain attention. Perennial rhizomatous grasses (PRGs) are herbaceous crops which have higher nutrient, water and solar radiation efficiencies than other plants, where greater nutrient use efficiencies are at least in part due to lower N fertilizer demands, since the N is internally recycled from above-ground biomass to the rhizomes, in autumn, where it is stored over winter, and then translocated to new emerging shoots in the following spring (Heaton et al., 2004; Lewandowski and Schmidt, 2006). Crop harvest after senescence (either late autumn/early winter or early spring) usually results in a better biofuel quality as the feedstock biomass has lower ash (Si), mineral (mainly N, Cl, K) and water contents (Heaton et al., 2004; Lewandowski and Schmidt, 2006). Perennial rhizomatous grasses (PRGs) have larger root systems, highly lignified stems and root and rhizome tissues through which they can store carbon in soil (Lewandowski et al., 2003). Among PRGs, SG (C_4 grass) was selected by the U.S. Department of Energy (DOE) Herbaceous Energy Crops Program (HECP) for ethanol and electricity production (Parrish and Fike, 2005; Sanderson et al., 2006), with yields in the 30 Mg DM ha^{-1} yr^{-1} range reported in parts of the US (Lewandowski et al., 2003) and in Canada (southwestern Québec) 10.9-13.0 Mg DM ha^{-1} (Madakadze et al., 1998; Mehdi et al., 2000).

Biochar is black carbon-rich material which has been demonstrated to increase soil pH and nutrient availability, leading to crop yield improvement (Glaser et al., 2002; Lehmann et al., 2011; Steiner et al., 2008). This carbon sequestering product can slow the rate of nitrification ($NH_4^+ \rightarrow NO_3^-$) and reduce N_2O emissions (Lehmann, 2007). Carbon input as biochar serves to offset the carbon removed in crops harvested as biofuel feedstocks. While biochar can be manipulated to contain critical plant nutrients (Radlein et al., 1997), even unmodified

biochars improve soil fertility because their high surface area retains water and nutrients (Marris, 2006). Discoveries of long abandoned biochar treated sites in the Amazon Basin show that these effects can last for 1000s of years (Liang et al., 2008). Thus, biochar amended soils reduce N fertilizer application and so can lessen groundwater water contamination and lead to reduced emissions of the very potent greenhouse gas (GHG) nitrous oxide (N_2O). Biochar enriched soils are associated with increased microbial dynamics due to sorption and inactivation of growth inhibiting substances (Lehmann et al., 2011), increased nutrient availability (N, P and metal ions) and direct physical protection from grazing predation within biochar pores (Warnock et al., 2007).

Microorganisms associated with the plant rhizosphere, either living in symbiosis or free-living and coexisting with plants, can have beneficial effects on plant growth. These PGPR can increase crop yields by facilitating nutrient uptake by plant roots, producing plant hormones that directly stimulate plant growth and by improving plant resistance to disease (Gray and Smith 2005). There is growing evidence that much of the resistant soil humus is a byproduct of microbial biosynthesis, and these substances can persist for several hundred years (Marschner et al., 2008; Kleber et al., 2007). It seems possible that an active PGPR community could contribute to long-term carbon sequestration, whereas plant residues are generally degraded within months to decades. Positive growth and yield responses to PGPR have been studied in several agronomically important crops, including sugarcane, wheat, rice and corn (Boddey et al., 2003; Dobbelaere et al., 2001, Rodrigues et al., 2008). An important part of the success of biofuel production from sugarcane in Brazil has been attributed to lower inputs of N fertilizer (Dobereiner, 1996; Pessoa-Jr et al., 2005). *Gluconacetobacter diaztrophicus* is the predominant diazotroph of sugarcane; it has also been shown to colonize rice, wheat, maize and *Arabidopsis thaliana* (Cocking et al., 2000).

During the last decade there have been successful isolations, identifications and characterizations of beneficial PGPR (P-solubilizing, N-fixing, etc.) (Bai et al., 2002; 2003; Duzan et al., 2004; Gray et al., 2005; Mabood et al., 2006) performed in the laboratory of D. L. Smith at McGill University. Recently Ker et al. (2012) reported on PGPR associated with SG (mixed PGPR) when inoculated onto SG plantations, caused positive growth and yield responses under field conditions. These mixed PGPR consist of a strain of *Paenibacillus*

polymyxa, two strains of *Pseudomonas* species, two strains of *Rahnella* species and three strains of *Serratia* species; where *Paenibacillus polymyxa* was confirmed to possess the nif H gene, a marker for N fixation capabilities (Ker et al., 2012).

While biochar and PGPR have the potential to improve the efficiency of bioenergy crop production and reduce greenhouse gas emissions, they have not been tested for effects and interactions on relevant crops in southern Québec. Our objective, therefore, was to test the effects of SG seed inoculation with some specific PGPR (N-fixing, P-solubilizing), along with biochar (20 t ha^{-1}) application to several soil types under field conditions. The successful establishment of SG depends upon a wide range of factors, such as seeding rate, planting method, soil type, weather, weed and pest control, fertilizer amendments, etc. (Parrish and Fike, 2005; Sanderson and Reed, 2000, Schemer et al., 2006). Here we present the growth responses and yield of SG inoculated with N-fixing, P-solubilizing PGPR, and a combination of the two PGPR (N-fixing and P-solubilizing), in soils amended with biochar, or not (0 or 20 t ha^{-1}). We were interested in determining whether the combination of specific PGPR and biochar would have positive growth effects on SG.

3.3 Material and methods

3.3.1 Field sites and experimental design

A field study was conducted on three soil types, at two research sites in southwestern Québec during the years 2010 to 2012: the Emile A. Lods Agronomy Research Station of the Macdonald Campus (42°28'N 73°45'W) of McGill University, Ste-Anne-de-Bellevue (2900 to 3100 corn heat units) and St-Augustin-de-Desmaures (2300 to 2500 corn heat units), associated with Laval University, and with a sandy loam soil (site 3). Two experiments were conducted at the Ste-Anne-de-Bellevue site, one on Saint Bernard loam (melanic Brunisol, loamy mixed, frigid typic Hapludalf) soil (field 1), one at the Saint Amable site (field 2) on a sandy loam soil (frigid typic Endoaquent (Dystric Gleysol .Field-site 1 was fallow during the previous year (2009) whereas corn and barley, respectively, were cultivated (without any fertilization) at field-site 2 (sandy soil) and field-site 3 (sandy loam) during 2009. Soil pH was 5.3, 5.5 and 6.4 for field sites 1, 2 and 3, respectively, during 2010. Details of soil classification and soil texture of the three field sites are presented in Table 3.2. The experiment was organized following a randomized complete block split-plot design with four

blocks (replicates). The treatments were comprised of factorial combinations of biochar (0 and 20 t ha^{-1}), with PGPR inoculation, where the inoculation levels were: an N-fixing set of PGPR (applied or not) and a P-solubilizing PGPR (applied or not); a combination of the N-fixing and P-solubilizing PGPR. Biochar was the whole plot factor Each block contained 6 aggregated plots with biochar added at 20 t ha^{-1} and 6 aggregated plots without biochar (the whole plots), plus N-fixing PGPR, P-solubilizing PGPR, a combination of both PGPR, a control and three levels of N fertilizer (0, 50 and 100 kg N ha^{-1}).

3.3.2 Seed inoculation and seeding rate

Seeds were inoculated {N-fixing and P-solubilizing bacterial cultures each at 10^8 colony forming units (cfu) per mL} by seed coating with peat, a rate of 8 g peat kg^{-1} seed 24 h before seeding. Detailed descriptions of the PGPR (N-fixing or P-solubilizing) are given in Table 3.3. The bacterial inoculants were added to SG (cv. Cave-in-Rock) seeds at the rate of 140 mL inoculants kg^{-1} seed, then vortex-mixed and allowed to sit at room temperature for 24 h (Ker et al., 2012). Inoculated seeds were then air dried (for at least 1 h) in a laminar air flow hood, before seeding in the field. Control plot SG seeds were also inoculated with an equivalent amount of sterile bacterial medium (LB medium for N-fixing and King's B medium for P-solubilizing PGPR) and peat to avoid the risk of cross-contamination. Seeding was conducted at the recommended rate of 10 kg ha^{-1}. Control plots were seeded first, in order to reduce the risk of contamination with bacterial inoculants. Each plot was 6 m^2 (1.5 x 4 m) at sites 1 and 3, and 7.5 m^2 (1.5 m x 5 m) at site 2. Seed were placed 1.5 cm below the soil surface, as recommended (Christensen et al., 2010); seeding, at a rate of 10 kg ha^{-1}, was conducted with a Plotman seeder (Fabro limited, Swift Current, Saskatchewan, Canada).

3.3.3 Cultural management and sampling

SG germinates well at soil temperatures above 10 ^oC, and seeding was conducted on 21st June, 2010, by which time the soils were well above the threshold temperature (Figure 1). Biochar (20 t ha^{-1}) was applied into the randomly selected whole-plot portions of each block. N fertilizer was broadcast as NH_4NO_3 (27:0:0 % N:P:K). This fertilizer was added to the soil in a split application with one third added during the spring (prior to seeding) and the rest (two thirds) at the inflorescence stage (Gustavsson, 2011). Herbicide (2, 4 D) was sprayed on the soil post-crop emergence (at the leaf development stage (10-11) – Gustavsson, 2011)

to control weeds. Additional weed control was conducted by hand throughout the establishment year and no irrigation water was applied. At the flowering stage, SG height and number of tillers in one meter or row (stand count/density) were measured. At the end of the year (before the first killing frost) plants from 1 m of three randomly selected row segments within each plot were cut at a 10 cm height. All harvested herbage was weighed immediately following harvest. Subsamples were oven-dried to a constant weight, at 60 ^{o}C, to determine moisture and dry matter content. During the early spring of the following year another sampling was conducted with a mechanical harvester (Model no. SMN WB WS 24-18-00-04, Swift Machine and Welding Ltd., Swift Current Saskatchewan, Canada, cut size 60 cm). During the second crop year (2011), sampling was conducted at three crop growth stages: inflorescence emergence (stage 50), flowering (stage 61) and harvest ripening/senescence (stage 89+) (Gustavsson, 2011). At each harvest, plants were cut from 50 cm segments of four randomly selected rows, then weighed, dried (sub-samples) and weighed again, as described above. About 100 g of dried sample material was harvested from each plot in the autumn of 2010, the spring of 2011, the fall of 2011 and the spring of 2012, at all three sites, and stored for further chemical analysis.

3.3.4 Elemental analysis

An elemental analyzer (Model no. NC 2500, Thermo Quest CE Instruments from Isomass Scientific Inc., Calgary, Canada) was used for determination of N concentration in aboveground dried grass samples. The principle of operation is the combustion of the sample: i t must be quantitative and instantaneous so that the combustion gases can be efficiently eluted through the chromatographic column and thermal conductivity detector (TCD), so it will give output signals proportional to the sample elemental composition. The instrument uses the dynamic flash combustion method, which ensures these conditions (Pella and Colombo, 1973). The SG samples (properly dried) were homogenized by careful grinding (Wiley Mill, model no. 4, Thomas Scientific, USA screen size 1mm). The sample size analyzed was generally 30-60 mg. A weighed amount of sample in a tin capsule was placed in the autosampler drum where it was deareated (to remove any atmospheric nitrogen), then introduced into a vertical quartz tube heated to 1000 ^{o}C, with a constant flow of helium (carrier gas). A few seconds before each sample dropped into the combustion tube, the helium stream was enriched with a measured amount of high purity oxygen to

achieve a strongly oxidizing environment, which guarantees near-complete combustion, even of thermally resistant substances. To achieve quantitative oxidation the combustion gas mixture is driven through an oxidation catalyst (Cr_2O_3) zone, then through a subsequent zone of copper which reduces nitrogen oxides formed during combustion or catalyst oxidation, to elemental nitrogen and scrubs excess oxygen. At the outlet of the reaction tube the gas mixture (N, CO_2, H_2O) meets a trap containing anhydrone, which adsorbs water. The resulting components of the combustion mixture are eluted and separated by a Porapack PQS column and subsequently detected by a TCD in the sequence N, CO_2.

3.3.5 Calculation and statistical analysis

The amount of N export through the removal of aboveground biomass at fall and spring harvests was calculated as follows:

N export (kg N ha^{-1}) = N concentration in aboveground tissues (kg N Mg^{-1}) x harvested biomass (Mg ha^{-1}) (Jung et al.,2011)

Statistical analyses were performed with the software package SAS 9.2 (SAS Institute Inc.). All raw data from all three field sites and both years were checked for normality and constant variance of errors before conducting statistical analyses. A split plot analysis (randomized complete block design) was conducted using the PROC GLM procedure of SAS to determine main effects and interactions of factors. Biochar was the whole plot factor and factorial combinations of the PGPR treatments made up the subplot factors. When a factor (e.g. biochar, N fixing PGPR, P solubilizing PGPR) or interaction (biochar x N fixing PGPR interaction, biochar x P solubilizing PGPR) was declared statistically significant the standard error of the mean was used to determine differences between means for three levels of significance ($P<0.05$, $P<0.01$and $P<0.001$) (Peterson,1985). T-tests were performed for the comparisons between mean pairs.

3.4 Results and discussion

Monthly mean temperature (^oC) and total precipitation (mm) from April through November in 2010, 2011 and 2012 are given for the three field sites in Table 3.1. The rate of temperature increase in the spring and the mean spring temperature were generally higher for 2010 than in 2011 and 2012. Higher than normal autumn temperatures occurred at both

western and eastern Quebec sites in 2012. The year 2012 as well 2011 was also characterized by very dry periods in June at Ste Anne de Bellevue; 2010 was very dry in May and July at the Ste –Augustin- de- Desmaures.

3.4.1 Switchgrass growth variables

3.4.1.1 Height

i) Biochar X N-fixing PGPR effect

An interaction between biochar and N-fixing PGPR (P = 0.0016) was observed at site 1 (Table 3.4, 3.5 and 3.6). There were positive effects due to inoculation with the N-fixing PGPR (P = 0.0007; P = 0.0001) at the site 2 in both years (Tables 3.4-3.5). There was also no interaction or main effect of inoculation with the N-fixing PGPR at site 3 for the years 2010 and 2011. Across the years 2010 to 2012, and across all three sites, the tallest SG occurred at the site 1. Site 1 consisted of loam soils that generally retain more water and available nutrients (Tisdale et al., 2005). With the addition of biochar and PGPR, SG roots tended to extract more available nutrients at this site, which resulted in the production of the tallest plants across all three sites. Recent field studies on SG indicated that a mixed PGPR inoculation affected SG growth and productivity during the establishment year (Ker et al., 2012), where taller grass was reported for PGPR-inoculated plots.

ii) Biochar X P-solubilizing PGPR effect

For crop height, there was an interaction between biochar and P-solubilizing PGPR at sites 1 (P = 0.0049) and 2 (P = 0.0020) during the establishment year (2010) and the second year (2011) (Table 3.4-3.5). The application of biochar (20 t ha^{-1}) increased SG height; however, biochar application to different soil types may have elicited different responses.

iii) Biochar X PN (combination of N-fixing and P-solubilizing PGPR) effect

There was no interaction between biochar and the combined PGPR inoculation (PN) for height at the three sites during both years (Tables 3.4-3.5). Combined PGPR (PN) inoculation increased plant height at sites 1 (P = 0.01 in 2010) and 2 ($P \leq 0.0001$ and P = 0.0084 in 2010 and 2011, respectively) (Tables 3.4-3.5). There was no effect due to inoculation with both sets of PGPR bacteria at site 3.

3.4.1.2 Tiller dynamics (Stand count)

i) Biochar X N-fixing PGPR effect

There was an interaction between biochar and N-fixing PGPR (P = 0.0140) at site 2 during the second year (Table 3.5). Recent field investigations regarding SG stand dynamics (inoculated with mixed PGPR) showed greater stand densities with greater tiller biomass under temperate conditions (Ker et al., 2012).

ii) Biochar X P-solubilizing PGPR effect

Inoculation with P-solubilizing PGPR increased stand count for SG (P = 0.04) at site 1 in 2011 (Table 3.5). Yield components related to stand density (i.e. number of plants per unit area, tillers per plant) for perennial grass systems can greatly affect overall yield (Parrish and Fike, 2005).

iii) Biochar X PN (combination of N-fixing and P-solubilizing PGPR) effect

There was an interaction (P = 0.0433 for 2010 and P = 0.0108 for 2011) between biochar and combined PGPR (PN) inoculation occurred at site 2 in both years for stand count (Table 3.4-3.5). Inoculation with PGPR increased number of tillers more in the second year than the first; PGPR-inoculated SG plants caused allocation of additional material for new shoot development and the incorporation of biochar into soil may encourage the growth of PGPR through increased nutrient availability and direct physical protection from grazing predation within biochar pores (Warnock et al., 2007).

3.4.1.3 Fresh biomass yield

i) Biochar X N-fixing PGPR effect

Interactions between biochar and N-fixing PGPR (P = 0.043) for fresh biomass production occurred at site 1 in 2011. During the establishment year no increases occurred for fresh biomass yield across all 3 sites. However for the second and third years, increasing fresh biomass occurred as a result of biochar and PGPR treatments. This suggests that biochar and PGPR (N-fixing or P-solubilizing) may take time to exert their effects (Major et al., 2010).

ii) Biochar X P-solubilizing PGPR effect

There was a weak positive interaction between biochar and P-solubilizing PGPR (P = 0.0393) at site 3 in 2011 at the spring harvest.

iii) Biochar X PN (combination of N fixing and P solubilizing PGPR) effect

Combined PGPR inoculation (P = 0.0267) increased fresh biomass yield during the first year at site 2. Overall, inoculation of SG with PGPR along with biochar application resulted in fresh biomass increases of 2 to 29%, where a 15% increase over the control was generally statistically significant, at the site 1, and similar increases of 2.6 to 14%, where 10% increases over the control were generally statistically significant, at the site 2 and increases of 2.6 to 19%, where 15% increases over the control were generally statistically significant, at site 3 for the years 2010 to 2012. This suggested that, during longer growing seasons, through better N acquisition (from BNF) or immobile P solubilization (P-solubilizing mechanism from P-solubilizing PGPR), or both, inoculated plants tended to produce more biomass when fully grown, than uninoculated control plants. Moreover, biochar boosted plant growth, in conjunction with PGPR inoculation, due to enhanced soil physical and chemical stability (Downie et al., 2009), moisture holding capacity and nutrient cycling (Lehmann et al., 2011).

3.4.1.4 Dry biomass yield

Biochar has been reported to increase crop yields, including rice (Asai et al., 2009), maize (Major et al., 2010) and radish (Chan et al., 2008), but field investigation of interactions with PGPR (N-fixing and P-solubilizing) along with the effects of biochar on energy grass productivity and growth have not been reported. In this field investigation, across nine site-years, increases due to treatments were observed for tiller biomass (Tables 3.4, 3.5 and 3.6) as well as aboveground total dry biomass for SG (Figure 3.1).

i) Biochar X N-fixing PGPR effect

There were no interactions between N-fixing PGPR and biochar treatments regarding biomass yield for any site-year. The N-fixing PGPR increased dry biomass production (P = 0.0136) at site 1 in 2010, at site 3 (P = 0.0016) in 2012, while biochar application increased dry biomass production (P = 0.039) at site 3 in the establishment year and in 2012 (P = 0.004) (Figure.3.1). Biochar amended soils may have enhanced availability of nutrients

(Steiner et al., 2008) and moisture (Glaser et al., 2002) leading to higher plant biomass yields.

ii) Biochar X P-solubilizing PGPR effect

There was an interaction between P-solubilizing PGPR and biochar (P = 0.0467) at site 3 for the spring harvest (2011). The P-solubilizing PGPR and biochar amended soil at site 3 resulted in the greatest dry biomass during the third year fall harvest (2012) (Figure.3.1). PGPR are also reported to increase with biochar application (Graber et al., 2010); this may be because biochar can serve as a habitat for PGPR and because nutrients may sorb to biochar surfaces, rendering them less susceptible to leaching in soil (Pietikäinen et al., 2000).

iii) Biochar X PN (combination of N-fixing and P-solubilizing PGPR) effect

The combined PGPR inoculation interacted (P = 0.0475) with biochar at site 2 during the second year. Overall, inoculation of SG with N-fixing PGPR, along with biochar application, resulted in a dry biomass increase of 10.9 to 30.3%, where 21% increases over the control were generally statistically significant, at site 1, increases of 1.3 to 16.5%, where a 10% increase over the control was generally statistically significant, at site 2 and increases of 1.4 to 5.5%, where 5% increases over control was statistically significant at site 3; inoculation with P-solubilizing PGPR along with biochar resulted increases of 9.1 to 20.5%, where 16% increases over there control were generally statistically significant, at site 1, increases of 2.4 to 6%, where 5% increases over control was statistically significant at site 2 and increases of 4 to 15%, where 11% increases over control was statistically significant at site 3; inoculation with combined PGPR inoculums (both N-fixing and P-solubilizing bacteria) resulted in increases of 9.6 to 24.4%, where increases of 19% over the control were generally statistically significant at site 1, increase of 2 to 15%, where 12% increases over control were generally statistically significant, at site 2 and increase of 3 to 13%, where 11% increases over the control were usually statistically significant, at site 3 for the years 2010 to 2012 (Figure 3.1). However two different types of PGPR (one N-fixing and one P-solubilizing) were used in this field study. The N-fixing PGPR have been shown to increase nutrient acquisition through BNF and has been reported to increase energy grass productivity (Ker et al., 2012) under field conditions, while P-solubilizing PGPR can enhance

productivity (e.g. lettuce and maize) by increasing phosphate solubilization and moderate phosphatase activity under field conditions (Chabot et al., 1996). Both of these mechanisms can stimulate SG root growth, which would expand the rhizosphere and enhance rhizosphere microbial activity (Glick, 1995; Dobbelaere et al., 2003). Thus the addition of N-fixing PGPR or P-solubilizing PGPR, alone or in combination , could result in better nutrient acquisition which, in turn, could produce larger rhizomes that lead to greater biomass yield.

3.4.2 N concentration and N export by aboveground biomass

The N concentration in harvested biomass and the amount of N export (kg N ha $^{-1}$) are shown in Tables 3.7 to 3.9. At site 1 the N export (kg N ha $^{-1}$) increased when SG plants were inoculated with PGPR (N-fixing and P-solubilizing) or biochar was applied to the soil, even though in some cases the aboveground biomass did not. The range of N export (kg N ha^{-1}) at sites 1, 2 and 3 (for two years) ranged from 22.6 kg N ha^{-1} to 180.1 kg N ha^{-1} , 27.4 kg N ha^{-1} to 125.6 kg N ha^{-1} and 42.6 kg N ha^{-1} to 205.6 kg N ha^{-1}, respectively. The concentration of N in aboveground biomass at harvest time influences the amount of N exported with the biomass removal. When aboveground biomass is harvested after nutrients are translocated to belowground tissues, it can reduce the amount of N export due to biomass removal (Jung et al., 2011). Seasonal variation occurred in N concentration of SG aboveground biomass from fall harvest and spring harvest (Tables 3.7 to 3.9). N export (kg ha^{-1}) generally increased due to PGPR (N-fixing and P-solubilizing) inoculation, along with biochar (20 t ha^{-1}) application (Tables 3.7 to 3.9). It may be that roots and rhizomes of SG increased uptake of soil N and other minerals due to the combined effects of N-fixing PGPR, P-solubilizing PGPR and biochar or the addition of each PGPR alone. A decline in N export occurred for spring aboveground biomass as shoots move N-containing components belowground in the fall, which can result in high quality biomass for biofuel production (cellulosic ethanol), with low N concentrations (Parrish and Fike, 2005). Some interactions occurred between biochar and combined PGPR (PN) ($P = 0.0051$) for N export at site 2 (Tables 3.7, 3.8 and 3.9), whereas inoculation with the N-fixing PGPR increased ($P = 0.0136$) N export at site 1. Inoculation with N-fixing PGPR contributed N to SG, possibly from BNF; in some cases, biochar may also improved soil nutrient availability (Steiner et al., 2007). Inoculation with P-solubilizing bacteria can increase the availability of soil phosphates for plant growth by solubilization (Kucey et al., 1989), as well as by better scavenging of soluble P, and these effects can

enhance plant growth by increasing the efficiency of BNF, enhancing the availability of other minerals (Zn, Fe, etc.) and by production of plant growth promoting substances (Kucey et al., 1989). Several studies have shown the beneficial influence of combined inoculation with P-solubilizing and N-fixing bacteria on rice and on barley (Kundu et al., 1984), as they provided more balanced nutrition for the plants. In this study, the combination of PGPR (N fixing and P solubilizing) inoculations resulted in a similar pattern of response for SG aboveground biomass N contents (Tables 3.7 to 3.9), with the highest rate of N export reported from combined PGPR inoculation and biochar-amended plots.

3.5 Conclusions

This study demonstrated a positive effect of biochar, along with PGPR, on plant growth variables (i.e. height, stand count, dry biomass), aboveground N concentration, aboveground N export (kg N ha^{-1}). Overall, PGPR and biochar resulted in 9-30 % increases in dry biomass and 10-65 % increases in N export (kg N ha^{-1}) as compared with the control treatment. However in the field, biochar and PGPR interact mostly during the growing season. Overall, yield increased approximately 30% with biochar application and PGPR inoculation, demonstrating a possible biochar by PGPR interaction or their main effects. These findings have the potential to accelerate the development of more sustainable low-input energy grass production systems for temperate regions. Further understanding of the influence of plant-microbe interactions on biomass yield and the effects of biochar on the mechanisms underlying both of these awaits further investigation.

Table 3.1 Mean temperatures (^{0}C) and total precipitation (mm) for 2010 and 2012 for April through November for two locations (Emile A. Lods Research Centre, McGill University and St. Augustin de Desmaures, Laval University, Québec City) under southern Québec region.

Month	McGill University			Laval University			McGill University			Laval University		
	Site 1 & Site 2			Site 3			Site 1 & Site 2			Site 3		
	2010	2011	2012	2010	2011	2012	2010	2011	2012	2010	2011	2012
	Mean temperature (^{o}C)						Total precipitation (mm)					
Apr	9.5	6.5	6.7	7.5	3.5	5.6	84.1	123.9	51.4	36.4	110.5	68.7
May	15.4	13.7	15.6	13.3	10.9	13.6	33.8	115.4	93.5	39.8	130.3	147.8
June	18	19.2	19.4	16.9	16.8	17.9	160.4	52.8	54.8	104.4	86.8	184.8
July	22.6	22.6	21.8	21.4	20.6	20.8	60.6	35.6	85.5	48.8	131	74.1
Aug	20.6	20.9	21.4	19.3	18.6	20.4	162.6	136	49.2	112	171.4	65.8
Sept	15.8	15.7	17.8	13.6	15.5	15.6	157.6	59.1	67.5	184.8	106.8	35.6
Oct	8.5	9.5	10.5	6.6	8.2	8.2	90	74.9	74.6	60.5	77.9	127
Nov	2.1	5.1	0.4	0.4	2.9	-0.1	78	38.5	5	131.9	62.9	11.6

Ref.:. www.weather environment canada.

Table 3.2 Description of soil types for SG field trial during 2010-2012 at Site 1, Site 2 and Site 3 in southern Québec region.

Field site	**McGill (Site 1)**	**McGill (Site 2)**	**Laval (Site 3)**
Soil types	Melanic Brunisol, loamy, mixed, frigid typic Hapludalf	Frigid typic Endoaquent (Dystric Glyeysol) of the St. Amable and Courval series	Sandy loam type
Soil quality	Sand 476 g kg^{-1} Silt 231 g kg^{-1} Clay 293 g kg^{-1} Organic carbon 28 g kg^{-1}	Sand 815 g kg^{-1} Silt 89 g kg^{-1} Clay 96 g kg^{-1} Organic carbon 19.9 g kg^{-1}	Sand 553 g kg^{-1} Silt 250 g kg^{-1} Clay 197 g kg^{-1} Organic carbon 27 g kg^{-1}
pH	5.5	5.3	6.4

Ref.: Soil sample (Site 1 and Site 2) analysis result obtained from the laboratory of Dr. Joann Whalen, McGill University and Site 3 from Bioenergy Research Laboratory, Laval University, Québec City.

Table 3.3 Brief description of N-fixing and P-solubilizing PGPR that were used (as treatment) for SG field trial during 2010-2012 at Site 1, Site 2 and Site 3 in southern Québec region.

Content	**N-fixing PGPR**	**P-solubilizing PGPR**
Bacteria species	*Paenibacillus polymyxa*, *Rahnella* sp., *Serratia* sp.	*Pseudomonas rhodesiae*
Media name	N free solidified LG medium[1]	King's B medium[2]
Media composition	ddH_2O – 1000 ml Sucrose – 10 g K_2HPO_4 – 0.5 g $MgSO_4.7H_2O$ – 0.2 g NaCl – 0.2 g $MnSO_4.H_2O$ – 0.001 g $FeSO_4$ – 0.001 g $NaMoO_4.2H_2O$ – 0.001 g $CaCO_3$ - 5 g Bacto agar – 15g	ddH_2O – 500ml Proteose peptone (Difco)-10 g K_2HPO_4 – 0.75 g $MgSO_4.7H_2O$ – 0.75 g Glycerol – 5 ml Agar – 7.5 g
Incubation	30°C for 2 days (10^8 cfu mL^{-1})	28°C (Shaker at 150 rpm for 2 days) (10^8 cfu mL^{-1})

[1]Dobereiner 1995; [2]Schaad 1980

Table 3.4 Effect of biochar and PGPR (N-fixing, P-solubilizing and their combination) inoculation on growth variables (stand count, tiller dry weight-DW, tiller distribution and height) of SG at 3 sites for first establishment year (2010).

			Stand count			Tiller DW			Tiller distribution			Height		
Year	**Biochar**	**Treatment**	**Site 1**	**Site 2**	**Site 3**	**Site 1**	**Site 2**	**Site 3**	**Site 1**	**Site 2**	**Site 3**	**Site 1**	**Site 2**	**Site 3**
2010	**0 t ha^{-1}**	**Control**	50.41	58.91	64.5	0.9	0.63	1.73	470.56	687.36	1161	96.75	97.43	64.5
		BacN[1]	55.83	48.41	63.12	1.22	1.02	1.53	521.11	564.86	1136.25	118.5	100.5	63.12
		BacP[2]	60.41	44.5	65.87	1.11	0.96	1.38	563.89	519.17	1185.75	112.75	106	65.87
		PN[3]	50.33	59.66	62.62	1.08	0.71	1.59	469.78	696.11	1127.25	108.75	108.81	62.82
	20 t ha^{-1}	**Control**	64.66	54.91	62.5	0.91	0.85	1.99	603.56	640.69	1125	116.25	103.37	62.5
		BacN	67	57.83	63.12	1.23	0.89	1.9	625.33	674.72	1136.25	122.75	110.68	63.12
		BacP	60.66	50.25	60.75	0.76	0.72	1.68	566.22	586.25	1093.5	116	106.25	60.75
		PN	56.91	63.58	63.37	1.14	0.77	1.99	531.22	741.81	1140.75	118.25	112.87	63.37
ANOVA (Split plot analysis)														
Biochar			NS	NS	NS	NS	NS	NS	NS	NS	NS	NA	NS	NS
BacN			NS	NS	NS	NS	*	NS	NS	NS	NS	NA	*	NS
Biochar*BacN			NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS
Biochar			NS	NS	NS	NS	NS	NS	NS	NS	NS	NA	NS	*
BacP			NS	NS	NS	NS	NS	*	NS	NS	NS	NA	*	NS
Biochar*BacP			NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS
Biochar			NS	NA	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PN			NS	NA	NS	NS	NS	NS	NS	NS	NS	*	***	NS
Biochar*PN			NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*** P < 0.001; ** P < 0.01; * P < 0.05; NS, not significant; NA, not applicable;

[1]BacN refers to N-fixing PGPR;

[2]BacP refers to P-solubilizing PGPR;

[3]PN refers to combination of N-fixing and P-solubilizing PGPR;

Table 3.5 Effect of biochar and PGPR (N-fixing, P-solubilizing and their combination) inoculation on growth variables (stand count, tiller dry weight, tiller distribution and height) of SG at 3 sites for second year (2011).

			Stand count			Tiller DW			Tiller distribution			Height		
Year	**Biochar**	**Treatment**	**Site 1**	**Site 2**	**Site 3**	**Site 1**	**Site 2**	**Site 3**	**Site 1**	**Site 2**	**Site 3**	**Site 1**	**Site 2**	**Site 3**
2011	**0 t ha^{-1}**	**Control**	143.5	120.17	76.12	1.82	0.76	6.16	1339.33	1401.94	1370.25	146.75	132.91	126.88
		BacN[1]	157.58	97.58	65	1.4	1.27	8.29	1470.78	1138.47	1170	146.83	141.66	124.38
		BacP[2]	128.17	113.75	63.87	2.38	0.95	7.9	1196.22	1327.08	1149.75	149.91	149.58	126.63
		PN[3]	123.75	90.08	73.25	2.15	1.06	6.95	1155	1050.97	1318.5	147.75	141.25	125.13
	20 t ha^{-1}	**Control**	139.67	101.17	74.75	3.58	1.28	6.8	1303.56	1180.28	1345.5	154.47	148.83	132.25
		BacN	128.58	103.75	51.75	2.54	0.94	10.83	1200.11	1210.42	931.5	154.41	155.16	131.25
		BacP	108.42	100.08	68.24	2.35	0.99	7.04	1011.89	1167.64	1228.33	149	147.91	131.5
		PN	109.83	101.75	58.07	2.32	0.78	9.49	1025.11	1187.08	1059.75	153.5	151.5	136.13
ANOVA (Split plot analysis)														
Biochar			NS	NA	NS	NS	NA	NS	NS	NA	NS	NS	NS	NS
BacN			NS	NA	*	NS	NA	NS	NS	NA	*	NS	***	NS
Biochar*BacN			NS	*	NS	NS	*	NS	NS	*	NS	NS	NS	NS
Biochar			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NA	NS
BacP			*	NS	NS	NS	NS	NS	*	NS	NS	NS	NA	NS
Biochar*BacP			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS
Biochar			NS	NA	NS	NS	NA	NS	NS	NA	NS	NS	NS	NS
PN			NS	NA	NS	NS	NA	NS	NS	NA	NS	NS	**	NS
Biochar*PN			NS	*	NS	NS	*	NS	NS	*	NS	NS	NS	NS

*** P < 0.001; ** P < 0.01; * P < 0.05; NS, not significant; NA, not applicable;

[1]BacN refers to N-fixing PGPR;

[2]BacP refers to P-solubilizing PGPR;

[3]PN refers to combination of N-fixing and P-solubilizing PGPR;

Table 3.6 Effect of biochar and PGPR (N-fixing, P-solubilizing and their combination) inoculation on growth variables (stand count, tiller dry weight, tiller distribution and height) of SG at 2 sites for third year (2012).

			Stand count		Tiller DWs		Tiller distribution		Height	
Year	**Biochar**	**Treatment**	**Site 1**	**Site 2**	**Site 1**	**Site 2**	**Site 1**	**Site 2**	**Site 1**	**Site 2**
2012	**0 t ha^{-1}**	**Control**	41.75	69	1.691	1.147	1169	1932	170.37	166.62
		BacN[1]	61.5	62.25	1.261	1.265	1722	1743	162.5	177.87
		BacP[2]	62.5	54	1.109	1.529	1750	1512	173	175.87
		PN[3]	61.75	53.25	1.193	1.517	1729	1491	166	170.12
	20 t ha^{-1}	**Control**	65.75	57.37	1.096	1.399	1841	1606.5	169	161.12
		BacN	69.75	60.62	1.107	1.325	1953	1697.5	172.5	172.62
		BacP	52.5	66.87	1.199	1.213	1470	1872.5	169.5	177.87
		PN	75.25	58.75	1.018	1.324	2107	1645	173.75	179.37
ANOVA (Split plot analysis)										
Biochar			NS	NS	NS	NS	NS	NS	NS	NS
BacN			NS	NS	NS	NS	NS	NS	NS	NS
Biochar*BacN			NS	NS	NS	NS	NS	NS	NS	NS
Biochar			NA	NA	NA	NA	NA	NA	NS	NS
BacP			NA	NA	NA	NA	NA	NA	NS	NS
Biochar*BacP			**	*	*	*	*	*	NS	NS
Biochar			NS	NS	NS	NS	NS	NS	NS	NS
PN			*	NS	NS	NS	*	NS	NS	NS
Biochar*PN			NS	NS	NS	NS	NS	NS	NS	NS

** P < 0.001; ** P < 0.01; * P < 0.05; NS, not significant; NA, not applicable;

[1]BacN refers to N-fixing PGPR;

[2]BacP refers to P-solubilizing PGPR;

[3]PN refers to combination of N-fixing and P-solubilizing PGPR;

Table 3.7 Effect of biochar and PGPR (N-fixing, P-solubilizing and their combination) inoculation on N dynamics (N concentration or % and N export) of SG at Site 1 for 2010 to 2012 years.

			N percentage				N export (Kg N ha^{-1})			
Site	**Biochar**	**Treatment**	**F-2010**	**Sp-2011**	**F-2011**	**Sp-2012**	**F-2010**	**Sp-2011**	**F-2011**	**Sp-2012**
Site 1	**0 t ha^{-1}**	**Control**	1.37	1.33	1.55	1.08	27.11	64.17	129.84	32.75
		BacN[1]	1.4	1.23	1.68	1.01	44.35	114.12	151.55	39.3
		BacP[2]	1.53	1.27	1.8	1.08	43.79	147.21	176.84	44.52
		PN[3]	1.34	1.19	1.79	0.99	35.27	147.03	152.63	37.04
	20 t ha^{-1}	**Control**	1.45	1.03	1.76	1.09	39.54	104.75	169.44	34.65
		BacN	1.38	0.932	1.51	1.07	43.56	120.3	150.49	29.53
		BacP	0.997	1.3	1.615	1.14	22.61	97.27	144.55	40.35
		PN	1.31	1.085	2.06	1.2	39.52	154.57	180.11	42.99
ANOVA (Split plot analysis)										
Biochar			NS	NS	NS	NS	NS	NS	NS	NS
BacN			NS	NS	NS	NS	NS	NS	NS	NS
Biochar*BacN			NS	NS	NS	NS	NS	NS	NS	NS
Biochar			NA	NS	NA	NS	NS	NS	NA	NS
BacP			NA	NS	NA	NS	NS	NS	NA	NS
Biochar*BacP			*	NS	*	NS	NS	NS	*	NS
Biochar			NA	NS	NS	NS	NS	NS	NS	NS
PN			NA	NS	NS	*	NS	*	NS	*
Biochar*PN			*	NS	NS	NS	NS	NS	NS	NS

*** P < 0.001; ** P < 0.01; * P < 0.05; NS, not significant; NA, not applicable;

[1]BacN refers to N-fixing PGPR;

[2]BacP refers to P-solubilizing PGPR;

[3]PN refers to combination of N-fixing and P-solubilizing PGPR;

Table 3.8 Effect of biochar and PGPR (N-fixing, P-solubilizing and their combination) inoculation on N dynamics (N concentration or % and N export) of SG at Site 2 for 2010 to 2012 years.

			N percentage				N export (Kg N ha^{-1})			
Site	**Biochar**	**Treatment**	**F-2010**	**Sp-2011**	**F-2011**	**Sp-2012**	**F-2010**	**Sp-2011**	**F-2011**	**Sp-2012**
Site 2	**0 t ha^{-1}**	**Control**	2.29	3.21	1.8	1.05	37.32	55.03	107.57	39.3
		BacN[1]	2.31	3.04	1.71	1.09	49.72	101.16	107.11	32.4
		BacP[2]	2.26	2.82	1.97	0.94	44.51	124.56	124.56	39.97
		PN[3]	3.11	2.85	1.77	1.01	61.8	114.61	114.61	27.42
	20 t ha^{-1}	**Control**	2.08	3.1	1.59	1.07	39.36	101.79	101.79	42.58
		BacN	2.58	3.22	1.87	1	56.01	117.3	117.3	40.32
		BacP	3.35	2.91	1.7	1.19	56.25	106.52	106.52	42.31
		PN	3.63	3.13	1.5	1.15	82.44	88.09	88.09	48.49
ANOVA (Split plot analysis)										
Biochar			NS	NS	NS	NS	NS	NS	NA	NS
BacN			NS	NS	NS	NS	NS	**	NA	NS
Biochar*BacN			NS	NS	NS	NS	NS	NS	*	NS
Biochar			NA	NS	NA	NS	NS	*	NS	NS
BacP			NA	NS	NA	NS	NS	NS	NS	NS
Biochar*BacP			*	NS	*	NS	NS	NS	NS	NS
Biochar			NS	NS	NS	NS	NS	NS	NS	NS
PN			*	NS	NS	*	***	NS	NS	NS
Biochar*PN			NS	NS	NS	NS	NS	NS	NS	NS

*** P < 0.001; ** P < 0.01; * P < 0.05; NS, not significant; NA, not applicable;

[1]BacN refers to N-fixing PGPR;

[2]BacP refers to P-solubilizing PGPR;

[3]PN refers to combination of N-fixing and P-solubilizing PGPR;

Table 3.9 Effect of biochar and PGPR (N-fixing, P-solubilizing and their combination) inoculation on N dynamics (N concentration or % and N export) of SG at Site 3 for 2010 to 2011 years.

			N percentage			N export (Kg ha^{-1})		
Site	**Biochar**	**Treatment**	**F-2010**	**Sp-2011**	**F-2011**	**F-2010**	**Sp-2011**	**F-2011**
Site 3	**0 t ha^{-1}**	**Control**	1.7	2.19	1.28	104.28	60.24	148.29
		BacN[1]	1.94	1.94	1.39	103.34	54.69	147.47
		BacP[2]	0.921	2.17	1.33	45.93	71.75	139
		PN[3]	0.781	2.13	1.44	42.59	69.68	172.86
	20 t ha^{-1}	**Control**	1.85	2.15	1.23	123.45	67.67	137.88
		BacN	1.84	2	1.36	121.6	66.52	171.54
		BacP	0.951	2.106	1.43	53.4	65.22	157.59
		PN	0.915	2.235	1.47	63.07	70.57	204.59
ANOVA (Split plot analysis)								
Biochar			NS	NS	NS	NS	NS	NS
BacN			*	NS	NS	*	NS	NS
Biochar*BacN			NS	NS	NS	NS	NS	NS
Biochar			NS	NS	NS	NS	NS	NS
BacP			NS	NS	NS	*	NS	NS
Biochar*BacP			NS	NS	NS	NS	NS	NS
Biochar			NS	NS	NS	NS	NS	NS
PN			*	NS	*	*	NS	NS
Biochar*PN			NS	NS	NS	NS	NS	NS

*** P < 0.001; ** P < 0.01; * P < 0.05; NS, not significant; NA, not applicable;
[1]BacN refers to N-fixing bacteria;
[2]BacP refers to P-solubilizing bacteria;
[3]PN refers to combination of N-fixing and P-solubilizing bacteria;

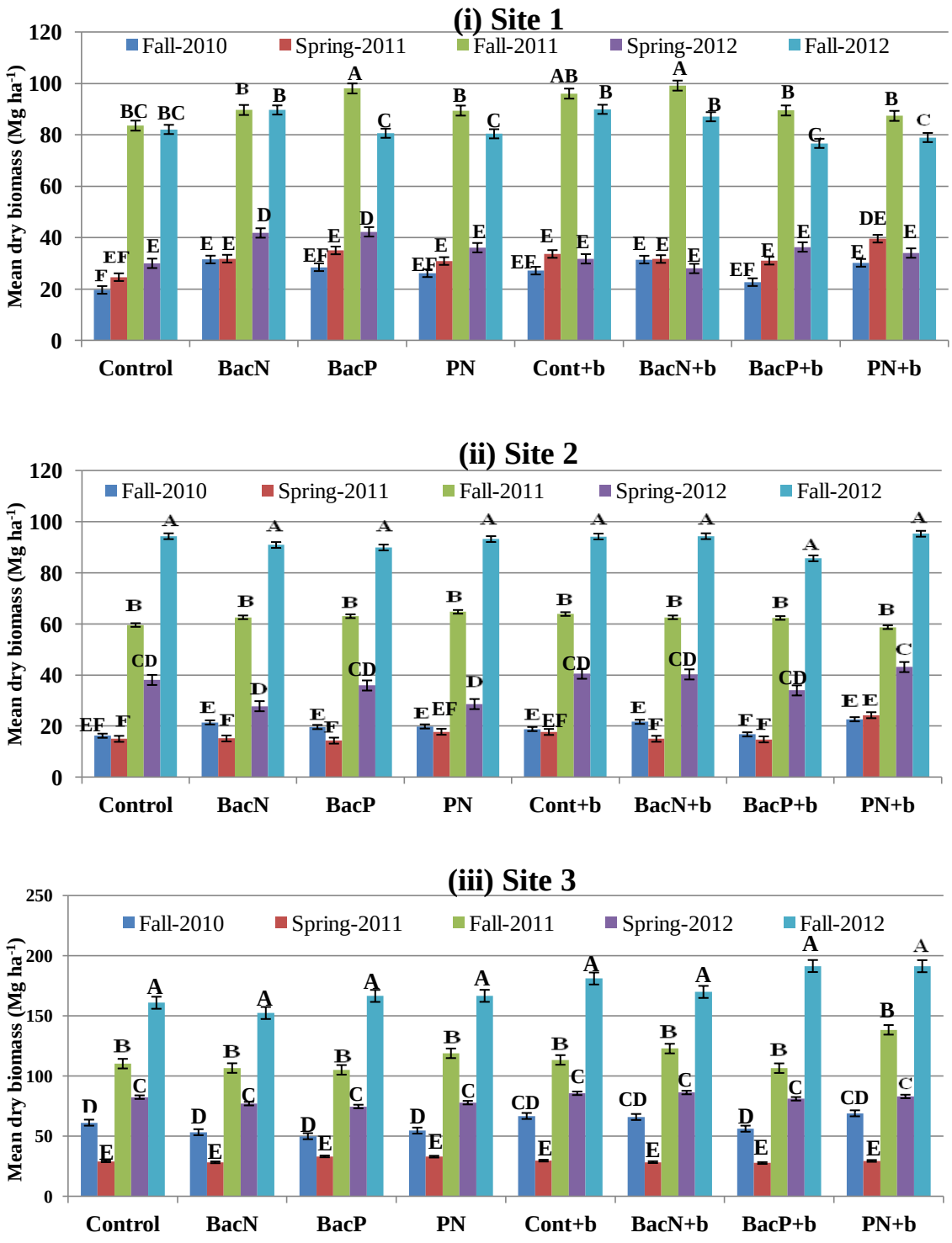


Figure 3.1 Mean dry biomass (Mg ha^{-1}) of switchgrass (inoculated or not) with standard error (SE) bars of control (biochar 0 t ha^{-1}) , N-fixing PGPR (BacN) without biochar, P-solubilizing PGPR (BacP) without biochar , combined PGPR (PN) culture without biochar, control (Cont+b) with biochar (20 t ha^{-1}) , N-fixing PGPR with biochar (20 t ha^{-1}) (BacN+b), P-solubilizing PGPR (BacP+b) with biochar (20 t ha^{-1}) and combined PGPR (PN+b) with biochar (20 t ha^{-1}) at three different sites (i) site 1, (ii) site 2 and (iii) site 3 under Southern Québec.

Connecting text to Chapter 4

In the previous chapter we evaluated the effects of biochar and PGPR inoculation on SG growth and N use efficiency at three field sites across southern Québec with a view toward development of a low input SG production system. Observed results (three years) across three field sites demonstrated positive growth responses of SG to soil amendment with biochar and inoculation with specific (N-fixing or P-solubilizing PGPR) or combined PGPR under high-latitude temperate zone field conditions.

In the following chapter, we have investigated the effects of soil amendment with biochar and/or N fertilizer additions on SG growth and biomass productivity, N export, NUE and apparent N recovery of aboveground dry biomass at three sites for three continuous years in two climatic region of southern Québec. Our objective was to determine whether or not biochar-amended soils results in better NUE than unamended soils for SG under field conditions, with a view to development of a low-input sustainable production for PRGs as biomass energy crops.

I have contributed to all the work contained in the following chapter, which includes reviewing the pertinent literature, conducting field experiments, analyzing the data and writing the chapter. The chapter will be submitted, as a manuscript, to a selected journal for publication. The manuscript is co-authored by the me, Dr. Donald L. Smith, Dr. Inna Teshler and Dr. Xiaomin Zhou of the Department of Plant Science, Macdonald Campus of McGill University and Dr. Suzanne Allaire from Department of Soil and Agroenvironment Engineering and Horticulture Research Center and Dr. Anne Vanasse of Department of Plant Science, Laval University. Dr. Teshler assisted in managing the field experiments at the McGill Univesity field site. Dr. Zhou provided important input regarding methods of statistical analysis. Drs. Allaire and Vanasse managed the field site at Laval University. This research was supported by funds from Fonds de Recherche Nature et Technologies (FQRNT).

CHAPTER 4

4 Title: Growth performance and N use efficiency of SG receiving biochar and N fertilizer treatments

4.1 Abstract

Sustainable low-input bioenergy feedstock production systems rely on maximizing the yield of crops while minimizing the use of N fertilizer. SG (*Panicum virgatum* L.) is a promising warm-season grass able to generate high biomass yields with minimal input of N fertilizer, in part due to its perennial nature. While maximum annual yields of established SG for bioenergy purposes are obtained with N fertilizer, the efficiency of N fertilizer use has been reported to be, in general less than 50% due to leaching, run-off, etc. Biochar, a carbonaceous porous substance, could slow the rate of nitrification by binding of ammonium so that biochar-amended soils would require less N fertilizer to achieve target crop yields. Despite the positive impact of biochar on crop yield, it has not been tested for interactions with N fertilizer under temperate field conditions. Therefore, the objective of this study was to determine if biochar amended soils result in better N use efficiency than control conditions (no biochar). For this purpose the variables biomass yield, N export, NUE and apparent N recovery of SG were studied at three field sites under two climatic conditions (warmer and cooler) in southern Québec. Treatments were comprised of N fertilizer (0, 50, 100 kg N ha^{-1}) as the split plot factor and biochar (0 and 20 t ha^{-1}) as the whole plot factor, in a split plot experimental design. Biochar by N fertilizer interactions (positive) occurred at two field sites in the warmer climatic region during the second year. The highest biomass yield resulted from 50 kg N ha^{-1} at site 1 and 100 kg N ha^{-1} along with biochar at sites 2 and 3. Biochar-amended soil had greater NUE at 50 kg N ha^{-1} while comparatively high apparent N recovery (ANR) was observed at 50 kg N ha^{-1} along with biochar at spring harvest. Overall, biochar with 50 kg N ha^{-1} enhanced SG biomass yield, N export and NUE across all three field sites over three years (nine site years), which indicated potential for biochar and PGPR in a sustainable low-input bioenergy crop production system under southern Québec (high-latitude temperate) conditions.

4.2 Introduction

SG (*Panicum virgatum*) is one of the most widely used bioenergy grasses because of its high biomass production, lower N fertilizer requirement and short growing season (Heaton et al., 2004). This high-utility warm-season C_4 grass species has already been identified as having good potential for biofuel production in eastern Canada (Madkadze et al., 1996; 1998). Recycling N for the subsequent year's growth is a beneficial trait of herbaceous perennial grasses, but the addition of N fertilizer can increase biomass production (Hogberg, 2007). Maximum annual yields of established SG for biomass energy production are generally obtained with N fertilizer application rates ranging from 120 to 168 kg N ha^{-1}(Sanderson et al., 1999; Vogel et al., 2002). However, the efficiency of N fertilizer use has been reported to be, in general, less than 50%, due to leaching, run-off and gaseous emission as N_2O, N_2 and NH_3 (Bouwman, 1996; Olarewaju et al., 2009). Optimizing NUE for yield could minimize N losses and maximize the efficiency of reuse of N contained in crop tissues.

Biochar is a highly porous material (Downie et al., 2009) which contains primarily C and inorganic matter (ash); it has the ability to help retain nutrients and water in soil. In the absence of biochar more nutrient can be leached through the soil profile, below the root zone (Marris, 2006) and lost to crop plants. The binding of NH_4^+ to the surface of biochar is of particular interest because this can slow the rate of nitrification, and hence the potential loss of N_2O and N_2 via denitrification. Thus, biochar-amended soil may require less N fertilizer to achieve target crop yields, leading to, for instance, less contamination of ground water by nitrates (Almarsi and Kaluarachchi, 2004), less production of the GHG gas N_2O (Almaraz et al., 2009), and economic benefits. Under greenhouse conditions interactions between biochar and N fertilizer have been observed, with higher yields at higher rates of biochar application, in the presence of N fertilizer (Chan et al., 2007). Steiner et al. (2008) reported increased uptake of N into crop biomass with the application of a low nutrient biochar. In general, the peer reviewed literature indicates that biochar is able to adsorb NH_3^+, and thus minimize its loss through volatilization.

Despite the positive impacts of biochar application on soils, it has not been tested for effects and interactions with N fertilizer on relevant crops under field conditions in the southern Québec region, a high-latitude temperate zone agricultural area. The objective of the present

study was to test for this interaction through field trials using two rates of biochar (0 and 20 t ha^{-1}) and three rates of N fertilizer application (0, 50 and 100 kg N ha^{-1}) at three sites in southern Québec. These experiments were conducted on the same site for three successive years, for a total of six site-years. The interest was in determining if biochar amendment of soils results in better NUE than in unamended soils; high NUEs are essential for low-input production of bioenergy crops.

4.3 Material and methods

4.3.1 Site selection and experimental design

The research was conducted from 2010 to 2012 using *Panicum virgatum* (SG, cv. Cave-In-Rock) grown at three sites in two regions of southern Québec (McGill University in southwestern Québec and Laval University in southeastern Québec. Two research sites (site 1: Saint Bernard loam soil (melanic Brunisol, loamy mixed, frigid typic Hapludalf that contains 47.6% sand, 23.1% silt, 29.3% clay and 2.8% organic C) and site 2: sandy loam soil (frigid typic Endoaquent - Dystric Gleysol, that contains 55.3% sand, 25% silt, 19.7% clay and 2.7% organic C) were located at the Emile A. Lods Research Institute of the Macdonald Campus ($42^{o}28'$N $73^{o}45'$W) of McGill University, St-Anne-de-Bellevue (2900 to 3100 corn heat units). There was also one experimental site at Saint Amable or St-Augustin-de-Desmaures (2300 to 2500 corn heat units), and associated with Laval University, Field-site 1 was fallow during the previous year (2009) whereas, corn and barley, respectively, were cultivated (without any fertilization) at field-sites 2 and 3 during 2009. Soil pH was 5.3, 5.5 and 6.4 for field sites 1, 2 and 3, respectively, during 2010. The experiment was organized following a split-plot randomized complete block design with four blocks (replicates). The treatments consisted of factorial combinations of N fertilizer application rates (0, 50 and 100 kg N ha^{-1}) and biochar addition levels (0 and 20 t ha^{-1}). The whole plot factor was biochar and the split-plot factor was N fertilizer.

4.3.2 Stand management

Seeds were placed at 1.5 cm below the soil surface, as recommended (Christensen et al., 2010); seeding, at a rate of 10 kg ha^{-1}, was conducted with a Plotman seeder (Fabro limited, Swift Current, Saskatchewan, Canada) on June 10, 2010. Biochar (20 t ha^{-1}) was incorporated into a randomly-selected whole-plot portion of each block early in the spring of

2010. In each case one third of the N fertilizer (broadcast as ammonium nitrate - 27:0:0% N: P: K) was applied shortly prior to seeding (spring) and the rest (two thirds) was applied at the inflorescence stage (Gustavsson, 2011). At the flowering stage, SG height and number of tillers in 1 m of row (stand count/density) was measured. After each sampling tiller biomass was determined as the total tiller dry weight (DW) divided by the total number of tillers for each research plot (Ker et al., 2012).

4.3.3 Biomass harvesting and elemental analysis of sample biomass

At the end of the establishment year (before the first killing frost) plants from 1 m of three randomly selected rows within each plot were cut at a 10 cm height. All harvested herbage was weighted immediately following harvest. Sub-samples were oven-dried at 60 ^{o}C to a constant weight, to determine moisture and dry matter contents. During the spring another sampling was conducted with a mechanical harvester (Model no. SMN WB WS 24-18-00-04, Swift Machine and Welding Ltd., Swift Current Saskatchewan, Canada, cut size 60 cm). During the second crop year (2011), sampling was conducted at three crop growth stages: inflorescence emergence (stage 50), flowering (stage 61) and harvest ripening/senescence (stage 89+) (Gustavsson, 2011). At each harvest, plants were cut from 50 cm segments of four randomly selected rows, then weighed, dried (sub-samples) and weighed again, as described above.

An elemental analyzer (Model no. NC 2500, Thermo Quest CE Instrument from Isomass Scientific Inc. Calgary, Canada), was used for simultaneous determination of nitrogen concentration in grass samples. The principle of operation is the combustion of the sample: it must be quantitative and instantaneous so that the combustion gases can be efficiently eluted through the chromatographic column and TCD so it will give output signals proportional to the sample elemental composition. The instrument uses the dynamic flash combustion method, which ensures these conditions (Pella and Colombo, 1973). The grass samples (properly dried) were homogenized by careful grinding (Wiley Mill, model no. 4, Thomas Scientific, USA screen size 1mm). The sample size analyzed was generally 30-60 mg. A weighed amount of sample in a tin capsule was placed in the autosampler drum where it was deareated (to remove any atmospheric nitrogen), then introduced into a vertical quartz tube heated to 1000 ^{o}C, with a constant flow of helium (carrier gas). A few seconds before each

sample dropped into the combustion tube, the helium stream was enriched with a measured amount of high purity oxygen to achieve a strongly oxidizing environment, which guarantees near-complete combustion, even of thermally resistant substances. To achieve quantitative oxidation the combustion gas mixture is driven through an oxidation catalyst (Cr_2O_3) zone, then through a subsequent zone of copper which reduces nitrogen oxides formed during combustion or catalyst oxidation to elemental nitrogen and scrubs excess oxygen. At the outlet of the reaction tube the gas mixture (N_2, CO_2, H_2O) meets a trap containing anhydrone, which adsorbs water. The resulting components of the combustion mixture were eluted and separated by a Porapack PQS column and subsequently detected, by a TCD, in the sequence N_2, CO_2. The amount of nitrogen exported through the removal of aboveground harvested biomass was calculated by multiplying the N concentration in aboveground tissues (kg N Mg^{-1}) by the total harvested dry biomass yield (Mg ha^{-1}) (Jung et al., 2011). Nitrogen-use efficiency (kg biomass (kg N)$^{-1}$) was calculated as the difference between fertilizer treatment plot biomass yield and control plot biomass yield and then divided by the rate of N fertilizer applied (kg ha^{-1}) while ANR (apparent N recovery) was calculated as the difference between fertilizer treatment plot total N export (kg N ha^{-1}) and control plot total N export (kg N ha^{-1}) and then divided by the rate of N fertilizer applied (kg ha^{-1}) (Jung et al., 2011).

4.3.4 Statistical analysis

Statistical analyses were performed with the software package SAS 9.2 (SAS Institute Inc.). Raw data from all three field sites and three years were checked for normality and constant variance of errors before conducting statistical analyses. A split plot analysis (randomized complete block design) was conducted using the PROC GLM procedure in SAS to determine main effects and interactions of biochar and N fertilizer. Biochar was considered as the whole plot factor and N fertilizer treatments as the subplot factor. When a factor (biochar, N fertilizer) or interaction (biochar x N fertilizer) was declared statistically significant the standard error of the mean was used to determine differences between means for three levels of significance ($P < 0.05$, $P < 0.01$and $P < 0.001$) (Peterson ,1985).

4.4 Results

4.4.1 Growing conditions

Site 3 experienced a very dry period in May and July, during the establishment year, 2010 (Fig. 4.1). At sites 1 and 2 the summer of 2011(June-July) was very dry. The rate of temperature increase in the spring and the mean spring temperature were generally higher for 2010 than 2011 for both these research sites (Fig. 4.1). In 2012 higher than normal autumn temperatures occurred for both eastern and western Québec sites.

4.4.2 Biomass yield

A meta-analysis (regarding effect of biochar to soils on crop productivity) revealed statistically significant increases in crop productivity were seen when biochar was applied concurrently with inorganic fertilizer, compared to applying inorganic fertilizer alone, as well as when biochar was applied to soil without any fertilizer ($P < 0.05$) addition (Jeffery et al., 2011). The biochar by N fertilizer interaction for dry biomass yield was significant for the summer sampling at site 1 ($P = 0.0171$) and the fall sampling at site 2 ($P = 0.0329$) (Table 4.1). Per tiller biomass, as well as total biomass, increased with N fertilizer and biochar application to soil at both of these sites during all three years of experimentation (Table 4.1).

At site 1, maximum biomass production occurred at 50 kg N ha^{-1} during first two years (2010-2011) while at site 2 maximum biomass production occurred at 100 kg N ha^{-1} and biochar (20 t ha^{-1}) during 2010 to 2011. During the third year (2012), the highest biomass yield occured with 100 kg N ha^{-1} with biochar (20 t ha^{-1}) was at site 1 and 50 kg N ha^{-1} with biochar (20 t ha^{-1}) at site 2. Biomass production varied with N fertilizer application rate at site 3; the greatest biomass yield occurred when biochar was applied (Table 4.1). During the third year, the highest biomass production occurred with biochar amendment and 0kg N ha^{-1}) during the spring of 2012, whereas during the autumn of 2012 harvest 100 kg N ha^{-1} along with biochar soil amendment resulted in the greatest dry biomass production at site 3. Overall, biochar application resulted in a dry biomass increase of 5-40%, where increases of 29% over the control were generally statistically significant, at site 1, 6-35%, where increasese of about 20% over the control were generally statistically significant, at site 2 and 4-10%, with increases of 9% over the control treatment generally being statistically significant, at site 3 (Table 4.1).

4.4.3 Tiller dynamics and plant height

A greater number of tillers was observed when biochar (20 t ha^{-1}) was incorporated into the soil of plots that received N fertilizer (Table 4.2). The highest number of tillers occurred with 50 kg N ha^{-1}along with biochar at site 1, 50 kg N ha^{-1} at site 2 (Table 4.2) during the second year (2011). Tiller dry weight varied across the three sites. However, the greatest tiller dry weights occurred for the 100 kg N ha^{-1} with biochar-amended soil at all sites in year 3 (2012) (Table 4.2). No interaction occurred between biochar and N fertilizer for the measured tiller variables (stand count, tiller dry weight) at any site. In addition, the number of tillers was found to be increased by biochar addition in the second and third years, which may suggest that biochar was able to improve soil properties by enhancing water, nutrient retention and leaching of inorganic N fertilizer over the course of time, which resulted in better agronomic performances of SG (Lehmann et al., 2011).

From the establishment year to year 3 (2010 to 2012), maximal mean height occurred for treatments that combined biochar and 100 kg N ha^{-1} at sites 1 and 2, whereas at site 3 this was the case for 100 kg N ha^{-1}alone. An interaction between biochar and N fertilizer occurred for height at sites 1 ($P = 0.0143$) and 2 ($P \leq 0.0001$). This field study showed trends similar to glasshouse studies by Chan et al. (2007), where a biochar x N fertilizer interaction was observed.

4.4.4 N concentration and N export

N export increased with N fertilizer application whether the soil was amended with biochar or not. N export ranged from 29.26 to 208.32 kg N ha^{-1} at site 1; 31.11 to 122.49 kg N ha^{-1} at site 2 and 61.06 to 211.69 kg N ha^{-1} at site 3 over the three years when testing occurred (Table 4.3).

4.4.5 Nitrogen Use Efficiency (NUE) and apparent N recovery (ANR)

Though NUE varied among sites there was a consistent yield increase with N fertilization as well as with biochar application to soil. Increased NUE was observed only at site 2 with 50 kg N ha^{-1} at the fall 2011 sampling and 50 kg N ha^{-1} along with biochar at the spring 2012 harvest, whereas the highest NUE occurred at site 3 with 100 kg N ha^{-1} along with biochar during spring harvest, 2012 (Table 4.4). Although the interaction between N and biochar

was not significant, biochar amendment tended to increase NUE at the 50 kg N ha^{-1} rate (Table 4.4).

Apparent N recovery, which discounts the amount of N removed by the control (0 kg N ha^{-1}) plants, varied among sites and harvest times. Hence increased ANR occurred with 50 kg N ha^{-1} for the fall-harvested aboveground dried biomass at sites 1and 2, whereas for spring harvested material the highest ANR was for the 100 kg N ha^{-1} treatment at the same sites. At site 3 the highest ANR was the result of the 100 kg N ha^{-1} application during the establishment year, whereas 50 kg N ha^{-1} resulted in the highest ANR in the second year of the study (2011) (Table 4.5).

4.5 Discussion

4.5.1 Biomass yield

In this study, biomass yield varied across site-years due to crop establishment, weather patterns and soil types. In general, there were increases in SG biomass production with biochar application and N fertilization. A significant biochar x N fertilizer interaction was observed during the second year at sites 1 and 2. Chan et al. (2007) found biochar x N fertilizer interactions where higher yield was observed with increasing rates of biochar application in the presence of N fertilizer. The observed interaction among N fertilizer and biochar could indicate that mineralization was taking place and, if it occurred, the N was retained in microbial biomass or leached from the system (Gaskin et al., 2010). Overall N fertilizer and biochar combinations increased yield in this study. Steiner et al. (2007) reported increased yield with compost/charcoal/fertilizer combinations under field conditions, whereas charcoal alone caused little increase in yields. In my study, biomass yields of SG increased at site 2 when the N fertilizer increased from 50 to 100 kg N ha^{-1}. At site three the greatest production of dry biomass, across the three years of field experimentation (2010 to 2012), was produced on biochar amended soil (Table 4.1). Furthermore, the water retention capacity of biochar can also influence NUE by reducing the leaching of N through the soil profile, an effect that is likely to be greatest for sandy soils (Major et al., 2009). Parrish and Woolf (1992) examined N response, with switchgrass cultivar Cave-In-Rock, and reported that 50 kg N ha^{-1} resulted in greater biomass production than did 0 or 100 kg N ha^{-1}. We found the same response at site 1; the highest biomass yield

for a sandy soil occurred with 100 kg N ha^{-1} along with biochar application. This suggests that biochar application to sandy soil improves water holding capacity, moreover biochar can sorb ions onto its surface, from the soil solution, by a combination of electrostatic and capillary forces on their surface and in pores (Major et al., 2009).

4.5.2 Tiller dynamics and height

In comparison with control plots, the combination of biochar (20 t ha^{-1}) and N fertilizer resulted in greater tiller number, as well as higher tiller biomass. Furthermore, N fertilized and biochar treated plants were taller than control plants (Fig. 4.3). Ker et al. (2012) reported that SG enhanced plant population and N dynamics with mixed PGPR inoculation with a combination of N fertilizer under field conditions. Some evidence regarding the agronomic efficiency of biochar has been reported for crops such as sorghum, maize and radishes, but perennial crops have received little attention. Chan et al. (2007, 2008) reported on the agronomic effects of poultry and green biomass biochar on radishes where fertilizer NUE was enhanced with biochar amendments. Though stand count was not affected by biochar/N fertilizer treatment, tiller biomass varied and tiller biomass was increased through biochar addition to soils. This suggests that the agronomic potential of biochar increased when combined with N fertilizer, with regard to SG growth and final productivity. As a result, higher tiller number and greater tiller biomass may lead to higher dry biomass yield at harvest time.

4.5.3 N concentration, N export, NUE and apparent N recovery

There was no difference in SG tissue N concentration across the three sites over three years. Though the highest N export (kg N ha^{-1}) occurred at site 1, some differences ($P \leq 0.05$) were observed at sites 2 and 3. Similarly, it was shown that more N was exported through harvested biomass from biochar amended soil than control plots without biochar. Van Zwieten et al. (2010) reported increased NUE through the application of biochar to soil in a glasshouse study. Similar results were reported by Chan et al. (2007) who found that low-nutrient biochar could increase fertilizer use efficiency. In this study the N export in aboveground biomass was less in fall than that in spring during the establishment year. The amount of N export associated with biomass removal was affected by the concentration of N in aboveground biomass at harvest time. When aboveground biomass is harvested after nutrients are translocated to belowground tissues, it can

reduce N export due to biomass removal (Jung et al., 2011). N concentration varied seasonally (Table 4.3) but higher N concentrations occurred for spring-harvested aboveground biomass, when some some regrowth material was included in the harvested plant matter, from the biochar amended soil and 100 kg N ha^{-1} treatment for the establishment year, across all three sites; at second year fall-harvested (2011) samples contained higher concentrations of N (N%) than spring-harvested (2012) samples. Perennial bioenergy crops showed good N recycling for the subsequent year's growth, and a resulting low N requirement for the growth plus high quality biomass with low N concentrations, making them very suitable for cellulosic biofuel production (Lemus et al., 2008). The lower N export in this study compared to the N dynamics study of Ker et al. (2012) was probably due to the lower aboveground biomass production rather than due to differences in N concentration of the biomass. Lemus et al. (2008) reported 25 to 80% ANR for SG (cv. Cave-in-Rock) biomass production with a one-time N application. They also reported higher ANR values for a lower N rate (90 kg N ha^{-1}). In the current study, higher ANR values were observed at 100 kg N ha^{-1} at spring harvest across all three sites, while at the fall harvest 50 kg N ha^{-1} along with biochar (20 t ha^{-1}) resulted in the highest ANR (Table 4.5). Steiner et al. (2008) reported that biochar application to soil increased the retention of N in soil and uptake of N into crop biomass whereas biochar water retention capacity also influenced NUE by indirectly reducing the leaching of N through the soil profile, especially for sandy soils with low clay contents (Major et al.,2009).

4.6 Conclusions

SG is thought to be the best adapted perennial grass for biomass production across much of North America due to its relatively high biomass yield, low nutrient removal and low N fertilizer requirement. This study has described the application of biochar into three soil types, which also received three rates of N fertilizer (0, 50 and 100 kg N ha^{-1}) for SG production. Biochar and N fertilizer interactions were observed for the loam soil (site1) and sandy loam soil (site 2) during the second year. The highest biomass yield resulted from the application of 50 kg N ha^{-1} at site 1and 100 kg N ha^{-1} plus biochar at sites 2 and 3. Biochar-amended soils tended to have greater NUEs at 50 kg N ha^{-1}. Higher ANR occurred at fall harvest when 100 kg N ha^{-1} was applied, while 50 kg N ha^{-1} along with biochar application resulted in the same trend for the spring harvest. Overall, biochar application with a lower rate of N fertilizer enhanced SG biomass yield, NUE and ANR indicating that this could represent a promising strategy for low-input energy grass production.

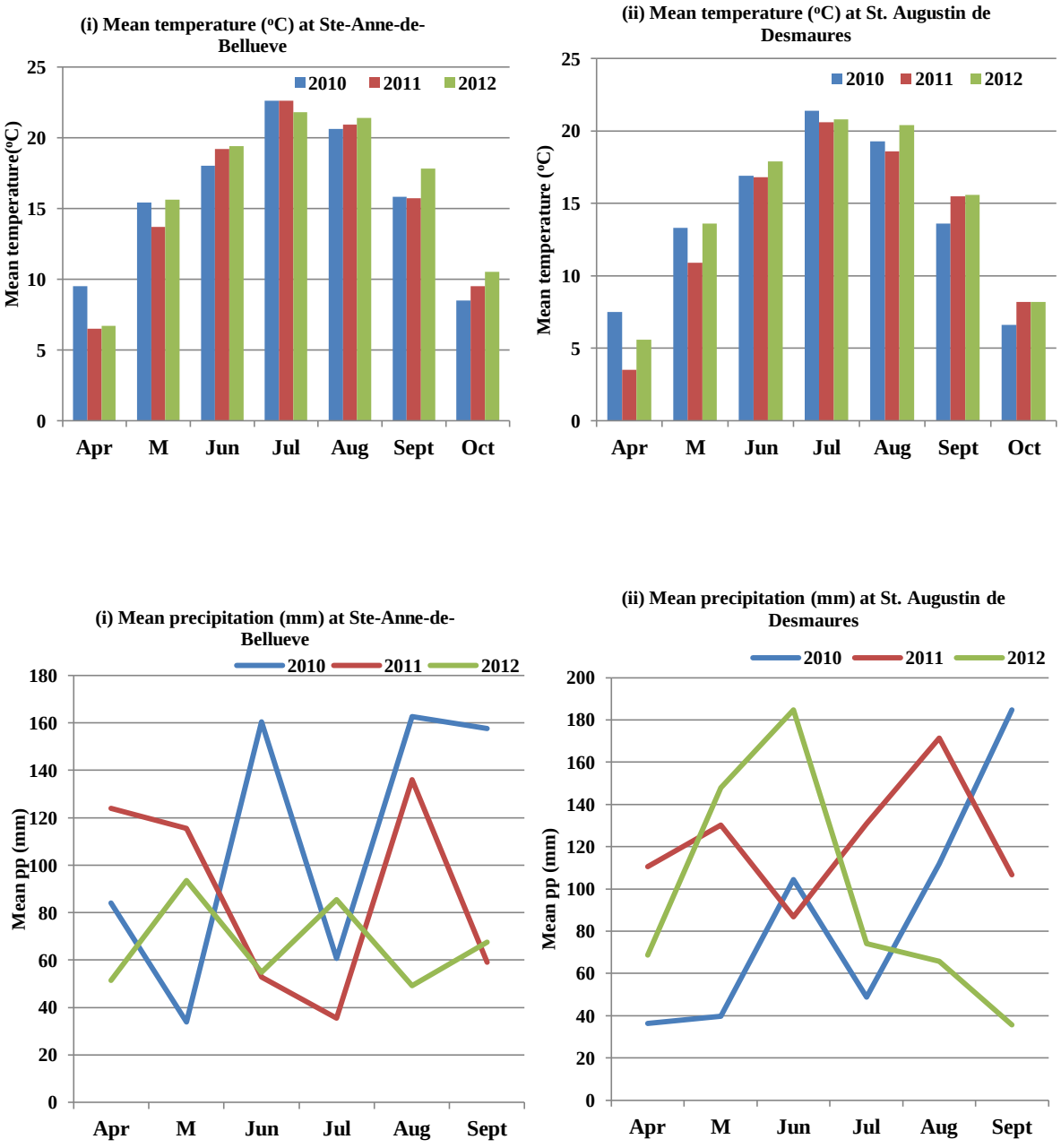


Figure 4.1 Mean temperatures (^{o}C) and total precipitation (mm) for 2010, 2011 and 2012 at two locations in southern Québec: (i) Emile A. Lods Research Centre, McGill University and (ii) St. Augustin de Desmaures, Laval University, Québec City.
(Ref. www.climate.weatheroffice.gc.ca)

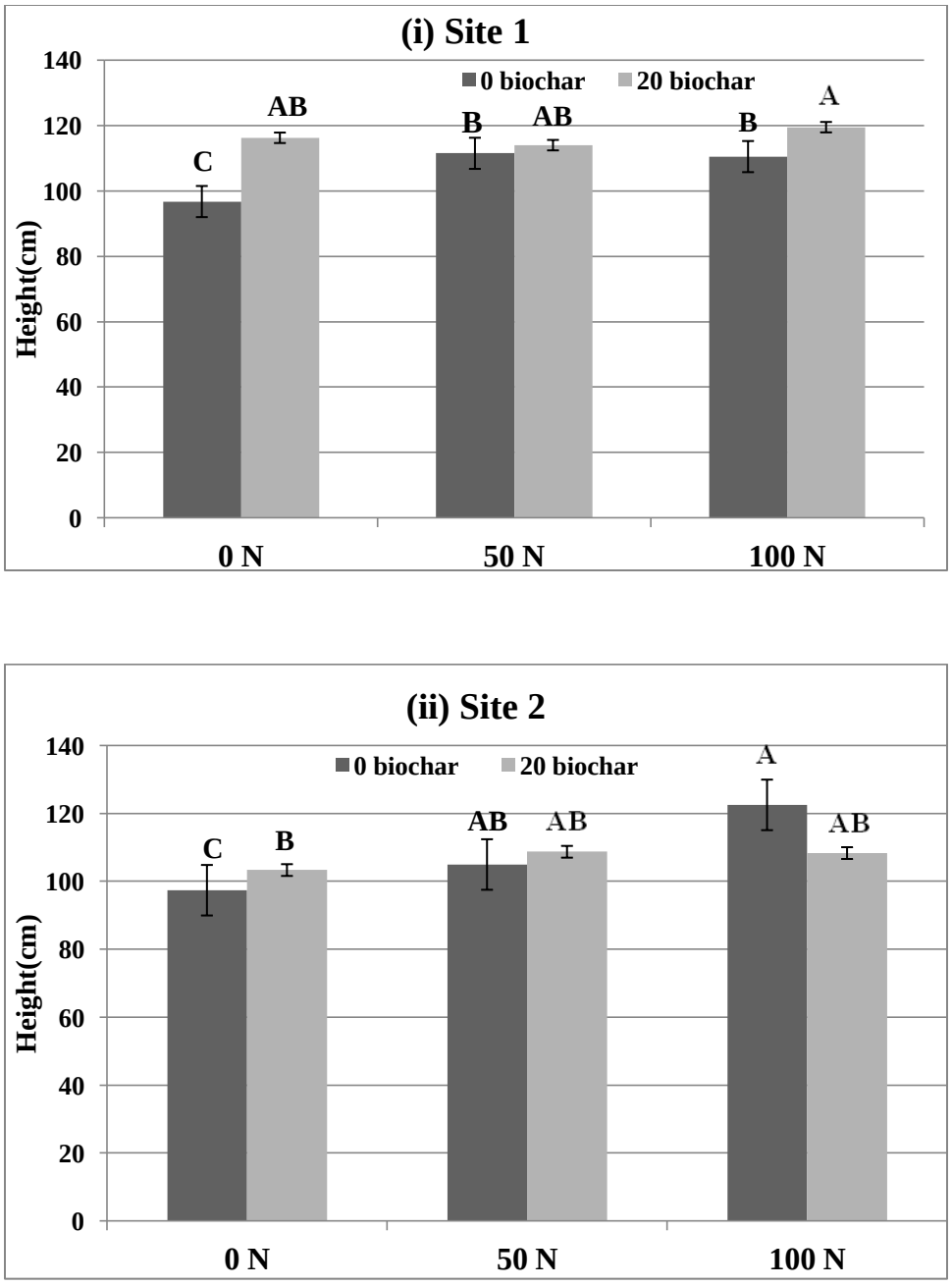


Figure 4.2 Mean height, with standard error (SE) bars, of control (biochar 0 t ha^{-1}) and biochar (biochar 20 t ha^{-1}) amended SG plants fertilized with N (0, 50 and 100 kg N ha^{-1}). Bars represent means of pooled data from sites 1 (i) and 2 (ii), where interactions occurred.

Table 4.1 The effect of N fertilizer (with 0 and 20 t ha^{-1} biochar) on dry biomass yields (Mg ha^{-1}) of SG over three years (2010 to 2012) at three sites in southern Québec.

Biochar	Treatment	Site 1					Site 2					Site 3				
		Fall 2010	Spring 2011	Fall 2011	Spring 2012	Fall 2012	Fall 2010	Spring 2011	Fall 2011	Spring 2012	Fall 2012	Fall 2010	Spring 2011	Fall 2011	Spring 2012	Fall 2012
0 t ha^{-1}	**0 N[1]**	20.98	23.21	83.6	29.99	82.12	16.05	13.38	59.57	38.07	94.32	61.45	27.78	108.1	82.33	160.80
	50 N[2]	35.06	41.54	105.04	35.73	89.94	27.72	21.74	62.35	42.6	94.72	64.02	31.33	141.02	82.55	174.71
	100 N[3]	28.42	24.05	91.5	36.81	96.71	30.66	16.77	67.73	39.58	95.79	62.22	30.24	135.16	85.84	184.37
20 t ha^{-1}	**0 N**	26.37	32.67	96.06	31.75	90.28	18.95	18.15	63.87	40.5	94.21	67.63	30.7	112.66	85.53	180.97
	50 N	29.86	37.28	94.32	39.41	93.76	19.41	15.87	65.16	45.38	99.44	64.09	30.99	138.1	81.90	181.30
	100 N	34.47	35.96	96.68	41.53	101.34	28.11	22.86	62.78	31.98	98.38	62.36	30.67	141.26	80.03	188.04
ANOVA (Split plot analysis)																
Biochar		NS	NS	NS	NS	NS	NS	NS	NA	NS	NS	NS	NS	NS	NS	**
N		*	NS	**	NS	*	***	NS	NA	NS	NS	NS	NS	*	NS	NS
Biochar X N		NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS

*** P < 0.001; ** P < 0.01; * P < 0.05; NS, not significant; NA, not applicable;

[1]0 N refers to control;

[2]50 N refers to 50 kg N ha^{-1} as ammonium nitrate;

[3]100 N refers to 100 kg N ha^{-1} as ammonium nitrate;

Table 4.2 Mean values of variables related to tiller dynamics {stand count, tiller dry weight (DWs; g), and height in cm} of control (biochar 0 t ha^{-1}) and biochar amended (20 t ha^{-1}) SG plants N fertilized (0, 50 and 100 kg N ha^{-1}). Means represent pooled data from each site at each sampling period.

Year	Biochar	Treatment	Stand count			Tiller DWs			Height		
			Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
2010	0 t ha^{-1}	**0 N[1]**	50.41	58.91	64.5	0.906	0.634	1.73	96.75	97.43	64.5
		50 N[2]	49.75	55.16	61.37	1.622	1.232	1.915	111.5	105	61.37
		100 N[3]	50.5	61.33	65.98	1.156	1.188	1.63	110.5	122.56	65.85
	20 t ha^{-1}	**0 N**	64.66	54.91	62.5	0.915	0.855	1.997	116.25	103.37	62.5
		50 N	54.58	59.16	64.5	1.197	0.808	1.805	114	108.75	64.5
		100 N	64.08	56.91	63.3	1.214	1.064	1.825	119.5	108.37	63.38
2011	0 t ha^{-1}	**0 N**	41.75	59	76.12	1.829	0.767	6.165	146.75	132.91	126.88
		50 N	63.75	68.37	69.12	2.946	1.432	8.251	147.66	135.16	129.75
		100 N	61.12	59.87	68.39	2.172	1.37	8.374	144.08	135.25	135.98
	20 t ha^{-1}	**0 N**	65.75	57.37	74.75	3.587	1.28	6.805	154.47	148.83	132.25
		50 N	70.62	59.87	60.12	2.504	1.449	9.039	149	146.41	130.38
		100 N	63.25	66.5	57.56	2.509	1.039	9.633	149.25	149.91	131.11
2012	0 t ha^{-1}	**0 N**	143.5	120.17	.	1.691	1.147	.	170.37	166.62	.
		50 N	133.17	105.83	.	1.148	1.227	.	184.75	187.5	.
		100 N	150.83	102.83	.	1.223	1.337	.	188.25	185.37	.
	20 t ha^{-1}	**0 N**	139.67	101.17	.	1.096	1.399	.	169	161.12	.
		50 N	128	89.33	.	1.124	1.374	.	183.62	174.5	.
		100 N	123.83	108.42	.	1.221	1.752	.	190.5	181.12	.
			ANOVA(Split plot)								
2010	Biochar		NS	NS	NS	NS	*	NS	NA	NA	NS
	N		NS	NS	*	*	NS	NS	NA	NA	NS
	Biochar X N		NS	NS	NS	NS	NS	NS	*	***	NS

2011	Biochar	NS	NS	NS	NS	NS	*	NS	NS	NS
	N	NS	NS	NS	*	*	NS	NS	*	NS
	Biochar X N	NS	NS	NS	NS	NS	NS	NS	NS	NS
2012	Biochar	NS	NS	.	NS	NS	.	NS	NS	.
	N	NS	*	.	NS	*	.	*	*	.
	Biochar X N	NS	NS	.	NS	NS	.	NS	NS	.

*** P < 0.001; ** P < 0.01; * P < 0.05; NS, not significant; NA, not applicable;
[1]0 N refers to control;
[2]50 N refers to 50 kg N ha^{-1}as ammonium nitrate;
[3]100 N refers to 100 kg N ha^{-1} as ammonium nitrate;

Table 4.3 Effect of biochar and N fertilizer (0, 50 and 100 kg N ha^{-1}) on N concentration (%) and N export (kg N ha^{-1}) in harvested aboveground biomass of SG at each site for three years. Means represent pooled data from each site at each sampling. ANOVA results are also presented.

Year	Biochar	Treatment	N concentration (%)			N export (kg N ha^{-1})		
			Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
2010(Fall)	**0 t ha^{-1}**	**0 N[1]**	1.38	2.01	1.79	29.26	31.11	110.69
		50 N[2]	1.48	2.26	1.26	46.48	61.52	80.46
		100 N[3]	1.53	2.14	2.1	40.39	66.79	130.81
	20 t ha^{-1}	**0 N**	1.46	2.15	1.9	33.31	40.69	127.6
		50 N	1.29	2.27	1.09	43.95	42.8	70.32
		100 N	1.5	2.12	1.95	51.87	60.83	121.57
2011(Spring)	**0 t ha^{-1}**	**0 N**	3.31	3.21	2.19	75.23	44.01	61.06
		50 N	2.73	3.02	2.19	110.35	66.03	68.62
		100 N	2.87	2.88	2.12	68.83	48.6	64.33
	20 t ha^{-1}	**0 N**	3.52	3.1	2.15	108.01	55.01	66.59
		50 N	3.11	3.2	2.27	111.1	51.56	70.52
		100 N	3.34	3.19	2.2	118.14	72.86	68.13
2011(Fall)	**0 t ha^{-1}**	**0 N**	1.64	1.82	1.28	137.35	107.57	139.35
		50 N	1.97	1.81	1.5	208.32	113.93	211.69
		100 N	1.83	1.8	1.41	168.59	122.49	190.79
	20 t ha^{-1}	**0 N**	1.86	1.59	1.23	179.45	101.8	138.88
		50 N	1.79	1.72	1.44	170.72	112.52	197.64
		100 N	1.88	1.52	1.2	182.2	95.53	172.35
2012 (Spring)	**0 t ha^{-1}**	**0 N**	1.08	1.17	.	32.75	39.3	.
		50 N	1.11	1.01	.	38.71	41.14	.
		100 N	1.13	1.12	.	41.05	43.99	.
	20 t ha^{-1}	**0 N**	1.09	1.07	.	34.65	42.58	.
		50 N	1.1	1.02	.	45.78	46.6	.
		100 N	1.02	1.06	.	42.34	34.26	.

ANOVA(Split plot analysis)							
2010(Fall)	**Biochar**	NS	NS	NS	NS	NS	NS
	N	NS	NS	NS	NS	NS	***
	Biochar X N	NS	NS	NS	NS	NS	NS
2011(Spring)	**Biochar**	NS	NS	NS	NS	NS	NS
	N	NS	NS	NS	NS	**	NS
	Biochar X N	NS	NS	NS	NS	NS	NS
2011(Fall)	**Biochar**	NS	NS	NS	NS	NA	NS
	N	NS	NS	NS	NS	NA	**
	Biochar X N	NS	NS	NS	NS	*	NS
2012(Spring)	**Biochar**	NS	NA	.	NS	NA	.
	N	NS	NA	.	NS	NA	.
	Biochar X N	NS	*	.	NS	*	.

*** P < 0.001; ** P < 0.01; * P < 0.05; NS, not significant; NA, not applicable;

[1]0 N refers to control;

[2]50 N refers to 50 kg N ha^{-1} as ammonium nitrate;

[3]100 N refers to 100 kg N ha^{-1} as ammonium nitrate;

Table 4.4 Nitrogen use efficiency (kg biomass (kg N)$^{-1}$) as affected by biochar (0 and 20 t ha^{-1}) and N fertilizer (0, 50, 100 kg N ha^{-1}) for SG production over 3 years (2010 through 2012) at three sites in southern Quebec.

Site	Treatment	Fall-2010		Spring-2011		Fall-2011		Spring-2012	
		0 biochar	20 biochar	0 biochar	20 biochar	0 biochar	20 biochar	0 biochar	20 biochar
Site 1	**0 N**[1]	20.98	26.37	23.21	32.67	83.6	96.06	29.99	31.75
	50 N[2]	35.06	29.86	41.54	37.28	105.04	94.32	35.73	94.32
	100 N[3]	28.42	34.47	24.05	35.96	91.5	96.68	36.81	96.68
Site 2	**0 N**	16.05	18.95	13.38	18.15	59.57	63.87	38.07	40.5
	50 N	27.72	19.41	21.74	15.87	62.35	65.16	42.6	45.38
	100 N	30.66	28.11	16.77	22.86	67.73	62.78	39.58	31.98
Site 3	**0 N**	61.45	67.63	27.78	30.7	108.1	112.66	.	.
	50 N	64.02	64.09	31.33	30.99	141.02	138.1	.	.
	100 N	62.22	62.36	30.24	30.67	135.16	141.26	.	.
ANOVA(Split plot analysis)									
Site 1	**Biochar**	NS		NS		NS		NS	
	N	*		NS		**		NS	
	Biochar X N	NS		NS		NS		NS	
Site 2	**Biochar**	NS		NS		NA		NS	
	N	***		NS		NA		NS	
	Biochar X N	NS		NS		**		NS	
Site 3	**Biochar**	NS		NS		NS		.	
	N	NS		NS		*		.	
	Biochar X N	NS		NS		NS		.	

*** P < 0.001; ** P < 0.01; * P < 0.05; NS, not significant; NA, not applicable;

[1]0 N refers to control;

[2]50 N refers to 50 kg N ha^{-1}as ammonium nitrate;

[3]100 N refers to 100 kg N ha^{-1} as ammonium nitrate;

Table 4.5 Apparent N recovery (ANR) as affected by biochar (0 t ha $^{-1}$ and 20 t ha^{-1}) and N fertilizer (0, 50 and 100 kg N ha^{-1}) for SG production over 3 years (2010 through 2012) at three sites in southern Québec.

Site	Treatment	Fall-2010		Spring-2011		Fall-2011		Spring-2012	
		0 biochar	20 biochar	0 biochar	20 biochar	0 biochar	20 biochar	0 biochar	20 biochar
Site 1	**50 N[1]**	127.41	144.64	266.43	305.09	193.94	175.95	109.06	108.57
	100 N[2]	140.62	149.21	284.46	330.94	182.36	186.79	111.93	101.12
Site 2	**50 N**	216.12	222.93	298.38	315.04	178.49	168.63	99.06	100.88
	100 N	212.77	210.5	286.12	313.33	178.95	150.38	111.15	105.04
Site 3	**50 N**	123.14	105.77	214.63	222.73	148.26	139.18	.	.
	100 N	208.53	193.48	210.64	218.44	140.26	119.69	.	.
		ANOVA(Split plot analysis)							
Site 1	**Biochar**	*		NS		NS		NS	
	N	NS		*		NS		NS	
	Biochar X N	NS		NS		NS		NS	
Site 2	**Biochar**	NS		NS		NS		NS	
	N	NS		NS		NS		NS	
	Biochar X N	NS		NS		NS		NS	
Site 3	**Biochar**	NS		NS		*		.	
	N	***		NS		NS		.	
	Biochar X N	NS		NS		NS		.	

*** P < 0.001; ** P < 0.01; * P < 0.05; NS, not significant; NA, not applicable;

[1]0 N refers to control;

[2]50 N refers to 50 kg N ha^{-1}as ammonium nitrate;

[3]100 N refers to 100 kg N ha^{-1} as ammonium nitrate;

Connecting text for Chapter 5

In the previous two chapters, we evaluated the effects of biochar and PGPR, as well as biochar and N fertilizer interactions, on the growth and biomass productivity, and nitrogen dynamics, of the bioenergy grass SG over three continuous field seasons, across three field sites in two regions of southern Québec. Observed results demonstrated generally positive responses of SG growth and N dynamics to biochar and PGPR treatments. However, in the second chapter we reported that at some sites, biochar amended soil with only 50 kg N ha^{-1} resulted in the greatest biomass production and the greatest maximum NUE.

In the following study, we have investigated either biochar and PGPR or biochar and N fertilizer affects on growth, development and NUE of the C_3 bioenergy grass RCG under high-latitude temperate zone field conditions. Two years of field experimentation with RCG, indicated positive yield responses resulting from soil amendment with biochar and/or inoculation with PGPR. Like earlier studies with the C_4 grass SG (chapter 4), biochar by N fertilizer interactions (more or less positive) existed although, in general, greater amounts of dry biomass yield resulted when soils were amended with biochar.

I have contributed to all the work contained in the following chapter, which includes reviewing the pertinent literature, conducting the field experiment, analyzing the data and writing the chapter. The chapter will be submitted, as a manuscript, to a selected journal for publication. The manuscript is co-authored by the me, Dr. Donald L. Smith, Dr. Inna Teshler and Dr. Xiaomin Zhou of the Department of Plant Science, Macdonald Campus of McGill University. Dr. Teshler assisted in managing the field experiments at the McGill Univesity field site. Dr. Zhou provided important input regarding methods of statistical analysis. This research was supported by funds from Fonds de Recherche Nature et Technologies (FQRNT).

CHAPTER 5

5 **Title:** Agronomic performance and NUE of RCG receiving biochar, PGPR and N fertilizer treatments

5.1 Abstract

Concern about global energy supplies has caused increased consideration of purpose grown biomass feedstock crops and the best low-input systems to produce them. Reed canarygrass (RCG - *Phalaris arundinacea* L.) is a C_3-cool season perennial that has received attention as a possible energy crop in recent years. Biochar is a black porous, carbonaceous product that shows promise for retaining nutrients and water in soil, leading to long-term improvement in soil fertility. Studies have reported beneficial effects of biochar soil amendment on plant growth. There have been no previous studies investigating the ability to improve nitrogen efficiency by RCG. PGPR are bacteria that live near, on or in plant roots and promote plant growth through a variety of mechanisms. There have been no investigations regarding the effects of biochar, in conjunction with PGPR inoculants on RCG productivity. The objective of this work was to test PGPR inoculants (either N fixing or P solubilizing) along with soil applied biochar (20 t ha^{-1}) and N fertilizer levels on RCG growth and nitrogen utilization variables under high-latitude temperate zone field conditions. Overall, biochar treatments increased NUE from 10 to 52 %, whereas the greatest N export (kg N ha^{-1}) occurred with combined PGPR (N-fixing and P-solubilizing PGPR) inoculation, which was about 90% greater than the control. A biochar by N fertilizer interaction existed for both years of field testing, whereby the greatest level of dry biomass was produced with 150 kg N ha^{-1} plus biochar applied as a soil amendment. The greatest carbon content (increase of 38.5% over the control) resulted from addition of 150 kg N ha^{-1} plus biochar. Biochar application in conjunction with PGPR inoculation enhanced RCG plant growth and biomass yield over the two years of field experimentation. The combination of increased biomass production plus improved NUE and N recovery can be an efficient low-input system for biomass feedstock production under Québec conditions.

5.2 Introduction

A bioenergy sector based on biomass could be one of the best ways to increase renewable energy as a share of total energy consumption. In comparison to biofuel crops based on potential food materials such as starch and sugars (e.g. corn, sugar cane) or seed oils (eg. soybean), perennial rhizomatous grasses have greater energy returns, because of smaller overall requirements for labor, equipment and fossil fuel energy during cultivation and seeding (Heaton et al., 2004, Lewandowski and Schmidt 2006). RCG - *Phalaris arundinacea*) is C_3 cool season perennial rhizomatous grass which is circumtemporal through the northern hemisphere, well adapted to wet conditions, but also very drought tolerant (Lewandowski et al., 2003). The development of RCG as an energy crop has been ongoing for the last 15 years, with breeding programs in Finland and Sweden aimed at improving its biomass production. In addition at China, some researchers have focused on increasing lignin content (Qin et al., 2012) of some bioenergy grasses as high lignin coupled with low moisture and ash contents make for efficient combustion of biomass; however in Europe, most researchers have been interested in the carbohydrate energy content of grasses carbohydrate (Finell et al., 2005), while lowering its mineral concentration (Lewandowski et al., 2003). Because of its high biomass production on poor soils and a short growing period, RCG has also been evaluated as a potential bioenergy crop in the USA (Wright, 1988) and in the south-eastern Canada (Wrobel et al., 2009).

Biochar is a promising carbon rich material which can be produced as a byproduct of biofuel production when thermochemical methods are used (Briens et al., 2008). The binding of ammonia to the surface of biochar slows the rate of nitrification and hence reduces N_2O production (Lehmann, 2007). In addition, biochar also binds the macronutrient phosphate by surface adsorption (Lehmann et al., 2008), thus biochar amended soils may require less fertilizer to achieve target crop yields, leading to, for instance, less contamination of surface and ground water by phosphate and nitrate (Almasri and kaluarachchi 2004; Elmi et al., 2005) and less production of the greenhouse gas N_2O (Almaraz et al., 2009). Soil applied biochar probably favors the growth of microorganisms (Warnock et al., 2007), although this remains to be demonstrated under field conditions.

Resistant soil humus, which is a byproduct of microbial biosynthesis, can persist for several years to decades (Marschner et al., 2008; Kleber et al., 2007). Studies have reported investigations of diazotrophic associations with SG (Brejda et al., 1998; Day et al., 1975) and the contribution of N fixing bacteria to the NUE of SG (Ker et al., 2012). Plant growth promotion by PGPRs, other than through biological nitrogen fixation (BNF), have also been reported, through the production of phytohormones, enhancement of enzymatic activities, increased nutrient uptake and other mechanisms (Dobbelaere et al., 2003; Dobereiner and Pedrosa, 1987). However, unlike the extensive body of literature pertaining to sugarcane, to our knowledge, no research has been published regarding the contribution of N-fixing bacteria to the NUE of the perennial rhizomatous grass (PRG) RCG. Ker et al. (2012) reported PRG (C_4 grass) growth promotion by mixed PGPR (*Paenibacillus polymyxa*, *Pseudomonas*, *Rahnella* sp., *Serratia* sp.) under field conditions in eastern Canada.

As RCG utilizes the C_3 carbon fixation pathway, like most other C_3 plants it has lower NUE and water use efficiency (WUE) values than plants utilizing the C_4 photosynthesis pathway (Bill et al., 1997). Only a few field trials have been conducted that compare the yields of C_3 and C_4 grasses in western Europe and northeastern North America. Both biochar application (appropriate rate) and PGPR inoculation have the potential to enhance the nutrient supply available to plants leading to increased DM productivity, and possibly to reduced GHG emissions, but they have not been tested with C_3 PRGs under high-latitude other temperate zone conditions, such as those prevalent in southern Québec. The present investigation was conducted to test specific PGPR (N-fixing, P-solubilizing) inoculated onto RCG seed grown in biochar amended soil (20 t ha^{-1}) under temperate zone field conditions. Here we present findings related to growth and yield responses of RCG to inoculation with N fixing and P solubilizing PGPR, and combinations of the two, in the presence and absence of biochar-amended soil. As our greater objective was to develop a low-input system for biofuel crop production, we also evaluated RCG growth and productivity under three rates of N fertilizer application (0, 75 and 150 kg N ha^{-1}) along with biochar application to soil.

5.3 Material and methods

5.3.1 Study site and experimental design

The research was conducted at a field site with a Chicot fine sandy loam soil (pH 6.03; Soil organic matter 3.18%) at the Emile A. Lods Agricultural Research Institute of the Macdonald Campus ($42^{o}28'$N $73^{o}45'$W) of McGill University, Québec, Canada in the years 2011-2012. Seeds of RCG (cultivar Bellevue) were obtained from a commercial seed vendor (Belcan, Saint Marthe, Québec). A germination test was performed to check the viability of seed before planting in the field trial. The experiment was organized following a randomized complete block split-plot design with four blocks (replicates). The treatments were comprised of factorial combinations of biochar (0 and 20 t ha^{-1}), an N-fixing PGPR (applied or not) and a P-solubilizing PGPR (applied or not); biochar was the whole plot factor. There was also a set of N fertilizer level plots (0, 75 and 150 kg N ha^{-1}), established to determine the fertilizer equivalency of the N-fixing PGPR and the potential interaction between N fertility and biochar. The N levels were combined factorially with the biochar levels. This resulted in a total of 12 treatments and 48 experimental plots (n = 48). Each block contained 6 aggregated plots (whole plot) with biochar added at 20 t ha^{-1} and 6 aggregated plots without biochar. The split-plot treatments were control, 75 kg ha^{-1} of N (half the recommended rate), 150 kg ha^{-1} of N (the recommended rate), the N-fixing PGPR, the P-solubilizing PGPR and the combination of both of these PGPR.

5.3.2 Seed inoculation and seeding

The group of added N fixing PGPR included *Paenibacillus polymyxa*, *Pseudomonas*, *Rahnella* sp., and *Serratia* sp. Each isolate was individually cultured in sterile Luria Bertani (LB) broth at 30 ^{o}C for 48 h with shaking. N-free solidified LG medium was used for screening and permanent preparation of pure isolates of N-fixing PGPR (modified *Azotobacter* medium; per L ddH_2O: 10.0 g sucrose, 0.5 g K_2HPO_4, 0.2 g $MgSO_4 \bullet 7H_2O$, 0.2 g NaCl, 0.001 g $MnSO_4 \bullet H_2O$, 0.001 g $FeSO_4$, 0.001 g $NaMoO_4 \bullet 2H_2O$, 5.0 g $CaCO_3$, 15.0 g Bacto-agar) (Döbereiner 1995). Colonies were chosen based on different colony morphologies and restreaked (twice) on N-free LG medium to obtain pure isolates (Ker et al., 2012). King's B media (500 ml ddH_2O: 10.0 g proteose peptone, 0.75 g K_2HPO_4, 0.75 g $MgSO_4 \bullet 7H_2O$, 5 ml glycerol, 7.5 g Bacto-agar) (Schaad, 1980) was used for P-solubilizing

PGPR (*Pseudomonas rhodesiae*). Seeds for the selected bacterial treatments (N-fixing PGPR and P-solubilizing PGPR cultures each at 10^8 colony forming units per mL) were inoculated by seed coating (with peat, at a rate of 8 g peat kg^{-1} seed) for 24 h before seeding. The bacterial inoculant was added to RCG seeds at 140 mL inoculant kg^{-1} seed, then vortex-mixed and allowed to sit at room temperature for 24 h (Ker et al., 2012). Inoculated seeds were then air dried (at least for 1 h) in a laminar air flow hood, before seeding in the field. Control plot RCG seeds were also inoculated with an equivalent amount of sterile bacterial medium (LB medium and King's B medium). Control plots were seeded first, in order to reduce the risk of contamination with bacterial inoculants. As RCG seeds germinate well at soil temperatures > 10 ^oC (Tahir et al., 2011) seeding was conducted on 16th June, 2011 when the mean soil temperature was 15 ^oC. Seeds were placed at 1.5 cm below the soil surface, as recommended (Christensen et al., 2010); seeding, at a rate of 12 kg ha^{-1}, was conducted with a Plotman seeder (Fabro limited, Swift Current, Saskatchewan, Canada).

5.3.3 Cultural management and sampling

N fertilizer (0, 75 and 150 kg N ha^{-1}) was broadcast as ammonium nitrate (27:0:0% N:P:K) and split into two times of application during the growing season. In each case one third of the N was applied shortly prior to seeding (spring) and the rest (two thirds) at the inflorescence stage (Gustavsson, 2011). Weed control was conducted by hand throughout the establishment year and no irrigation water was applied. At the flowering stage, RCG height and number of tillers in 50 cm per row (stand count/density) was measured. At the end of the year (before the first killing frost) plants from 50 cm of four randomly selected row segments within each plot, were cut at a 10 cm height. All harvested herbage was weighed immediately following harvest. Subsamples were oven-dried, at 60 ^oC, to a constant moisture level, to determine moisture and dry matter contents of harvested material. During the early spring (2012) and then at mid-summer (2012), additional samplings were conducted. About 100 g of dried sample material was stored for further chemical analysis.

5.3.4 Elemental analysis for the samples

An elemental analyzer (Model no. NC 2500, Thermo Quest CE Instruments from Isomass Scientific Inc. Calgary, Canada) was used for simultaneous determination of nitrogen present in RCG samples. The principle of operation is the combustion of the sample; it must be

quantitative and instantaneous so that the combustion gases can be efficiently eluted through the chromatographic column and thermal conductivity detector TCD, so it will give output signals proportional to the sample elemental composition. The instrument uses the dynamic flash combustion method, which ensures these conditions (Pella and Colombo, 1973). The grass samples (properly dried) were homogenized by careful grinding (Wiley Mill, model no. 4, Thomas Scientific, USA, screen size 1mm). The sample size analyzed was generally 30-60 mg. A weighed amount of sample in a tin capsule was placed in the autosampler drum where it was deareated (to remove any atmospheric nitrogen), then introduced into a vertical quartz tube heated to 1000 ^{o}C, with a constant flow of helium (carrier gas). A few seconds before each sample dropped into the combustion tube, the helium stream was enriched with a measured amount of high purity oxygen to achieve a strongly oxidizing environment, which guarantees near-complete combustion, even of thermally resistant substances. To achieve quantitative oxidation the combustion gas mixture is driven through an oxidation catalyst (Cr_2O_3) zone, then through a subsequent zone of copper which reduces nitrogen oxides formed during combustion or catalyst oxidation to elemental nitrogen and scrubs excess oxygen. At the outlet of the reaction tube the gas mixture (N_2, CO_2, H_2O) meets a trap containing anhydrone, which adsorbs water. The resulting components of the combustion mixture were eluted and separated by a Porapack PQS column and subsequently detected, by a thermal conductivity detector, in the sequence N_2, CO_2. The amount of N exported through the removal of aboveground harvested biomass was calculated by multiplying the N concentration in aboveground tissues (kg N Mg^{-1}) by the total harvested dry biomass yield (Mg ha^{-1}) (Jung et al.,2011). Nitrogen-use efficiency (kg biomass (kg N)$^{-1}$) was calculated as the difference between fertilizer treatment plot biomass yield and control plot biomass yield and then divided by the rate of N fertilizer applied (kg ha^{-1}) and ANR (apparent N recovery) was calculated as the difference between fertilizer treatment plot total N export (kg N ha^{-1}) and control plot total N export (kg N ha^{-1}) and then divided by the rate of N fertilizer applied (kg N ha^{-1}) (Jung et al.,2011). Carbon pool/C content (Mg C ha^{-1}) of aboveground harvested biomass was calculated by multiplying the carbon percentage on aboveground tissues by the total harvested dry biomass yield (Mg ha^{-1}) and then divided by 100 (McLauglin et al., 1998).

5.3.5 Statistical analyses

Statistical analyses were performed with the software package SAS 9.2 (SAS Institute Inc.). Raw data from both years were checked for normality and constant variance of errors before conducting statistical analyses. A split plot analysis (randomized complete block design) was conducted using the PROC GLM procedure of SAS to determine main effects and interactions of factors. Biochar was the whole plot factor and factorial combinations of the PGPRs made up the subplot factor treatments. When a factor (e.g. biochar, N-fixing PGPR, P-solubilizing PGPR) or interaction among factors (e.g. biochar x N-fixing PGPR interaction, biochar x P solubilizing PGPR interaction) was declared statistically significant the standard error of the mean was used to determine differences between means for three levels of significance ($P < 0.05$, $P < 0.01$and $P < 0.001$) (Peterson, 1985). T-tests were performed for the comparison of mean-pairs. The following linear additive model describes the independent variables used to analyze the response to treatment for each growth variable:

$$Y_{ijk} = \mu + \text{biochar}_i + \text{block}_j + (\text{biochar x block})_{ij} + \text{treatment}_k + (\text{biochar x treatment})_{ik} + \text{error}_{ijk}$$

where Y_{ijk} is the observed value of our dependent variable from the i^{th} biochar, j^{th} block and k^{th} treatment. The overall mean is μ. Biochar_i is the fixed effect of the i^{th} biochar, block_j is the random effect of the j^{th} block and treatment_k is the fixed effect of the k^{th} treatment on growth parameter of RCG. The term $(\text{biochar x treatment})_{ik}$ is the fixed effect interaction between the k^{th} treatment and the i^{th} biochar. The term error_{ijk} is the random residual error associated with the i^{th} biochar, j^{th} block and k^{th} treatment.

A similar linear additive model describes the independent variables which were used to analyze the responses to N fertilizer level treatments for each growth variable:

$$Y_{ijk} = \mu + \text{biochar}_i + \text{block}_j + (\text{biochar x block})_{ij} + \text{treatment}_k + (\text{biochar xtreatment})_{ik} + \text{error}_{ijk\backslash}$$

where everything is the same as the above equation, except we used N fertilizer treatments (0, 75 and 150 kg N ha^{-1}) instead of PGPR treatments.

5.4 Results and discussion

5.4.1 Growth response

5.4.1.1 Biochar X PGPR (N-fixing, P-solubilizing or combination) effect on plant height

Biochar by PGPR (N-fixing or P-solubilizing) interactions were observed for RCG plant height (Table 5.1). A biochar by P solubilizing PGPR interaction (P = 0.0009) was observed during the summer of 2012 while a biochar by combined PGPR (combination of N-fixing and P-solubilizing PGPR) interaction (P = 0.0259) was observed in the sampling of the fall of the establishment year (2011) sampling. Ker et al. (2012) reported taller SG (C_4 grass) during the establishment year when the seeds were inoculated with mixed PGPR.

5.4.1.2 Biochar X PGPR (N-fixing, P-solubilizing or combination) effect on stand density

Selection of RCG with larger tillers and leaf blades has shown the potential for yield increases (Davis 1960, Carlson et al., 1996). Overall, the yield of perennial grasses can be greatly affected by their stand density (number of tillers per unit area) (Parrish and Fike, 2005). Recent field studies of C_4 grass stand dynamics have found increased tiller number with mixed PGPR inoculation under temperate region conditions (Ker et al., 2012). In this study, no significant difference was observed for stand density during the establishment year (2011), whereas there was a biochar by P-solubilizing PGPR interaction (P = 0.0032) observed for the second year (2012) (Table 5.1). Besides this, the number of tillers was found to be increased, in the second year when the seeds had been inoculated with PGPR (N-fixing or P-solubilizing or combination), which indicates that PGPR inoculation results in the allocation of additional nutrients to new shoot development of RCG and the soil conditioner biochar may encourage the growth of PGPR by alternating soil physio-chemical properties through increased nutrient availability (Lehmann et al., 2011; Warnock et al., 2007).

5.4.1.3 Biochar X N fertilizer effect on plant height

Interactions between biochar and N fertilizer (P = 0.0036; $P \leq 0.0001$) were observed under field conditions (Table 5.3). This study showed patterns of response similar to glasshouse studies by Chan et al. (2007) where a biochar x N fertilizer interaction was observed. The tallest plants, mean height 178.16 cm, occurred with 150 kg N ha^{-1} and biochar (20 t ha^{-1}) soil application, and the lowest mean height resulted when biochar was not applied (0 t ha^{-1})

(Table 5.3). Canopy height has also been reported to be positively correlated with yield (Christensen et al., 1984).

5.4.1.4 Biochar X N fertilizer effect on stand density

A significant biochar by N fertilizer interaction (P = 0.0008) was observed during the second year, when tiller distribution was found to be increased by 44% with 75 kg N ha^{-1} along with biochar (20 t ha^{-1}) and 53% with 150 kg N ha^{-1} along with biochar (20 t ha^{-1}), as compared with the control treatment with biochar alone (20 t ha^{-1}) (Table 5.3). However, without biochar (0 t ha^{-1}) there was no increase over the control treatment, even with N fertilizer (75 and 150 kg N ha^{-1}).

5.4.2 Biomass yield

5.4.2.1 Biochar X PGPR (N-fixing, P-solubilizing or combined) effects

Positive effects of biochar on crop yield have been reported, including maize (Major et al., 2010; Lentz and Ippolito, 2012), various types of bean, banana and carrot (Lehmann et al., 2003), but not for ryegrass biomass production (Kammann et al., 2012). There has been no field investigation of PGPR inoculation effects along with biochar application on C_3 cool season RCG production. In this study we found a biochar by combined N fixing and P solubilizing PGPR interaction for the second year (Table 5.1). While PGPR inoculation or biochar application alone did not result in any yield response, they did not have negative effects. Yields of 1.32 to 2.13 Mg DM ha^{-1} for RCG were measured for PGPR inoculated treatments during the spring of 2012, whereas yields of 2.37 to 2.80 Mg DM ha^{-1} were determined for the same treatment during summer sampling (August, 2012).

5.4.2.2 Biochar X N effects

RCG produced more biomass when nitrogen was supplied at 50 and 100 kg N ha^{-1}, becoming 35 and 195% more productive, respectively (Kercher and Zedler, 2004). Rather than split applications of N fertilizer, the single application of N fertilizer during spring (early season treatment) increased RCG yield, leading to a doubling of biomass production (Vetsch et al., 1999). In a glasshouse pot trial a biochar by N fertilizer interaction was observed with increasing rates of biochar application along with N fertilizer application (Chan et al., 2007). In the study reported here, a biochar by N fertilizer interaction was observed during fall of

2011 (P = 0.0155) and during the summer of 2012 ($P \leq 0.0001$) (Table 5.3). Coulman (1996) reported 7.3 Mg DM ha $^{-1}$ yield for RCG in southwestern Québec. A study with a compost/charcoal/fertilizer combination under field conditions reported enhanced yield, compared to charcoal or N fertilizer alone (Steiner et al., 2007). A similar trend was observed in my study where the highest DM yield resulted from 150 kg N ha^{-1} with biochar application, which resulted in 8 Mg ha^{-1} (Table 5.4). This may suggest that biochar application could result in greater mobilization or mineralization, making more N available to the crop plants (Gaskin et al., 2010).

5.4.3 N export, NUE and ANR

5.4.3.1 Biochar X PGPR (N-fixing, P-solubilizing or combined) effects

A previous study showed that the respiration rate, microbial biomass and diversity were higher ($P < 0.03$; 1.5 to 3 fold) for RCG invaded soils than in soils supporting native species (Jaccinthe et al., 2012). A recent field investigation of the N dynamics of C_4 grasses showed greater N export with mixed PGPR inoculated onto seeds under high-latitude temperate zone conditions (Ker et al., 2012). In the current study, N export ranged from 29.52 to 87.62 kg N ha^{-1} and 8.64 to 22.34 kg N ha^{-1} for the fall (2011) and spring (2012) harvests, respectively (Table 5.2) with PGPR treated RCG. Interactions between biochar and P-solubilizing bacteria (P = 0.0086), and biochar and combined PGPR (P-solubilizing and N-fixing) (P = 0.009) were observed for the spring harvest in 2012 (Table 5.2). The greatest N export (kg N ha^{-1}) was observed for the combined PGPR inoculated treatment, which resulted in an increase of about 90% over the control. Several studies have reported the beneficial influence of combined inoculation of P-solubilizing and N-fixing bacteria on rice and barley (Kundu et al., 1984), as they provided more balanced nutrition for the plants. Overall, PGPR inoculation has been found to increase the N export rate of RCG under temperate zone field conditions.

5.4.3.2 Biochar X N effect

The N content of RCG shoots in autumn was about half of that for summer and during winter this content was as low as 9-20% before N was remobilized back into the shoots for spring regrowth (Partala et al., 2001), which was largely from N applied as fertilizer during the previous year. It is preferable if RCG production for biofuel purposes is carried out with

lower amounts of N fertilization because higher N fertility can result in increased the amounts of N, P, K and S in plant tissues at harvest (Kätterer et al., 1998), which is undesirable for bioenergy production systems. In addition, biochar is able to bind N so that less is lost to leaching and denitrification, likely leading to a reduced requirements for N fertilizer application and, therefore, improved net energy ratio (NER - the ratio between energy consumed and energy gained) as well as reduced groundwater contamination and GHG emissions. In the current study, a biochar by N fertilizer interaction (P = 0.0132) was observed during second year (Spring-2012) (Table 5.4). The amount of N export was 13.47 to 32.75 kg N ha^{-1} and 7.65 to 20.23 kg N ha^{-1} with N fertilizer application for the fall (2011) and spring (2012) samplings, respectively (Table 5.4). In addition, N concentration (%) of aboveground tissues of RCG was much lower during spring sampling, especially for plants grown on biochar amended soils, which is very desirable for biofuel crop production .Delaying the RCG harvest until spring decreases the ash concentration of RCG which leads to increased biomass quality, at least from the perspective of biofuel production (Burvall, 1997); this resulted biomass with lower levels of K, Cl and N, which have been translocated from the aboveground biomass to the roots or leached from above ground tissues or leached out of the tissues during the winter (Kätterer et al., 1998).

A yield advantage with N fertilization plus biochar application was observed in the current work. The recommended rate of N fertilization is 140 kg N ha^{-1} to maximize RCG NUE and energy use efficiency (net energy yield/energy input) (Lewandowski and Schmidt, 2006). Here, the highest NUE occurred at 150 kg N ha^{-1} along with biochar application (20 t ha^{-1}), and this was greater than for the same fertilizer level without biochar (Fig. 5.1.i). Overall biochar application increased NUE from 10 to 52% compared to the control treatment.

Apparent N recovery (ANR), which discounts the amount of N removed in the control treatment (0 kg N ha^{-1}), followed the same pattern of response observed for NUE i.e. ANR was greater at 150 kg N ha^{-1} with biochar (20 t ha^{-1}) applied than 150 kg N ha^{-1} alone (Fig 5.1.ii). The effect of biochar (P = 0.0143) in the spring of 2012 resulted in a 54 and 225% increase over the non-biochar (0 t ha^{-1}) treatments at 75 and 150 kg N ha^{-1}, respectively. Van Zwieten et al. (2010) reported increased NUE following the application of biochar to

soil in a greenhouse study while Chan et al. (2007) reported that low nutrient biochar increased fertilizer use efficiency.

5.4.4 Carbon dynamics (C%, Carbon pool and N:C ratio)

5.4.4.1 Biochar X PGPR (N-fixing, P-solubilizing or combined) effects

For the green parts of plants, C % has generally has been estimated to be within the range of 45 (Kobak 1988) to 50% (Birdsey 1992, Kurz et al., 1992) of the biomass. Allison et al. (2009) reported the C % of the RCG as ranging from 40.7 to 47.4%. In my study C % values were similar to these estimates (Table 5.5). The mean C % for RCG inoculated with PGPR ranged from 39.07 to 41.42%, whereas the mean C pool (Mg C ha^{-1}) ranged from 0.3641 to 8.44 Mg C ha^{-1} and the mean N:C ratio ranged from 0.0140 to 0.024 over two years under field conditions (Table 5.5). The C pool/content of RCG aboveground biomass was much lower later in the season (e.g. fall 2011) than in spring (e.g. 2012). Perennial grasses added 1.1 Mg of C ha^{-1} yr^{-1} to the upper 100 cm of the soil profile over a 5 year period (Mclaughlin et al., 1998), which replaced 23% of the soil C lost during prior tillage. A significant effect of combined PGPR (P-solubilizing and N-fixing) on C content was observed during fall (2011) with the highest mean C content occurring for the PGPR inoculated treatment (Fig 5.2.i).

5.4.4.2 Biochar X N effects

The mean C percent for N fertilized RCG ranged from 38.73 to 41.77%, whereas the mean C pool (Mg C ha^{-1}) ranged from 0.6560 to 11.72 Mg C ha^{-1} and mean N:C ratio ranged from 0.0155 to 0.024 over two years under field conditions (Table 5.6). Bransby et al. (1998) reported C_4 warm season grass yields of 4.22 Mg C ha^{-1} from aboveground biomass. In this study, the highest C pool/content (Mg C ha^{-1}) resulted from the treatment that combined 150 kg N ha^{-1} and biochar amended soil, where C content increased 38.53% over control plot. A biochar by N fertilizer interaction occurred for C% and N:C ratio in that biochar and N fertilizer each increased these variables in isolation during the fall of 2011 but, with C content biochar and N fertilizer main effects were significant during both the establishment (2011) and second years (2012) (Table 5.6, Fig 5.2.ii).

5.5 Conclusions

RCG has potential as a biofuel crop for production in cool season areas, such as northern Europe and northern North America. Among the cool-season grasses, it matures quickly and is well adapted to cool conditions and has high biomass yield, a wide range of adaptation and low N fertilizer requirement. This study focused on the effects of biochar application, along with PGPR inoculation, on RCG production under temperate climatic conditions. Yields of 1.32 to 2.13 Mg DM ha^{-1} for RCG were measured during the spring of 2012 when the crop was inoculated with PGPR, whereas yields were 2.37 to 2.80 Mg DM ha^{-1} for the same treatment at the summer sampling. In addition to this, RCG receiving three rates of N fertilizer (0, 75 and 150 kg N ha^{-1}) along with biochar soil application resulted in biochar and N fertilizer interactions; the highest DM yield occurred at 150 kg N ha^{-1} with biochar (Table 5.3) applied to the soil, but at 150 kg N ha^{-1} without biochar the overall yield was greater with biochar. The greatest N export (kg N ha^{-1}) was observed with combined PGPR inoculation, which was about 90% greater than the control. Overall, biochar treatment increased NUE from 10 to 52% compared to the control treatment. The highest C content (Mg C ha^{-1}) resulted from application of 150 kg N ha^{-1} and biochar amendment of the soil, where C content increased 39% over the control plot. These results suggested two scenarios pertaining to the increased RCG growth response and biomass yield with the application of biochar plus PGPR inoculation, and biochar plus N fertilization. One (biochar and PGPR) possibility was enriched availability of soil nutrients as well as enhanced N and P in the soil because of PGPR inoculation, which would have resulted in higher absorption of nutrients leading to vigorous vegetative growth and higher biomass yield. The other (biochar and N fertilizer) possibility is increased biomass yield through binding of ammonium N to biochar leading to greater NUE than biochar unamended soils. This is an important attribute of low-input production systems for bioenergy crops.

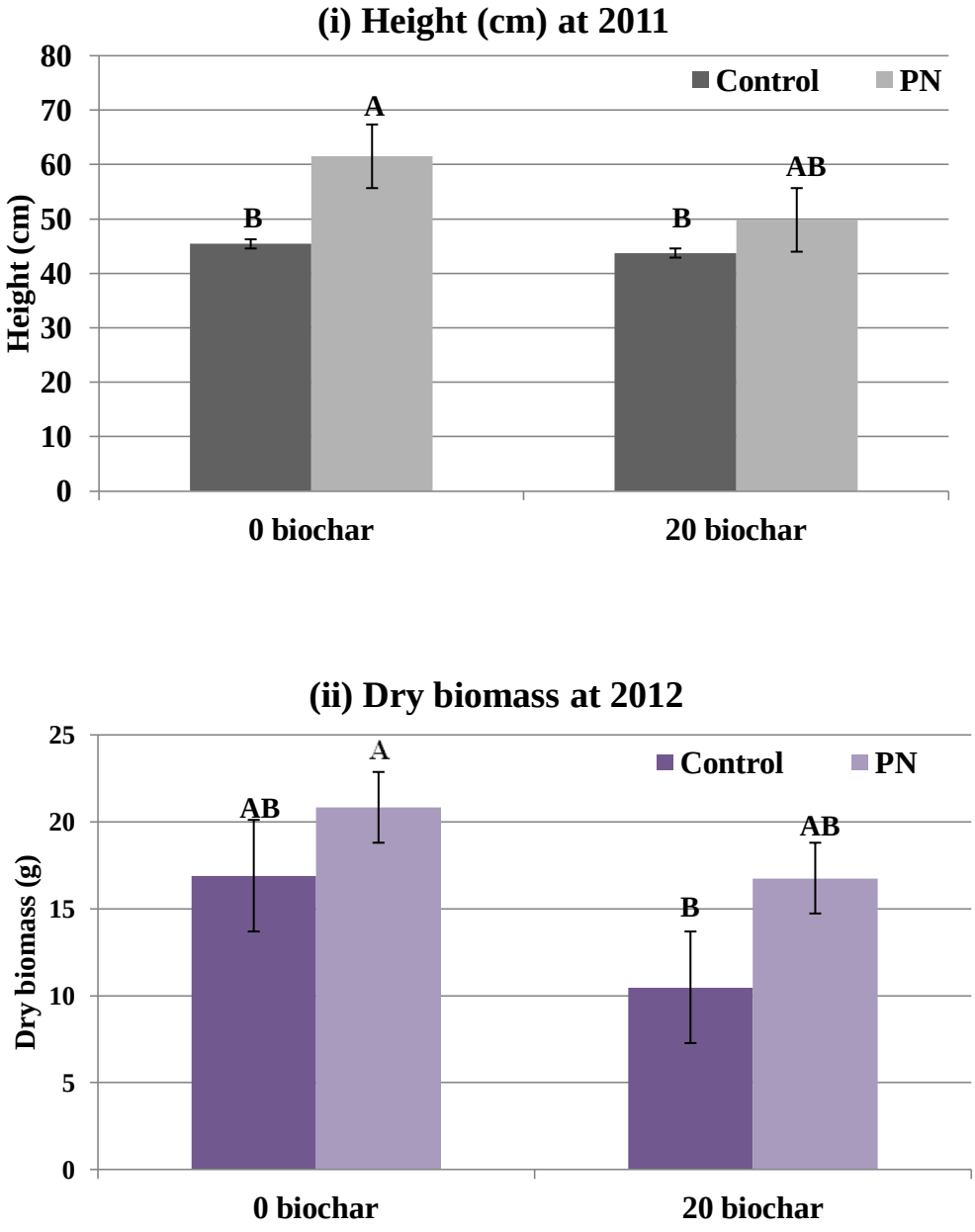


Figure 5.1 (i) Mean height (cm) with standard error (SE) bars of control (biochar 0 t ha^{-1}) and control with biochar (biochar 20 t ha^{-1}) amended RCG inoculated or not (control) with combined PGPR (PN) culture. **(ii)** Mean dry biomass (g) with standard error (SE) bars of control (biochar 0 t ha^{-1}) and control with biochar (biochar 20 t ha^{-1}) amended RCG inoculated or not (control) with combined PGPR (PN) culture. Means represent pooled data from first year after establishment (2011) where significant interactions were observed between biochar and PGPR inoculation.

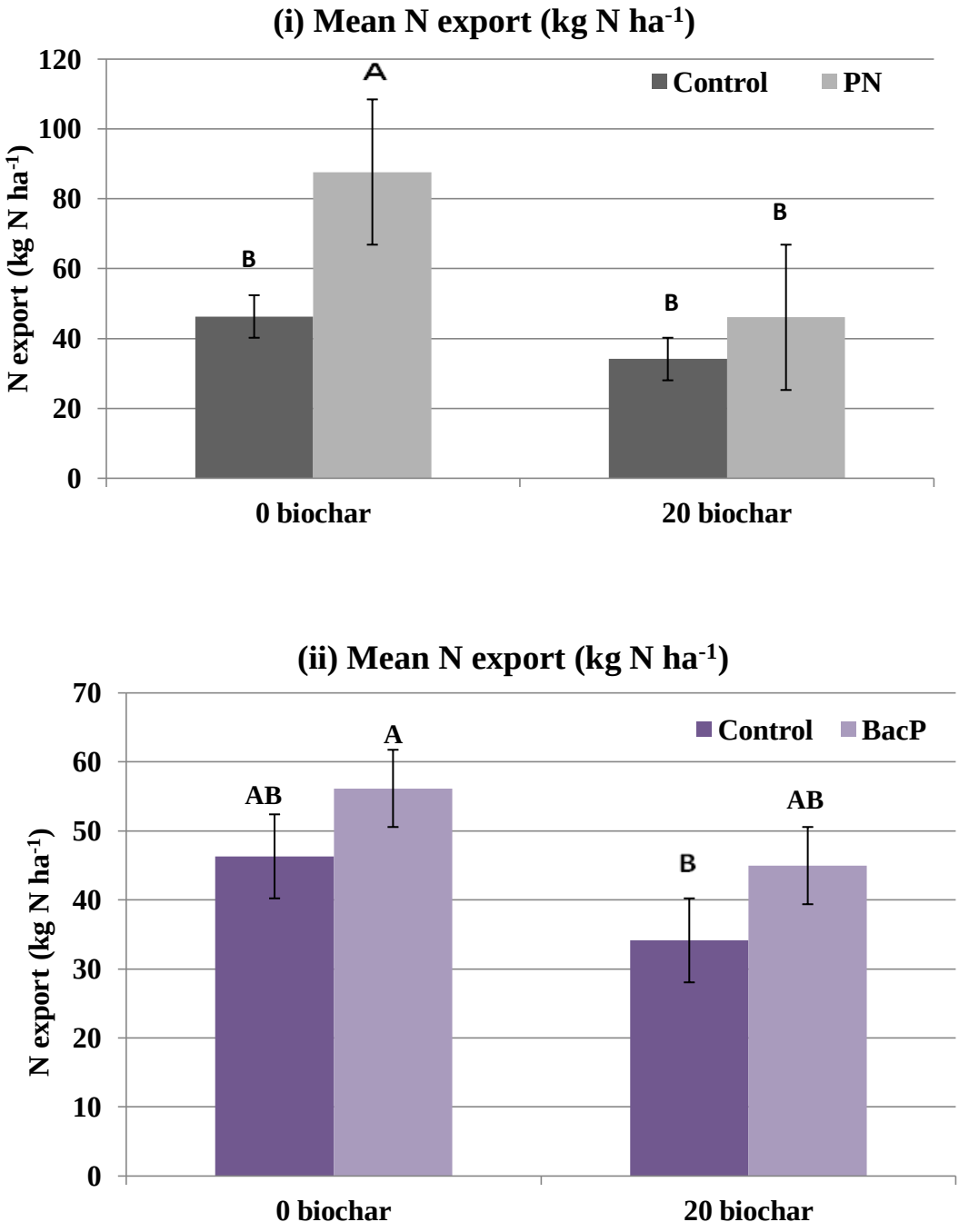


Figure 5.2 (i) Mean N export (kg ha^{-1}) with standard error (SE) bars of control (biochar 0 t ha^{-1}) and control with biochar (biochar 20 t ha^{-1}) amended RCG inoculated or not (control) with combined PGPR (PN) culture. **(ii)** Mean N export (kg ha^{-1}) with standard error (SE) bars of control (biochar 0 t ha^{-1}) and control with biochar (biochar 20 t ha^{-1}) amended RCG inoculated or not (control) with P-solubilizing PGPR (BacP) culture. Means represent pooled data from first year after establishment (2011) where significant interactions were observed between biochar and PGPR inoculation.

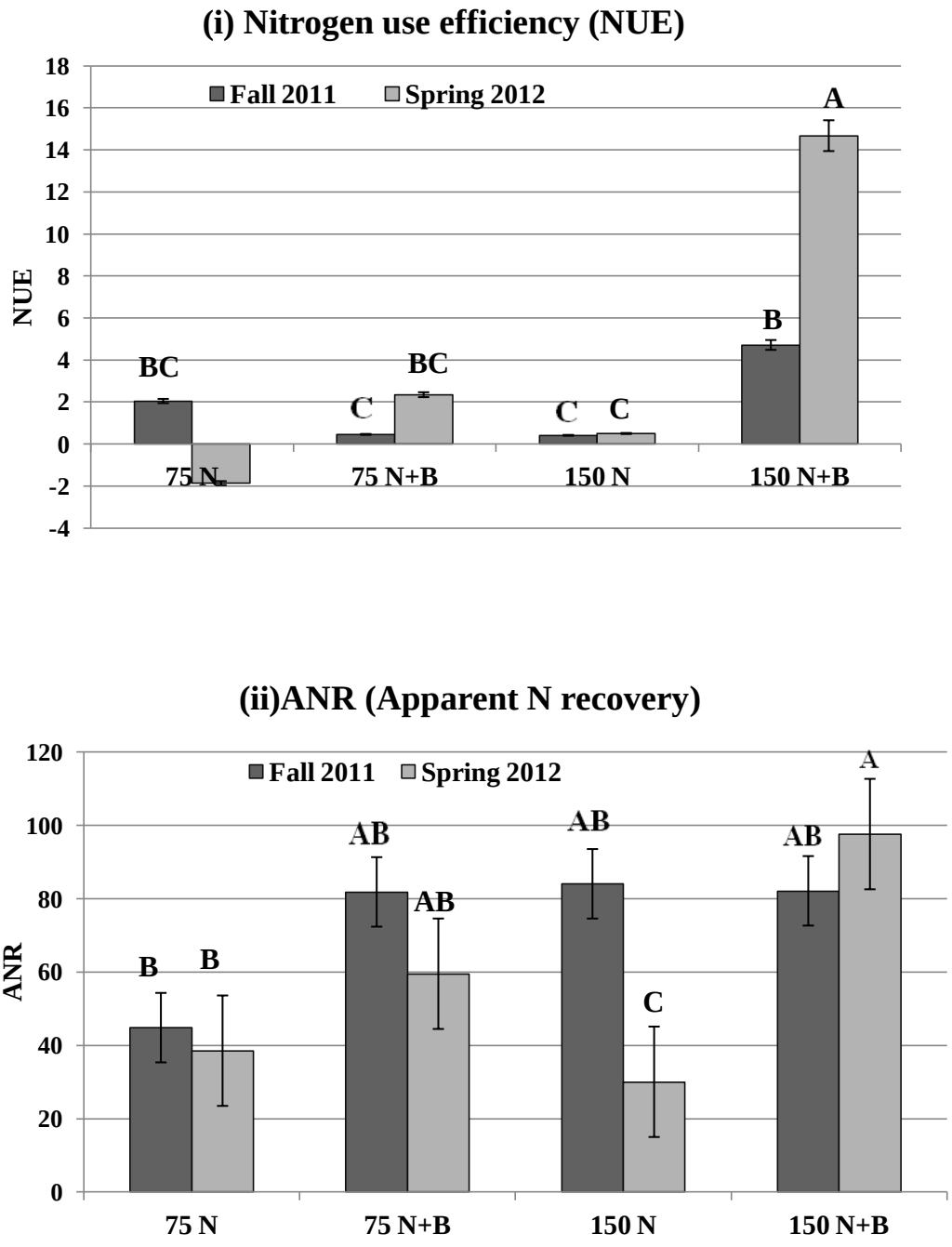
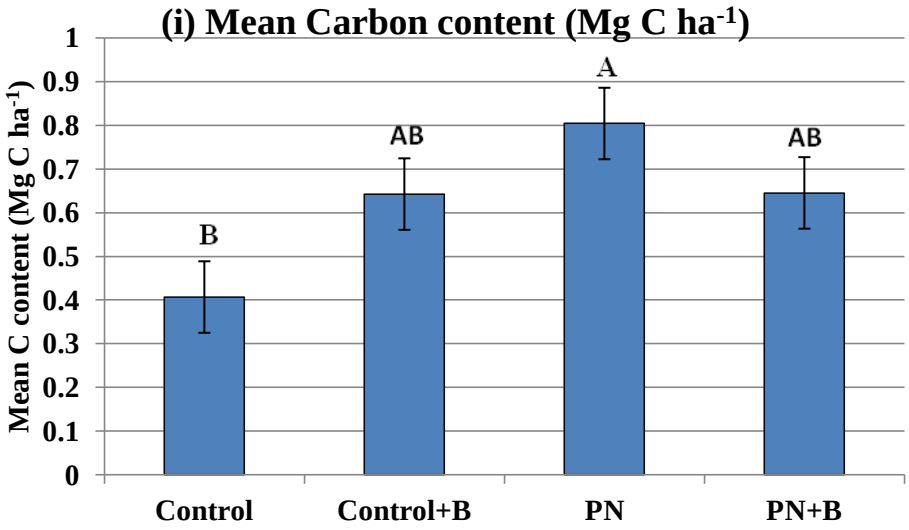


Figure 5.3 (i) Mean NUE (nitrogen use efficiency) and **(ii)** mean ANR (apparent nitrogen recovery) of control (biochar 0 t ha^{-1}) and control with biochar (biochar 20 t ha^{-1}) amended RCG plants fertilized or not (control) with N fertilizer (0 N refers to control ; 75 N refers to 75 kg N ha^{-1}; 150 N refers to 150 kg N ha^{-1}). Means represent pooled data from one year, either 2011 or 2012.



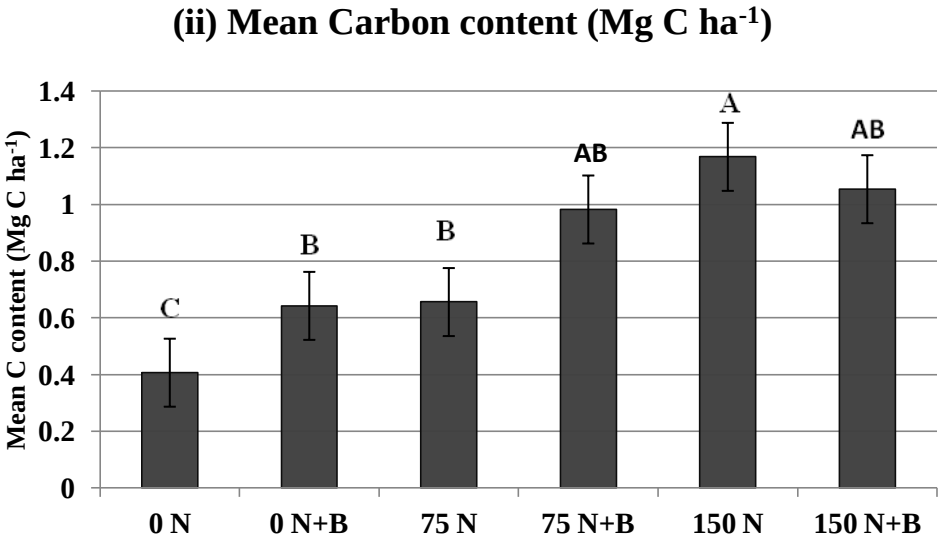


Figure 5.4 (i) Mean C content (Mg C ha^{-1}) with standard error (SE) bars of control (biochar 0 t ha^{-1}) and control with biochar (biochar 20 t ha^{-1}) amended RCG inoculated or not (control) with combined PGPR (PN) culture (Control +B refers to control with biochar; PN+B refers to combined PGPR inoculated with biochar). **(ii)** Mean C content (Mg C ha^{-1}) with standard error (SE) bars of control (biochar 0 t ha^{-1}) and control with biochar (biochar 20 t ha^{-1}) amended RCG plants fertilized or not (control) with N fertilizer (0 N refers to control ; 75 N refers to 75 kg N ha^{-1}; 150 N refers to 150 kg N ha^{-1}; 0 N+B refers to control with biochar ; 75 N+B refers to 75 kg N ha^{-1} with biochar and 150 N+B refers to 150 kg N ha^{-1} with biochar).

Table 5.1 Effect of biochar and PGPR (N-fixing, P-solubilizing and their combination) inoculation on growth variables (height, stand count, fresh weight and dry weight) of reed canary grass (RCG) during the first two years after establishment.

Biochar	Treatment	Fall-2011				Spring-2012		Fall-2012			
		Height (cm)	Stand count	FW (g)	DW (g)	FW (g)	DW (g)	Height (cm)	Stand count	FW (g)	DW (g)
0 t ha^{-1}	Control	45.43	56	50.61	16.89	37.05	29.47	166.08	61.81	271.14	98.23
	BacN[1]	49	50.91	31.67	16.11	28.55	19.95	154.33	52.81	168.64	77.71
	BacP[2]	47.87	43.33	49.56	16.45	20.17	14.2	131.75	55.37	156.75	76.60
	PN[3]	61.53	48.66	72.87	20.82	30.77	22.55	153.17	57.75	165.09	71.75
20 t ha^{-1}	Control	43.75	42.75	31.58	10.47	30.7	21.92	152.5	53.81	188.93	80.40
	BacN	45.53	45.5	29.28	9.45	20.2	14.52	148.33	51.31	153.69	76.54
	BacP	46.09	46.08	42.66	14.15	30.32	22.5	149.25	51.25	190.92	95.41
	PN	49.84	45.42	49.89	16.75	15.47	10.9	149.67	56.5	142.71	75.55
ANOVA (Split plot analysis)											
Biochar		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
BacN		NS	NS	NS	NS	NS	NS	NS	NS	**	NS
Biochar X BacN		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Biochar		NS	NS	NS	NS	NS	*	NA	NA	NA	NA
BacP		NS	NS	NS	NS	NS	NS	NA	NA	NA	NA
Biochar X BacP		NS	NS	NS	NS	NS	NS	**	**	**	**
Biochar		NA	NS	NS	NS	NS	NS	NS	NS	NS	NS
PN		NA	NS	NS	NS	NS	NS	*	NS	**	*
Biochar X PN		**	NS	NS	NS	NS	NS	NS	NS	NS	NS

*** P < 0.001; ** P < 0.01; * P < 0.05; NS, not significant; NA, not applicable;

[1]BacN refers to N-fixing PGPR;

[2]BacP refers to P-solubilizing PGPR;

[3]PN refers to combination of N-fixing and P-solubilizing PGPR;

Table 5.2 Effect of biochar and PGPR (N-fixing, P-solubilizing and their combination) inoculation on N dynamics (dry biomass production, N concentration and N export) of RCG in the two years after establishment (2011 and 2012).

Biochar	Treatment	Fall-2011			Spring-2012		
		Dry biomass (kg ha^{-1})	N (%)	N export (kg ha^{-1})	Dry biomass (kg ha^{-1})	N (%)	N export (kg ha^{-1})
0 t ha^{-1}	**Control**	916.9	2.94	46.29	2750.8	2.4	22.04
	BacN[1]	620.6	3.36	71.8	1861.9	1.99	13.39
	BacP[2]	441.7	2.97	56.13	1325.2	1.96	8.636
	PN[3]	701.5	3.07	87.62	2104.5	1.99	14.18
20 t ha^{-1}	**Control**	682.1	3.24	34.12	2046.2	2.24	11.86
	BacN	451.9	3.34	29.52	1355.6	1.88	13.76
	BacP	710.8	3.38	44.95	2132.5	2.19	16.42
	PN	308.8	2.96	46.08	926.3	1.96	22.12
ANOVA (Split plot analysis)							
Biochar		NS	NS	NS	NS	NS	NS
BacN		NS	NS	NS	NS	**	NS
Biochar X BacN		NS	NS	NS	NS	NS	NS
Biochar		NS	NS	NS	*	NA	NA
BacP		NS	NS	NS	NS	NA	NA
Biochar X BacP		NS	NS	NS	NS	**	**
Biochar		NS	NS	*	NS	NS	NA
PN		NS	NS	NS	NS	**	NA
Biochar X PN		NS	NS	NS	NS	NS	**

*** P < 0.001; ** P < 0.01; * P < 0.05; NS, not significant; NA, not applicable;
[1]BacN refers to N-fixing PGPR;
[2]BacP refers to P-solubilizing PGPR;
[3]PN refers to combination of N-fixing and P-solubilizing PGPR;

Table 5.3 The effect of N fertilizer (0 N, 75 kg N ha^{-1} and 150 kg N ha^{-1}) and biochar on RCG growth variables (height, stand count, fresh weight and dry weight) in the two years after establishment (2011 and 2012).

Biochar	Treatment	Fall-2011				Spring-2012		Fall-2012			
		Height (cm)	Stand count	FW (g)	DW (g)	FW (g)	DW (g)	Height (cm)	Stand count	FW (g)	DW (g)
0 t ha^{-1}	**0 N[1]**	45.43	56	50.61	16.89	37.05	29.47	166.08	61.81	271.44	98.22
	75 N[2]	51.37	52.75	59.91	16.87	24.2	15.07	156.33	55.93	175.98	78.93
	150 N[3]	67.4	47.41	101.01	30.2	18.32	11.25	134.67	53.68	226.62	90.82
20 t ha^{-1}	**0 N**	45.75	42.75	31.58	10.47	30.7	21.92	152.5	53.81	188.93	80.40
	75 N	67.4	45.33	81.98	25.7	27.72	17.82	136.58	55.25	190.49	91.68
	150 N	74.25	54.84	98.27	27.03	36.95	30.95	178.16	52.93	283.83	109
ANOVA (Split plot analysis)											
Biochar		NA	NS	NS	NA	NS	*	NA	NA	NA	NA
N		NA	NS	***	NA	NS	NS	NA	NA	NA	NA
Biochar X N		**	NS	NS	*	NS	NS	***	**	*	*

*** P < 0.001; ** P < 0.01; * P < 0.05; NS, not significant; NA, not applicable;

[1]0 N refers to control;

[2]75 N refers to 75 kg N ha^{-1} added as ammonium nitrate;

[3]150 N refers to 150 kg N ha^{-1} added as ammonium nitrate.

Table 5.4 Effect of N fertilizer (0 N, 75 kg N ha^{-1} and 150 kg N ha^{-1}) and biochar on RCG N dynamics (Dry biomass, N concentration and N export) in the two years after establishment (2011 and 2012).

Biochar	Treatment	Fall-2011			Spring-2012		
		Dry biomass (kg ha^{-1})	N (%)	N export (kg ha^{-1})	Dry biomass (kg ha^{-1})	N (%)	N export (kg ha^{-1})
0 t ha^{-1}	**0 N[1]**	916.9	2.94	26.97	7971.69	1.99	22.34
	75 N[2]	929	2.85	13.47	7956.14	2.24	9.32
	150 N[3]	350	3.36	11.99	7976.15	2.4	7.65
20 t ha^{-1}	**0 N**	682.1	3.24	22.89	7965.02	2.18	11.86
	75 N	554.5	3.49	19.31	7973.91	1.99	12.3
	150 N	962.8	3.35	32.75	7995.02	1.96	20.23
ANOVA (Split plot analysis)							
Biochar		NA	NS	NS	*	NA	NA
N		NA	*	*	NS	NA	NA
Biochar X N		*	NS	NS	NS	**	**

*** P < 0.001; ** P < 0.01; * P < 0.05; NS, not significant; NA, not applicable;
[1]0 N refers to control;
[2]75 N refers to 75 kg N ha^{-1} added as ammonium nitrate;
[3]150 N refers to 150 kg N ha^{-1} added as ammonium nitrate.

Table 5.5 Effect of biochar and PGPR (N-fixing, P-solubilizing and their combination) inoculation on RCG carbon dynamics (C%, C content and N: C ratio) in the two years after establishment (2011 and 2012).

		Fall-2011			Spring-2012		
			C content			C content	
Biochar	**Treatment**	**C(%)**	**(ha^{-1})**	**N/C**	**C(%)**	**(ha^{-1})**	**N/C**
0 t ha^{-1}	**Control**	41.64	0.4069	0.0205	40.88	11.21	0.0182
	BacN[1]	40.53	0.6055	0.0205	40.25	7.44	0.015
	BacP[2]	41.42	0.631	0.0187	40.2	5.33	0.0151
	PN[3]	41.03	0.804	0.024	39.67	8.42	0.0152
20 t ha^{-1}	**Control**	41.02	0.6425	0.0235	41.38	8.46	0.018
	BacN	41.26	0.3641	0.0212	39.82	5.39	0.014
	BacP	41.3	0.5453	0.0182	39.87	8.44	0.0177
	PN	41.33	0.6452	0.0207	39.07	3.625	0.0155
ANOVA(Split plot analysis)							
Biochar		NS	NS	NS	NS	NS	NS
BacN		*	NS	**	NS	NS	NS
Biochar x BacN		NS	NS	NS	NS	NS	NS
Biochar		NS	NS	NS	NS	NS	NS
BacP		*	NS	NS	NS	NS	NS
Biochar x BacP		NS	NS	NS	NS	NS	NS
Biochar		NS	NS	NS	NS	NS	NS
PN		**	*	**	NS	NS	NS
Biochar x PN		NS	NS	NS	NS	NS	NS

*** P < 0.001; ** P < 0.01; * P < 0.05; NS, not significant; NA, not applicable;
[1]BacN refers to N-fixing PGPR;
[2]BacP refers to P-solubilizing PGPR;
[3]PN refers to combination of N-fixing and P-solubilizing PGPR;

Table 5.6 Effect of N fertilizer (0 N, 75 kg N ha^{-1} and 150 kg N ha^{-1}) and biochar on RCG carbon dynamics (C%, C content and N:C ratio) in the two years after establishment (2011 and 2012)

		Fall-2011			Spring-2012		
			C content			C content	
Biochar	**Treatment**	**C (%)**	**(ha^{-1})**	**N/C**	**C (%)**	**(ha^{-1})**	**N/C**
0 t ha^{-1}	**0 N[1]**	41.64	0.4069	0.0205	40.88	11.21	0.0182
	75 N[2]	41.33	0.6055	0.024	39.32	5.519	0.0157
	150 N[3]	41.23	0.631	0.0197	38.73	4.056	0.0185
20 t ha^{-1}	**0 N**	41.02	0.6425	0.0235	41.38	8.46	0.018
	75 N	41.33	0.3641	0.0187	40.34	6.7	0.0171
	150 N	41.77	0.5453	0.024	40.22	11.72	0.0155
ANOVA (Split Plot analysis)							
Biochar		NA	*	NA	NS	NS	NS
N		NA	NS	NA	NS	*	NS
Biochar X N		*	NS	*	NS	NS	NS

*** P < 0.001; ** P < 0.01; * P < 0.05; NS, not significant; NA, not applicable;
[1]0 N refers to control;
[2]75 N refers to 75 kg N ha^{-1} added as ammonium nitrate;
[3]150 N refers to 150 kg N ha^{-1} added as ammonium nitrate.

CHAPTER 6

6 General Discussion and Conclusions

6.1 General Discussion

Perennial rhizomatous grasses (PRGs) are promising bioenergy crop as they have several desirable characteristics in this regard, such as high biomass yield, low N fertilizer demand, a large root system and adapted to stressful environmental conditions. Numerous research articles have focused on their energy crop potential, maximum biomass yield, NUE, fertilizer recovery, growth response to PGPR and biochar effects on growth. There is no doubt that biochar and PGPR can have positive effects on the growth of fast-growing C_3 and C_4 grasses, but their combinations have not been tested in low-input, sustainable energy grass production systems with a range of soil types and climatic conditions in high-latitude temperate zone conditions, such as those prevalent in southern Québec.

In this study, I have shown that application of biochar and specific PGPR (N-fixing or P-solubilizing), or their combined inoculation, to both C_3 (RCG) and C_4 (SG) energy grasses enhanced growth, biomass productivity and NUE at three field sites in two climatic regions (southwestern - Ste-Anne-de-Bellevue-warmer and southeastern – St-Augustin-de-Desmaures-cooler) of Québec. In this study, we report that biochar interacted with specific PGPR (either N-fixing or P-solubilizing), or their combined inoculation, at the warmer field sites (sites 1 and 2, in southwestern Québec, those associated with McGill University) resulting in higher levels of specific growth variables over 3 years of field study. There was no biochar by PGPR interaction at the comparatively cooler site 3 (southeastern Québec, associated with Laval University). A higher dry biomass yield of SG at site 2 (sandy soil) and site 3 (sandy loam soil) occurred on biochar-amended soils (statistically significant increases averaging about 11% over the control). This increased dry biomass could be related to the enhanced moisture holding capacity of biochar amended soils, and/or the enhanced nutrient availability to SG plants growing on sandy and sandy loam soils. An increased number of tillers resulted from PGPR inoculation at site 1 for all 3 years, whereas, combinations of both PGPR , P-solubilizing and N-fixing PGPR, resulted in increased yields at sites 2 and 3 only in the establishment year of the field study. This may, at site 1, have

been due to the soil type (loamy) facilitating PGPR growth so that those plots inoculated with PGPR caused allocation of additional nutrients for SG new shoot development during the growing season. Nitrogen export (kg N ha^{-1}) increased following biochar and PGPR treatments, resulting in a higher level with combined PGPR inoculation and biochar soil amendment at site 1 (180.1 kg N ha^{-1}) and site 3 (205.6 kg N ha^{-1}). Combined PGPR inoculated SG plants may export more N, through better N acquisition (from BNF) or immobile P solubilization (leading to improved root growth and therefore N uptake), than the uninoculated control treatment. However, at site 2, inoculation with P-solubilizing PGPR resulted in the export of more N to aboveground biomass, which may suggest that they enhance the efficiency of BNF through nutrient supplement or by production of plant growth stimulators.

In the second study, I have shown increases in biomass production and growth variables of SG with added biochar that combined with low rate of N fertilizer at different field sites, in keeping with our goal of establishing a low-input sustainable bioenergy crop production system. Significant biochar by N fertilizer interactions occurred for dry biomass at site 2, whereas, at sites 1 and 3 N fertilizer effects were clear. There was an interaction for N export in the second year on the sandy soil, where biochar may have facilitated more moisture retention or binding of ammonium to the surface of biochar particles, in both cases leading to reduced leaching, therefore interacting with available N in soil. Across the three sites, 100 kg N ha^{-1} along with biochar application resulted greater dry biomass production at sites 1 and 3; however, at site 2, 50 kg N ha^{-1} along with biochar resulted in maximum dry biomass production. Fifty kg N ha^{-1} was the optimum amount of N needed for C_4 PRG growth along with biochar at site 2 (sandy soil). This would allow more effective use of nutrients, and N in particular, with smaller losses to leaching. The same pattern was observed for N export for spring-harvested aboveground dry biomass during the second year at site 3 and the third year at sites 1 and 2. Biochar amended soils tended to have the greatest NUE at 50 kg N ha^{-1} across all three sites. Maximum N recovery (apparent) resulted with application of 100 kg N ha^{-1} for fall-harvested material, whereas at spring harvest 50 kg N ha^{-1}, along with biochar application, resulted in similar yields at all three field sites. This suggests that biochar addition to soil systems may be used on marginal lands where nutrients like N, P, etc. are limiting.

Based on the above studies, the evaluation of SG as a bioenergy crop, using biochar and specific PGPR as sustainable agronomic inputs, at three field sites in two regions, indicated that low-input environmentally friendly production systems can be developed. In the third study, I have shown that these same treatments affect RCG - a promising C_3 PRG that is well adapted to higher-latitude temperate zone conditions. In addition to this, two N fertilizer rates (75 and 150 kg N ha^{-1}), along with biochar application, were able to meet the low-input demand. Taller RCG plants resulted with the combined PGPR inoculation treatment, following a response pattern similar to C_4 SG during the growing period; this resulted in higher dry biomass production at the end of growing season. Tiller dynamics did not vary with either biochar or PGPR inoculation treatments. Combined PGPR inoculated RCG plants exported the most N, probably through better N acquisition (from BNF) or P-solubilizing PGPR may facilitate N-fixing PGPR growth by secreting a growth stimulator such as auxin analogues, as was the case with SG in the studies already described. Biochar by PGPR interactions occurred for stand count and N export during the second year, which could be due to increasing PGPR colonization of developing PRG root systems such that the PGPR interact increasingly with biochar in the soil. During my continuous two year field study, biochar by N fertilizer interactions occurred for almost all RCG growth variables as well as N export. The highest dry biomass yield (8 Mg ha^{-1}) resulted from 150 kg N ha^{-1} plus biochar application, which may suggest that biochar in soil could result in greater mineralization of N, making more of it available to plant roots, and if this occurred, this may reduce leaching loses of N. The N concentration (%) of aboveground tissues of RCG was much lower during the spring sampling, especially for plants grown on biochar amended soils, which is very desirable for bioenergy crop production. The greatest RCG NUE occurred at 150 kg N ha^{-1} when biochar was applied (20 t ha^{-1}), and this was greater than the same fertilizer level without biochar. Over the two years of field study, biochar application increased NUE an average of 30% over the control treatment, which could be due to CEC binding of ammonium from N fertilizer, slowing the rate of nitrification and subsequent leaching and/or denitrification. Further study is needed to determine the exact mechanism that reduces N leaching from biochar amended soils, but the findings of my studies showed that both C_3 and C_4 PRGs have greater NUEs when biochar is applied to the soil. The C content (Mg C ha^{-1}) of aboveground biomass of RCG followed the same pattern in that it was

maximal for 150 kg N ha^{-1} plus biochar amendment; at this level the C content was 39% greater than the control treatment. In addition, combined PGPR inoculation with biochar soil application followed a similar pattern of response regarding C content. Perennial grasses tend to add 1.1 Mg of C ha^{-1} yr^{-1} to the upper 100 cm over the long-term, which replace 23% of the soil C lost during prior tillage. Moreover, biochar is a carbon-rich material; as a soil amendment it can sequester significant amounts of C into agricultural soils; in addition the extensive root systems of PRGs also facilitate sequestration of C into soils.

In conclusion, the results of this study indicate the potential of PRGs as promising bioenergy feedstock crops in southern Québec, and suggest that, with the addition of biochar and PGPR a low-input system can be developed that will possess a very good energy balance, due to reduced need for N fertilizer application; this should also result in reduced GHG emissions. Research related to biochar-microbe interactions under stressful environmental conditions, or biochar and signal compound effects on plant growth and yield, could have potential to further develop low input production systems for these crops.

6.2 Acceptance and Rejection of Hypotheses

<u>Hypothesis 1</u> Biochar, N-fixing PGPR and P-solubilizing PGPR inoculation has positive effect on C_4 perennial warm-season grass growth and productivity under temperate field condition.

Result: Both biochar application and inoculation with PGPR (N-fixing PGPR and P-solubilizing) resulted in positive effects on SG (C_4 grass) growth variables (i.e. height, stand count, dry biomass), aboveground N concentration and aboveground N export (kg N ha^{-1}) under field conditions. Overall I observed 9-30% increases in dry biomass and 10-65% increases in N export (kg N ha^{-1}) due to PGPR inoculation and biochar application, when compared with the appropriate controls.

Thus we accept hypothesis 1 when the test crop was SG (warm season C_4 grass).

<u>Hypothesis 2</u> The positive effect of biochar, N-fixing and P-solubilizing PGPR were reasonably constant across soil/climate condition within southern Québec.

Result: Across nine site-years (3 sites in each of 3 years, from 2010 to 2012) increases due to treatments (biochar, N-fixing PGPR and P-solubilizing PGPR) were observed for SG height, stand count, tiller biomass, (Tables 3.4, 3.5 and 3.6; Chapter 3) aboveground total dry biomass and N export (Tables 3.7, 3.8 and 3.9; Chapter 3).

Thus we accept hypothesis 2 when the test crop was SG (C_4 perennial grass).

<u>Hypothesis 3</u> Biochar and N fertilizer increased C_4 grass growth, NUE and apparent N recovery under three different soil types and two different climatic conditions in Québec.

Result: A greater number of tillers, taller plants and higher dry biomass production and increased N export were observed when biochar (20 t ha^{-1}) was incorporated into soils that received N fertilizer (Table 4.2; Chapter 4), across nine site-years (3 sites in each of 3 years, from 2010 to 2012). Although the responses of NUE and ANR varied among sites and years there was a consistent yield increase with N fertilization as well as with biochar application to soil.

Thus we accept hypothesis 3 when the test crop was SG (C_4 grass).

<u>Hypothesis 4</u> Biochar, N-fixing PGPR and P-solubilizing PGPR inoculation improve C_3 cool-season perennial grass growth at field condition.

Result: The field data for RCG (a C_3 grass) growth variables indicated that biochar along with PGPR treatment promoted RCG height, dry biomass yield and N export (Tables 5.1 and 5.2, Chapter 5) under high-latitude temperate zone field conditions. The greatest N export (kg N ha^{-1}) was observed with combined PGPR inoculation (both N-fixing and P-solubilizing strains), which was about 90% greater than the control.

Thus we accept hypothesis 4.

<u>Hypothesis 5</u> Biochar along with N fertilizer promote C_3 grass biomass yield, N export, NUE and apparent N recovery in southwestern Québec field condition.

Result: RCG receiving three rates of N fertilizer (0, 75 and 150 kg N ha^{-1}) along with biochar (20 t ha^{-1}) soil application showed positive effects on the growth and N related variables, dry biomass yield, N export, NUE and ANR (Tables 5.3 and 5.4) under high-latitude temperate zone field conditions.

Thus we accept hypothesis 5.

6.3 Contributions to knowledge

1. We have determined the impacts of biochar, specific PGPR (N-fixing or P-solubilizing) and their interactions on PRG growth and biomass production under the high-latitude temperate zone conditions of southern Québec.
2. This study provides novel information regarding growth and N dynamics responses of SG (C_4 grass) and RCG (C_3 grass) to biochar application along with PGPR inoculation at three field sites (three soil types and two climatic conditions) under field conditions in southern Québec.
3. We determined the interaction of biochar and N fertilizer application rate on growth performance, NUE and apparent N recovery of SG and RCG at three sites over each of three years (a total of nine site-years) under high-latitude temperate zone conditions.
4. We have provided agronomic information related to tools for the development of low-input systems (e.g. PGPR and biochar) for maximizing bioenergy crop yield and minimizing environmental risk, as well as fertilizer cost.

6.4 Suggestions for future research

1. Determine if biochar –PGPR effects differ among varieties of SG and RCG under field conditions as it is possible that the plant response to microbial inoculation is variety specific.
2. Determine and investigate the effects of the biochar and PGPR inoculations on the development, cellular and biochemical properties of C_3 and C_4 PRGs under stressful conditions (water deficit stress or salt stress, etc.).
3. Determine the optimum levels of biochar required to maximize cost-benefit ratios for production of biofuel feedstock crops.

4. Assess the survival of nutrient mobilizing PGPR in biochar amended soils under controlled environment and field conditions.
5. Investigate the effects of biochar and PGPR on gene expression of C_3 and C_4 PRGs, specifically with regard to water retention and nutrient availability, using microarray technology.
6. Determine if RCG associated with plant growth promoting rhizobia and a RCG-microbe production system could be developed to enhance efficient bioenergy feedstock production through low N input systems.
7. Longer term studies are required to address questions such as how long the PGPR inoculation effects or biochar effects, or their combined effect, will last, and whether re-application of biochar or re-inoculation or spraying of PGPR inoculums is more effective in enhancing the growth of SG and RCG, under a high-latitude temperate zone climatic conditions.

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