The Effect of Fertilizer Application on Greenhouse Gas Emissions from Willow Short Rotation Coppice Systems in Southern Ontario, Canada

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available.

Abstract

Willow (*Salix* spp.) short-rotation coppice (SRC) systems on marginal lands are effective at providing carbon (C) neutral bioenergy. However, nitrogen (N) fertilizer application, used to enhance aboveground biomass productivity, can result in greater soil-derived carbon dioxide (CO_2) and nitrous oxide (N_2O) emissions and negate the C neutrality of the willow biofuels. This study presents the effect of fertilizer application on GHG emissions, soil characteristics, winter freeze-thaw emissions and total annual emissions in willow SRC systems.

Mean CO_2-C emissions were 95.1 and 111.0 mg CO_2-C m^{-2} h^{-1} in 2014, and 69.4 and 92.7 mg CO_2-C m^{-2} h^{-1} in 2015, in fertilized and unfertilized treatments, respectively. Soil CO_2-C emissions exhibited seasonality, with the greatest emissions occurring in the summer and decreasing in autumn. Elevated summer emissions were due to preferable soil temperature and moisture regimes stimulating microbial respiration, and elevated air temperatures and sunlight availability increasing root respiration. Soils under willow clone SX67 (*Salix miyabeana*) consistently emitted more CO_2-C emissions than clone SV1 (*S. dasyclados*), as SX67 was more efficient at SOC accrual, which is an energy substrate for microbes. Total annual CO_2 emissions were 19.73 Mg CO_2 ha^{-1} yr^{-1} from fertilized treatments, and 26.30 Mg CO_2 ha^{-1} yr^{-1} from unfertilized treatments. Of this, only 7.2 and 13.4% were derived annually from winter emissions, respectively, and freeze-thaw cycles did not create a pulse of CO_2 emissions.

Mean N_2O-N emissions were 26.5 and 17.2 μg N_2O-N m^{-2} h^{-1} in 2014, and 22.9 and 18.2 μg N_2O-N m^{-2} h^{-1} in 2015, from fertilized and unfertilized treatments, respectively. In both years, fertilizer application increased NH_4^+ and NO_3^- availability in

the soil, resulting in a pulse of N_2O-N emissions. Thus, elevated N_2O-N emissions were due to inorganic N availability, which stimulated microbial nitrification following fertilizer application. The fertilizer amendment did not result in a substantial increase in willow biomass yields, which was 10.24 ± 1.86 odt ha^{-1} in fertilized treatments and 8.33 ± 0.97 odt ha^{-1} in unfertilized treatments; thus, willow SRC systems exhibited very low N use efficiency. There was no pulse of N_2O-N following spring thaw events.

The willow SRC systems had total annual emissions (expressed as CO_2 equivalents) of 20.43 Mg CO_2-eq ha^{-1} yr^{-1} from fertilized treatments, and 26.90 Mg CO_2-eq ha^{-1} yr^{-1} from unfertilized treatments. N_2O-N emissions only accounted for 2.2 to 3.4% of total emissions, whereas CO_2-C emissions accounted for 97.8 and 96.6%. When C sequestration in above and belowground biomass, and litter fall contribution to SOC were quantified, willow SRC systems acted as a C sink in fertilized treatments, with a C sequestration potential of 10.79 Mg CO_2-eq ha^{-1} yr^{-1}. Unfertilized treatments acted as a slight C source, with net emissions of 1.19 Mg CO_2-eq ha^{-1} yr^{-1}, but may become a C sink as willow SRC systems mature and accrue more C.

This thesis proposes that fertilizer application be limited in willow SRC systems, as willows exhibited very low N uptake, to eliminate the annual pulse of N_2O-N following fertilizer application, but to maintain willow yields at ~10 odt ha^{-1} and ensure that willow SRC systems are a net C sink. Willow SRC systems are potential C sinks, and can ameliorate atmospheric GHG emissions. This research contributes to a comprehensive understanding of N fertilizer dynamics in willow SRC systems.

Dedication

This work is dedicated to my grandparents:

Kevin Gerard MacNeil

and

Caroline Ann MacNeil

My Bamp spent many days sitting with me in the willow, while I collected greenhouse gas and soil samples. He has supported me throughout the entirety of my experiment, and has actively inquired about the results and meanings of my study.

Through his sharing of knowledge, constant support and intrigue, he truly was an invaluable field assistant. One day we will figure out what those little insects on the willow shoots were up to.

My grandparents have always supported me, and all of their grandchildren, no matter what our interests are. They may not have echoed my love of soil and insects, but they always encouraged me to succeed. My Mam has always highlighted the importance of family, and in doing so has created the strongest support system I could have asked for.

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I am thankful for my research groups at the University of Waterloo and at the University of Guelph for providing camaraderie and friendship during my Master's. My sincerest thank you is extended to the Biogeochemistry lab at the University of Waterloo, especially during my "ammonium conundrum". Their constant brainstorming support and friendship has been invaluable. I am also very grateful to all of my friends that I have

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1. General Introduction

Global consumption of fossil fuels and associated consequences, including climate change, have heightened demand for more sustainable alternative energy sources. Biomass is an energy resource that has the potential to mitigate global greenhouse gas (GHG) emissions and is projected to account for at least 50% of global renewable energy resources by 2050 (Hangs et al., 2014; Smith et al., 2014). This is because carbon (C) that is taken up through photosynthesis and stored in biomass tissue offsets C released from fuel consumption to be a C neutral source of energy. Woody short rotation coppice (SRC) plantations, used for biofuel production are fast-growing, densely planted perennial species that are particularly effective at providing high yields of biomass, and can greatly contribute to meeting these energy requirements (Dickmann, 2006; Smith et al., 2014). Willow (*Salix* spp.) SRC systems established on marginal lands are particularly effective at providing environmental services including long-term C sequestration (Amichev et al., 2014; Hangs et al., 2014). However, concerns have emerged surrounding ecosystem disservices in willow SRC systems (Crutzen et al., 2007). This is because nitrogen (N) fertilizers, which are used to enhance aboveground biomass productivity, affect soil C and N transformations and may lead to greater carbon dioxide (CO_2) and nitrous oxide (N_2O) emissions. These are powerful greenhouse gases that contribute to global warming; in particular, N_2O has a warming potential 296-298 times that of CO_2 and can also destroy stratospheric ozone (Senbayram et al., 2009; Yu et al., 2008). Without considering N_2O emissions, there is an overestimation of net GHG reductions, which negates C neutrality of willow biofuels. To date, little knowledge exists on the spatial and temporal variations of GHG emissions from woody biomass plantations, especially those

established on marginal lands (Amichev et al., 2014). Furthermore, winter and freeze-thaw cycles often result in N_2O-N and CO_2-C pulses, accounting for a significant proportion of annual emissions, but vary highly in the literature and are often absent from willow SRC GHG budgets.

The objectives of this study are to 1) determine the effect that fertilizer application has soil C and N nutrient cycling and GHG production under willow SRC (*Salix miyabeana* [SX67] and *S. dasyclados* [SV1]) systems, 2) quantify GHG emissions during winter months and freeze thaw cycles, and 3) estimate total annual emissions from a willow (*S. miyabeana*) SRC system. This research will contribute to the thorough understanding of nutrient cycling in willow SRC systems.

This thesis is divided into 6 chapters, which will address the research objectives at the University of Guelph Turfgrass Institute in the Mixed Wood Plains ecozone in Guelph, Ontario, Canada. Chapter 2 is a literature review of global bioenergy, focusing willow SRC systems in Canada, highlighting soil-derived greenhouse gas emission losses from willow SRC systems after fertilizer application. Chapter 3 outlines the study site and historical site management at the willow biomass plantations within the University of Guelph Turfgrass Institute. Chapter 4 highlights the effect of fertilizer application and seasonality on CO_2-C and N_2O-N emissions, and soil NH_4^+ and NO_3^- concentrations from two willow clones (*Salix miyabeana* [SX67] and *S. dasyclados* [SV1]). This study was published in Agroforestry Systems in 2016 (Lutes et al., 2016). Chapter 5 describes total annual emissions, including N_2O-N and CO_2-C emissions in winter and following spring thaws, while focusing on intermittencies between two field seasons of data. Chapter 6 summarizes the key findings and outlines management recommendations of willow SRC

systems for bioenergy production in Southern Ontario, Canada.

2. Literature Review

2.1 Renewable Energy

Fossil fuels stored in geologic repositories, like oil, coal and gas provide upwards of 85% of the world's energy supply (Srirangan et al., 2012). However, resource scarcity and uncertainty, energy insecurity and environmental deterioration associated with fossil fuels all threaten their continued widespread use as the global primary energy resource (Manazano-Agugliaro et al., 2012; Panwar et al., 2011; Ellebban et al., 2014). Of particular concern, fossil fuel burning is a main global factor for rising atmospheric greenhouse gas (GHG) concentrations, especially with regard to CO_2 emissions, which contribute to global warming. Atmospheric CO_2 equivalent (CO_2-eq) concentrations have reached unprecedented levels in recent years (IPCC, 2014). Reduction of GHG emissions from fuel sources is necessary to ensure that atmospheric CO_2 does not exceed proposed mitigation regimes, which aim for concentrations of 450 ppm CO_2 by 2100 to limit global warming to 2°C relative to preindustrial levels (Smith et al., 2014; IPCC, 2014). To achieve this, mitigation scenarios suggest that there needs to be a 40-70% reduction in anthropogenic GHG emissions by 2050, and a 100% reduction by 2100 (IPCC, 2014). Thus, current global energy consumption patterns are not sustainable (Naik et al., 2010). This has facilitated the growing interest and need to develop alternative, renewable resources for energy production, especially as world energy demands increase due to global population growth and economic development (Manzano-Agugliaro et al., 2012; Koh and Ghazoul, 2008; Baños et al., 2011).

Renewable resources are defined as naturally derived materials, which are replenished by the earth's biogeochemical and natural processes over a relatively short

period of time (Ellebban et al., 2014). Sustainable long-term renewable resources are also inexpensive to produce, and are not associated with negative externalities when used (Manzano-Agugliaro et al., 2012). Sustainable renewable energy resources must also be associated with zero or minimal net GHG emissions. These energy resources include solar, wind and geothermal energy, hydropower and liquefied biofuels derived from biomass (Baños et al., 2011). In Canada, 18.9% of total primary energy supply is currently derived from renewable energy resources (Natural Resources Canada, 2016).

Renewable energy resources have the potential to reduce fossil fuel consumption, and can ameliorate C emissions to the atmosphere (Baños et al., 2011). Furthermore, they presently have the potential to satisfy current energy demands; in 2007, the hypothetical production of renewable energy exceeded global demands by at least 2.6 times (Bruckner et al., 2014), which may further increase with technological advancements. However, merely substituting energy sources will likely not be enough to reduce atmospheric GHG emissions and mitigate climate change; long term C capture and storage will also be necessary.

2.2 Renewable Energy: Biomass

Biomass is a source of renewable energy derived from biological organic material, which uses photosynthesis to store the sun's energy in vegetative material (Ellabban et al., 2014). Energy derived from biomass, or bioenergy, refers to the conversion of this biomass into useable forms of energy (Ellabban et al., 2014). Biofuel sources include first generation herbaceous energy crops, second generation biofuels, like purpose grown and dedicated woody crops, agricultural and forest residues, and municipal solid wastes, and tertiary biofuel sources, like algae. Sources are converted

into useable forms including liquid (bioethanol or biodiesel), gaseous (biogas) and solid (biomass) biofuels.

Derivatives of biofuel can be stored and transported and can be available instantly; this sets them apart from other renewable energies including wind and solar energy, which are dependent on natural phenomenon and are not consistently available (Ellabban et al., 2014). Biofuels can also be blended with other fuel sources and used with current infrastructure and engines. Furthermore, biomass sources are more globally widespread than fragmented and scarce fossil fuel sources, thus, their adoption can increase energy security. Biomass can also be easily exploited without high levels of technology, so that they are widely accessible and provide employment opportunities in rural areas (Naik et al., 2010).

Solid, liquid and gaseous products of biofuels supply approximately 50 EJ (exajoules) of energy globally, which represents 10 – 14% of the world's primary energy consumption (Keoleian and Volk, 2005; Srirangan et al., 2012; Liu et al., 2014). This accounts for approximately 75 – 80% of energy derived from renewable energy resources, constituting the largest proportion of renewable energy to global energy demands (Smith et al., 2014; Srirangan et al., 2012; Liu et al., 2014). However, energy derived from renewable resources still accounts for less than 15% of the worldwide energy demand (Hangs et al., 2014a; Smith et al., 2014), and this energy is mainly expended during wood burning for food preparation and heat production in developing nations (Manzano-Agugliaro et al., 2011).

Biomass plantations will be relied upon in the future as a substantial source of renewable energy for fossil fuel substitution, and are projected to comprise at least 10 –

50% of global primary energy supplies by 2050 (Keoleian and Volk, 2005; Hangs et al., 2014a; Smith et al., 2014), which will amount to approximately 1500 EJ of energy from biomass (Srirangan et al., 2012). Consumption of biofuels is expected to triple from 2010 to 2035, from 1.3 million barrels of oil equivalent per day to 4.5 million barrels of oil equivalent per day, respectively (Ellabban et al., 2014).

2.3 Biofuels as Carbon Neutral Fuel Sources

Theoretically, C that is captured and stored through photosynthesis and growth of biofuels offsets all of the CO_2 emissions that are released through biofuel burning, making biofuels C neutral (Koh and Ghazoul, 2008; Naik et al., 2010). Replacing traditional fossil fuels with biofuels can reduce GHG emissions by approximately 31% for bioethanol substitutes, 54% for biodiesel and 71% for lignocellulosic ethanol (Koh and Ghazoul, 2008).

As biofuels require land area for production, biomass plantations are at the epicenter of land-use debates. Firstly, land for biofuel production is in direct competition with agricultural land needed for food production (Amichev et al., 2014; Gauder et al., 2011; Koh and Ghazoul, 2008). Reducing local agricultural production results in larger C footprints associated with food production because of the need for more imported goods (Koh and Ghazoul, 2008). This can also decrease food security for the surrounding area, and contribute to rising food costs (Koh and Ghazoul, 2008). This competition is often referred to as “food vs. fuel”. Secondly, urban sprawl and land adjacent to metropolitan areas also place agricultural land under pressure for urban development, which increases land competition for biofuel production (Smith et al., 2014). Thirdly, biomass sources must be located close to processing plants, to reduce transportation costs. Finally, when

forested land is converted to monoculture cropland, there can be an increase in the net soil CO_2 emissions associated with the land (Popp et al., 2012). Without considering GHG emissions that arise from land-use and land management, there can be overestimation of net GHG reductions associated with biofuels.

Canadian regulations stipulated in 2010-2011 specified that gasoline fuel must be comprised of 5% renewable content, while diesel fuel and heating oil must be comprised of at least 2% renewable content (Liu et al., 2014). In 2013, Canada produced 2% of global biofuels, ranking 5th in the world (Natural Resources Canada, 2016). However, to date, Canadian biofuels have largely been derived from conventional biofuels, which are primarily derivatives of ethanol and biodiesel from first generation grains and vegetable oils (Liu et al., 2014).

2.4 First Generation Biofuels

First generation biofuels are edible, herbaceous crops, including maize, sugar cane or wheat, which are used to produce bioethanol, biodiesel and biogas (Naik et al., 2010, Srirangan et al., 2012). The most common first generation biofuel crop in North America is maize (*Zea mays* L.), which is converted to bioethanol. It is preferred due to its high starch content as a C4 plant, ability to be genetically modified, and wide scale production and availability (Srirangan et al., 2012). However, maize has a low root to shoot ratio, such that there is proportionally more aboveground biomass than belowground, which is taken offsite during harvest. Thus, harvest results in a nutrient deficit that is compensated for with high levels of N-based fertilizer amendments, which can negatively affect C and N cycling in soil (Gauder et al., 2011). First generation biofuel monocultures are criticized for reducing biodiversity (Weih et al., 2011),

contributing to the aforementioned "food vs. fuel" debate and rising food prices (Naik et al., 2010; Sims et al., 2010; Srirangan et al., 2012), increasing agricultural soil GHG emissions (Crutzen et al., 2007; Gauder et al., 2011), and reducing soil fertility. Furthermore, fertilizer application can result in N_2O emissions and nitrate (NO_3^-) leachates, negating the GHG reductions associated with fossil fuel substitution, and accelerating climate change (Crutzen et al., 2007; Farquharson and Baldock, 2008). Thus, although first-generation biofuels are currently the most common source of renewable energy in Canada, they are not sustainable.

2.5 Second Generation Biofuels

Second generation biofuels are derived from non-edible materials, including lignocellulosic biomass, crop and forest residues, and waste materials (Naik et al., 2010; Srirangan et al., 2012). Although second generation biofuels are globally accessible, they are not as widespread as first generation biofuels because production is not cost-effective due to high concentrations of recalcitrant material (Keoleian and Volk, 2005). In particular, dedicated short-rotation coppice woody energy crops can meet the demand for renewable energy in the future (Keoleian and Volk, 2005; Carriquiry et al., 2011; Srirangan et al., 2012; Budsberg et al., 2012), while simultaneously sequestering C to ameliorate atmospheric GHG concentrations (Smith et al., 2014).

2.6 Willow Short Rotation Coppice Systems

Short-rotation coppice (SRC) systems are intensively managed sustainable systems comprised of fast-growing, densely planted woody perennials. Hardwood species of the Salicaceae family (including willow [*Salix* spp.] and poplar [*Populus* spp.]) are common SRC selections due to their ability to rapidly produce biomass (Dickmann,

2006; Carriquiry et al., 2011; Srirangan et al., 2012). Willow is a particularly effective perennial cultivar for SRC systems in Ontario because it is a native species with a wide Canadian geographic range, it has a large amount of genetic variability allowing for the creation of hybrid clones, and it easily propagates after coppice and from dormant cultivars to form dense shrubbery, even on marginal lands (Keoleian and Volk, 2005; Amichev et al., 2014). When established on marginal sites, willow SRC systems can increase C storage associated with abandoned agricultural soil and act as a C sink (Keoleian and Volk, 2005).

Although willows typically require little nutrient inputs to maintain growth, fertilizer application is common in willow SRC systems (Amichev et al., 2014). Willow SRC systems differ from typical silvicultural systems; the willows are grown intensively and are therefore considered analogous to agricultural crops (Dickmann, 2006). Thus, they are typically exposed to agricultural management practices, including fertilization, weed control and irrigation, and are considered agroforestry systems (Heller et al., 2003, Keoleian and Volk, 2005, Amichev et al., 2014).

Weed control is typically only required in the first year of willow establishment, after which the canopy shade prevents weed growth (Dickmann, 2006; Heller et al., 2003). After the first field season of growth, perennial stems in willow SRC systems are coppiced (cut to <5 cm tall), just before the dormant season in winter (Heller et al., 2003). This timing allows for litter fall to contribute to soil C sequestration and soil nutrient cycling, and for plant nutrients to be transferred to the root systems for overwintering (Keoleian and Volk, 2005), providing a nutrient pool for new growth to exploit in the

following spring. Fertilizer amendments typically occur at the beginning of the growing season following winter coppice, in spring (Heller et al., 2003).

In spring, the coppiced plant regrows rapidly due to the pre-established root system, which readily provides nutrients to the plants (Dickmann, 2006), allowing the willows to double in density per unit area (Konecsni, 2010). Rotations are 3 years long (excluding rotation 1 of willow establishment and coppice, which totals 4 years), and harvested in the winter of year 3, when the willows reach the maximum mean annual incremental growth, after which rapid growth plateaus (Keoleian and Volk, 2005; Dickmann, 2006). Willows are harvested following this model (3-4 year rotation) for 6-7 rotations, totaling approximately 23 years, at which point the preexisting willow stools are removed, and new willow plants can be re-planted (Heller et al., 2003).

Willow SRC systems are associated with more ecosystem services relative to first generation energy crops and offer a greater amount of ecological, social and economic benefits (Hangs et al., 2014b; Naik et al., 2010) (Table 2.1). In particular, willow SRC systems offer many soil benefits. Relative to annual species, perennial species, like willow, improve soil microorganism biodiversity by providing optimal temperature and moisture conditions (Keoleian and Volk, 2005; Hangs et al., 2014a). Coppiced willow rapidly develop a full canopy, which creates a microclimate and shading buffer to reduce wind and water soil erosion and high temperature fluctuations (Clinch et al., 2009; Gauder et al., 2011). Perennial biofuels also develop large coarse root networks early after establishment, which remain for the duration of the plants lifecycle (Amichev et al., 2014; Pacaldo et al., 2011). This rooting network extends deeper into the soil than the superficial rooting systems of annual crops, permitting more cation and nutrient uptake

(Amichev et al., 2014) and reducing NO_3^- leaching (Heller et al., 2003). The root masses also contribute to 10-20 years of C storage and to SOC enhancement, especially on marginal lands (Keoleian and Volk, 2005; Pacaldo et al., 2011), while annual leaf litter inputs provide internal nutrient cycling (Hangs et al., 2014a).

Currently, there are ~147 million ha of marginal land in North America, which can be rehabilitated and potentially converted to willow SRC plantations (Amichev et al., 2014). In Ontario, production on marginal lands alleviates pressure on agricultural lands, and diminishes land competition between food production and biofuels, thereby contributing to food and fuel security (Figure 2.1). Willow as a biofuel has been associated with a negative global warming potential (GWP), and can effectively be C neutral (Budsberg et al., 2012). When used as a fuel, willow has been suggested to have a warming potential 120 times less than that of gasoline (Budsberg et al., 2012). Furthermore, Keoleian and Volk (2005) suggested that substituting willow bioenergy for traditional electricity can result in GHG reductions of 78-90%.

Table 2. 1 Benefits of second generation willow SRC systems relative to first generation biofuels

	Second Generation Biofuels: Willow SRC Systems	
	Potential Benefits	Limitations
Environmental	• Can be grown on marginal lands and on a variety of soils while maintaining productivity and does not contribute to the food vs. fuel debate (Carriquiry et al., 2011; Sriranagan et al., 2012) • High tolerance to many different environments including cold wet and temperate soils (Sriranagan et al., 2012) • Fast growth rate (Sriranagan et al., 2012) • High energy content and large, positive net energy ratios (amount if biomass energy harvested: amount of energy consumed in harvest) (Heller et al., 2003; Keoleian and Volk, 2005; Sriranagan et al., 2012) • Ease of cultivation compared to grain crops (Sriranagan et al., 2012) • High annual biomass yield • Reduces soil erosion (Carriquiry et al., 2011) • Improves soil fertility (Carriquiry et al., 2011) • High biodiversity and wildlife habitat provision (Carriquiry et al., 2011)	• Fertilizer application can result in overestimation of GHG reductions and increase in N_2O-N emissions (Crutzen et al., 2007) • Competes with agricultural land use when grown on arable lands • Monoculture plantations • Are intensively managed, requiring fertilizer, weed management and irrigation depending on site specific locale (Keoleian and Volk, 2005)
Economic	• Large pool of genetic material that can be exploited for hybrids (Amichev et al., 2014) • Harvested on regular intervals to ensure a consistent return on investments, with less fluctuation in yield than annual crops (Kahle et al., 2007) • Diversify income for farmers and provide more employment opportunities for rural farmers (Naik et al., 2010)	• Biorefineries are not cost-competitive with petroleum based refineries (Sriranagan et al., 2012) • Lack of large scale biorefineries (Carriquiry et al., 2011) • Transportation of biofuels is too high (Sriranagan et al., 2012) • Potential costs associated with storage, feedstock acquisitions and opportunity costs (Carriquiry et al., 2011) • High start- up costs with high density biomass plantations (Dickmann, 2006)
Technical	• Can be blended with conventional fuels and used in present infrastructure with minimal capital investment	• Recalcitrant and lignin based material makes it very difficult to break down and convert to liquid fuels • Transportation of fuels to distant refineries increases GHG emissions; refineries must be located close to the feedstock production site

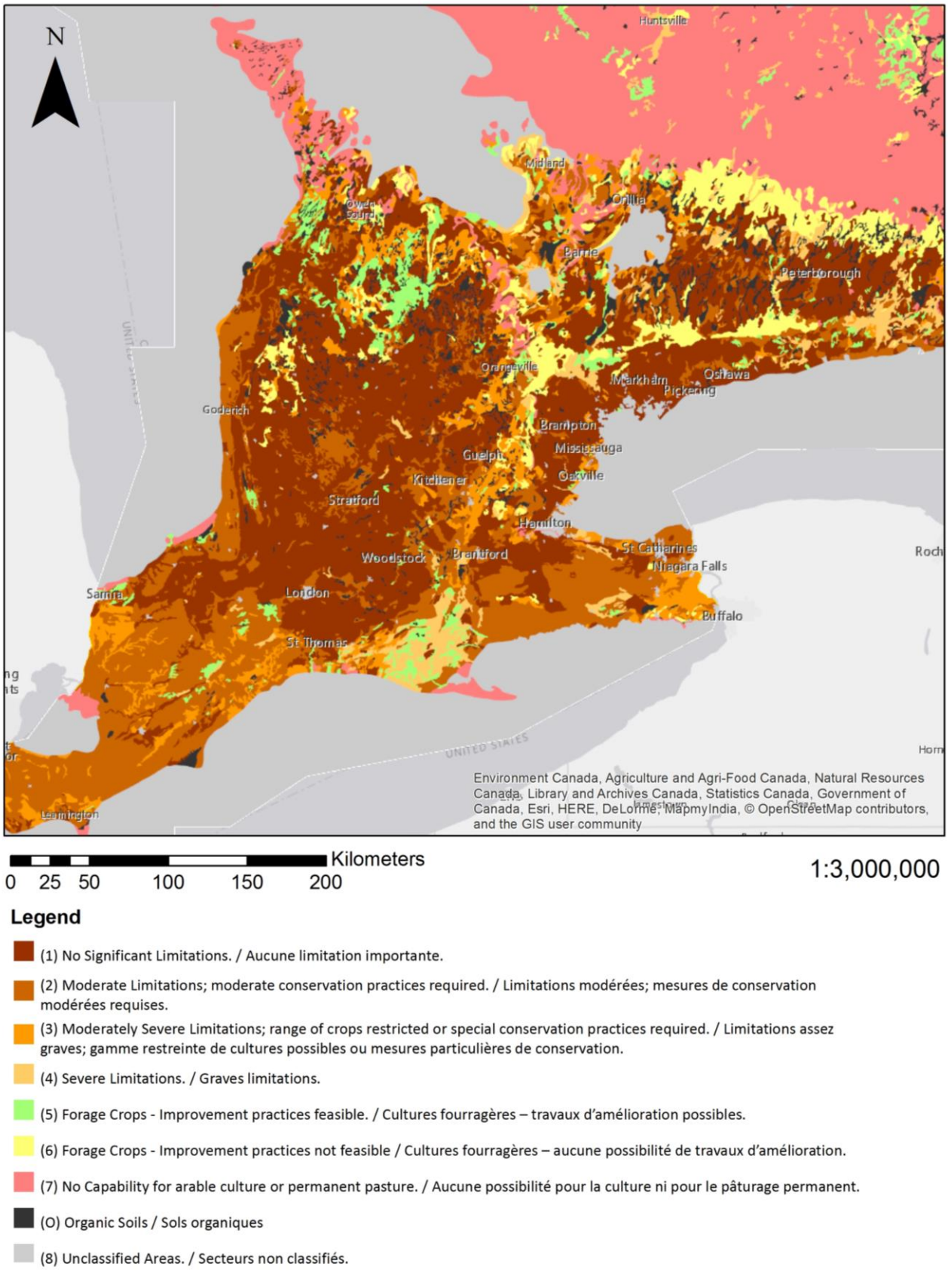

Figure 2.1 Canada Land Inventory for southern Ontario, expressing the suitability for growing agricultural crops. Willow SRC systems can be established on marginal lands with substantial limitations. The study site is located at Guelph, Ontario, on lands that are Class 3 – 4 (moderate to severe limitations).

2.7 Nitrogen Fertilizer

Anthropogenic N production through combustion of fossil fuels, production of N-based fertilizers and the cultivation of legumes have increased the amount of excess N in the global N cycle, such that the critical load of N has been exceeded (Erisman et al., 2015). Of this, anthropogenic N fixation, derived from synthetic fertilizer production, has contributed the most to this imbalance (Yu et al., 2008). Nitrogen fertilizer in agricultural fields improves yields and feeds the global population, however, excess N can result in environmental disservices including atmospheric N deposition, reduced biodiversity via acidification of water bodies (Gundersen et al., 2006), NO_3^- cation leaching causing eutrophication of surface water (Bouwman et al., 2002; Gundersen et al., 2006; Yu et al., 2008), terrestrial biodiversity loss favouring acid resistant and nitrophilic species (Erisman et al., 2015), and altered soil C and N transformations resulting in higher CO_2 and N_2O emissions (Crutzen et al., 2007; Farquharson and Baldock, 2008; Hellebrand et al., 2008; Hangs et al., 2014b). Although conventional fuel combustion releases NO_x gases, models of N pollution that substituted biofuels for fossil fuel inputs predict greater global N pollution (Winiwarter et al., 2013). This is because biomass requires high levels of fertilizer input, which results in excess reactive N, causing N atom cascades through the biosphere and atmosphere (Erisman et al., 2015). Global use of fertilizers, and alteration of the global N cycle have already exceeded critical loads, and studies suggest that fertilizer use needs to be reduced by 50% (De Vries et al., 2013; Steffan et al., 2015).

The effect of fertilizer application on willow SRC biomass yields is contested. Nitrogen is limiting in temperate ecosystems, thus, inorganic N fertilizer can be applied to increase aboveground biomass of willow plantations (Dickmann, 2006). Nutrient amendments support rapidly growing perennial woody structures, as harvest of aboveground biomass removes C and

N from the nutrient cycle (Adegbidi et al., 2001; Amichev et al., 2014; Hangs et al., 2014b). This creates a nutrient imbalance that can be amended with inorganic N fertilizers to maintain productivity, which can also increase soil GHG emissions. Conversely, it has been suggested that fertilizer application may have negligible impacts on willow growth, and may not result in significantly greater biomass yields (Smith et al., 2014; Hangs et al., 2014b).

2.8 Carbon Dioxide Emissions

Heterotrophic and autotrophic respiration from agricultural soils contribute to global CO_2 emissions, rivaling quantities produced by fossil fuel combustion (Smith et al., 2003). Soil CO_2 efflux is positively correlated with soil moisture as it creates optimal conditions for microbes (Smith et al., 2003). However, saturated soils can limit respiration due to a reduction of air-filled pores, and therefore gaseous diffusion from the soil (Smith et al., 2003). Alternatively, absence of water in the soil profile can also limit respiration through the creation suboptimal conditions for microbe and plant growth, which may be even more limiting to soil respiration than saturated soil conditions (Smith et al., 2003).

Generally, soil respiration is positively correlated with soil temperature. Ambient temperatures have a daily sinusoidal pattern, resulting in diurnal waves of respiration, with the highest respiration values lagging behind the highest temperatures (Smith et al., 2003). CO_2 emissions also follow seasonal patterns with the highest emissions occurring in the spring and summer months, affected by annual soil temperature fluctuations (Gauder et al., 2011). Soil CO_2 emissions can accelerate climatic change through positive feedback loops (Smith et al., 2003); elevated temperatures increase respiration from microorganisms and vegetation, which further contribute to the GHG effect and elevate temperature in a cyclical way.

Some studies have shown that soil moisture availability is the most important factor for soil respiration (Garten et al., 2009), while others highlight temperature as the primary control (Gauder et al., 2011). However, soil temperature and moisture interact, and are generally negatively correlated; increased temperature in summer months may not result in increased respiration, when coupled with low precipitation (Garten et al., 2009).

2.9 Effect of Fertilizer Application on Carbon Dioxide Emissions in Willow SRC Systems

Fertilizer application may result in elevated CO_2 emissions due to increased autotrophic and heterotrophic respiration from root growth and microbial activity in N limited systems (Inselsbacher et al., 2011; Gauder et al., 2011). However, it is difficult to separate the contributions of roots and microbes to the total CO_2-C efflux from the soil. Although fertilizer application increases aboveground net primary productivity (NPP), C allocation to fine roots and to surrounding mycorrhizal microbes can actually decrease with greater available N; the excess N can suppress microbial respiration from communities adapted to N-limited temperate soils (Ramirez et al., 2010).

Soil respiration rates are expected to be strongly positively correlated with soil moisture and temperature values (Raich and Schlesinger, 1992; Smith et al., 2003), which may have more control over CO_2 emissions than fertilizer application. Gauder et al. (2011) found that CO_2 emissions were positively correlated to soil temperature rather than added N, which caused strong seasonality in emissions. Soil moisture was also positively correlated with CO_2 emissions (Gauder et al., 2011). Drewer et al. (2011) found strong seasonal soil temperature controls over CO_2 emissions in soils under willow, with elevated CO_2 emissions in the summer and low emissions in the winter.

Willows are able to grow dense root networks, which contribute to the accumulation of SOC and can stimulate microbial respiration. Willow fine roots are labile material that is readily decomposed after senescence and become a part of the SOC pool (Amichev et al., 2014), which can act as an energy substrate for decomposing microbes. Gauder et al. (2011) found that perennial willow emitted higher soil CO_2 emissions than conventional, annual biofuel plantations, due to the higher concentration of SOC. They also noted that the constant canopy cover reduced temperature fluctuations, and resulted in more stable soil moisture conditions than soils under annual crops, which further promoted CO_2 emissions (Gauder et al., 2011). Soil respiration may peak following litter fall from willow SRC systems in autumn, with decomposition of labile C.

2.10 Nitrous Oxide Emissions

In recent years, life cycle assessments of biofuels have recognized the need to include changes in soil C stocks and N_2O emissions in GHG budgets, instead of solely basing C neutrality on fossil fuel inputs (Reijnders and Huijbregts, 2011). This is because N_2O molecules have a warming potential 296-298 times greater than CO_2 and can also destroy stratospheric ozone (Bouwman et al., 2002; Kavdir et al., 2008; Yu et al., 2008; Senbayram et al., 2009; Abdalla et al., 2010; Reijnders and Huijbregts, 2011). Agricultural soil emissions of N_2O can contribute to 65-70% of total N_2O emissions from the biosphere (Heller et al., 2003; Farquharson and Baldock, 2008). However, N_2O emissions vary greatly depending on many factors, including feedstock type, agricultural management, soil C and N stocks, soil temperature and moisture, biome climate, and soil freeze thaw cycles (Bouwman et al., 2002; Heller et al., 2003; Abdalla et al., 2010; Reijnders and Huijbregts, 2011).

N_2O emissions are a major byproduct of N fixation in soils, and N amendments to soil can cause widespread environmental externalities and deteriorate air quality (Bouwman et al., 2002; Yu et al., 2008; Aballa et al., 2010; Reijnders and Huijbregts, 2011). IPCC Guidelines suggest that the amount of fertilizer-derived N_2O emissions from the amount of N added to the soil is 1 – 1.25% (Heller et al., 2003; Crutzen et al., 2007; de Klein et al., 2006; Inselsbacher et al., 2011). However, Crutzen et al. (2007) suggested that the amount of N_2O derived from newly fixed N was actually higher, at 3 – 5% from biofuels like maize (*Zea mays* L.), sugarcane (*Saccharum officinarum* L.) and rapeseed (*Brassica napus* L.) (Reijnders and Huijbregts, 2011), and may be even more variable, reaching 8% (Abdalla et al., 2010; Inselsbacher et al., 2011).

Denitrification is typically the main biological process for N_2O production in anaerobic soils (Weier et al., 1993), while nitrification contributes to N_2O emissions in aerobic soils (Bouwman et al., 2002).

2.10.1 Denitrification

Denitrification rates are dependent on SOC, NO_3^- and soil O_2 availability (Bouwman et al., 2002). Soil denitrifying bacteria perform denitrification; they are facultative anaerobes that typically produce energy in aerobic conditions, but can survive in anaerobic conditions if required (Chapin et al., 2011). Dentrification can be inhibited by the presence of oxygen (O_2) in the soil profile (Bouwman et al., 2002), as O_2 has a greater redox potential to obtain electrons from organic substrates (Yu et al., 2008). In anaerobic conditions, NO_3^- readily acts as an electron acceptor to oxidize SOC for microbial energy (Senbayram et al., 2009; Smith et al., 2003). A simplified equation of denitrification is shown in Equation 2.1.

$$NO_3^- \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2 \quad \text{[Eq. 2.1]}$$

(Chapin et al., 2011; Smith et al., 2003)

The production of N_2O through denitrification is a difficult process to model; denitrification can be complete or incomplete and can result in the production of N_2 or N_2O gas (Equation 2.1). Denitrification can also result in NO production. Although NO is an intermediate gas that can diffuse into the atmosphere, it is often preferentially consumed by denitrifying bacteria quickly, and very little is released (Smith et al., 2003).

Both SOC and NO_3^- availability need to be considered with regard to N_2O production via denitrification. Heterotrophic microorganisms rely on the availability of C substrates, including SOC, for energy provision (Senbayram et al., 2009). Thus, as SOC depletes in a soil profile denitrification is limited. When NO_3^- is proportionally higher than labile SOC, more N_2O is produced than gaseous N_2 (Farquharson and Baldock, 2008). However, fertilizer application may not produce substantial N_2O emissions without a source of labile C as a microbial energy source (Weier et al., 1993). Denitrification is also dependent on soil moisture and porosity, as N_2O production requires a direct pathway of air-filled soil pores for the N_2O molecule to diffuse into the atmosphere (Farquharson and Baldock, 2008; Smith et al., 2003). N_2O that cannot readily diffuse has a greater likelihood of being converted into N_2 gas. Thus, the amount of N_2O released from denitrification is highly dependent on the heterogeneous patterns of soil moisture, temperature, porosity, NO_3^- and SOC availability (Bouwman et al., 2002; Chapin et al., 2011).

2.10.2. Nitrification

Nitrification is an aerobic process, whereby NH_4^+ is oxidized to NO_3^-. Equation 2.2 models the nitrification process.

$$NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^- \text{ [Eq. 2.2]}$$

(Farquharson and Baldock, 2008; Smith et al., 2003)

N_2O can be released as a byproduct due to a reaction of intermediate compounds (including HNO and NH_2OH) (Khalil et al., 2004). The availability of NH_4^+ in the soil profile is the most important factor to determine nitrification rates (Bouwman et al., 2002). NH_4^+ can be derived from organic matter decomposition through N mineralization or fertilizer application, which can result in nitrification by microbes, and subsequent release of N_2O (Farquharson and Baldock, 2008). The proportion of N_2O produced (rather than NO_3^-) through nitrification generally increases as soil moisture increases, until soil moisture becomes limiting by creating anaerobic conditions (Smith et al., 2003).

Nitrification is carried out by ammonia-oxidizing bacteria and archea which require SOC as an energy source (Farquharson and Baldock, 2008; Hangs et al., 2016). Thus, SOC concentrations are positively correlated with microbial activity (Hangs et al., 2016). However, consumption of SOC by heterotrophic microbes can act as a negative feedback loop as they respire and increase anaerobic conditions, decreasing nitrification rates (Farquharson and Baldock, 2008).

Nitrification increases the amount of NO_3^- available in the soil, which is a prerequisite for the denitrification pathway, and can result in more N_2O as a product of denitrification (Farquharson and Baldock, 2008). However, the two processes occur under completely different moisture and porosity conditions. Precipitation can reduce O_2 availability in an aerobic sites and result in an increase of denitrification, using the products of nitrification (Farquharson and Baldock, 2008).

2.11 Effect of Fertilizer Application on Nitrous Oxide Emissions in Willow SRC Systems

Generally there are two opposing schools of thought with regard to N_2O emissions from fertilized willow biomass plantations. The first theory is that the application of inorganic N,

which provides a direct source of NO_3^- and NH_4^+, will result in elevated N_2O emissions due to greater microbial nitrification and denitrification rates (Bouwman et al., 2002; Hellebrand et al., 2008; Inselsbacher et al., 2011). Hellebrand et al. (2008) found elevated N_2O emissions under willow, which persisted for 4 weeks following N fertilizer application and significantly contributed to the annual N_2O budget. Kavdir et al. (2008) also observed a peak in N_2O emissions following fertilizer application in perennial SRC systems. Kavdir et al. (2008) further noted that N_2O emissions peaked in autumn, due to greater SOC availability derived from plant residues (leaf senescence and fine root turnover) and decaying soil organisms, which increased microbial nitrification and denitrification (Farquharson and Baldock, 2008; Hellebrand et al., 2008; Gauder et al., 2011; Hangs et al., 2016).

The second school of thought is that fast-growing willows are able to cycle nutrients effectively, exhibiting high N-use efficiency (Amichev et al., 2014). The large root expanses of perennial willows are able to effectively take up NO_3^- and NH_4^+ (Kavdir et al., 2008; Inselsbacher et al., 2011; Gauder et al., 2011), and therefore may not result in a peak in N_2O emissions following fertilizer application because of the plants ability to outcompete microbes for the N (Abdalla et al., 2010). Gauder et al. (2011) did not see a significant effect of N fertilizer on N_2O emissions under willow. This was due to efficient N uptake by willow and weeds, as fertilizer was applied early in the growing season, when vegetation is efficiently cycling nutrients for biomass accrual (Gauder et al., 2011). Drewer et al. (2011) also found that N_2O emissions under willow were unaffected by fertilizer, and were unrelated to soil moisture or temperature, or ambient temperature. Microbial N immobilization can also contribute to N use in the soil profile, but has been suggested to play a minor role in inhibiting N_2O emissions (Yu et al., 2008).

2.12 Winter Freeze Thaw Cycles

In the temperate biome, soils are seasonally snow covered and pedological temperatures fall to values below 0°C for at least 4 months in the winter (Brooks et al., 2011; Wertz et al., 2012). However, predicted warmer winter temperatures and more frequent rain due to climate change will reduce snow accumulation, shorten the seasonal persistence of snow cover, and reduce the spatial coverage of snow, which can increase soil freezing (Maljanen et al., 2007; Groffman et al., 2010; Brooks et al., 2011). Thus, climate change will likely indirectly increase the frequency and severity of soil freeze-thaw cycles (FTCs) (Kurganova and Lopes de Gerenyu, 2015). Soil FTCs are commonly coupled with pulses of CO_2 and N_2O emissions (Ludwig et al., 2004; Teepe and Ludwig, 2004; Mørkved et al., 2006; Kurganova et al., 2007; Maljanen et al., 2007; Hentschel et al., 2008; Kavdir et al., 2008; Kurganova and Lopes de Gerenyu, 2015). The impact of soil FTCs on C and N soil transformations generally depend on the thickness of the overlying snow pack, ambient temperatures, soil moisture (Hentschel et al., 2008), nitrification and denitrification rates, and SOC availability (Groffman et al., 2010).

2.12.1 Winter CO_2 Emissions

Winter CO_2 emissions can account for 5-10% of yearly ecosystem respiration in deciduous forests (Brooks et al., 2011). Therefore, without considering potential winter CO_2 emissions, net C sequestration can be overestimated (Brooks et al., 2011). Generally, following a soil FTC, there is a CO_2 pulse, due to the increased access to labile C released in the soil (Kavdir et al., 2008; Brooks et al., 2011). Decaying microbes, fine root senescence, and fractured soil aggregates following FTCs all provide additional SOC and soil nutrients to living microbes (Ludwig et al., 2004; Henstchel et al., 2008), promoting heterotrophic respiration (Brooks et al., 2011; Kurganova and Lopes de Gerenyu, 2015). This suggests that N-limited temperate

ecosystems become C-limited in the onset of winter, and microbes require accessible C substrates to respire (Brooks et al., 2011).

Snow acts as an insulator, buffering extreme atmospheric temperature fluctuations so that soil temperature remains constant (Brooks et al., 2011), and steep ambient temperature fluctuations may not result in soil FTCs. Therefore, microbial activity in winter months, when the soil temperature remains constant, is strongly dependent on soil water availability rather than being positively correlated to ambient temperature (Brooks et al., 2011). In the growing season, CO_2 emissions are positively correlated with soil temperature, thus, true soil FTCs in the spring, when soil temperatures increase above ~5°C, will inherently increase soil CO_2 emissions due to warming and microbial decomposition (Kurganova and Lopes de Gerenyu, 2015). It is also important to note that dormant vegetation roots contribute very little to winter soil respiration, reducing winter CO_2 emissions (Groffman et al., 2006).

2.12.2 Winter N_2O emissions

Winter emissions can account for up to 50-70% of annual N_2O emissions (Ludwig et al., 2004; Mørkved et al., 2006; Maljanen et al., 2007). In particular, FTCs in the spring are often coupled with a marked increase in N_2O emissions (Ludwig et al., 2004; Teepe and Ludwig, 2004; Groffman et al., 2006; Mørkved et al., 2006; Maljanen et al., 2007; de Bruijn et al., 2009). Many factors have been suggested to control N_2O emissions from frozen soil.

As aforementioned, freeze-thaw disturbances break apart aggregates, and provide a pool of labile C from microbial and fine root death for living microbes to readily consume and produce N_2O (Ludwig et al., 2004; Groffman et al., 2006; Mørkved et al., 2006; Malajanen et al., 2007; de Brujin et al., 2009; Mohn et al., 2013; Kurganova and Lopes de Gerenyu, 2015). Thus, the amount of labile C available for consumption impacts N_2O emissions, which depends on the

balance of N that is both consumed (immobilization) and produced (denitrification) by microbes (Groffman et al., 2010; Brooks et al., 2011). However, fine roots are generally low in N, and therefore their supposed contribution of supplemental N is not likely to explain a presumed N_2O pulse during spring FTCs (de Bruijn et al., 2009). Winter N_2O emissions have also been attributed to reduced competition for inorganic N from dormant vegetation, making more N accessible for microbes for transformation into N_2O (Groffman et al., 2006; de Bruijn et al., 2009; Brooks et al., 2011; Mohn et al., 2013). Furthermore, snow insulation can provide a preferable environment for microbes, so they can continue to respire and release N_2O through winter (de Bruijn et al., 2009). Snowpack depth also influences the rate of N_2O release, as more N is immobilized with greater snowpack depths (Brooks et al., 2011).

Elevated N_2O emissions may be due to increased rates of denitrification, which increase because of saturated, anaerobic soils due to melt water during FTCs (Mørkved et al., 2006; Maljanen et al., 2007; Brooks et al., 2011). This is consistent with studies that find that denitrification is the main source of N_2O emissions following a thaw (Mørkved et al., 2006; Maljanen et al., 2007; Kavdir et al., 2008; Brooks et al., 2011). Therefore, this pulse of N_2O may also be limited by NO_3^- availability (Mørkved et al., 2006; Maljanen et al., 2007), which may decrease during snowmelt with high NO_3^- leaching (Hentschel et al., 2008).

Groffman et al. (2006) attributed increased N_2O emissions from FTCs to the release of an accumulation of trapped N_2O from soil pores under ice. However, gaseous diffusion slows when the majority of soil pores are filled with frozen water, which can limit the release of N_2O from the soil profile (de Bruijn et al., 2009; Mohn et al., 2013). Overall, FTC pulses can be substantial from willow SRC systems, and need to be directly quantified in total annual emissions, to ensure that willow is C neutral.

2.13 Primary Thesis Objectives

The primary goal of bioenergy is to ameliorate GHG emissions, which is largely achieved by being a C neutral fuel source (Smith et al., 2014). Willow SRC systems can achieve this by growing on marginal land for C sequestration, and can produce high yields to supply current biomass demands for bioenergy (Dickmann, 2006; Carriquiry et al., 2011; Srirangan et al., 2012). Nitrogen fertilizer application can increase biomass productivity to create greater biomass yields, but can also be associated with greater N_2O-N emissions (Crutzen et al., 2007; Kavdir et al., 2008; Hellebrand et al., 2008; Senbayram et al., 2009; Lutes et al., 2016). Without considering N_2O-N emissions associated with fertilizer application, there can be an overestimation of net GHG reductions in willow SRC systems, and therefore, the biofuel may not actually be C neutral (Crutzen et al., 2007). Little is known about the spatial and temporal variability of annual CO_2-C and N_2O-N emissions from willow SRC systems, especially after fertilizer application, freeze-thaw events in the spring and winter emissions. Therefore, the objectives of this thesis are to:

1) Determine the effect of fertilizer application on soil CO_2-C and N_2O-N emissions and soil characteristics under willow SRC (*Salix miyabeana* [SX67] and *S. dasyclados* [SV1]) systems,
2) Directly quantify soil CO_2-C and N_2O-N emissions from frozen winter soils and spring freeze thaw cycles, and
3) Estimate the total annual emissions and net annual emissions (in CO_2-eq) from a willow (*S. miyabeana*) SRC system.

2.14 Thesis Hypotheses

Given the objectives, the hypotheses of this thesis are:

1) There will be no significant differences in GHG emissions (N_2O-N and CO_2-C) or soil characteristics between willow (*Salix miyabeana* [SX67] and *S. dasyclados* [SV1]) clones,
2) There will be a significant increase in N_2O-N emissions following fertilizer application and no significant increase in CO_2-C emissions following fertilizer application,
3) There will be a pulse of CO_2-C and N_2O-N emissions following freeze thaw cycles in the spring and,
4) Fertilized willow SRC systems will have a positive net CO_2-eq efflux.

3. Study Site

3.1 Study Site

The experimental site was located at the University of Guelph Turfgrass Institute (GTI), Guelph, Ontario, Canada (43°33'03.41" N, 80°12'49.56" W). The site was located 334 m above sea level in the Mixed Wood Plains ecozone. The soil at GTI was classified as a Grey Brown Luvisol with a fine sandy loam texture (Clinch et al., 2009; Cardinael et al., 2012), and a bulk density of 1.32 g cm^{-3}. The soils were heavily eroded, and the site was classified as Class 3 and 4 agricultural lands, which are considered marginal (Clinch et al., 2009). Class 3 agricultural lands have moderately severe limitations, while class 4 lands have severe limitations (OMAFRA, 2016). These land classes limit the selection of crops and crop management on the land and have lower productivity of common field crops with conventional management (OMAFRA, 2016).

The experimental period extended from May 2014 to September 2015. There were 2 overlapping field seasons of data for May to September. The site had an average annual daily temperature of 16.8°C, and an annual precipitation of 543.0 mm from May to September 2014. In 2015, the average annual daily temperature was 17.8 °C, and the annual precipitation was 415.0 mm from May to September (Appendix A). The 30 year (from 1981-2010) mean temperature (May to September) was 16.7°C, while the 30 year mean annual precipitation (May to September) was 434.5 mm (Environment Canada, 2016). Thus, 2014 can be considered a wet year with average temperatures and 2015 can be considered a relatively warm year with average precipitation, relative to historical data.

3.2 Willow Establishment and Management

Prior to willow establishment, the land was under a conventional corn (*Zea mays* L.) – soybean (*Glycine max* L.) – wheat (*Triticum aestivum* L.) rotation. Monocropped plots of willow

clone *Salix miyabeana* (SX67) and *S. dasyclados* (SV1) were established on May 3, 2006 using a Step Planter (Salix Maskiner AB, Sweden) to plant double rows of 18-25 cm long willow cuttings in 10 m by 50 m plots (Figure 3.1, Table 3.1) (Clinch et al., 2009). Methods in Clinch et al. (2009) stated that the newly established willow plantations were treated with a Dual® II Magnum® (Syngenta Canada) herbicide applied at a rate of 0.92 kg ha^{-1} (S-metalachlor) to prevent weed growth in 2006 (Clinch et al., 2009). In 2007, the pre-emergent herbicide Goal 2XL® (Dow AgroSciences) was applied at a rate of 1.12 kg ha^{-1} (oxyfluorofen). Weeds that emerged prior to herbicide treatments were mechanically removed by hand (Clinch et al., 2009).

After one field season of growth, the willows were coppiced; stems were cut 2-4 cm from the soil surface, allowing new growth in subsequent growing seasons to propagate rapidly and at a greater density due to the pre-established rooting system (Dickmann, 2006). The preliminary coppice occurred in 2007, and each subsequent harvest took place on a 3-year rotation, which is typical for this land class. The initial stem density at the time of site establishment in 2006 was 12,000 to 15,000 stems ha^{-1}, which has now increased to ~100,000 stems ha^{-1}. The most recent biomass harvest occurred in December 2015.

3.3 Field Design

The study design was a randomized complete block split-plot design. Two levels of the main plot factor (willow clones) were replicated 3 times in 10 m x 25 m plots. Two levels of the subplot factor (fertilized and unfertilized treatments) were randomly assigned to 10 m x 12.5 m subplots within the main plots. In the 2014 field season, fertilizer was applied at 75 kg N ha^{-1}, 42 kg P ha^{-1}, 62 kg K ha^{-1} on June 3, 2014. In the 2015 field season, fertilizer was applied at the same rate on June 5, 2015. A two meter buffer around the border of each treatment subplot accounted for shading heterogeneity and edge effects, as higher light availability around the

perimeter may be associated with more optimal conditions for soil respiration (Smith et al., 2003); no measurements occurred in this area.

After one field season, clone SV1 was removed from sampling, due to the sheer time required to process the quantity of GHG samples. Greenhouse gas emissions and soil sampling for clone SV1 occurred from May 14, 2014 to November 6, 2014, and clone SX67 was sampled from May 14, 2014 to September 30, 2015. Soil samples were collected in 2014 until November 6, 2014, and resumed April 10, 2015; soil samples were not collected during winter when the soil surface was frozen.

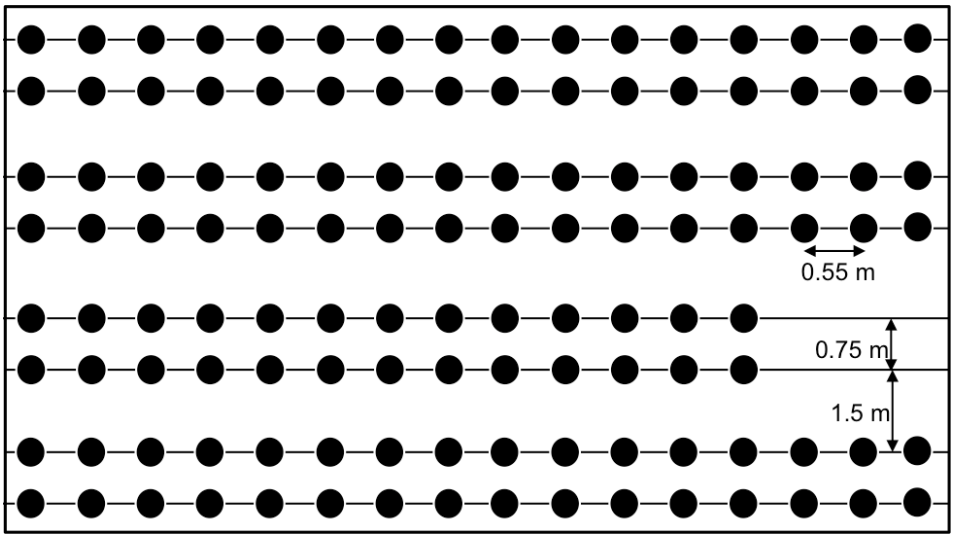

Figure 3. 1 Schematic diagram of double-row willow planting at the University of Guelph Turfgrass Institute biomass plantations.

Table 3. 1 Management of SRC willow biomass plantations from 2006-2015 at the Guelph Turfgrass Institute

Year	Season	Treatment
2006	Spring*	Willow establishment, pre-emergent herbicide, mechanical/by-hand weed control
2006	Winter	1st year coppice
2007	Spring*	Fertilizer application, pre-emergent herbicide, mechanical/by-hand weed control
2008		
2009	Winter	1st harvest
2010	Spring	Fertilizer application
2011		
2012	Winter	2nd harvest
2013	Spring	Fertilizer application
2014		
2015	Winter	3rd harvest

*Weeds that emerged later in each growing season were removed by hand
(Heller et al., 2003; Keoleian and Volk, 2005; Clinch et al., 2009; Koscenci, 2010)

4. Effect of Nitrogen Fertilizer on Greenhouse Gas Emissions in two Willow Clones (*Salix miyabeana* and *S. dasyclados*) in Southern Ontario, Canada

This chapter is an adaptation of the following published article: Lutes, K., Oelbermann, M., Thevathasan, N.V. and Gordon, A.M. (2016). Effect of nitrogen fertilizer in two willow clones (*Salix miyabeana* and *S. dasyclados*) in Southern Ontario, Canada. Agrofor Syst doi: 10.1007/s10457-016-9897-z

4.1 Introduction

Energy derived from lignocellulosic biomass will account for ~50% of global renewable energy resources by the year 2050 (Volk et al., 2004; Hangs et al., 2014, Smith et al., 2014). Lignocellulosic biomass is an effective renewable energy option because carbon (C) that is taken up through photosynthesis results in biomass accrual, and offsets the C released from fuel consumption during harvest (Lopez-Bellido et al., 2014). Therefore, the energy derived from biomass is C neutral (Palmer et al., 2014).

Biomass is commonly produced through short rotation coppice (SRC) systems, which are fast-growing and densely planted woody perennials capable of generating high productivity (Dickmann 2006; Smith et al., 2014). Willow (*Salix* spp.) is a common bioenergy crop used in SRC systems due to its high degree of genetic diversity, allowing for the development of hybrid clones (Volk et al., 2004; Amichev et al., 2014). Willow also has a broad native geographic range in temperate regions, making it an ideal crop that can be produced successfully under various climatic conditions (Amichev et al., 2014).

Establishing SRC willow systems on marginal soil prevents direct competition with land required for food production, while providing environmental services such as C sequestration (Amichev et al., 2014; Hangs et al., 2014; López-Bellido et al., 2014). Harvesting willow biomass, however, causes a net loss of nutrients, requiring the addition of nitrogen (N) fertilizer (Adegbidi et al., 2001; Dickmann 2006; Sevel et al., 2014). As a result, concerns have emerged

surrounding ecosystem disservices caused by N fertilizers due to its influence on soil C and N transformations that lead to greater greenhouse gas (GHG) emissions in SRC willow systems (Crutzen et al., 2008; Kavdir et al., 2008; Hellebrand et al., 2008; Senbayram et al., 2009). Fertilizer transforms N via nitrification and denitrification pathways due to a greater availability of ammonium (NH_4^+) or nitrate (NO_3^-) (Hellebrand et al., 2008). Carbon dioxide emissions can also escalate because the added fertilizer increases C availability and microbial activity (Gauder et al., 2011). However, on marginal and N-limited soil, perennial crops have a high N-use efficiency, and are able to cycle nutrients efficiently (Weih et al., 2010; Amichev et al., 2014), minimizing the emissions of GHG. This is because perennial crops have a large root network that effectively takes up excess N and therefore reduces the quantity of N transformed via nitrification or denitrification, limiting soil N_2O emissions (Kavdir et al., 2008; Gauder et al., 2011). As such, fertilizer application to SRC bioenergy crops, including willow, may not result in elevated N_2O emissions (Kavdir et al., 2008; Gauder et al., 2011). Additionally, soil respiration is primarily dependent on seasonal fluctuations driven by temperature and moisture changes, and thus CO_2 emissions do not necessarily increase with fertilizer application (Davidson et al., 1998; Laganière et al., 2012; Rowlings et al., 2012).

Both CO_2 and N_2O are powerful GHG that contribute to global warming (Kavdir et al., 2008; Senbayram et al., 2009). Therefore, without considering both CO_2 and N_2O emissions associated with biofuels, there is likely an overestimation of net GHG reduction from SRC systems relative to fossil fuel consumption, which negates the C neutrality of energy derived from biomass (Crutzen et al., 2008; Hellebrand et al., 2008; Gauder et al., 2011). To date, little knowledge exists on the spatial and temporal variations of soil GHG emissions from SRC systems, especially those established on marginal lands in temperate biomes (Amichev et al.,

2014). Carbon dioxide and N_2O emissions vary greatly among agricultural soils, depending on soil characteristics, microbial community diversity and activity, the type of perennial crop produced, land management practices and climate and soil characteristics (Smith et al., 2003; Kavdir et al., 2008; Hellebrand et al., 2008; Gauder et al., 2011; Hale et al., 2014). These factors can affect biomass productivity, and tree structure and growth patterns differently (Tharakan et al., 2005; Hangs et al., 2014; Amichev et al., 2014), and therefore influence CO_2 and N_2O emissions, especially after the application of fertilizer. Thus, quantifying GHG emissions from fertilized and unfertilized SRC systems provides new knowledge on their C-neutrality (Gauder et al., 2011).

A field study was conducted to quantify GHG emissions from SRC systems established on marginal land in southern Ontario, Canada. The objective of this study was to determine the effect of fertilizer application on CO_2 and N_2O emissions from SRC systems using two clones [*Salix miyabeana* (SX67), *S. dasylacos* (SV1)]; and to concurrently quantify soil chemical characteristics. Information gained from this study will further contribute to our comprehensive understanding of C and N transformations leading to GHG emissions from SRC willow systems. It was hypothesized that fertilized treatments will have significantly higher N_2O emissions than unfertilized treatments, but fertilizer application will not significantly influence CO_2 emissions, and clone type will not influence GHG emissions.

4.2 Materials and Methods

The study took place at the University of Guelph Turfgrass Institute in Guelph, Ontario, Canada (cf. Chapter 3).

4.2.1 Greenhouse gas sampling

Within each fertilized and unfertilized subplot per willow clone replicate, two GHG chambers were installed randomly on April 28, 2014. Chambers consisted of a permanent anchor and a removable chamber cap. Chamber anchors were constructed from white PVC pipe (10 cm in diameter and 25 cm in length) and installed to a 10 cm depth, leaving a headspace of 15 cm (Smith et al., 2003). Chamber caps were constructed from PVC covered with reflective insulation and contained a sampling port fitted with a rubber septum (1 cm diameter) for air extraction, and a 10 cm long vent tube (9 mm inner diameter) to reduce pressure differences during sample collection. Chambers were inspected 24 hours prior to sampling to remove any vegetation or litter fall that could interfere with emission results. Between sampling intervals, chambers were left open to the air.

GHG emissions exhibit a large temporal variability (Parkin and Venterea 2010; Rowlings et al., 2012). Thus, sampling occurred on a biweekly basis (Parkin and Venterea 2010). To account for the effect of sinusoidal diurnal temperature fluctuations, which can increase respiration following the highest daily temperatures (Smith et al., 2003), sampling took place between 10 a.m. to 3 p.m. to minimize bias (Parkin and Venterea, 2010). Initially, gas samples were taken at 0, 10, 20 and 30 min after deploying chamber caps, however this was reduced to 0, 15 and 30 min mid-way through the sampling season because the extra sample did not provide a more accurate rate of change of the gas' concentration. Gas samples were removed from the chamber headspace and stored in 3 mL over-pressurized evacuated vials (LabCo Ltd., High Wycombe, UK) (Parkin and Venterea 2010). Samples were collected biweekly from the beginning of May 2014 to the beginning of November 2014, for a total of 26 weeks. Sampling

frequency was increased after fertilizer application, where sampling took place 1, 3, 6, 10, 13 and 16 days after fertilizer application.

GHG samples were analyzed on a Gas Chromatograph 6890-N (Agilent Technologies Inc., Santa Clara, CA, USA), using a capillary column attached to a micro-electron capture detector (ECD) to quantify N_2O emissions (ppm), and a thermo conductivity detector (TCD) for CO_2 measurement. The GHG emission was calculated using the equation established by Hutchinson and Mosier (1981). The first equation is used to determine if the GHG flux is a linear or curvilinear response.

$[\frac{C_1-C_0}{C_2-C_1}]$ Eq. [4.1]

Where C_0, C_1 and C_2 are the flux values at T= 0, 15 and 30 minutes [ppm (v)], respectively. If Eq. 4.1 is <1, a linear regression slope is used to determine GHG flux. If Eq. 3.1 yields a result >1, an algorithm developed by Hutchinson and Mosier (1981) is used (Equation 3.2).

$$f_0 = \frac{V(C_1-C_0)^2}{[A*t_1(2*C_1-C_2-C_0)]*\ln[\frac{C_1-C_0}{C_2-C_1}]} \qquad \text{Eq. [4.2]}$$

Where C_0, C_1 and C_2 are the flux values at T= 0, 15 and 30 minutes (ppm(v)), respectively, V is the chamber volume (L), A is the soil surface area that the chamber covers (m^2), and t_1 is the evenly spaced interval between sampling times (min) (Parkin and Venterea 2010). The answer yields a GHG flux (f_0) in μL of trace gas (CO_2 or N_2O) m^{-2} min^{-1} (Parkin and Venterea 2010). The ideal gas law is used to convert this value into μmol of trace gas, and molecular mass is used to convert the flux to μg of trace gas.

4.2.2 Soil characteristics and photosynthetic photon flux density

Soil samples were collected biweekly for the analysis of NH_4^+ and NO_3^-, consistent with GHG sampling, to a 10 cm depth using a spade (Estefan et al., 2013). Soil samples were taken at a random location between the two GHG chambers in each treatment replicate. Soils were frozen immediately following sampling. Prior to soil sample analysis, soils were removed from the freezer, thawed in a fridge and then air-dried, ground using a mortar and pestle, and sieved (2 mm). Soil organic C and total N values are based on mean soil samplings taken on May 28, June 4, June 6, and June 9, 2014 at the same location as samples for NH_4^+ and NO_3^-.

Prior to soil analysis, 3 g of soil was acid washed with 30 mL of 0.5 M HCl, shaken at 300 rpm on a reciprocating shaker for 1 hour to remove carbonates. This process was repeated 3 times. Acid-washed soils were then rinsed with water, and oven baked at 50°C for 24 hours. Sieved soils were ground with a ball-mill (Retsch, Haan, Germany), and packed into tin capsules and analyzed for soil organic C and total N on a Costech CHNS-O 4010 Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, Italy).

Soil extracts for NH_4^+ and NO_3^- were prepared using 10 g of air-dried soil mixed with 50 mL of 2.0 M KCl. The solution was shaken on a reciprocating shaker for 15 minutes at 180 rpm, and extracted using Whatman 42 filter paper. Soil extracts were analyzed for NH_4^+ on a Shimadzu 1800 UV-Vis Spectrophotometer (Shimadzu Corp., Kyoto, Japan) at a 650 nm wavelength after 1 hour of color development (Verdow et al., 1978; Foster 1995). Soil NO_3^- was extracted according to Miranda et al., (2001) and Doane and Horwath (2003) and analyzed at a 540 nm wavelength 12 hours after color development using a UV-Vis Spectrophotometer.

Soil temperature and moisture were determined at the same time as GHG sampling using a HH2-WET Sensor (Delta T Devices, Cambridge, UK). Soil moisture and temperature values

are missing on October 23rd, 2014 due to faulty equipment. Photosynthetic photon flux density (PPFD) was quantified at ground level at each GHG chamber location using a quantum meter (Apogee Electronics Corporation, California, USA) on July 15, 2014; a time when peak sunlight conditions occur in the temperate zone. PPFD readings were recorded every 15 seconds over a period of a 90 seconds. Ambient air temperature and precipitation was determined from the GTI weather station (43°33'03.41" N, 80°12'49.56" W), while air pressure was obtained from the Waterloo Airport (43°27'39.00" N, 80°22'43.00" W) and adjusted for the vertical pressure gradient using the difference in height above sea level at Guelph.

4.2.3 Statistical analysis

All data was tested for homogeneity of variance and normality, and were found to have normal distributions (Steel et al., 1997). A repeated measure analysis of variance (ANOVA) was used to quantify the differences between fertilized and unfertilized treatments and between clones for soil CO_2 and N_2O emissions, NH_4^+, NO_3^- and soil temperature and moisture over time. The univariate linear general model (ANOVA) in SPSS was used to quantify differences in soil CO_2 and N_2O emissions, NH_4^+, NO_3^-, soil temperature and moisture among seasons [spring (May 14 to June 19, 2014), summer (July 3 to September 11, 2014) and autumn (September 25 to November 6, 2014)]. Significantly different main effects were further tested using the Tukey's multiple comparison test (Steel et al., 1997). Significant simple effects were tested using the estimated marginal means function in SPSS. The student *t* test was used to quantify differences in SOC and PPFD. Linear regression was used to determine the relationship between N_2O and CO_2 emissions and soil temperature or soil moisture. The type I error rate for all analyses was $p < 0.05$. All statistical analysis was conducted on SPSS Statistics for Windows Version 22.0 (IBM Corp. 2013).

4.4 Results

4.4.1 Greenhouse gas emissions

The interaction effect of time-by-treatment, time-by-clone, and time-by-treatment-by-clone was not significant for CO_2-C emissions. CO_2-C emission (mg CO_2-C m^{-2} h^{-1}) was significantly different with time over the 26-week sampling period [$F(6,126) = 10.2$, $p < 0.0001$] (Figure 4.1). Mean CO_2-C emissions ranged from 72 to 91 mg CO_2-C m^{-2} h^{-1} in fertilized, and from 63 to 105 mg CO_2-C m^{-2} h^{-1} in unfertilized treatments for SV1 and SX67, respectively (Table 4.1). CO_2-C emissions were significantly different among seasons in fertilized and unfertilized treatments in SV1 and SX67; and had significantly greater emissions in the spring and summer, except for the SX67 unfertilized treatment (Table 4.1). CO_2-C emission was significantly correlated to soil temperature ($r^2 = 0.202$), and had a significant negative correlation to soil moisture ($r^2 = 0.104$) in the autumn for fertilized and unfertilized treatments and both clones (Figure 4.2).

Table 4. 1 Mean seasonal CO_2 emissions (mg CO_2-C m^{-2} h^{-1}) from 2 willow clones (*Salix miyabeana* 'SX67' and *S. dasyclados* 'SV1') as effected by treatment (fertilized and unfertilized) in 2014.

	SV1		SX67	
Season	Fertilized	Unfertilized	Fertilized	Unfertilized
Spring	88.0 (5.9)^{A,a}	68.2 (4.2)^{A,b,*}	107.8 (6.3)^{A,a}	113.4 (7.0)^{AB,a,*}
Summer	76.9 (6.0)^{A,a,*}	75.9 (3.4)^{A,a,*}	107.1 (6.0)^{A,b,*}	129.2 (7.0)^{A,a,*}
Fall	52.0 (4.1)^{B,a,*}	43.3 (3.3)^{B,a,*}	67.9 (8.1)^{B,a,*}	94.0 (13.6)^{B,a,*}

[A] Means followed by the same uppercase letter are not significantly different among seasons
[a] Means followed by the same lowercase letter are not significantly different between treatments within clone
* Means are significantly different between the same (fertilized and fertilized or unfertilized and unfertilized) treatments between different clones

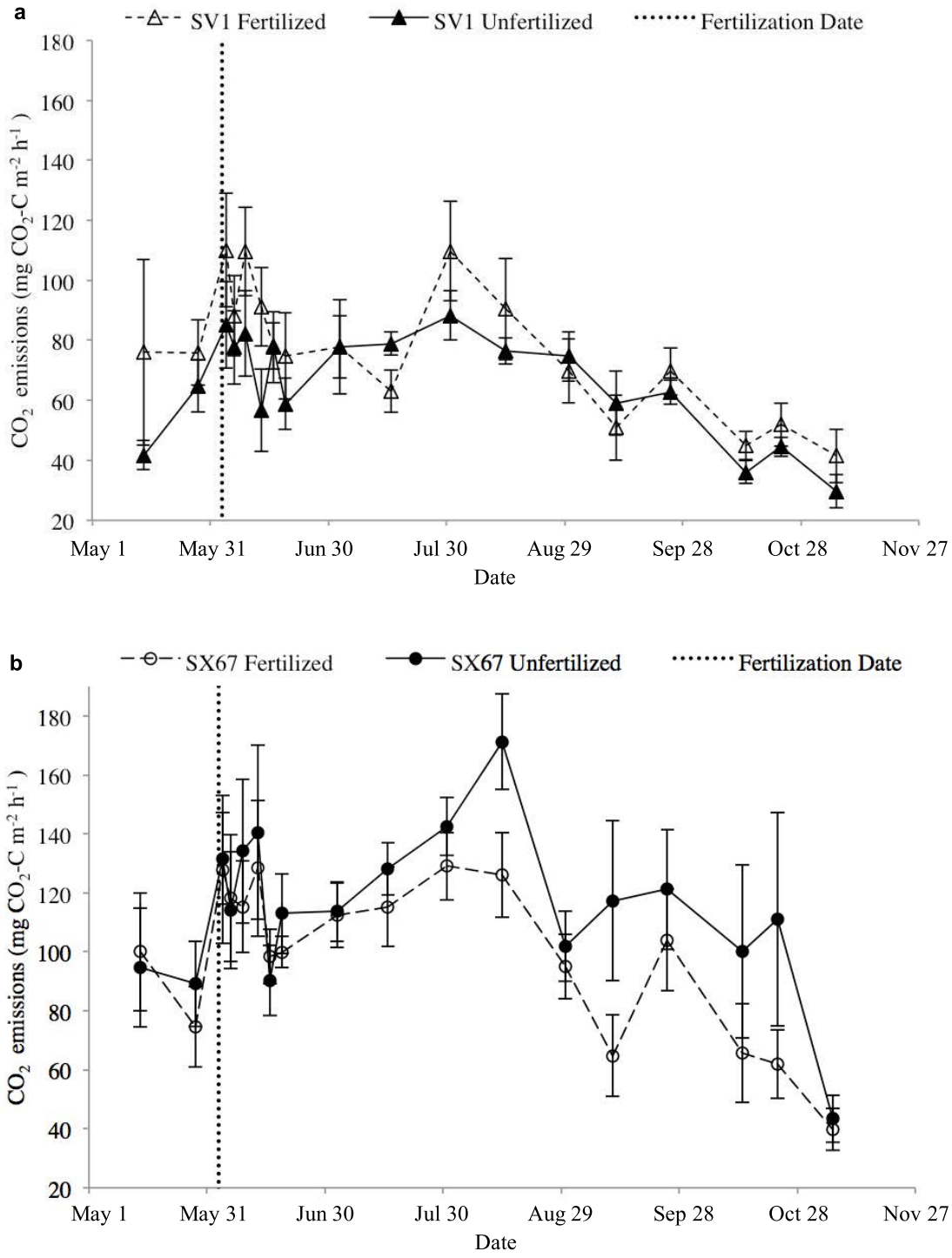

Figure 4. 1 Mean CO_2 emissions (mg CO_2-C m^{-2} h^{-1}) from willow clones (a) SV1 (*S. dasyclados*) and (b) SX67 (*S. miyabeana*) before and after fertilization at the Guelph Turfgrass Institute within the University of Guelph in Guelph, Ontario, Canada in 2014.

The interaction effect of time-by-treatment, time-by-clone, and time-by-treatment-by-clone was not significant for N_2O-N emissions. Mean N_2O-N emissions (μg N_2O-N m^{-2} h^{-1}) were significantly different with time [F(6,112) = 4.66, p = 0.0004] (Figure 4.2). Mean N_2O-N emissions ranged from 22 to 26 μg N_2O-N m^{-2} h^{-1} in fertilized, and from 16 to 17 μg N_2O-N m^{-2} h^{-1} in unfertilized treatments for SV1 and SX67, respectively (Table 4.2). Spring N_2O-N emissions were significantly greater compared to summer and autumnal emissions for both clones (Figure 4.2; Table 4.2). Willow clone SX67 had significantly greater emission of N_2O-N than SV1 in the fertilized treatment over the summer (Table 4.2). Soil N_2O-N emissions were significantly correlated to soil temperature (r^2=0.053) in the summer, and to soil moisture (r^2=0.061) in the autumn (Figure 4.2).

Table 4. 2 Mean seasonal N_2O emissions (μg N_2O-N m^{-2} h^{-1}) from two willow clones (*Salix miyabeana* 'SX67' and *S. dasyclados* 'SV1') as effected by treatment (fertilized and unfertilized) in 2014.

	SV1		SX67	
Season	Fertilized	Unfertilized	Fertilized	Unfertilized
Spring	36.8 (4.7)^{A,a}	18.9 (2.3)^{A,b}	38.3 (5.9)^{A,a}	20.2 (2.0)^{A,b}
Summer	14.1 (2.1)^{B,a,*}	12.5 (1.8)^{A,a}	23.4 (3.4)^{B,a,*}	18.3 (4.1)^{A,a}
Fall	14.3 (2.6)^{B,a}	17.4 (3.3)^{A,a}	16.0 (2.5)^{B,a}	11.6 (3.0)^{A,a}

[A] Means followed by the same uppercase letter are not significantly different among seasons
[a] Means followed by the same lowercase letter are not significantly different between treatments within clone
* Means are significantly different between the same (fertilized and fertilized or unfertilized and unfertilized) treatments between different clones

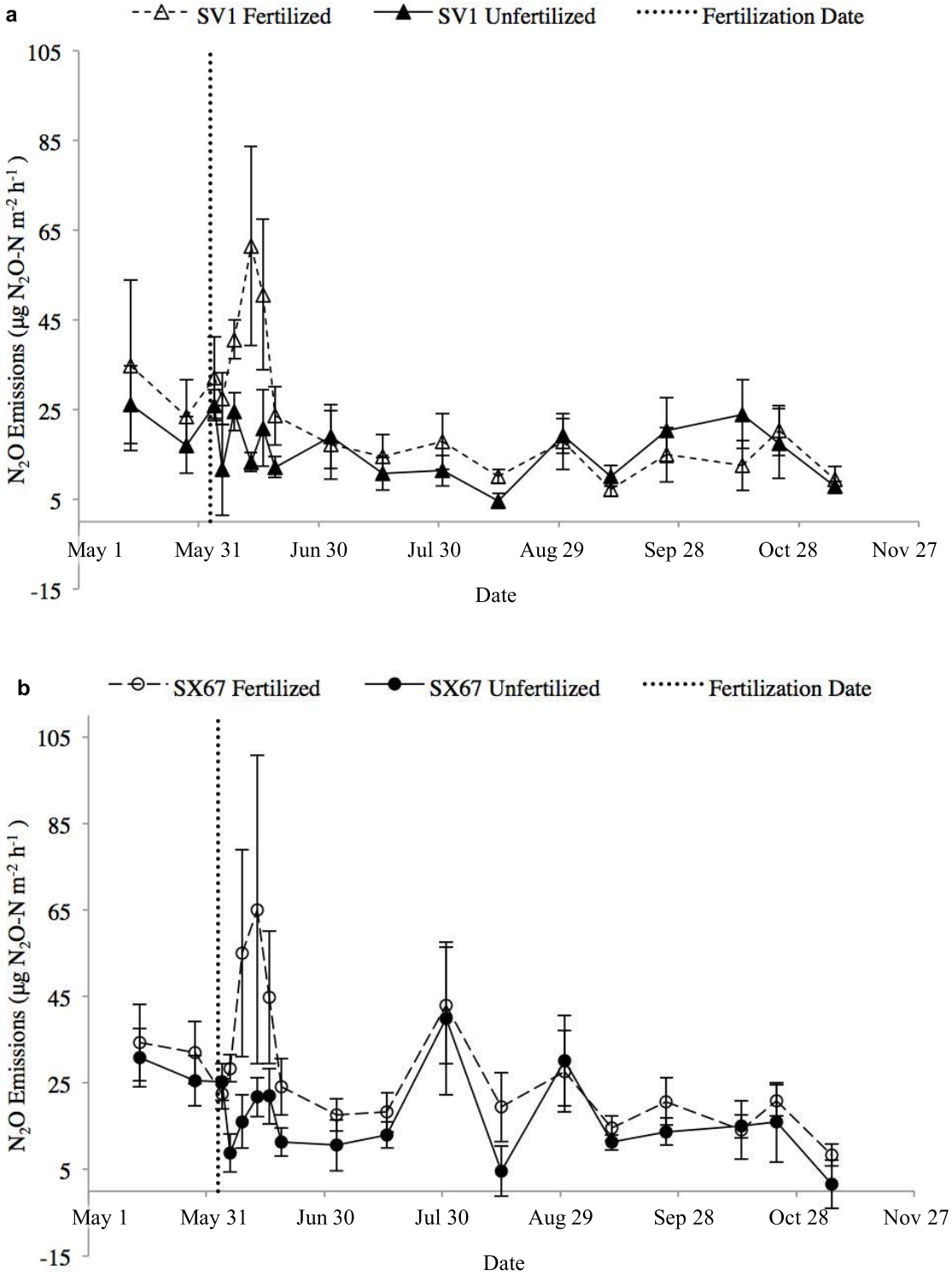

Figure 4. 2 Mean N_2O emissions (µg N_2O-N m^{-2} h^{-1}) from willow clones (a) SV1 (*S. dasyclados*) and (b) SX67 (*S. miyabeana*) before and after fertilization at the Guelph Turfgrass Institute within the University of Guelph in Guelph, Ontario, Canada in 2014

4.4.2 Soil chemical characteristics and photosynthetic photon flux density

Soil organic C concentration (%) did not differ significantly between fertilized and unfertilized treatments, however SOC was significantly greater in the fertilized SX67 treatment compared to that of both SV1 treatments (Table 4.3). Soil total N was not significantly different between treatments, but was significantly different between fertilized clones (Table 4.3).

There was a significant interaction effect for time-by-treatment for NH_4^+ [$F(3,21) = 6.396$, $p = 0.008$]. Soil NH_4^+ concentration (g N kg_{soil}^{-1}) varied significantly over the 26-week sampling period [$F(3,21) = 9.182$, $p = 0.002$], with an overall mean value of 4.10 g N kg_{soil}^{-1}. Soil NH_4^+ concentration was higher in fertilized than in unfertilized treatments from June 6 to June 13, directly following fertilizer, and on July 16 and September 11, 2014 (Table 4.4). The interaction effect of clone-by-treatment was not significant for soil NH_4^+ concentration. Soil NH_4^+ concentration was not significantly different between clones. The interaction effect of time-by-treatment was significant for soil NO_3^- concentration. Soil NO_3^- concentration (g N kg_{soil}^{-1}) varied significantly [$F(3,21) = 5.881$, $p = 0.006$] over the 26 week sampling period, with a mean value of 5.26 g N kg_{soil}^{-1}. Soil NO_3^- concentrations were significantly greater in fertilized treatments than unfertilized treatments from June 6 to July 3, and on October 10 and November 6, 2014 (Table 4.4). The interaction effect of clone-by-treatment for soil NO_3^- concentration was not significant. Clone type did not have a significant effect on soil NO_3^- concentration.

Soil NH_4^+ and NO_3^- concentrations were significantly influenced by season. For example, SX67 had significantly greater soil NH_4^+ and NO_3^- concentrations in fertilized treatments than unfertilized treatments in the spring and summer, and significantly greater NO_3^- concentration in the autumn (Table 4.4). In the autumn, fertilized SV1 had a significantly lower NO_3^-

concentration compared to SX67. However, in the summer, soil NH_4^+ concentration was significantly greater in the unfertilized treatment with SV1 (Table 4.4).

Soil NO_3^- concentration was significantly correlated to soil moisture (r^2=0.471) and temperature (r^2=0.198) in unfertilized SX67 treatments in the autumn. Soil NH_4^+ concentrations in SX67, in the summer for fertilized (r^2=0.407) and unfertilized (r^2=0.744) treatments, yielded significant negative relationships with soil moisture. Only the NH_4^+ concentration in SX67, unfertilized in the spring, was significantly correlated (r^2=0.209) to soil temperature. Soil NH_4^+ and NO_3^- concentrations from SV1 were not significantly correlated to soil moisture and temperature.

Photosynthetic photon flux density (μmol m^{-2} s^{-1}) was significantly different between treatments. A significantly lower rate of solar radiation (PPFD) reached the understory in fertilized treatments for both SX67 and SV1 clones (Table 4.3). PPFD decreased (relative) by 141% in the fertilized SX67 clone, and by 352% in the fertilized SV1 clone.

Table 4. 3 Mean soil characteristics from two willow clones (SV1 and SX67) and two treatments (fertilized and unfertilized) over the 26-week sampling period in 2014.

Soil Characteristic	SX67		SV1	
	Fertilized	Unfertilized	Fertilized	Unfertilized
Soil Organic C (%)	2.13 (0.10)[a,*]	2.11 (0.08)[a]	1.78 (0.12)[a,*]	1.98 (0.15)[a]
Total N (%)	0.18 (0.01)[a,*]	0.18 (0.01)[a]	0.14 (0.01)[a,*]	0.16 (0.01)[a]
Photosynthetic Photon Flux Density (μmol m^{-2} s^{-1})	48.80 (10.92)[b]	117.73 (21.99)[a]	21.60 (4.93)[b]	97.73 (22.62)[a]
Soil Moisture (% vol)	23.60 (0.52)[a]	24.44 (0.59)[a,*]	23.73 (0.53)[b]	25.65 (0.55)[a,*]
Soil Temperature (°C)	17.77 (0.41)[a]	17.73 (0.40)[a]	17.32 (0.36)[a]	17.60 (0.38)[a]

[a] Means followed by the same lowercase letter are not significantly different between treatments within clone

* Means are significantly different between the same (fertilized and fertilized or unfertilized and unfertilized) treatments between different clones

Table 4. 4 Mean soil NO_3^- and NH_4^+ concentrations (g N kg_{soil}^{-1}) from two willow clones (SV1 and SX67) and two treatments (fertilized and unfertilized) over the 26-week sampling period in 2014.

		SV1		SX67	
NO_3^-	Season	Fertilized	Unfertilized	Fertilized	Unfertilized
	Spring	11.8 (1.9)^{A,a}	3.8 (0.5)^{A,b}	9.4 (1.5)^{A,a}	4.2 (0.6)^{A,b}
	Summer	4.2 (0.4)^{B,a}	3.3 (0.3)^{A,a}	4.0 (0.4)^{B,a}	3.0 (0.2)^{A,b}
	Fall	3.0 (0.2)^{B,a,*}	2.9 (0.4)^{A,a}	5.2 (0.2)^{B,a,*}	3.2 (0.3)^{A,b}
NH_4^+					
	Spring	11.6 (2.0)^{A,a}	3.2 (0.2)^{A,b}	9.2 (1.8)^{A,a}	3.1 (0.1)^{A,b}
	Summer	2.3 (0.2)^{B,a}	2.1 (0.2)^{B,a,*}	2.4 (0.2) ^{B,a}	1.7 (0.1)^{B,b,*}
	Fall	1.7 (0.2)^{B,a}	1.8 (0.2)^{B,a}	2.1 (0.38) ^{B,b}	1.6 (0.1)^{B,b}

^{A} Means followed by the same upper letter are not significantly different among seasons
^{a} Means followed by the same lowercase letter are not significantly different between treatments within clone
* Means are significantly different between the same (fertilized and fertilized or unfertilized and unfertilized) treatments between different clones

4.5 Discussion

4.5.1 Soil CO_2 emissions

Soil CO_2-C emissions were within range of that reported by Oelbermann et al. (2015), Gauder et al. (2011), and Laganière et al. (2012) in temperate environments. Soil CO_2-C emissions, in natural and managed systems, are influenced by the availability and quality of C substrates for microorganisms, biomass accrual, plant root density, microbial population levels, and soil physical and chemical properties including soil temperature and moisture (Zanchi et al., 2014). Soil moisture and temperature are key factors regulating CO_2-C emissions (Davidson et al., 1998; Pacific et al., 2008; Laganière et al., 2012); paralleling seasonal changes in the temperate environments (Soosaar et al., 2011). Our study also showed a seasonal pattern of CO_2-C emissions, which was strongly influenced by soil temperature. The highest CO_2-C emission

was associated with the spring and summer months, where optimal temperature conditions prevail for maximum microbial activity. Short rotation willow crops grow quickly, have a high stem density, and are able to provide adequate soil shading with canopy closure; creating a favorable environment for soil microbes. Shading creates a microclimate that buffers changes in soil temperature and moisture, providing uniform conditions for soil respiration in the summer (Clinch et al., 2009; Gauder et al., 2011).

The lowest CO_2-C emissions in our study were associated with autumn (September 21 to November 6), when less suitable conditions for microbial and plant activity occurred (Pacaldo et al., 2014). In temperate climates, soil is moist and cool in the spring and autumn, which are unfavorable conditions for microbial activity (Mander et al., 2008). Additionally, differences in light availability contributed to differences in soil temperature and moisture among seasons (Laganière et al., 2012). For example, both willow clones had significantly lower PPFD at ground level in the fertilized treatment. This is because the greater availability of N from the applied fertilizer caused greater biomass productivity, closing the canopy more rapidly, and provided more shade compared to the unfertilized treatment. It was not possible to assess the aboveground biomass in 2014 as the 3rd harvest was scheduled for December, 2015. However, from visual observation it was obvious that fertilized willows had a denser canopy and prevented light penetration to the soil's surface.

Although physical factors including soil temperature, moisture, and light availability were similar between clones and treatments, the consistently greater soil CO_2-C emission under SX67 was due to a greater concentration of SOC. Soils with a greater concentration of SOC have greater rates of soil respiration (Gauder et al., 2011). In this study, on a hectare basis and to 20 cm depth (furrow soil depth), SOC stock in SX67 was 6.34 t ha^{-1} higher than in SV1, based in a

soil bulk density of 1.32 g cm^{-3}. Baseline SOC concentration, at the time of SRC willow system establishment in 2006, was 1.43% (Clinch et al., 2009), and increased to 2.12% (mean of fertilized and unfertilized treatment) by 2014, whereas SOC for SV1 only rose to 1.88% (mean of fertilized and unfertilized treatment). Although biomass productivity in SX67 was significantly lower in 2009 compared to SV1 (Cardinael et al., 2012), 3 years after site establishment, the greater accumulation of SOC in SX67 was due to differences in endogenous rates of nutrient cycling and due to variation in the structure and activity of the microbial community (Hale et al., 2014; Hu et al., 2014).

Fertilizer application can increase soil respiration in N-limited systems, leading to a greater microbial activity (Gauder et al., 2011). However, adding fertilizer to N-limited systems was also associated with negligible CO_2 emissions (Gauder et al., 2011). This is because in N-limited systems, any available N will be immediately taken-up by vegetation, minimizing leaching, and instead the available N is used in the production of biomass (Kavdir et al., 2008; Gauder et al., 2011). Our study showed that the addition of fertilizer did not influence CO_2-C emissions. This is because fertilizer was applied in the spring, a time in the temperate zone when trees are placing their energy into the accrual of woody and leaf biomass, therefore maximizing their N uptake (Volk et al., 2004).

4.5.2 Soil N_2O emissions

Results from N_2O-N emission reported in this study are comparable to those from other studies using SRC systems in temperate environments. However, results of peak N_2O-N emissions after fertilizer application in SRC willow systems remain controversial. For example, Gauder et al. (2011) did not observe increased N_2O-N emissions as a result of fertilizer application in SRC willow bioenergy crops under a similar fertilization regime as our study.

Kavdir et al. (2008), however, reported N_2O-N emissions ranging from 0 to 50 μg N_2O-N m^{-2} h^{-1}. Similar to our study, Kavdir et al. (2008) observed peak N_2O-N emissions after the application of inorganic N fertilizer in a *S. viminalis* SRC system in Germany. Hellebrand et al. (2008) reported that elevated N_2O-N emissions occurred for 4 weeks following fertilizer application, which was also observed in our study, in a *S. viminalis* bioenergy crop plantation in Germany.

N_2O-N emissions are dependent on soil biological, chemical and physical characteristics including soil temperature and moisture, SOC stocks and the availability of NO_3^-, NH_4^+ (Smith et al., 2003, Hellebrand et al., 2008). Among these factors, NH_4^+ availability is the most important component driving N_2O-N emissions because of its role in the process of nitrification (Senbayram et al., 2009). In our study, there is strong evidence that N_2O-N emissions were derived from nitrification because soil available N (NO_3^- and NH_4^+) reserves were low when N_2O production peaked (Kavdir et al., 2008). Additionally, our study takes place in well-drained soils, further supporting nitrification rather than denitrification-derived N_2O-N emissions, as nitrification occurs under aerobic soil conditions (Smith et al., 2003; Senbayram et al., 2009).

N_2O-N emissions peaked in fertilized treatments for both willow clones in the spring, at the time of N fertilizer application, which coincided with high NH_4^+ and NO_3^- availability. Correlations between N_2O-N emissions and soil moisture or soil temperature were weak, suggesting that these factors did not play a major role in controlling N_2O-N emissions in our study during the spring, summer and autumn. However, this does point to the important role of land management practices in SRC willow systems in controlling N_2O-N emissions; suggesting that practices like fertilizer application play an important role in the production of this GHG. Additionally, the selection of willow clones can also influence the emission of N_2O-N when combined with fertilizer in N-limited systems. Although SX67 had a lower biomass productivity

in 2009 than SV1 (Cardinael et al., 2012), SOC accumulation was greater in SX67, which was due to differences in vegetation patterns, including canopy structure, and the structure and activity of the microbial community (Hale et al., 2014) that contributed to different N transformation processes, leading to the emission of N_2O-N, than in SV1 (Senbayram et al., 2009).

4.6 Conclusions

This study showed that CO_2-C emissions were not influenced by the application of N fertilizer but were strongly controlled by fluctuations in soil temperature and moisture among spring, summer and fall. In addition, the greater accumulation of SOC in treatments with the willow clone SX67 suggests that differences in tree structure, vegetation patterns, and the structure and activity of the microbial community caused greater emissions of this GHG. The greater availability of NH_4^+ and NO_3^-, as a result of fertilizer application, rather than soil moisture and temperature, affected N_2O-N emissions. Our data contributed to a more thorough understanding of soil GHG emissions from two different willow clones produced on marginal soil; and provided new knowledge on the effect of N fertilizer application on CO_2 and N_2O emissions. It is recommended that further long-term perennial SRC systems for bioenergy production be established on marginal land, using a variety of different fertilizer application rates among other land management practices. This will help to bridge our current knowledge gap on soil C and N interactions and the processes that lead to GHG emissions in these land-use systems.

5. Interannual Soil CO_2 and N_2O Emissions from a Temperate Willow (*Salix miyabeana*) Short Rotation Coppice System

5.1 Introduction

Fossil fuels constitute ~85% of the global energy supply and are the world's primary energy source (Srirangan et al., 2012). However, their continued and extensive use is threatened by non-renewable resource scarcity and uncertainty, concern for long-term national energy security, and greenhouse gas (GHG) emissions associated with fossil fuel combustion, which have all driven the rapid development of renewable energy (Volk et al., 2004; Manazano-Agugliaro et al., 2012; Panwar et al., 2011; Ellebban et al., 2014). Bioenergy derived from biomass is a particularly effective carbon (C) neutral renewable energy source, which presently supplies ~50 EJ of energy worldwide, representing 10 – 14% of global primary energy (Keoleian and Volk, 2005; Panwar et al., 2011; Srirangan et al., 2012; Liu et al., 2014). Currently, the majority of bioenergy is derived from first generation biofuels produced from herbaceous food crops, which are criticized because they require high N fertilizer inputs and increase competition for agricultural land (Naik et al., 2010; Sims et al., 2010; Srirangan et al., 2012).

Second generation lignocellulosic biofuels produced from non-food crops are more sustainable renewable bioenergy sources than their first generation counterparts (Berndes et al., 2003; Volk et al., 2004; Budsberg et al., 2012). Woody perennial biofuels are capable of long-term C storage, contributing to GHG abatement (Keoleian and Volk, 2005; Konecsni, 2010; Amichev et al., 2014), and reduce CO_2 emissions relative to petroleum-based fossil fuels by an estimated 70 – 90%, excluding emissions derived from land-use change (Aylott et al., 2007; FAO, 2008; Grafton et al., 2013).

Willow (*Salix miyabeana*) grown in short rotation coppice (SRC) systems are particularly effective to provide consistent, high biomass yields with little field maintenance or inputs (Lemus and Lal, 2005; Kahle et al., 2007; Konecsni, 2010), even when cultivated on marginal lands (Verwijst, 2001; Amichev et al., 2016). Furthermore, willow feedstocks have a high net energy ratio, containing approximately 29 – 55 units of stored energy per every unit of fossil fuel energy input during production (Matthews et al., 2001; Heller et al., 2003; Volk et al., 2004; Keoleian and Volk, 2005; Aylott et al., 2007), are genetically variable, and have a large native geographic range in North America (Verwijst, 2001; Amichev et al., 2016).

Fertilizer applied to willow SRC systems supply adequate nutrients to maintain yields in N-limited temperate systems (Adegbidi et al., 2001; Adegbidi et al., 2003; Lemus and Lal, 2005; Dickmann, 2006; Sevel et al., 2014; Amichev et al., 2016). However, this increases N_2O-N emissions, due to a direct source of inorganic N elevating microbial activity in willow SRC systems (Lutes et al., 2016). This is particularly problematic as N_2O has a global warming potential 296-298 times that of CO_2 and can destroy stratospheric ozone (Bouwman et al., 2002; Kavdir et al., 2008; Senbayram et al., 2009; Abdalla et al., 2010; Reijnders and Huijbregts, 2011). Conversely, fertilizer application does not result in elevated CO_2-C emissions, which are related to seasonal temperature fluctuations during the growing season (Lutes et al., 2016). Consideration of both N_2O-N and CO_2-C emissions is necessary when assessing the C neutral potential of willow SRC systems.

Temperate biomes are characterized by soils that are seasonally snow covered, in which pedological temperatures are below 0°C for at least 4 months in the winter (Brooks et al., 2011; Wertz et al., 2012). Winter soil-derived emissions can account for up to 5-10% of annual CO_2-C respiration (Brooks et al., 2011), and 50 - 70% of annual N_2O-N emissions (Ludwig et al., 2004;

Mørkved et al., 2006). In particular, freeze-thaw cycles (FTCs) in the spring contribute substantially to both annual CO_2-C emissions (Kavdir et al., 2008; Brooks et al., 2011) and N_2O-N emissions (Ludwig et al., 2004; Teepe and Ludwig, 2004; Groffman et al., 2006; Mørkved et al., 2006; de Bruijn et al., 2009).

Pulses in heterotrophic respiration, and thus, CO_2-C emissions, are largely attributed to higher quantities of labile C following FTCs (Kavdir et al., 2008; Brooks et al., 2011), obtained from microbial death, fractured soil aggregates and fine root senescence, which are energy sources to active microbes (Ludwig et al., 2004; Henstchel et al., 2008; Kurganova and Lopes de Gerenyu, 2015). Additionally, root respiration increases CO_2-C emissions as plants become active nearing spring (Hopkins et al., 2013). Winter and FTC CO_2 emissions are variable, ranging from negligible to values rivalling that of summer CO_2 emissions. (Brooks et al., 2011; Groffman et al., 2006).

Winter N_2O-N emissions have been attributed to many different biogeochemical processes including: elevated denitrification due to soil saturation during thaws (Mørkved et al., 2006; Brooks et al., 2011), elevated SOC from microbe and root mortality, acting as an energy substrate for live microbes, which consume and produce N compounds (Ludwig et al., 2004; Groffman et al., 2006; Mørkved et al., 2006; Kurganova and Lopes de Gerenyu, 2015; de Brujin et al., 2009; Mohn et al., 2013), reduced competition for N due to vegetation dormancy, allowing microbes to convert N to N_2O-N (Groffman et al., 2006; de Bruijn et al., 2009; Brooks et al., 2011; Mohn et al., 2013), snow insulation creating preferable microbial conditions (de Bruijn et al., 2009) and release of built-up N_2O trapped in soil pores under ice (Groffman et al., 2006). However, winter N_2O-N emissions are inconstant, and may even be negligible (Groffman et al., 2010). Therefore, there is much uncertainty in assessing the importance of winter GHG

emissions on annual budgets to assess net GHG emissions in willow SRC systems (Brooks et al., 2011).

Total annual emissions, expressed as CO_2-equivalents (CO_2-eq), encompass both fertilizer-derived and FTC emissions of trace gases from willow SRC systems. To date, there is no study quantifying total annual emissions, including directly quantified winter emissions, from soils under fertilized willow SRC systems. The objectives of this study were 1) to quantify CO_2-C and N_2O-N emissions, and NH_4^+ and NO_3^- soil concentrations following fertilizer application over two field seasons, determining if there is intermittency between field seasons, 2) to determine winter and FTC CO_2-C and N_2O-N emissions and, 3) to quantify total annual emissions (CO_2-eq) from the willow SRC systems. It is hypothesized that there will be significantly higher emissions of N_2O-N following fertilizer application, while CO_2-C emissions will follow a seasonal trend, that there will be a pulse of N_2O and CO_2 emissions following FTCs, and that fertilized willow SRC systems will have a positive net emission budget.

5.2 Methods

The study site was located at University of Guelph Turfgrass Institute willow biomass plantations in Guelph, Ontario, Canada (cf. Chapter 3).

5.2.1 GHG Emissions

GHG emissions were measured using the static chamber method (Parkin and Venterea, 2010; Zhu-Barker et al., 2016). Within each fertilized and unfertilized subplot of *Salix miyabeana* (SX67), two GHG chambers were installed randomly on April 28, 2014. Permanent chamber anchors (10 cm diameter, 25 cm in length), constructed of white PVC pipe, were installed 10 cm into the surface of the ground, leaving a headspace of 15 cm (Smith et al., 2003). Chamber anchors were left open to the air and uncapped between sampling intervals. During

sampling events, anchors were covered with a cap for air sample extraction. Chamber caps were constructed from PVC covered with reflective insulation, which minimized internal chamber heating. Each chamber cap had a central sampling port fitted with a rubber septum (1 cm diameter) for air extraction, and a 10 cm long vent tube (9 mm diameter) to reduce pressure differences during sample collection. Rubber septa were replaced biweekly. Vegetation, litter fall or debris within the chamber, which could interfere with emission results, was removed 24 hours before sampling.

GHG sampling occurred biweekly to capture temporal variability (Parkin and Venterea, 2010). Sampling occurred between 10 a.m. to 3 p.m. on each sampling day to minimize respiration bias associated with daily temperature variations (Smith et al., 2003; Parkin and Venterea, 2010). Gas samples were taken at three 15-minute intervals (0, 15 and 30 min) after deploying chamber caps. Initially, GHG samples were collected at four 10-minute time intervals (0, 10, 20 and 30 min); however, this did not increase the accuracy of GHG flux measurement, and was reduced mid-way through summer 2014. Gas samples were removed from the chamber headspace using a 10 mL syringe and stored in 3 mL pre-evacuated over-pressurized vials (LabCo Ltd., High Wycombe, UK) (Parkin and Venterea, 2010).

GHG emission samples were collected over 72 weeks, totaling 48 sampling days, spanning from May 14, 2014 to September 30, 2015. GHG sampling occurred biweekly during the spring (March 20 to June 19), summer (June 20 to September 21) and autumn (September 22 to December 20), and monthly during the winter (December 21 to March 19). GHG samples were not collected in January due to extreme cold temperatures. Sampling frequency increased following fertilizer application on June 3, 2014 and June 5, 2015, and after freeze-thaw events in

March and April 2015. Sampling took place 1, 3, 6, 10, 13 and 16 days after fertilizer application in 2014, and 1, 3, 6, 10, 14 and 18 days after fertilizer application in 2015.

GHG samples were analyzed on a Gas Chromatograph 6890-N (Agilent Technologies Inc., Santa Clara, CA, USA), equipped with a capillary column attached to a micro-electron capture detector (ECD) and a thermo conductivity detector (TCD). The GHG emissions were calculated using the equation established by Hutchinson and Mosier (1981). First, the GHG flux was determined to be a linear or a curvilinear response. If the response was linear, linear regression was used to calculate the GHG flux. If the response was curvilinear, an algorithm was used, which considers the surface area and volume of the chamber, and the rate of change in GHG concentrations at each sampling point, yielding a GHG flux in in μL of trace gas (CO_2 or N_2O) (Hutchinson and Mosier, 1981; Parkin and Venterea, 2010; Zhu-Barker et al., 2016). The ideal gas law converted μL into μmol of trace gas, and molecular mass was used to convert to μg of trace gas.

5.2.2 Total Annual N_2O and CO_2 Emissions

Total annual N_2O-N and CO_2-C emissions were calculated using the trapezoidal rule adjusted for unequal intervals to approximate area under the curve, with the assumption that the measured GHG value represented the mean daily emission value for that day, and that mean daily emissions changed linearly between sampling measurements (Equation 5.1) (Thorman et al., 2007; Zhu-Barker et al., 2016).

$$Total\ Annual\ GHG\ Emission = \sum \Delta day \cdot \left(\frac{f_{day\,1} + f_{day\,2}}{2}\right) \quad \text{Eq. [5.1]}$$

Where Δ day is the number of sampling days between sampling intervals and *f(x)* represents the average quantified daily flux (in kg CO_2-eq ha^{-1} day^{-1}) on each respective

sampling day. Total annual emissions were quantified from May 14, 2014 to May 22, 2015, and therefore represent a slight overestimation.

Total annual N_2O-N emissions were normalized using IPCC 100 year global warming potential (GWP) values and expressed as CO_2-equivalents (CO_2-eq) (Appendix B). GWPs quantify the relative amount of heat that a particular GHG can capture relative to a reference gas (usually CO_2), in order to compare the potential impact of emissions across different GHGs (Forester et al., 2007). The conversion factor was 1 for CO_2 (as a reference gas), while total annual N_2O emissions were multiplied by the IPCC 100 year GWP of 298 (Forester et al., 2007).

Net annual emissions (CO_2-eq) were calculated by subtracting the total C sequestration from the total annual emissions from willow SRC systems. Aboveground, belowground and litter fall C (odt ha^{-1} yr^{-1}) contributions in fertilized and unfertilized treatments were summed to quantify willow C sequestration. Aboveground C in the willow was calculated as 50% of the annual yield (Winans et al., 2016; Pipatti et al., 2006; Smith et al., 2000), the belowground C allocation was quantified as 60% of the aboveground C (Kumar and Nair, 2011), and the contribution of litter fall C to SOC was quantified as 20% of annual litter fall (Ngao et al., 2005).

5.2.3 Freeze Thaw Emissions

Winter freeze thaw emissions were collected in two sampling events. Sampling durations were based on daily atmospheric temperature highs. The first sampling occurred when the maximum daily atmospheric temperature was consecutively 5°C, spanning 8 days from March 2 to March 16, 2015. The second sampling occurred when the daily maximum atmospheric temperature reached 10°C, spanning over 3 days from April 1 to April 3, 2016. April 10, 2016 and April 26, 2016 are also included in the second FTC data, as snowmelt was concluding.

5.2.4 Soil Characteristics

Soil samples were collected biweekly, at the same time as GHG collection to assess NH_4^+ and NO_3^- concentrations to a 10 cm depth (Estefan et al., 2013). Soil samples were collected from SX67 plots from May 14, 2014 to November 6, 2014, and from April 10, 2015 to September 30, 2015. Soil samples were taken at a random location between the two GHG chambers in each treatment subplot, totaling 6 soil samples biweekly. There were no soil samples collected during the winter months when the soil was frozen.

Soils were frozen immediately following sampling. Prior to soil sample analysis, soils were initially thawed in a fridge and then air-dried, ground with a mortar and pestle, and sieved using a 0.5 mm sieve. Soil extracts for NH_4^+ and NO_3^- were prepared using 10 g of soil mixed with 50 mL of 2.0 M KCl. The solution was shaken on a reciprocating shaker for 15 minutes at 180 rpm, and extracted using Whatman 42 filter paper. Soil extracts were analyzed on a Shimadzu 1800 UV-Vis Spectrophotometer (Shimadzu Corp., Kyoto, Japan). NH_4^+ concentrations were analyzed at 650 nm after 1 hour of color development (Verdow et al., 1978; Foster, 1995), while NO_3^- concentrations were analyzed at 540 nm 12 hours after color development (Miranda et al., 2001; Doane and Horwath, 2003).

Soil temperature and moisture were determined at the same time as GHG sampling using a HH2-WET Sensor (Delta T Devices, Cambridge, UK). Soil temperatures in the winter were assessed with thermocouples, constructed from type T thermocouple wire, and measured using an Acorn® Temp JKT Thermocouple Thermometer (Oakton Instruments, Vernon Hills, USA). There were no soil moisture recordings in winter. Ambient air temperature and precipitation from May 2014 to January 2015, and March 2015 to September 2015 was determined from the GTI weather station (43°33’03.41” N, 80°12’49.56” W). Air pressure and missing ambient

temperature and precipitation data was obtained from the nearest Environment Canada weather station at the Waterloo Airport (43°27'39.00" N, 80°22'43.00" W) and adjusted for the vertical pressure gradient using the difference in height above sea level at Guelph (Appendix A).

5.2.5 Statistical Analysis

Data was assessed for homogeneity of variance (Levene's test) and normality (Shapiro-Wilk Test for normality) (Steel et al., 1997). Data that violated the assumption of normality were log-transformed. Repeated measures analysis was conducted to determine if N_2O-N and CO_2-C emissions, and NH_4^+ and NO_3^- concentrations were significantly different between treatments and over time. In total, there were 48 sampling days from 12 sampling units (GHG chambers), resulting in an insufficient sample size to have adequate power for statistical inferences in repeated measures analysis. Thus, values from each sampling unit were averaged among seasons (spring 2014, summer 2014, autumn 2014, winter 2015, spring 2015 and summer 2015) and repeated measures analysis was conducted among seasons. Mauchley's test was conducted to determine if the data passed the assumption of sphericity. If the assumption of sphericity was violated, a Greenhouse-Geisser correction was applied to adjust the degrees of freedom.

Univariate ANOVA analyses were used to determine and compare the effect of fertilizer application, sampling year and season on N_2O-N and CO_2-C emissions, and NH_4^+ and NO_3^-. Tukey's HSD test was used as a multiple comparison test to determine significant differences among seasons (Steel et al., 1997). Significant differences between treatments and years were determined according to the F statistic. Linear regression was used to assess the relationship between N_2O-N and soil temperature, soil moisture and NO_3^- and NH_4^+ concentrations, CO_2-C emissions and soil temperature, soil moisture and NO_3^- and NH_4^+, and NO_3^- and NH_4^+ and soil temperature and moisture. The student *t* test was used to quantify differences between soil

temperature and soil moisture. All statistical analysis was conducted on SPSS Statistics for Windows Version 22.0 (IBM Corp., 2013). The significance threshold for all analyses was $p < 0.05$.

5.3 Results

5.3.1 Carbon dioxide Emissions

Carbon dioxide emissions differed significantly over time [$F(5,50) = 72.197$, $p < 0.001$], ranging from -4.87 to 226.54 mg CO_2-C m^{-2} h^{-1} with a mean of 79.56 ± 8.51 mg CO_2-C m^{-2} h^{-1} in fertilized treatments, and from -25.33 to 292.71 mg CO_2-C m^{-2} h^{-1} with a mean of 99.92 ± 10.0 mg CO_2-C m^{-2} h^{-1} in unfertilized treatments (Figure 5.1). Over the 76-week sampling period, there was a significant treatment-by-time interaction [$F(5,50) = 2.485$, $p = 0.044$], with elevated CO_2-C emissions in summer months. Treatment also had a significant effect [$F(1,10) = 6.171$, $p = 0.032$], with higher emissions from unfertilized treatments.

The main effect of season on CO_2 emissions was significant [$F(3,553) = 106.976$, $p < 0.001$] (Table 5.2). The highest CO_2-C emissions occurred in the summer in both treatments in 2014 and 2015. There were significantly higher CO_2-C emissions in unfertilized treatments [$F(1,553) = 14.251$, $p < 0.001$] than in fertilized treatments. Year was not significant, and the only significant difference within similar treatments across years was between the spring unfertilized treatments in 2014 and 2015 (Table 5.1).

The treatment-by-season-by-year interaction was not significant. The interaction of season-by-treatment [$F(3,553) = 3.669$, $p = 0.012$], season-by-year [$F(1,553) = 6.642$, $p = 0.010$], and treatment-by-year [$F(1,553) = 4.379$, $p = 0.037$] were all significant (Table 5.2). Simple effects showed significantly greater CO_2-C emissions in the spring and summer from unfertilized treatments than from fertilized treatments in both 2014 and 2015, but not in autumn or winter

(Table 5.1). There were significantly greater CO_2-C emissions in summer 2015 than in summer 2014 (Table 5.1).

There was a significant relationship between soil temperature and CO_2-C emissions over the entire sampling period ($r^2 = 0.429$, $p < 0.001$). In particular, CO_2-C emissions decreased with temperature, and were significantly related to autumn temperatures in 2014 in both fertilized and unfertilized treatments (Table 5.3). In 2015, soil temperature was significantly positively related to both fertilized and unfertilized emissions in the spring, while there were no significant relationships between soil temperature and CO_2-C emissions in the summer (Table 5.3).

There was a significant relationship between soil moisture and CO_2-C emissions over the entire sampling period ($r^2 = 0.021$, $p = 0.003$). When separated by season, treatment and year, there was only one occurrence of a significant relationship between soil moisture and CO_2-C emissions in 2014, which was in the autumn in fertilized treatments (Table 5.3). However, in 2015, soil moisture was significantly positively related to CO_2-C emissions in all seasons and treatments (Table 5.3).

In 2014, soil CO_2-C emissions in fertilized treatments were significantly related to soil NO_3^- concentrations in summer and spring, while emissions were only significantly related to NH_4^+ in the summer. CO_2-C emissions were not significantly related to NO_3^- or NH_4^+ in unfertilized treatments across all seasons (Table 5.3). In 2015, soil CO_2-C emissions were significantly related to soil NO_3^- concentrations in spring and summer in both treatments, with the exception of the fertilized treatments in the summer (Table 5.3). Soil CO_2-C emissions were significantly related to soil NH_4^+ in the unfertilized spring treatment and the fertilized summer treatment (Table 5.3).

Table 5. 1 2014 and 2015 mean seasonal CO_2 emissions (mg CO_2-C m^{-2} h^{-1}) from *S. miyabeana* (SX67) in fertilized and unfertilized treatments in southern Ontario, Canada.

	2014		2015	
Season	Fertilized	Unfertilized	Fertilized	Unfertilized
Spring	107.8 (6.3) ^{A,a}	113.4 (7.0) ^{AB,a}	88.9 (10.7) ^{A,a}	113.4 (10.7) ^{B,a}
Summer	107.1 (6.0) ^{A,b}	129.2 (7.0) ^{A,a,} *	115.1 (7.0) ^{A,b}	167.0 (10.9) ^{A,a} *
Autumn	60.4 (7.2) ^{B,a}	85.18 (11.5) ^{B,a}	n/a	n/a
Winter	n/a	n/a	1.2 (1.8) ^{B,a}	0.4 (0.9) ^{C,a}

^{A} Means followed by the same uppercase letter are not significantly different among seasons
^{a} Means followed by the same lowercase letter are not significantly different between treatments within year
* Means are significantly different between the same (fertilized and fertilized or unfertilized and unfertilized) treatments between different years

Table 5. 2 P-values of analysis of variance (ANOVA) and interactions for CO_2-C, N_2O-N, NH_4^+ and NO_3^- concentrations in willow (*S. miyabeana*) biomass plantations in Guelph, Ontario, Canada

Factors	CO_2-C	N_2O-N	NH_4^+	NO_3^-
Season (S_N)	< 0.001	< 0.001	0.001	< 0.001
Treatment (T_R)	< 0.001	0.035	< 0.001	< 0.001
Year (Y_R)	n.s.	n.s.	< 0.001	< 0.001
$S_N \times T_R \times Y_R$	n.s.	n.s	n.s.	n.s.
$T_R \times Y_R$	0.037	n.s	n.s.	n.s.
$S_N \times T_R$	0.012	0.001	0.037	n.s.
$S_N \times Y_R$	0.010	n.s.	< 0.001	0.006

Table 5. 3 Linear regression coefficient of determination (**r^2**) values for CO_2-C and N_2O-N emissions and soil characteristics in spring, summer and autumn in willow (*S. miyabeana*) SRC biomass plantations. There were no GHG emission data for autumn 2015.

Season	Treatment		2014				2015			
			NH_4^+	NO_3^-	Soil Temperature	Soil Moisture	NH_4^+	NO_3^-	Soil Temperature	Soil Moisture
Spring	Fertilized	CO_2-C	0.001	0.008	0.014	0.008	0.002	**0.108***	**0.357***	**0.100***
		N_2O-N	0.008	0.000	0.008	0.000	0.000	0.007	**0.126***	0.001
	Unfertilized	CO_2-C	0.001	0.019	0.048	0.001	**0.213***	**0.098***	**0.216***	**0.115***
		N_2O-N	0.044	0.028	0.050	0.048	0.011	0.021	**0.077***	**0.063***
Summer	Fertilized	CO_2-C	**0.150***	**0.123***	0.005	0.067	**0.161***	0.021	0.025	**0.136***
		N_2O-N	0.003	0.022	0.003	0.000	0.003	0.004	0.030	0.000
	Unfertilized	CO_2-C	0.008	0.014	0.080	0.001	0.036	**0.172***	0.054	**0.310***
		N_2O-N	0.009	0.000	0.104	0.067	0.009	0.001	0.001	0.005
Autumn	Fertilized	CO_2-C	0.009	**0.309***	**0.387***	**0.420***				
		N_2O-N	0.031	0.022	**0.358***	0.066				
	Unfertilized	CO_2-C	0.918	0.150	**0.221***	0.195				
		N_2O-N	0.018	0.000	0.009	0.116				

***Denotes a statistically significant relationship ($p < 0.05$)

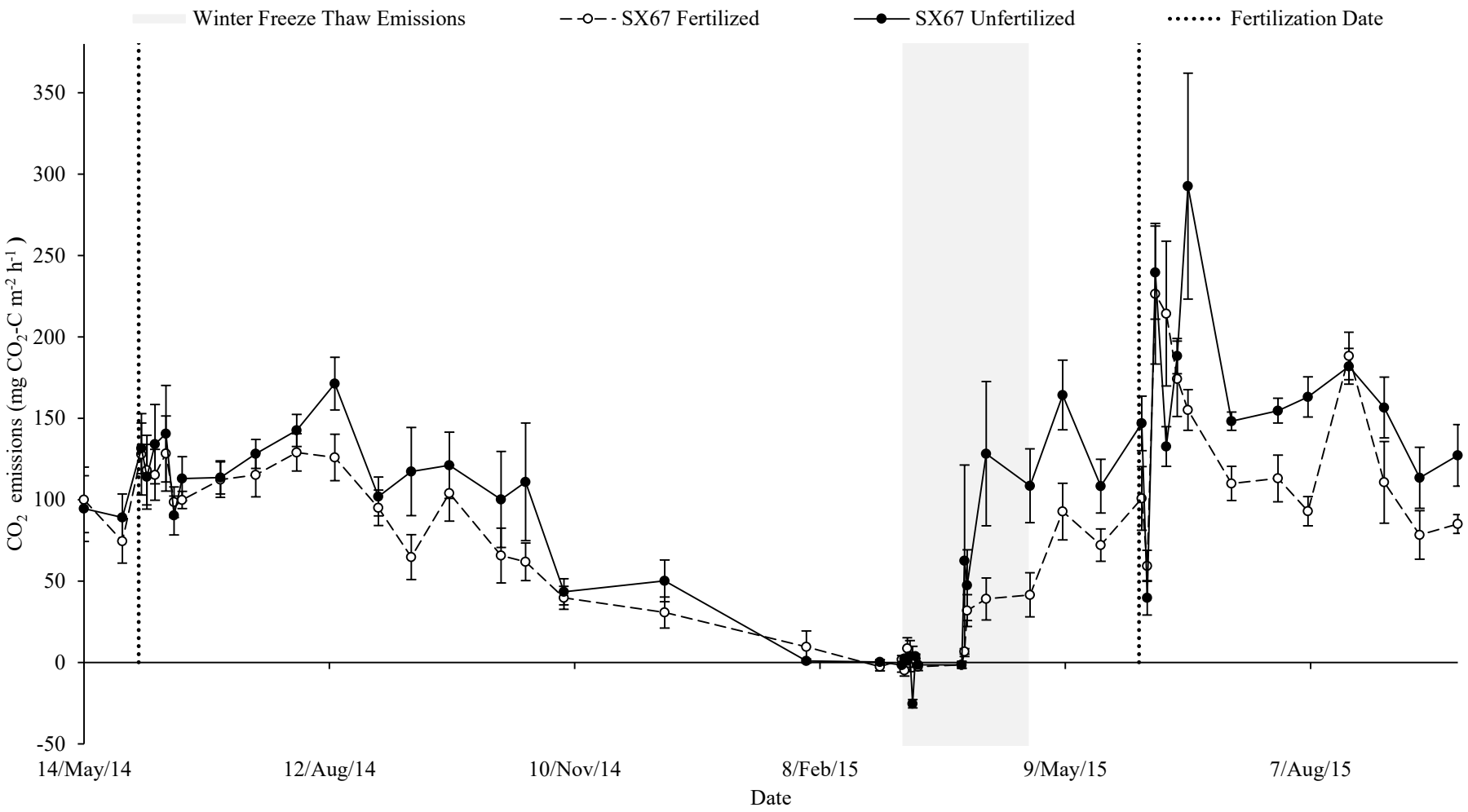

Figure 5. 1 Mean soil CO_2-C emissions (mg CO_2-C m^{-2} h^{-1}) from May 14, 2014 to September 30, 2015 from fertilized and unfertilized willow (*S. miyabeana*) plantations at the University of Guelph Turfgrass Institute in Guelph, Ontario, Canada

5.3.2 Nitrous Oxide Emissions

N_2O-N values ranged from -6.9 to 87.54 μg N_2O-N m^{-2} h^{-1} in fertilized treatments and from -2.72 to 39.9 μg N_2O-N m^{-2} h^{-1} in unfertilized treatments. The mean value of N_2O-N emissions in fertilized treatments was 24.33 ± 2.46 μg N_2O-N m^{-2} h^{-1}, and was 17.82 ± 1.33 μg N_2O-N m^{-2} h^{-1} in unfertilized treatments. N_2O-N emissions differed significantly over the 76-week sampling period [$F(2.383, 23.827) = 8.079$, $p = 0.001$] (Figure 5.2); there were significantly higher emissions in fertilized treatments following fertilizer applications [$F(1,10) = 6.913$, $p = 0.025$]. The effect of season on N_2O-N emissions was significant [$F(3,561) = 14.786$, $p < 0.001$], such that emissions were significantly greater in the spring when fertilizer was applied. The treatment effect was also significant; fertilized treatments had significantly greater N_2O-N emissions than unfertilized treatments [$F(1,561) = 4.476$, $p = 0.035$]. There were no significant differences in N_2O-N emissions between years (Table 5.4).

Only the season-by-treatment interaction was significant for N_2O-N emissions [$F(3,561) = 5.469$, $p = 0.001$] (Table 5.2). In spring, fertilizer treatment resulted in significantly higher N_2O-N emissions [$F(1,561) = 27.388$, $p < 0.001$] than from unfertilized treatments in both years (Table 5.4). During summer, autumn and winter, there were no differences in N_2O-N emissions between treatments (Table 5.4).

There was a significant relationship between soil temperature and N_2O-N emissions ($r^2 = 0.035$, $p < 0.001$) throughout the entire sampling period. When divided by treatment, year and season, there was only a significant relationship between temperature and N_2O-N emissions in spring 2015 fertilized and unfertilized treatments, and in the autumn 2014 fertilized treatment (Table 5.3). Over the entire experiment, N_2O-N

emissions were not related to soil moisture ($r^2 = 0.001$, $p = 0.545$). Comparisons across seasons, treatments and years, revealed only one significant relationship between N_2O-N emissions and soil moisture, in the spring 2015 unfertilized treatment (Table 5.3).

There was a significant relationship between NH_4^+ concentrations and N_2O-N emissions ($r^2 = 0.024$, $p = 0.002$) during the entire sampling period. However, when divided by season, treatment and year, there were no significant relationships between NH_4^+ concentrations and N_2O-N emissions. N_2O-N emissions were not significantly related to NO_3^- concentrations ($r^2 = 0.010$, $p = 0.051$) (Table 5.3).

Table 5. 4 2014 and 2015 mean seasonal N_2O emissions (μg N_2O-N m^{-2} h^{-1}) from *Salix miyabeana* (SX67) in fertilized and unfertilized treatments in southern Ontario, Canada.

	2014		2015	
Season	Fertilized	Unfertilized	Fertilized	Unfertilized
Spring	38.3 (5.9)^{A,a}	20.2 (2.0)^{A,b}	34.1 (4.2)^{A,a}	19.7 (1.9)^{A,b}
Summer	23.4 (2.7)^{B,a}	18.3 (4.1)^{A,a}	19.6 (2.1)^{B,a}	20.2 (2.3)^{A,a}
Autumn	11.5 (2.7)^{C,a}	9.5 (6.7)^{A,a}	n/a	n/a
Winter	n/a	n/a	10.6 (2.4)^{B,a}	14.6 (3.1)^{A,a}

[A] Means followed by the same uppercase letter are not significantly different among seasons

[a] Means followed by the same lowercase letter are not significantly different between treatments within year

* Means are significantly different between the same (fertilized and fertilized or unfertilized and unfertilized) treatments between years

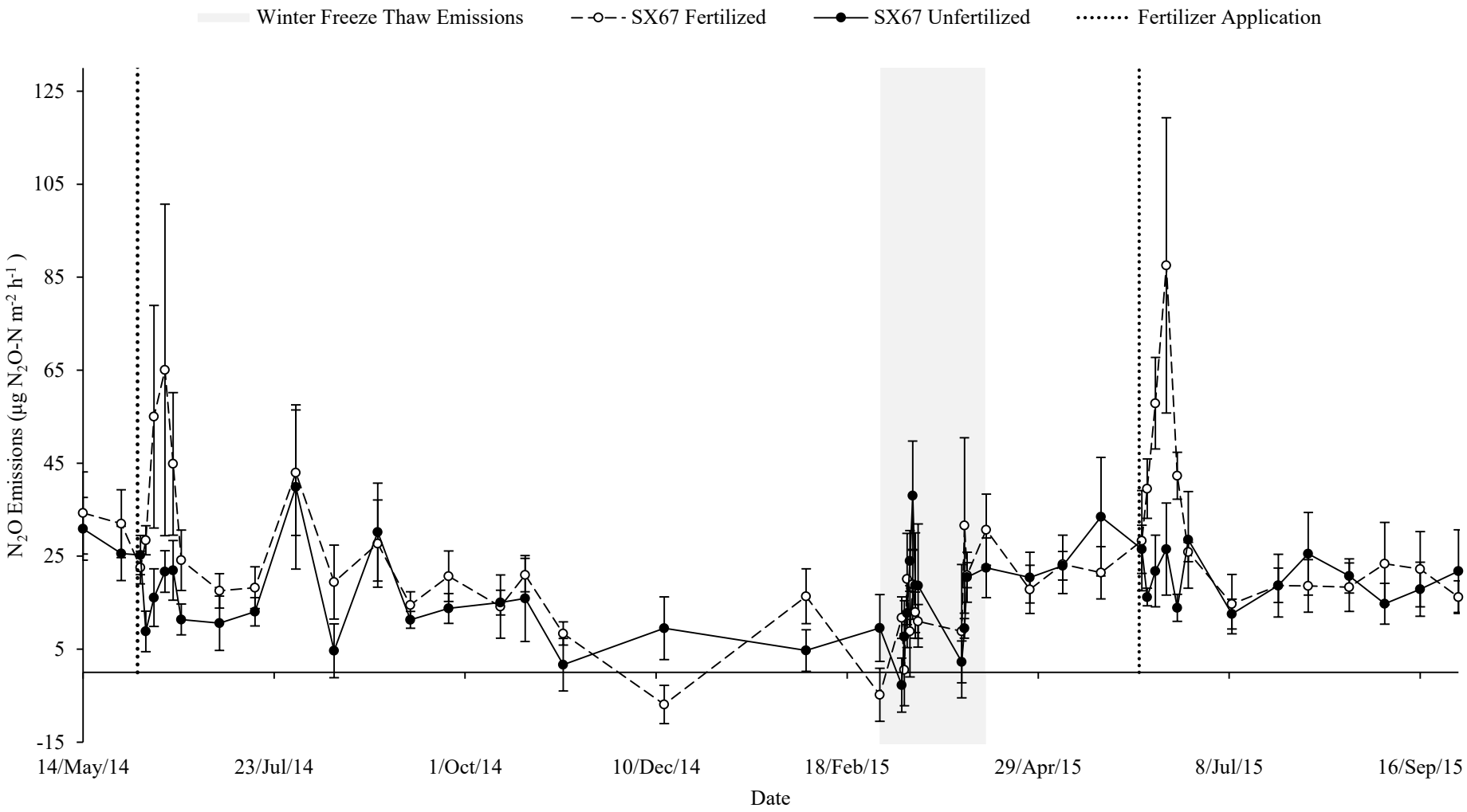

Figure 5. 2 Mean soil N_2O-N emissions (µg N_2O-N m^{-2} h^{-1}) from fertilized and unfertilized treatments from willow (*S. miyabeana*) plantations in Guelph, Ontario, Canada, spanning over two field seasons, from May 14 2014 to September 30 2015.

5.3.3 Freeze Thaw and Winter Emissions

Winter emissions of CO_2 (from February 3, 2015 to April 26, 2015) from fertilized treatments ranged from -4.87 to 39.04 mg CO_2-C m^{-2} h^{-1} with a mean of 9.79 ± 4.20 mg CO_2-C m^{-2} h^{-1}. Emissions from unfertilized treatments ranged from -25.32 to 128.29 mg CO_2-C m^{-2} h^{-1} with a mean of 23.53 ± 13.11 mg CO_2-C m^{-2} h^{-1}. Instantaneous N_2O-N winter emission from fertilized treatments ranged from 4.81 to 31.61 μg N_2O-N m^{-2} h^{-1}, with a mean of 14.69 ± 2.69 μg N_2O-N m^{-2} h^{-1}. Unfertilized treatments ranged from -2.71 to 38.04 μg N_2O-N m^{-2} h^{-1}, with a mean of 14.75 ± 2.77 μg N_2O-N m^{-2} h^{-1}.

The first FTC spanned over 8 sampling dates from March 2 to March 16 2015 (Figure 5.3). For CO_2-C emissions, there was no significant time effect, no significant treatment effect, and no significant time-by-treatment interaction. With regard to N_2O-N emissions for the first freeze thaw event, there was a significant effect of time [$F(7,70) = 2.288$, $p = 0.037$], but no significant treatment effect or time-by-treatment interaction.

The second freeze thaw event was spread across 5 sampling days from April 1 to April 26, 2015 (Figure 5.3). CO_2-C emissions differed significantly over time [$F(1.553,15.533) = 4.031$, $p = 0.048$], and there was a significant treatment effect [$F(1,10) = 5.590$, $p = 0.040$], with significantly greater emissions from unfertilized treatments (Figure 5.3c). There was no significant time-by-treatment interaction. For N_2O-N emissions there was no significant effect of time or treatment, and no significant time-by-treatment interaction. During the entire winter, soil temperature was not significantly related to CO_2-C fertilized ($r^2 = 0.019$) or unfertilized ($r^2 = 0.012$) treatments, or N_2O-N fertilized ($r^2 = 0.008$) or unfertilized ($r^2 = 0.004$) treatments.

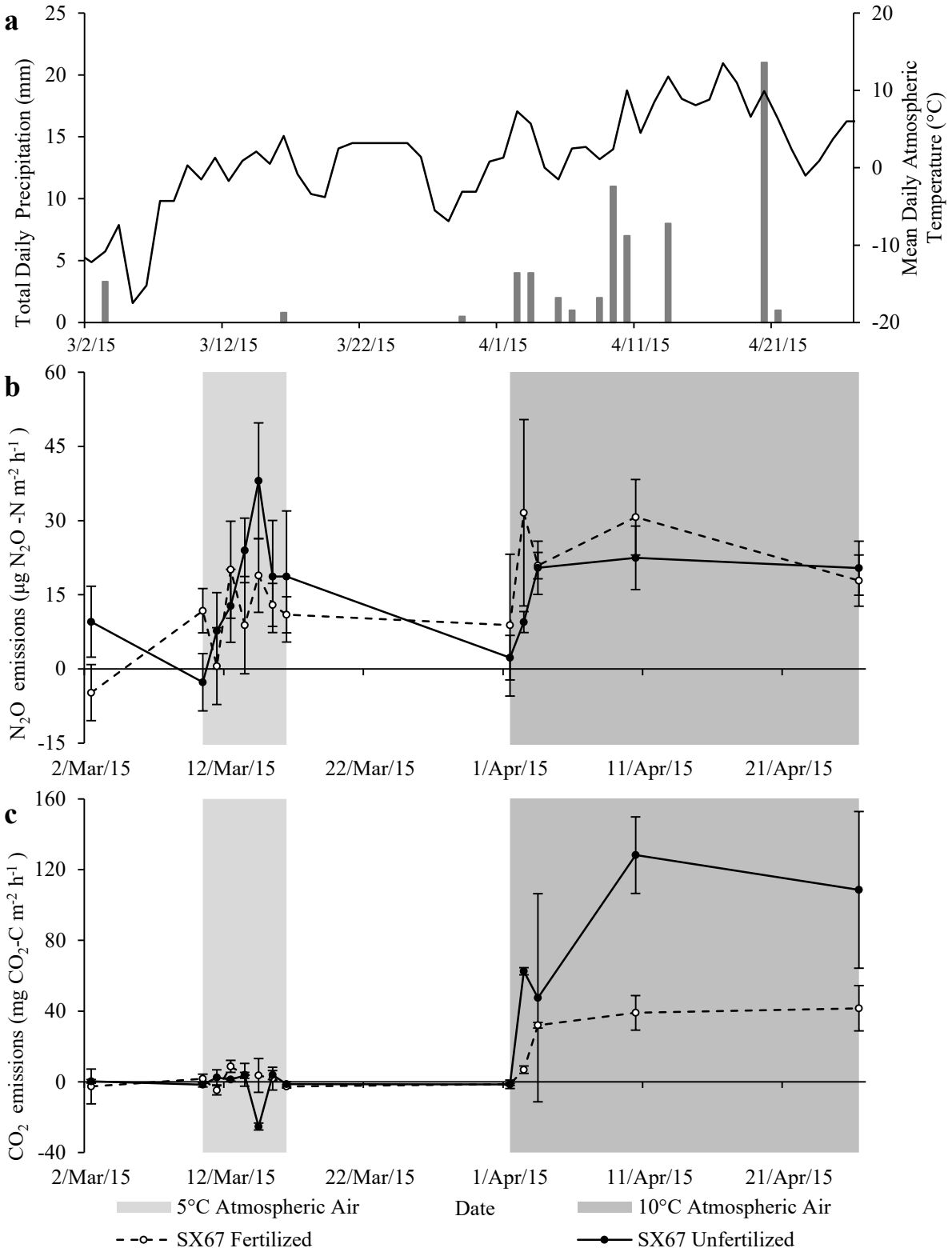

Figure 5. 3 Winter temperatures and precipitation (**a**), and CO_2-C (mg CO_2-C m^{-2} h^{-1}) (**c**) and N_2O-N (µg N_2O-N m^{-2} h^{-1}) (**b**) emissions from two freeze thaw cycle events (5°C and 10°C atmospheric daily high) from soils under willow (*S. miyabeana*).

5.3.4 NO_3^- Concentrations

Soil NO_3^- concentrations differed significantly over time [$F(4,16) = 25.626$, $p < 0.001$]. There were higher NO_3^- concentrations from fertilized than unfertilized treatments in both years [$F(1,192) = 45.497$, $p < 0.001$], spring had significantly higher NO_3^- concentrations than the summer and autumn [$F(2,192) = 31.458$ $p < 0.001$], and there were significantly greater soil NO_3^- concentrations in 2014 than in 2015 [$F(1,192) = 112.533$ $p < 0.001$]. There was a significant season by year interaction [$F(1,192) = 7.708$ $p = 0.006$] (Table 5.2). Assessment of simple effects determined that there were significant differences between 2014 and 2015 NO_3^- concentrations in both the spring [$F(1,192) = 34.773$, $p < 0.001$] and the summer [$F(1,192) = 80.116$, $p < 0.001$] (Table 5.5; Figure 5.4).

Soil NO_3^- concentrations were significantly related to soil temperature in the fall 2014 unfertilized treatment ($r^2 = 0.415$, $p = 0.001$), in the spring 2014 unfertilized treatment ($r^2 = 0.086$, $p = 0.049$), and in the spring 2015 fertilized treatment ($r^2 = 0.107$, $p = 0.016$). NO_3^- concentrations were significantly related to soil moisture in autumn of 2014 in both fertilized ($r^2 = 0.261$, $p = 0.030$) and unfertilized ($r^2 = 0.417$, $p = 0.004$) treatments, and in summer 2015 in both fertilized ($r^2 = 0.142$, $p = 0.008$) and unfertilized treatments ($r^2 = 0.116$, $p = 0.022$).

NH_4^+ concentration was a significant predictor of NO_3^- concentration in spring fertilized treatments in both 2014 ($r^2 = 0.094$ $p = 0.002$), and 2015 ($r^2 = 0.190$ $p = 0.024$). There was also a significant relationship between NH_4^+ concentrations and NO_3^- concentrations in the summer 2015 fertilized ($r^2 = 0.289$, $p < 0.001$) unfertilized ($r^2 = 0.098$, $p = 0.034$) treatments.

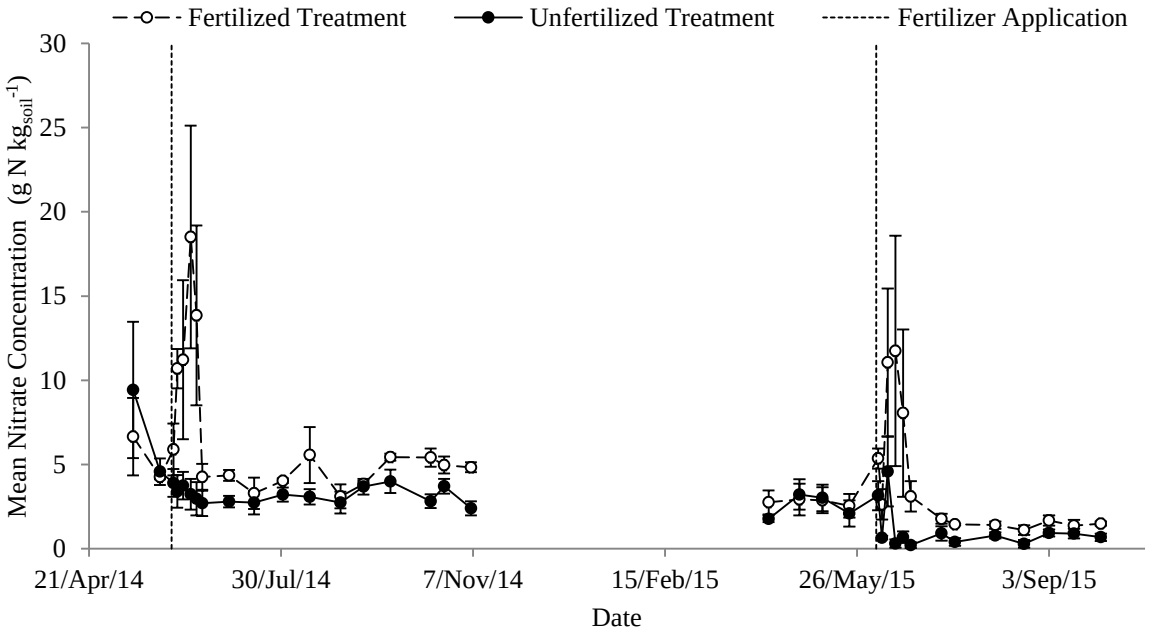

Figure 5. 4 2014 and 2015 mean soil NO_3^- concentrations (g N kg_{soil}^{-1}) from fertilized and unfertilized SX67 (*S. miyabeana*) treatments at the University of Guelph Turfgrass Institute in Guelph, Ontario.

Table 5. 5 Mean soil NO_3^- and NH_4^+ concentrations (g N kg_{soil}^{-1}) from *Salix miyabeana* fertilized and unfertilized treatments over 26 weeks in 2014 and 25 weeks in 2015 in southern Ontario, Canada.

		2014		2015	
NO_3^-	Season	Fertilized	Unfertilized	Fertilized	Unfertilized
	Spring	9.4 (1.5)^{A,a,*}	4.2 (0.6)^{A,b,*}	5.6 (1.4)^{A,a,*}	2.2 (0.4)^{A,b,*}
	Summer	4.0 (0.4)^{B,a,*}	3.0 (0.2)^{A,b,*}	1.7 (0.2)^{B,a,*}	0.6 (0.1)^{B,b,*}
	Fall	5.2 (0.2)^{B,a,}	3.2 (0.3)^{A,b,}	n/a	n/a
NH_4^+					
	Spring	9.2 (1.8)^{A,a}	3.1 (0.1)^{A,b,*}	14.4 (3.5)^{A,a}	6.8 (1.1)^{B,b,*}
	Summer	2.4 (0.2) ^{B,a,*}	1.7 (0.1)^{B,b,*}	13.6 (0.6)^{A,a,*}	12.3 (0.5)^{A,a,*}
	Fall	2.1 (0.38) ^{B,b}	1.6 (0.1)^{B,b}	n/a	n/a

[A] Means followed by the same upper letter are not significantly different among seasons
[a] Means followed by the same lowercase letter are not significantly different between treatments within year
* Means are significantly different between the same (fertilized and fertilized or unfertilized and unfertilized) treatments between different years

5.3.5 NH_4^+ Concentrations

Soil NH_4^+ concentrations significantly differed over time [$F(1.480, 5.920) = 23.219$, $p = 0.002$]. Concentrations were significantly higher in spring than in summer and autumn [$F(2,200) = 6.774$, $p = 0.001$], and there were greater NH_4^+ concentrations occurring in 2015 than in 2014 [$F(1,200) = 155.868$, $p < 0.001$]. Overall, there were significantly greater concentrations in fertilized treatments [$F(1,200) = 14.069$, $p < 0.001$] (Table 5.5).

The interactions of season-by-treatment [$F(2,200) = 3.347$, $p = 0.037$] and season-by-year were significant [$F(1,200) = 62.644$, $p < 0.001$] (Table 5.2). Contrast of simple effects between treatments determined that there were significantly greater [$F(1,200) = 26.843$, $p < 0.001$] NH_4^+ concentrations in fertilized treatments in the spring (Figure 5.5), while there were no significant differences between treatments in the summer and autumn in both years. There were significantly greater NH_4^+ concentrations in 2015 than 2014 in both spring [$F(1,200) = 11.670$, $p = 0.001$] and summer [$F(1,200) = 188.253$, $p < 0.001$] (Table 5.5; Figure 5.5).

In 2014, NH_4^+ concentrations were significantly correlated to temperature in the spring unfertilized ($r^2 = 0.203$, $p = 0.002$) treatment, and to soil moisture in both treatments in summer ($r^2_{fertilized} = 0.396$, $p < 0.001$; $r^2_{unfertilized} = 0.705$, $p < 0.001$). In 2015, soil NH_4^+ concentrations were significantly related to soil temperature in spring fertilized ($r^2 = 0.165$, $p = 0.002$) and unfertilized ($r^2 = 0.314$, $p < 0.001$) treatments, and in summer fertilized ($r^2 = 0.286$, $p < 0.001$) and unfertilized ($r^2 = 0.146$, $p = 0.008$) treatments. NH_4^+ concentrations were not related to soil temperature or moisture in the autumn.

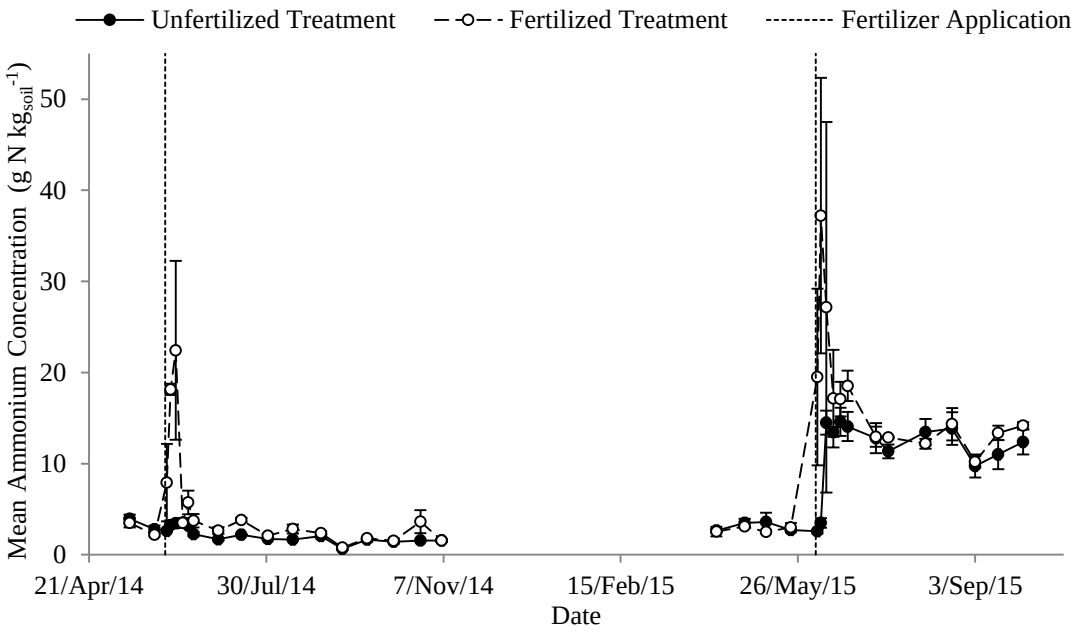

Figure 5. 5 Mean soil NH_4^+ concentrations from fertilized and unfertilized willow clone SX67 (S. miyabeana) treatments over 2014 and 2015 at the University of Guelph Turfgrass Institute in Guelph, Ontario, Canada.

5.3.6 Soil Temperature and Moisture

Over the duration of the entire study, there were no significant differences between soil moisture from fertilized and unfertilized treatments in 2014 [t(198) = -1.067, p = 0.287], or 2015 [t(225) = -1.546, p = 0.124] (Table 5.6). However, when separated by season and year, unfertilized treatments had significantly higher soil moisture concentrations than fertilized treatments in summer 2015 [t(93) = -2.199, p = 0.030] and in fall 2014 [t(34) = -2.464, p = 0.019]. Overall, there were no significant differences between soil temperature from fertilized and unfertilized treatments in 2014 [t(222) = 0.050, p = 0.960] and 2015 [t(345) = 0.007 p = 0.995] (Table 5.6), and no significant differences in soil temperature between treatments across seasons.

There were significant differences in soil temperature between 2014 and 2015 fertilized treatments [t(282.005) = 8.274, p < 0.001] and unfertilized treatments [t(282.088) = 8.232, p < 0.001] (Table 5.6). There were also significant differences

between soil moisture in 2014 and 2015 in fertilized treatments [$t(178.212) = -2.997$, $p < 0.001$], and unfertilized treatments [$t(195.066) = -4.077$, $p < 0.001$] (Table 5.6). Although soil temperatures appear to be significantly lower in 2015 (Table 5.6), this is due to the inclusion of winter temperature data, as winter sampling occurred in 2015.

Table 5. 6 Mean soil temperature and moisture from 2014 and 2015 in the willow clone SX67 in fertilized and unfertilized treatments over 76 weeks.

Soil Characteristic†	2014		2015	
	Fertilized	Unfertilized	Fertilized	Unfertilized
Soil Moisture (% vol)	23.60 (0.52) [a,*]	24.44 (0.59)[a,*]	26.73 (0.91) [a,*]	28.65 (0.85) [a,*]
Soil Temperature (°C)	16.86 (0.54) [a,*]	16.82 (0.53) [a,*]	10.11 (0.62) [a,*]	10.10 (0.62) [a,*]

†Soil temperature readings were taken from May 2014 to September 2015. No soil moisture readings were taken from December 2014 to April 2015 as the ground was frozen.
[a] Means followed by the same lowercase letter are not significantly different between treatments within year
* Means are significantly different between the same (fertilized and fertilized or unfertilized and unfertilized) treatments between different years

5.3.7 Total Annual Emissions

Over the duration of the entire experiment (from May 14, 2014 to September 30, 2015), total CO_2 emissions were 33.55 Mg CO_2 ha^{-1} from fertilized treatments, while the total CO_2 emissions were 44.62 Mg CO_2 ha^{-1} from unfertilized treatments. Total N_2O emissions through the entire experiment measured 1.1 Mg CO_{2eq} ha^{-1} from fertilized treatments, of which 25.4% was occurred following fertilizer applications. Conversely, N_2O from unfertilized treatments was 0.90 Mg CO_2-eq ha^{-1}. In total, 34.60 Mg CO_2-eq ha^{-1} was emitted from fertilized treatments, and 45.53 Mg CO_2-eq ha^{-1} from unfertilized treatments.

The total annual CO_2 emissions (May 14, 2014 to May 22, 2015) from fertilized and unfertilized treatments were 19.73 Mg CO_2 ha^{-1} yr^{-1} and 26.30 Mg CO_2 ha^{-1} yr^{-1}, respectively, while the average daily instantaneous emissions from fertilized and unfertilized treatments during this period were 221.00 mg CO_2 m^{-2} h^{-1} and 283.98 mg CO_2 m^{-2} h^{-1}. 7.2% and 13.4% of total CO_2 emissions from fertilized and unfertilized treatments were derived from winter FTCs. Total annual N_2O emissions from fertilized and unfertilized treatments were 0.70 Mg CO_2-eq ha^{-1} yr^{-1} and 0.60 Mg CO_2-eq ha^{-1} yr^{-1}, respectively, while the average daily instantaneous emission of N_2O was 10.09 mg CO_2-eq m^{-2} h^{-1} from fertilized treatments and 7.90 mg CO_2-eq m^{-2} h^{-1} from unfertilized treatments. On an annual basis, 17.6% and 21.7% of N_2O-N emissions occurred in winter in fertilized treatments and unfertilized treatments, respectively.

The total annual emissions from May 14, 2014 to May 22, 2015 were 20.43 Mg CO_2-eq ha^{-1} yr^{-1} from fertilized treatments, and 26.90 Mg CO_2-eq ha^{-1} yr^{-1} from unfertilized treatments. Annual willow biomass was 10.24 ± 1.86 odt ha^{-1} from fertilized treatments, with 18.78 Mg CO_2-eq ha^{-1} yr^{-1} as aboveground C, and 11.27 Mg CO_2-eq ha^{-1} yr^{-1} as belowground C in roots. Total litter fall C amounted to 1.17 Mg CO_2-eq ha^{-1} yr^{-1} (D. Walter, personal communication, August 26, 2016). Thus, total C sequestration in fertilized treatments was 31.23 Mg CO_2-eq ha^{-1} yr^{-1}. Net emissions from fertilized treatments amounted to -10.79 Mg CO_2-eq ha^{-1} yr^{-1}. Unfertilized treatments biomass amounted to 8.33 ± 0.97 odt ha^{-1}, with 15.29 Mg CO_2-eq ha^{-1} yr^{-1} as aboveground C, and 9.17 Mg CO_2-eq ha^{-1} yr^{-1} as belowground C. Litter fall C amounted to 1.25 Mg CO_2-eq ha^{-1} yr^{-1} (D. Walter, personal communication, August 26, 2016). Thus, total C

sequestration amounted to 25.71 Mg CO_2-eq ha^{-1} yr^{-1}, while net emissions under unfertilized treatments were 1.19 Mg CO_2-eq ha^{-1} yr^{-1}.

5.4 Discussion

5.4.1 Soil CO_2 Emissions

Carbon dioxide emissions reported here are similar to temperate willow plantation emissions in other studies. Drewer et al. (2012) quantified mean respiration under unfertilized willow (*Salix* spp.) from June 2008 to November 2010, as 81.8 mg CO_2-C m^{-2} h^{-1}, while this study's mean CO_2 efflux (from May 2014 to September 2015) was 89.74 mg CO_2-C m^{-2} h^{-1}. Gauder et al. (2011) found that annual respiration values from fertilized and unfertilized willow (*S. schwerinii* x *S. viminais*) in Germany ranged from 0.3 to 217.1 mg CO_2-C m^{-2} h^{-1}, while this study ranged from -25.32 to 171.31 mg CO_2-C m^{-2} h^{-1}. Pacaldo et al. (2013) estimated the range of soil respiration under unfertilized willow (*S. dasyclados*) during the growing season (May to September) as 155.5 to 203.0 mg CO_2-C m^{-2} h^{-1}. Growing season emissions had a broader range in this study, varying from 64.8 to 171.3 mg CO_2-C m^{-2} h^{-1} in 2014, and 39.7 to 292.7 mg CO_2-C m^{-2} h^{-1} in 2015. Drewer et al. (2012), Pacaldo et al. (2013), and Lutes et al. (2016) described the same overall trend in annual CO_2-C effluxes (Figure 5.1) with elevated CO_2-C emissions in the growing season relative to the dormant seasons (autumn and winter).

Seasonal soil CO_2 effluxes are often solely attributed to heterotrophic respiration, which is cited to depend on soil temperatures (Ryan and Law, 2005; Gomez-Casanovas et al., 2012). In the present study, there were elevated soil CO_2-C emissions in summer 2015 relative to summer 2014, which was significantly greater in unfertilized treatments (Table 5.1). Mean ambient temperatures were ~2°C higher in summer 2015 than in 2014

(Appendix A). However, there was no significant difference between mean summer soil temperatures (which were 19.53 ± 1.07°C in 2014, and 18.37 ± 1.19°C in 2015), due to the dense willow canopy acting as a shading buffer (Clinch et al., 2009; Gauder et al., 2011). Furthermore, CO_2-C emissions did not have a significant relationship with soil temperature in summer 2015 fertilized ($r^2 = 0.025$) or unfertilized treatments ($r^2 = 0.054$). Thus, significant differences between CO_2-C emissions in summer 2014 and 2015 cannot be solely attributed to soil temperature differences affecting microbial respiration. Soil respiration is also often attributed to the availability of SOC (Ludwig et al., 2004; Henstchel et al., 2008; Kurganova and Lopes de Gerenyu, 2015; Hangs et al., 2016). However, SOC changes little annually, and short-term changes in belowground C do not largely contribute to yearly changes in soil respiration (Ryan and Law, 2005). Therefore elevated 2015 CO_2-C emissions relative to 2014 CO_2-C emissions cannot be attributed to greater SOC availability.

Total soil respiration is generally comprised of heterotrophic respiration from microbial biomass and soil fauna, and autotrophic respiration from roots and root-associated organisms (Hopkins et al., 2013; Ngao et al., 2005). Generally, root respiration can contribute 50-65% of total soil respiration (Högberg et al., 2001; Bhupinderpal-Singh et al., 2003; Ngao et al., 2005), but is highly variable, with estimates ranging from 10 to 90% (Hanson et al., 2000; Bhupinderpal-Singh et al., 2003). Photosynthesis, which increases allocation of C to tree roots, is positively correlated to root respiration (Janssens et al., 2000; Högberg et al., 2001; Ryan and Law, 2005; Hopkins et al., 2013). In temperate systems, maximum vegetative productivity and photosynthesis occurs when ambient temperatures are highest (Hopkins et al., 2013), resulting in greater root

respiration (Högberg et al., 2001). Therefore, as ambient air temperatures were ~2°C higher in 2015 than in 2014, rates of photosynthesis were enhanced, which contributed to greater summer soil CO_2-C emissions in 2015.

Additionally, soil moisture availability and precipitation patterns are also considered main drivers of soil respiration (Wu et al., 2010; Gomez-Casanovas et al., 2012; Hopkins et al., 2013). Soil CO_2-C emissions had a significantly positive relationship with soil moisture in fertilized and unfertilized treatments during summer 2015, which was not observed in the summer of 2014. Furthermore, soil moisture was significantly greater in both treatments in 2015 than in 2014. Thus, preferable soil moisture conditions contributed to elevated CO_2-C emissions in 2015 than in 2014. In the summer of both years, there was also greater soil moisture in unfertilized treatments than in fertilized treatments, which was significantly different in 2015. Therefore, this likely contributed to increased CO_2-C emissions from unfertilized treatments relative to fertilized treatments.

5.4.2 Soil N_2O Emissions

Soil N_2O-N emissions are similar to values previously reported in willow biomass plantations (Gauder et al., 2011; Lutes et al., 2016). Gauder et al. (2011) reported values of annual N_2O-N efflux from willow ranging from -7.1 to 6.7 μg N_2O-N m^{-2} h^{-1}. In the present study, N_2O-N emissions ranged from -6.9 to 65.05 μg N_2O-N m^{-2} h^{-1} from May 2014 to May 2015, and had a much higher maximum value of N_2O emissions. However, Gauder et al. (2011) did not see any effect to willow emissions following fertilization, whereas there was a marked increase in N_2O-N emissions following fertilizer application

in this study (Figure 5.2). N_2O-N emissions are not significantly different from values reported by Lutes et al. (2016) (Table 5.4).

N_2O-N emissions peaked following fertilizer application in spring in 2014 and 2015, and there were no significant differences in N_2O-N emissions between both years (Table 5.4, Figure 5.2). Thus, there was a consistent response of N_2O-N emissions to fertilizer in both years. There was a significant relationship between NH_4^+ concentrations and N_2O-N emissions during the entire sampling period, and the highest N_2O-N emissions coincided with high levels of NH_4^+, frequent precipitation and increasing temperatures in the springtime (Abalos et al., 2015), creating favourable conditions for microbial activity (Figure 5.2 – 5.4). Other studies have also found that N_2O-N emissions are related to NH_4^+ emissions, rather than NO_3^- (Bremner and Blackmer, 1981; Khalil et al., 2004), suggesting that the emissions are nitrification-derived rather than denitrification-derived.

There were significant differences in NO_3^- and NH_4^+ concentrations in 2014 and 2015. Generally, the NH_4^+ concentrations were higher in 2015 than in 2014, while the NO_3^- concentrations were lower in 2015 than in 2014 (Table 5.5). NH_4^+ concentrations increased in both fertilized and unfertilized treatments in summer 2015, and therefore cannot be attributed to excess fertilizer-derived NH_4^+ build-up. However, the presence of elevated NH_4^+ can increase root activity, and may further explain why there were more CO_2-C emissions in summer 2015 (Rewald et al., 2014).

Ammonification or mineralization converts organic nitrogen into ammonia (NH_3), while NH_3 oxidizing bacteria increases NH_4^+ in the soil. Thus, there were likely higher levels of mineralization in 2015, resulting in higher NH_4^+ concentrations. Increased root

activity, indicated by the elevated soil respiration observed in 2015, may have increased willow root exudates and therefore N mineralization (Yin et al., 2014). However, NH_4^+ was not converted into NO_3^-, as NO_3^- levels remained low from July to September 2015 (Figure 5.4). Thus, nitrification or microbial nitrifier activity was seemingly inhibited during this period. As microbial pools and mineralization were not assessed in this study, this theory warrants further research.

5.4.3 Winter and FTC CO_2 Emissions

Low CO_2-C emissions from snow-covered soils in winter (Table 5.1) have been previously reported in willow SRC systems (Pacaldo et al., 2011). Here, mean dormant season respiration (October to April) ranged from -25.32 to 128.29 mg CO_2-C m^{-2} h^{-1}. Pacaldo et al. (2013) reported winter effluxes that were less variable, ranging from 38.4 to 51.8 mg CO_2-C m^{-2} h^{-1}. However, in their study the dormant season ranged from October to December (Pacaldo et al., 2013), excluding emissions from January to March, and therefore did not encompass low CO_2 effluxes from snow-covered soils, or higher CO_2 values from spring thaws. The mean CO_2-C emission in the dormant season was 16.66 mg CO_2-C m^{-2} h^{-1}.

Winter CO_2-C emissions only contributed to 7.2 to 13.4% of total annual emissions in this study, which is slightly above the proportion of winter emissions suggested by Brooks et al. (2011). The first FTC, when ambient air temperatures reached a daily maximum of 5°C (mean soil temperature 0.69°C), did not show marked increases in CO_2-C efflux (Figure 5.3c). During the second FTC, CO_2-C emissions began to substantially increase, as daily maximum temperatures reached 10°C (mean soil

temperature 6.32°C) (Figure 5.3c), suggesting that soil temperature must exceed 5°C to substantially increase CO_2-C emissions (Kurganova and Lopes de Gerenyu, 2015).

5.4.4 Winter and FTC Soil N_2O Emissions

In Southern Ontario, FTCs are often cited to create a pulse of N_2O-N from agricultural soils, which when combined with winter emissions, comprise 30% of total annual N_2O-N emissions from Canadian agricultural soils (Smith et al., 2004; Rochette et al., 2008). Generally, the mean N_2O-N emissions during winter and spring thaw in southern Ontarian agricultural soils were cited to range from 0.45 to 1.2 kg N_2O-N ha^{-1} (Wagner and Thurtell, 1998; Rochette et al., 2008; Wagner-Riddle et al., 2007). Emissions reported in this study represent lower emissions than average, as only 17.6 to 21.7% of total annual N_2O-N emissions were derived from winter emissions and FTCs from fertilized and unfertilized treatments, respectively. This amounted 0.31 kg N_2O-N ha^{-1} from fertilized treatments and 0.31 kg N_2O-N ha^{-1} from unfertilized treatments. Lower than average N_2O-N emissions were attributed to the perennial presence of willow as a cover crop preventing FTC emission pulses compared to annual agricultural systems. Wagner-Riddle and Thurtell (1998) found that the presence of an over-wintering plant can result in negligible emissions of N_2O-N during freeze thaw events.

Hellebrand et al. (2008) found that frost-induced emissions from willow (*S. viminalis*) plantations in Germany had a negligible effect on the total annual N_2O budget. Similarly, the present study did not see a pulse in N_2O-N emissions following freeze thaw emissions in the spring (Figure 5.3b); emissions throughout the winter and during FTCs remained similar to N_2O-N emissions during the growing season, which was also observed by Groffman et al. (2006). Hellebrand et al. (2008) found that winter emissions

(from October to March) usually remained below 19 μg N_2O-N m^{-2} hr^{-1}. In our study, winter emissions from October to March were more variable, but had a similar average of 19.47 ± 6.62 μg N_2O-N m^{-2} hr^{-1}.

Generally, fertilizer applications in the autumn can elevate N_2O-N emissions during the spring thaw, while spring nutrient amendments allow mineral N to be taken up by actively growing plants, reducing availability during FTCs (Wagner-Riddle and Thurtell, 1998; Abalos et al., 2015). As fertilizer was applied on June 3, 2014 and June 5, 2015, respectively, mineral N was readily consumed by growing plants and active microbes, which resulted in elevated N_2O-N emissions directly following application in both years (Figure 5.2a, Table 5.4), reducing N available for conversion to N_2O-N during the spring thaw. Hellebrand et al. (2008) suggested that winter N_2O emissions were unrelated to fertilizer application in willow (*S. viminalis*) plantations, as FTC-derived emissions were exhibited at both fertilized and unfertilized sites. In the present study, there were no significant differences between N_2O-N emissions between treatments in the winter (Figure 5.3b; Table 5.4), thus, fertilizer application did not influence winter and spring FTC N_2O-N emissions.

5.4.5 Total Annual Emissions

Pacaldo et al., (2011) found that total CO_2-C annual emissions from a 13-year-old willow (*S. dasyclados*) clone amounted to 30.6 ± 2.8 Mg CO_2 ha^{-1} yr^{-1}, while Drewer et al. (2012) quantified annual CO_2-C efflux as 26.0 Mg CO_2 ha^{-1} yr^{-1}. These results are similar to our study, where total annual emissions equated 19.73 Mg CO_2 ha^{-1} yr^{-1} from fertilized treatments, and 26.29 Mg CO_2 ha^{-1} yr^{-1} from unfertilized treatments.

Annual values of N_2O-N emissions reported in the present study amount to 0.70 Mg CO_2-eq ha^{-1} yr^{-1} from fertilized treatments and 0.60 Mg CO_2-eq ha^{-1} yr^{-1} from unfertilized treatments. Drewer et al. (2012) found that willow (*Salix* sp.) N_2O-N emissions only accounted for 0.008 Mg CO_2-eq ha^{-1} yr^{-1}; however, they did not fertilize their willow biomass plantations. Abalos et al. (2015) determined that annual N_2O-N emissions from perennial grass-legumes in Elora, Ontario, treated with broadcasted manure applications, ranged from 0.3 to 2.1 Mg CO_2-eq ha^{-1} yr^{-1}. This study's values are within the range of values reported by Abalos at al. (2015).

The SX67 biomass, harvested from the Guelph Turfgrass Institute in December 2015 was 10.23 ± 1.86 odt h^{-1} yr^{-1} from fertilized treatments and 8.33 ± 0.97 odt h^{-1} yr^{-1} from unfertilized treatments (D. Walter, personal communication, December 15, 2015). Thus, fertilizer application did not result in measurable increases in biomass yield. A lack of response from willow SRC systems to fertilization was also reported by Kosencni (2010), Quaye and Volk (2013) and Amichev et al. (2014). This is because willow nutrient requirements can potentially be met from mineralization of leaf litter (Hangs et al., 2014), which contributes 33-66% of annual willow nutrient requirements (Ericsson et al., 1992; Keoleian and Volk, 2005). Thus, willow biomass plantations may be able to be a self-sustaining system, supplying adequate nutrients to the soil through foliar cycling. However, N_2O-N emissions represent a very small fraction of the annual emissions (CO_2-eq) of willow. In fertilized treatments, N_2O-N emissions only constituted 3.4% of the total emissions, while unfertilized constituted even less, at 2.2%. Drewer et al. (2012) also found the CO_2-C emissions accounted for the largest proportion of total annual emissions.

Net annual emissions, which encompassed rates of C sequestration in biomass and in the soil, were -10.79 Mg CO_2-eq ha^{-1} yr^{-1} from fertilized and 1.19 Mg CO_2-eq ha^{-1} yr^{-1} from unfertilized willow SRC systems. Overall, fertilized willow SRC systems represent a C sink, with a negative emission value, whereas emissions were slightly positive from unfertilized treatments. Rytter (2012) found that willow SRC systems in Sweden also had the potential to sequester C in woody biomass and soils, but they did not account for the soil-derived trace gas emissions. Fertilized treatments exhibited the potential to sequester more C than unfertilized treatments by increasing willow biomass productivity. Although unfertilized treatments had a slightly positive net emission value, as more C is accrued belowground through subsequent willow rotations, C sequestration may increase in the next 3-year cycle of the willow SRC system.

Willow SRC systems still exhibited low N uptake as demonstrated by the pulse of N_2O-N from the system following N application; excess reactive N atoms in the environment cascade through ecosystems resulting in environmental externalities, which were unmeasured in this study (Erisman et al., 2015). Additionally, Ruan et al. (2016) suggested that the application of excess N should be limited in willow SRC systems if the crop is unresponsive to fertilization; fertilizer should only be applied as necessary to increase willow yields.

5.5 Conclusions

This study shows that CO_2-C emissions are not affected by fertilizer application, and follow seasonal patterns (Gauder et al., 2011; Lutes et al., 2016). However, SOC and soil temperature were not responsible for differences between summer emissions in 2014 and 2015. Instead, there were likely higher rates of photosynthesis, C allocation to roots

and root respiration in 2015, which resulted in elevated CO_2-C efflux. However, the proportions of heterotrophic and autotrophic respiration remain unknown. Values of N_2O-N emissions were consistent in 2014 and 2015, with no significant differences. In both 2014 and 2015, elevated N_2O-N emissions corresponded with higher levels of inorganic N (NH_4^+ and NO_3^-) from fertilizer application, thus, emissions increased due to elevated levels of microbial nitrification (Lutes et al., 2016). Furthermore, winter FTCs did not result in pulses of CO_2-C or N_2O-N emissions in fertilized or unfertilized willow SRC systems.

Although N_2O-N emissions significantly increase following fertilizer application, the proportion of N_2O-N emissions in total annual emissions is very small (2.2-3.4%). CO_2-C emissions accounted for the majority of emissions. Fertilized treatments were C sinks, when aboveground, belowground and litter fall C were considered in addition to soil emissions, and have the potential to sequester 10.79 Mg CO_2-eq ha^{-1} yr^{-1}, whereas unfertilized treatments were slight C sources, with a net emissions value of 1.19 Mg CO_2-eq ha^{-1} yr^{-1}. Unfertilized treatments may also become net C sinks throughout future willow rotations, as more C is accrued. Overall, willow SRC systems enhance belowground SOC in marginal lands, and energy derived from willow biomass is more sustainable than conventional energy sources. It is recommended that fertilizer use be reduced in willow SRC systems, and only be applied to maintain biomass yields at 10 odt ha^{-1} to be a C sink, which is also the typical rate of biomass production per year in these systems (Heller et al. 2003; Keoleian and Volk, 2005). This will limit excess N outputs from willow SRC systems.

6. Conclusion

Willow SRC systems can provide high, consistent yields of second generation biofuels, which can reduce global reliance on fossil fuels for energy. Nitrogen fertilizer application to willow SRC systems is a common management practice, and can result in elevated N_2O-N emissions, which can potentially negate the C neutrality of biofuels derived from these systems.

CO_2-C emissions comprised the majority (96.9 - 98.0%) of total annual emissions, thus, reduction of CO_2-eq emissions should be a primary management concern in willow SRC systems. Willow clone selection can influence CO_2-C emissions; Lutes et al. (2016) found that clone SX67 had lower emissions than SV1, due to greater SOC accrual since clone establishment. This study also found that there was greater CO_2-C emissions when ambient air temperatures and soil moisture were high, which was attributed to possible elevated photosynthesis, C allocation to roots and root respiration. There was also greater total respiration with increases in SOC and soil temperature, which can elevate microbial respiration. Quantifying the relative contributions of heterotrophic and autotrophic respiration to total respiration in willow SRC systems represent an area for further research. Furthermore, fertilizer application did not have an influence on CO_2-C efflux, and on an annual basis, emissions followed seasonal trends. In this study, winter FTC emissions were did not create a pulse of CO_2-C or N_2O-N emissions.

Despite elevated N_2O-N emissions following fertilizer application, fertilized willow SRC plantations acted as a C sink, with the potential to sequester 10.79 Mg CO_2-eq ha^{-1} yr^{-1}. Unfertilized willow SRC systems were a slight C source, with net emissions of 1.19 Mg CO_2-eq ha^{-1} yr^{-1}. However, this value can become negative with greater

belowground and soil C sequestration as the willow SRC systems mature, and therefore warrants further research and monitoring.

Overall, C is sequestered in willow shoots, by taking up atmospheric CO_2 to store in woody tissue, and is also accrued in the soil, offsetting N_2O-N emissions released following fertilizer application, and annual CO_2-C emissions from willow respiration. However, excess N has surpassed critical thresholds globally such that fertilizer use needs to be reduced by 50% (De Vries et al., 2013; Steffan et al., 2015; Erisman et al., 2015). Therefore, willow SRC systems exhibited a low N uptake, and increased inorganic N (NH_4^+ and NO_3^-) in the soil profile and N_2O-N efflux to the atmosphere, fertilizer should be limited in willow SRC systems, and only be applied to maintain a yield of 10 odt ha^{-1}. Further research is needed to determine how much fertilizer is appropriate to achieve this biomass production with different land and clone combinations. Long-term studies of GHG emissions from the soils under willow SRC systems are recommended to better understand the impact of climate change on willow biofuels.

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Appendix

Appendix A 2014, 2015 and 20-year average environmental data from the Guelph Turfgrass Institute willow biomass plantation research sites. Highlighted area indicates the months in which there are two field seasons of data

Month	Cumulative monthly rainfall (mm)†			Mean monthly temperature (°C)†		
	30-year average	2014	2015	30-year average	2014	2015
January	29	-	11	-6.5	-	-8.6
February	30	-	8	-5.5	-	-14.8
March	37	-	5	-1.0	-	-3.5
April	68	-	61	6.2	-	6.2
May	82	57	72	12.5	13.3	15.9
June	82	59	136	17.6	18.8	17.0
July	99	130	45	20.0	18.5	20.0
August	84	121	118	18.9	18.3	18.8
September	88	176	44	14.5	15.0	17.7
October	66	72	-	8.2	9.4	-
November	75	45	-	2.5	0.7	-
December	38	23	-	-3.3	-1.2	-

†Cumulative monthly rainfall and mean temperature for the GTI willow biomass plantation. Weather data was obtained from the GTI weather station in Guelph, Ontario. Missing data (February – March 2015) was supplemented from the nearest Environment Canada weather station in the Region of Wellington, Ontario. 20 year means were obtained from the nearest Environment Canada weather station in Wellington (Environment Canada 2016)

Appendix B Sample calculation of conversion of 20 µg N_2O-N m^{-2} hr^{-1} to CO_2 equivalents (kg CO_2-eq ha^{-1} day^{-1}), and 1500 mg CO_2-C m^{-2} hr^{-1} to CO_2 equivalents (kg CO_2-eq ha^{-1} day^{-1}).

Converting µg N_2O-N m^{-2} hr^{-1} to kg CO_2-eq ha^{-1} day^{-1}

1. To convert from emissions of N_2O-N to emissions of N_2O, multiply by the molecular mass of N_2O (44 g mol^{-1}) divided by the molecular mass of N in N_2O (28 g mol^{-1}).

$$20\ \mu g\ N_2O - N\ m^{-2}hr^{-1}\ \times \frac{44}{28} =\ 31.43\ \mu g\ N_2O\ m^{-2}hr^{-1}$$

2. Multiply by the IPCC 100 year GWP of 298 to convert emissions in N_2O to emissions as CO_2-eq.

$$31.43\ \mu g\ N_2O\ m^{-2}hr^{-1}\ \times\ 298 =\ 9366.14\ \mu g\ CO_2 - eq\ m^{-2}hr^{-1}$$

3. Convert from hours to days.

$$9366.14\ \mu g\ CO_2 - eq\ m^{-2}hr^{-1}\ \times\ 24\ hr = 224787.36\ \mu g\ CO_2 - eq\ m^{-2}day^{-1}$$

4. Convert from meters squared to hectares.

$$224787.36\ \mu g\ CO_2 - eq\ m^{-2}day^{-1}\ \times\ 10000\ m^2 = 2247873600\ \mu g\ CO_2 - eq\ ha^{-1}day^{-1}$$

5. Convert from micrograms to kilograms.

$$224787.36\ \mu g\ CO_2 - eq\ ha^{-1}day^{-1}\ \times \frac{1\ kg}{1000000000\ \mu g} = 2.25\ kg\ CO_2 - eq\ ha^{-1}day^{-1}$$

Converting mg CO_2-C m^{-2} hr^{-1} to kg CO_2-eq ha^{-1} day^{-1}

1. To convert from emissions of CO_2-C to emissions of CO_2, multiply by the molecular mass of CO_2 (44 g mol^{-1}) divided by the molecular mass of C in CO_2 (12 g mol^{-1}). This converts the gas into CO_2 equivalents, as the IPCC GWP of CO_2 is 1.

$$150\ mg\ CO_2 - C\ m^{-2}hr^{-1}\ \times \frac{44}{12} =\ 550\ mg\ CO_2 - eq\ m^{-2}hr^{-1}$$

2. Convert from hours to days.

$$550\ mg\ CO_2 - eq\ m^{-2}hr^{-1}\ \times\ 24\ hr\ =\ 13200\ \ CO_2 - eq\ m^{-2}day^{-1}$$

3. Convert from meters squared to hectares.

$$13200 \text{ mg } CO_2 - \text{eq m}^{-2}\text{day}^{-1} \times 10000 \text{ m}^2 = 132000000 \text{ mg } CO_2 - \text{eq ha}^{-1}\text{day}^{-1}$$

4. Convert from micrograms to kilograms.

$$132000000 \text{ mg } CO_2 - \text{eq ha}^{-1}\text{day}^{-1} \times \frac{1 \text{ kg}}{1000000 \text{ mg}} = 132 \text{ kg } CO_2 - \text{eq ha}^{-1}\text{day}^{-1}$$