Assessment of soil carbon storage and greenhouse gas emissions in perennial bioenergy crops on low productive agricultural land in southwestern Ontario, Canada

by

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Examining Committee Membership

The following served on the Examining Committee for this thesis. The decision of the Examining Committee is by majority vote.

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Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the 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 to the public.

Statement of Contributions

The dissertation is manuscript-based, with some material resulting from a joint research effort.

I am the first author of all the manuscripts and am mainly responsible for the preparation, development, data analysis, and collection found in this dissertation. The contributions from co-authors are as follows:

Chapter 1: Provides literature review, outlines the research needs, the objectives, hypotheses, information on research site and experimental layout. This chapter was edited by Dr. Maren Oelbermann. The original experimental design is a joint effort by Dr. Maren Oelbermann and Dr. Naresh Thevathasan (University of Guelph).

Chapter 2: This chapter will be submitted to the Canadian Journal of Soil Science.

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Dr. Maren Oelbermann provided guidance and edited the chapter.

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Dr. Oelbermann and Dr. Fereidoun Rezanezhad provided guidance and supervision on the project design and data collection. All co-authors reviewed the final manuscript and provided editorial advice and guidance.

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Chapter 7: This chapter covers the overall conclusion, including key research findings and contributions.

Dr. Oelbermann edited the chapter.

Abstract

Low productive agricultural lands with less suitability for annual row crop production have been recommended globally for growing perennial bioenergy crops. This is due to the low input requirement of perennial bioenergy crops and their ability to produce large biomass on low productive agricultural lands as well as rejuvenating these lands for possible future annual row crop production. In addition to eliminating competition with food crops for land occupation, growing perennial bioenergy cops on low productive agricultural lands provides climate change mitigation benefits through soil carbon (C) sequestration and reduced greenhouse gas (GHG) emissions. In this dissertation, four studies were conducted to evaluate soil C pools and GHG (N_2O and CO_2) emissions in unfertilized perennial bioenergy crops of miscanthus (*Miscanthus giganteus* L.), switchgrass (*Panicum virgatum* L.), and willow (*Salix miyabeana* L.) on low productive agricultural land in southern Canada to understand the climate benefit of growing perennial bioenergy crops on low productive agricultural land relative to a natural regrowth vegetation (successional) site.

The first study in Chapter 2 quantified the nitrous oxide (N_2O) and carbon dioxide (CO_2) emissions in miscanthus, switchgrass, willow, and a successional site over two growing seasons to understand the GHG mitigation benefits of the bioenergy crops compared to the successional site. The static chamber method which consisted of installing two polyvinyl chloride (PVC) chambers (25 cm height, 10 cm radius) to a 10 cm depth in each bioenergy crop replicate and three chambers in the successional site was used to quantify N_2O and CO_2 emissions in the perennial bioenergy crops and successional site. Gas from each chamber headspace was sampled at time t = 0, 15 and 30 minutes once every two weeks for the 2020 and 2021 growing seasons (May to October). The sampled gas was brought to the laboratory and analyzed on a gas chromatograph for N_2O and CO_2

in each bioenergy crop and successional site. A repeated measure and univariate ANOVA was used to quantify differences between N_2O and CO_2 fluxes and cumulative emissions from the bioenergy crops and successional site over the two growing seasons. Mean N_2O and CO_2 fluxes during the two growing seasons ranged from –0.022 to 0.087 μg N_2O-N m^{-2} hr^{-1} and 0.010 to 0.266 mg CO_2-C m^{-2} hr^{-1}, respectively. Whereas mean N_2O fluxes did not differ ($p>0.05$) among land use type and between years, mean CO_2 fluxes significantly differed ($p<0.05$) among land use types and between years. The 2-year total cumulative N_2O emissions ranged from 93.75 μg N_2O-N m^{-2} to 174.50 μg N_2O-N m^{-2} and was significantly higher ($p<0.05$) in switchgrass than the successional site. Of the 2-year total cumulative N_2O emissions, 51% in the successional site and switchgrass occurred in 2020, while 63% and 69% in willow and miscanthus, respectively, occurred in the same year. The 2-year cumulative CO_2 emissions on the other hand ranged from 760.68 mg CO_2-C m^{-2} to 1009.04 mg CO_2-C m^{-2} and was not significantly different ($p>0.05$) among land use type. Although soil parameters such as temperature and available nitrogen (N), which can provide favorable conditions for CO_2 emissions from soil microbial respiration, were significantly higher ($p<0.05$) in 2020 compared to 2021, the absence of any statistically significant ($p>0.05$) differences in CO_2 emissions between both years suggests that the majority of the emitted CO_2 may have been contributed by root respiration rather than soil microbial respiration. This is because root respiration can constitute the majority of CO_2 emissions from the soil. Moreover, the overall combined average emissions which ranged from 4.96 Mg CO_2-eq ha^{-1} 6-$month^{-1}$ to 8.18 Mg CO_2-eq ha^{-1} 6-$month^{-1}$ were significantly higher ($p<0.05$) in switchgrass compared to the successional site. The implication of this is that, unlike miscanthus and willow, converting low productive agricultural land to switchgrass may result in an overall higher emission. This means

that the intended benefits of reduced GHG may actually worsen emissions – if what occurs at this experimental scale is true at a global scale with switchgrass cultivation.

The objective of the second study (Chapter 3) was to evaluate the impact of repeated soil freeze-thaw on soil C and N cycling and resultant N_2O and CO_2 emissions in low productive agricultural land under different perennial bioenergy crops and a successional site to understand their GHG mitigation benefits under a changing climate. In this study, a 49-day laboratory incubation experiment was conducted to compare the impact of freeze-thaw cycles on N_2O and CO_2 emissions in miscanthus, switchgrass, and willow to a successional site and to understand the processes controlling the N_2O and CO_2 emissions in these crops. The results showed that freeze-thaw cycles caused a decline in dissolved organic C (DOC) and dissolved inorganic N (DIN) concentrations but enhanced the dissolved organic N (DON) and nitrate (NO_3^-). Although freeze-thaw decreased water stable soil aggregates in all the bioenergy crops and successional site, this did not have any statistically significant ($p>0.05$) impact on N_2O and CO_2 emissions, suggesting that the N_2O and CO_2 emitted during the freeze-thaw cycles may have originated mostly from cellular materials released from lysis and death of microbial biomass rather than from soil aggregate disruption. Cumulative N_2O emissions measured over the 49-day incubation period ranged from 148 mg N_2O-N m^{-2} to 17 mg N_2O-N m^{-2} and were highest ($p<0.05$) in miscanthus followed by willow, switchgrass, and successional site. Cumulative CO_2 on the other hand was highest ($p<0.05$) in the successional site than any of the bioenergy crops and ranged from 25,262 mg CO_2-C m^{-2} to 15,403 mg CO_2-C m^{-2} after the 49 days. Higher N_2O emissions in the miscanthus and willow than switchgrass and successional site were attributed to accelerated N losses as N_2O due to possible changes in soil microbial biomass and structure as influenced by the different crop species. Results from this study point to a potential influence of soil microbial biomass and

microbial diversity in regulating C and N cycling in the different bioenergy crop species resulting in the varied responses of these crops to N_2O and CO_2 emissions during soil freeze-thaw. Hence, reducing soil freezing intensities in perennial bioenergy crops on low-productive agricultural lands may be important for decreasing freeze-thaw-related N_2O and CO_2 emissions in miscanthus, switchgrass, and willow. This reduction can lead to climate change mitigation benefits under a changing climate scenario, characterized by more frequent soil freeze-thaw events in cold temperate regions where these crops are cultivated.

To understand how miscanthus, switchgrass, and willow influence long-term soil organic carbon (SOC) storage and stabilization compared to a successional site, the third study (Chapter 4) evaluated and compared C storage in whole soil and different soil-size fractions in the perennial bioenergy crops to a successional site. The proportion of C contributions from the different perennial bioenergy crops to different soil-sized fractions were also determined. Soil sampled to 30 cm depth from the different bioenergy crops were physically fractionated into macro-sized (250-2000 μm), micro-sized (53-250 μm), and silt + clay (<53 μm) fractions using the dry-sieving method. SOC concentrations and stocks were determined in whole and fractionated soil using an elemental analyzer while the proportion of C contributions from the different perennial bioenergy crops to different soil-sized fractions were determined using the ^{13}C natural abundance technique. Due to the absence of fertilization and potential decreased organic residue input to the soil from lower biomass yield, after 12 years of cultivation, the miscanthus and willow only posted marginal gains in SOC concentrations that were 2.5% higher compared to the baseline SOC concentrations in 2009, with the switchgrass recording 3.7% lower SOC concentrations. Soil organic C stocks ranged from 5686 to 7002 g C m^{-2} and were significantly higher ($p<0.05$) in the successional site than switchgrass and willow but not in miscanthus. Of the three bioenergy crops, only the

miscanthus maintained SOC stocks comparable to the successional site owing to the ability of unfertilized miscanthus to produce higher biomass on low productive agricultural lands compared to switchgrass and willow. The distribution of soil-sized fractions and SOC in the soil-sized fractions under the perennial bioenergy crops were consistent with the aggregate hierarchy model proposed by Tisdall and Oades (1982) and followed an increasing order of silt + clay < micro- < and macro-sized fractions. Significantly higher ($p<0.05$) SOC in miscanthus in micro-sized and silt + clay fractions were contained in 20–30 cm depth compared to surface soil layers. These higher micro-sized and silt + clay-associated SOC in miscanthus in deeper soil depths compared to the switchgrass and willow indicates the vital contribution miscanthus could play in long-term C stabilization in deeper soil layers. The $\delta^{13}C$ signatures of the different soil-sized fractions under the perennial bioenergy crops to 30 cm revealed a higher proportion of C contribution by the crops were contained in the micro-sized fractions compared to macro-sized fractions, indicating their significant contribution to more stabilized C pools with long-term positive impact on climate change mitigation.

The objective of the fourth study (Chapter 5) was to use the Century model to predict and compare long-term SOC dynamics in miscanthus, switchgrass, and willow to a successional site and a row crop system to understand the soil C storage potential of the different bioenergy crops over the long-term. The Century model was calibrated using the site-specific soil biophysical characteristics, climate, and land management practices of the study site to predict SOC stocks for 162 years. Average SOC stocks over the 162-year simulation period were highest in miscanthus (8521 g C m^{-2}), followed by the successional site (6877 g C m^{-2}), switchgrass (6480 g C m^{-2}), willow (5448 g C m^{-2}) and lowest in the row crop system (3995 g C m^{-2}). Higher SOC stocks in the miscanthus than the successional site indicates that, despite frequent biomass harvest, perennial

bioenergy crops can accumulate higher C in soil over the long-term than when a low productive agricultural land is left to undergo secondary regrowth. This may, however, depend on the crop species, since the miscanthus was the only bioenergy crop that reached pre-cultivation (1911) SOC levels of 8288 g C m^{-2}. Moreover, the perennial bioenergy crops enhanced SOC in the slow fraction, whereas row crops depleted organic C in this fraction. This indicates the vital contribution of perennial bioenergy crops in long-term SOC sequestration and their role in climate change mitigation, especially when grown on low productive agricultural lands.

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Dedication

This dissertation is dedicated to my late mother – Mary Osei, who died on April 02, 2024, just before the completion of this dissertation and had always been a strong pillar in my life. Rest well mom, you will forever be missed!

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GENERAL INTRODUCTION

I. Problem Context and Research Rationale

The "safe operating space for humanity" within the "Earth's System" is currently threatened by climate change (Rockström et al., 2009). A major contributor to this is the reliance on fossil fuels as the major source of global energy (Jia et al., 2019). Fossil fuels provide about 84% of the global primary energy supply and the production and consumption of this energy contributes approximately 75% of the total global greenhouse gas (GHG) emissions (Ge et al., 2022; Looney, 2020). The projected increase in global population in the coming decades and advancement of industrialization in developing countries for economic growth increases the likelihood of more global energy consumption (Jia et al., 2019). This also implies that more GHG emissions are to be expected and a possible catastrophic consequence from climate change if fossil fuels are to remain the primary global energy source. Moreover, the resource scarcity surrounding the use of fossil fuels has begun to question the sustainability of fossil fuel as the primary energy source (Khan et al., 2019). These concerns about the sustainability and negative impact of fossil fuels on the earth's climate has called for the need to consider more sustainable and 'clean' energy alternatives such as bioenergy or energy produced from biomass sources (Lemus & Lal, 2005).

The sustainability of using plant biomass for energy stems from the understanding that plants are renewable as they can be continuously replenished (Chimento et al., 2016). Moreover, the CO_2 emitted from producing and burning energy from biomass comes from the carbon stored by those biomasses during their lifetime (Perea-Moreno et al., 2019). Hence, energy from biomass is carbon neutral (Chimento et al., 2016; Lutes et al., 2016; Perea-Moreno et al., 2019). There is, however, the argument that, growing biomass purposely for energy production can result in net GHG emissions, food insecurity, and other environmental consequences (Pool, 2013; Reid et al.,

2020; Searchinger & Heimlich, 2015). This is due to the multitude of sources of bioenergy feedstocks and the different management methods that go into their production (Blanco-Canqui, 2010; Gasparatos et al., 2013; Norgrove, 2010).

In major bioenergy producing countries such as the USA, Brazil, Indonesia, and in Europe, conventional crops, also known as 'first-generation' crops such as corn (*Zea mays* L.), sugarcane (*Saccharum officinarum* L.), wheat (*Triticum aestivum*), sorghum (*Sorghum* spp), soybeans (*Glycine max* L.), oil palm (*Elaeis guineensis* L.), canola (*Brassica napus* L.) and other oilseeds and cereals have largely been used (Koizumi, 2015; Langeveld et al., 2014). In addition to being food crops, these cops are grown on prime agricultural lands, thereby, competing with food crops for land occupation (Searchinger & Heimlich, 2015). Also, the intensive use of synthetic fertilizers and the direct and indirect land-use changes resulting from increased production of these crops results in increased GHG emissions and degradation of natural vegetations (Pool, 2013). These problems associated with bioenergy crops from food sources can be addressed by using dedicated perennial crops, also known as 'second-generation' bioenergy crops which do not compete with food crops for productive resources and end-use (Jia et al., 2019).

Growing low-input and fast-growing perennial bioenergy crops such as herbaceous and short-rotation woody crops on low productive agricultural lands for energy is an innovative approach to meeting current and future energy demands whilst eliminating the food insecurity and environmental threats associated with energy from food crop sources (Agostini et al., 2015; German et al., 2011; Norgrove, 2010). Additionally, perennial bioenergy crops such as willow (*Salix miyabeana* L.), switchgrass (*Panicum virgatum* L.), and miscanthus (*Miscanthus giganteus* L.) (Table A1) have the ability for high biomass productivity on low productive agricultural lands with little requirement for fertilizer inputs (Chimento et al., 2016; Nanda et al., 2018; Perea-

Moreno et al., 2019). Establishing these perennial crops on low productive agricultural lands could also regenerate these lands and offer climate change mitigation benefits through soil carbon (C) sequestration and reduced GHG emissions (Blanco-Canqui, 2010; Chimento et al., 2016; Koh & Ghazoul, 2008).

To fully understand the contribution of perennial bioenergy crops in soil C sequestration and their potential GHG mitigation impact, long-term studies are needed (Sartori et al., 2006). This will help advance our knowledge on the role bioenergy crops play in mitigating climate change. Several studies have evaluated soil C sequestration potentials in different perennial bioenergy crops (McCalmont et al., 2017; Coleman et al., 2018; Bazrgar et al., 2020). However, majority of these studies have been limited to just quantifying and comparing the soil C pools in different perennial crops and/or under different management systems without going further to explore the dynamics and actual contribution of these crops to the soil C pools in different soil-sized fractions.

The ^{13}C natural abundance stable isotope techniques have been successfully used to study and quantify proportion of soil organic carbon (SOC) contribution from perennial bioenergy crops in whole soils (Collins et al., 2020; Dondini et al., 2009). This technique has been applied only to a limited extend in perennial bioenergy crops on low productive agricultural land to determine the proportion of C contribution in different soil sized fractions. Considering that the nature of soil fractions associated with C influences its mean residence time and long-term storage (Nair et al., 2010), it is crucial to understand how different perennial bioenergy crops contribute to soil C storage in different soil-sized fractions and how this may result in long-term C storage. This information will also be crucial in providing insight into management strategies such as knowledge on bioenergy crop types capable of yielding the most benefit in mitigating climate change through soil C sequestration and reduced GHG emissions.

II. General Research Goal and Objectives

The overall goal of this research was to determine and compare the climate benefit of unfertilized miscanthus, switchgrass, and willow on low productive agricultural land to a successional site by quantifying SOC pools and GHG (N_2O and CO_2) emissions in these different land use types in southern Canada.

The general objectives of this study were:

- ✓ To quantify N_2O and CO_2 emissions in unfertilized perennial bioenergy crops of switchgrass, miscanthus, and willow on low productive agricultural land.
- ✓ To evaluate the impact of freeze-thaw cycles on N_2O and CO_2 emissions on low productive agricultural land under different perennial bioenergy crops.
- ✓ To quantify SOC pools and dynamics in different soil-sized fractions in different perennial bioenergy crops on low productive agricultural land.
- ✓ To evaluate and quantify long-term SOC dynamics in perennial bioenergy crops using the Century model.

III. Dissertation Structure

The research in this dissertation is organized in a manuscript format. Chapter 1 reviews the literature on how land-use change has caused GHG emissions and how sustainable climate change solutions are needed to combat climate change mitigation. Among these sustainable climate change solutions is the cultivation of bioenergy crop on low productive agricultural lands. Additionally, the sustainability challenges associated with bioenergy crop production, particularly, those from first-generation biomass sources are reviewed and how growing perennial bioenergy (second-generation) crops address the sustainability challenges associated with growing first-

generation bioenergy crops such as reduced GHG emissions and increased SOC storage. Finally, the impact of winter freeze-thaw on GHG emissions in perennial bioenergy crops is reviewed. Chapter 1 concludes with a description on the study site and provides details on the perennial bioenergy crop establishment in 2009. Chapter 2 presents the first study on quantifying N_2O and CO_2 emissions in different perennial bioenergy crops and compare these emissions to that of a successional site to understand how these bioenergy crops may contribute to climate change mitigation through reduced GHG emissions. In Chapter 3, a laboratory incubation study on soil samples from the research site under the different land-use types were evaluated under repeated freeze-thaw cycles and N_2O and CO_2 emissions were quantified to understand how increased freeze-thaw events would impact GHG emissions from these crops. Chapter 4 of this dissertation evaluates the SOC stocks in different soil-sized fractions in the perennial bioenergy crops in relation to the successional site. The ^{13}C and ^{15}N natural abundance stable isotope technique was also employed to understand C and N dynamics in soil-sized fractions as influenced by the bioenergy crops. Chapter 5 quantified long-term SOC dynamics in the bioenergy crops using the Century model to understand the C sequestration benefit of these crops. The synthesis and conclusion of the research chapters as well as future research opportunities are presented in chapter 6.

CHAPTER 1

1.0 Literature Review

1.1 Land-Use and Climate Change

Human induced-land-use change, which refers to any modification of the earth's natural landscape from human actions (Erle and Pontius, 2007), is considered a major contributor to global climate change (Jia et al., 2019). Within the last millennium, about three-quarters of the global ice-free land surface has been impacted by human use (Luyssaert et al., 2014; Shukla et al., 2019). Key among the drivers for these human-induced land-use changes are increased societal demand for food, fibre, fuel, and shelter (Foley et al., 2005; Winkler et al., 2021). These land-use changes continue to drive changes in the global climate through changes in biogeochemical processes such as C and N cycling, resulting in the emission of GHGs (Luyssaert et al., 2014). Key among these GHGs are CO_2, N_2O, and methane (CH_4) (Carlson et al., 2017; Jia et al., 2019; Hong et al., 2021).

Whereas removal of natural vegetation, peat drainage and burning are some of the major causes of CO_2 emissions (Pongratz et al., 2021), majority of N_2O emissions come from N fertilizer use and manure management from agricultural activities (Tian et al., 2020). Methane emissions, on the other hand, mainly originate from enteric livestock fermentation, biomass burning under incomplete combustion, and rice cultivation (Saunois et al., 2020). A recent study (Hong et al., 2021) estimates that land-use emissions represent ~25% of net anthropogenic GHG emissions. Land-use change such as deforestation can be a key source of anthropogenic GHG emissions, but conservation land-use practices such as afforestation/reforestation (e.g. biomass plantations with carbon capture and storage) can be important GHG sinks (Jia et al., 2019; Lemus & Lal, 2005). For instance, a study by Guo & Gifford (2002) showed that converting pasture and forest to

croplands led to 59% and 42% decline in SOC stocks, respectively. Meanwhile, converting from cropland to forest and pasture, respectively, yielded 53% and 19% gains in SOC.

Although, land-use change is an important driver of climate change, equally, a changing climate can lead to changes in land use and land cover (Qiao et al., 2021). For instance, climatic factors such as temperature and precipitation are greatly influenced by climate change which in turn affect vegetation cover (Zheng et al., 2021). Significant increase in vegetation greening has been associated with factors such as precipitation, temperature, concentration of CO_2, and soil characteristics (Peng et al., 2019; Piao et al., 2015; Qiao et al., 2021; Tucker et al., 2001; Wang et al., 2017). Considering that these factors are influenced by climate change, it shows the key impact a change in the earth's climate can have on land-use and land cover change. Hence, sustainable climate solutions such as reforesting low productive agricultural lands are required to enhance the GHG sink capacity of terrestrial ecosystems while avoiding the negative consequences of climate change.

1.2 Sustainable Climate Change Solutions

The rise in atmospheric GHG emissions and the need to mitigate their impact on the earth's climate have led to increased calls for sustainable solutions to address both the effects and causes of this global problem (Ellis et al., 2024; Griscom et al., 2020). Climate change solutions refer to the innovative combination of strategies that offer climate change mitigation and adaptation by offsetting atmospheric GHGs and providing the capability to adapt to the effects of climate change (Kabisch et al., 2016).

Using nature-based solutions such as improved land management pathways that generate climate change mitigation outcomes through avoided GHG emissions and/or increase C

sequestration offer sustainable solutions to climate change (Ellis et al., 2024; Griscom et al., 2017). Aside being a natural process, the practicality of land management strategies in climate solutions are based on their cost-effectiveness and the added benefits associated with their adoption (Griscom et al., 2020; Lal, 2018). Among some of these land management strategies for climate solutions are agroforestry, afforestation and reforestation of agricultural and degraded lands which enhances their C sequestration capacity in vegetation and soil (Busch et al., 2019; Oelbermann et al., 2004). Shukla et al. (2019) estimates that afforestation and reforestation have the potential to mitigate 0.5 to 10.1 $GtCO_2$-eq annually. Griscom et al. (2020) suggested three nature-based pathways for climate change solutions – the 'protect', 'manage', and 'restore' pathways. Whilst the 'protect' pathway comprise the prevention of loss of native ecosystems such as peatlands, mangroves, and forests, the 'manage' pathway refers to management practices that avoid GHG emissions or enhance C sinks such as agroforestry practices which introduce trees in agricultural landscapes. The 'restore' pathway on the other hand refers to the re-establishment of forests and non-forest ecosystems to areas from where they had previously been lost as a result of human activity (e.g., reforestation) (Griscom et al., 2020).

Additionally, the use of Bioenergy with Carbon Capture and Storage (BECCS) offers sustainable solution to climate change (Griscom et al., 2019; Jia et al., 2019). This is because energy from biomass sources offer sustainable energy option and contributes to decarbonizing our energy systems, which are major contributors to CO_2 emissions (Perea-Moreno et al., 2019). Moreover, these bioenergy crops can enhance C sequestration, particularly, in the soil which provide mitigation and adaptation benefits (Chimento et al., 2016; Lemus & Lal, 2005). Jia et al. (2019) estimates 0.4 to 11.3 Gt CO_2-eq yr^{-1} GHG mitigation potential through BECCS. Bossio et al. (2020) showed that soil C represents 25% of the potential of natural climate solutions,

suggesting the crucial role maintaining and rebuilding soil C stocks could play in mitigating climate change.

1.2.1 Soil organic carbon sequestration as a sustainable climate change solution

Soil C sequestration is the process of removing C from the atmosphere in the form of CO_2 into soil C pools (Lemus & Lal, 2005; Nair et al., 2009). Of the total 2860 Pg C in terrestrial ecosystems, ~80% (2300 Pg) is contained in the soil to 1 m depth, with SOC constituting 1550 Pg C (Lal, 2008). Batjes (2014) estimates SOC to 0.3 m depth at 700 Pg C. The 2300 Pg total C contained in the soil to a 1 m depth is 3 times greater than that in the atmosphere (760 Pg C) and 4.1 times greater than that stored in vegetation (560 Pg C) (Lal, 2008). Therefore, the soil represents an important component for C storage in terrestrial ecosystems.

The recognition and acceptability of SOC sequestration as a sustainable climate solution is due to the ecosystem services that parallel this cost-effective climate mitigation and adaptation strategy compared to other engineering techniques used for C sequestration (Griscom et al., 2017; Chung et al., 2010; Lal, 2008). For instance, SOC sequestration comes with added benefits such as improved soil health, biodiversity habitat, and enhanced climate resilience (Amin et al., 2020; Griscom et al., 2020). Building soil C improves soil health via enhanced soil fertility, increased microbial activity, and resilience to degradation by erosion and climate change (Billings et al., 2021; Blanco-Canqui, 2010; Lemus & Lal, 2005; Singh, 2018). This inherent ability of a healthy soil to have greater resilience to environmental change will lead to its greater capacity to adapt to a changing climate by maintaining their productive capacity while contributing to climate change mitigation through C sequestration (Lal, 2008).

The amount of C in the soil is determined by the balance between inputs from net primary productivity and losses through organic matter decomposition, leaching, and erosion (Janzen, 2004; Plante et al., 2014). According to Bossio et al. (2020), climate change mitigation through soil C management necessitates the protection of existing soil C and the rebuilding of depleted stocks. To achieve this, adopting management practices that prevent losses and facilitate the return of C to the soil in excess of what is lost is crucial (Lal, 2003). The capabilities of a land management type in mitigating climate change through soil C sequestration depends on several biophysical and ecological factors relating to climate, soil, vegetation, management practices and disturbances (Baldocchi & Penuelas, 2019; Buczko et al., 2023; Gebeyehu et al., 2019; Sharma et al., 2021). Based on the type of management system, a land use type could serve as net sink or source for CO_2 and other GHG emissions (Chang et al., 2021). For instance, land management practices such as agricultural activities can reduce C inputs and increase losses compared to natural vegetation (Toru & Kibret, 2019; Wei et al., 2014). This reduction in SOC over time can create a soil C deficit relative to the levels of C that previously existed in the soil (Fargione et al., 2008; Ontl & Schulte, 2012). This soil C deficit represents opportunities to store C using land management options that can increase C inputs or decrease losses (Ontl & Schulte, 2012).

Reforestation of trees or grass on former croplands can reduce C deficits caused from years of crop production and sequester C through higher above and belowground biomass productivity compared to annual row crops (Bárcena et al., 2014; Guo & Gifford, 2002; Laganière et al., 2010). Poeplau et al. (2011) observed an 83% increase in SOC stocks in afforestation of cropland after 100 years, but a 7% decrease was observed with afforestation of grasslands within the same period in temperate environments. Li et al. (2024) also observed a 5.7% decrease in SOC following the conversion of a grassland into planted forest in a temperate region in Northeast China after 15

years. Bárcena et al. (2014) explained that afforestation on soils depleted in soil C such as former croplands may become net C sinks compared to more C-rich soils such as grasslands which could even experience C loss after conversion to forest land. This implies that low productive agricultural lands present greater opportunities for soil C sequestration potential compared to fertile lands.

1.2.2 The role of low productive agricultural lands in sustainable climate change solutions

Various parameters have been used to define what constitutes a 'marginal' (low productive agricultural) land and these categories differ across regions, countries, and organizations (Csikós & Tóth, 2023; Dauber et al., 2012; Jin et al., 2019; Khanna et al., 2021). For instance, economic (*e.g.*, land value), geographic (e.g., temperature and precipitation), and soil suitability (*e.g.*, yield capability, physical and chemical soil properties) are some of the criteria used to define 'marginal' lands (Csikós and Tóth, 2023). Jin et al. (2019) define 'marginal' croplands as low productivity lands whose historical crop yields are 25% below the regional average. The Canadian Land Inventory on soil capability for agriculture system also classifies 'marginal' lands as those with moderate to severe limitations such as slope and stoniness which limit their productive capacity. These lands are categorized as those in Class 4-6 and even sometimes those in Class 3 (Liu et al., 2017). However, Milbrandt & Overend (2009) argue that a land may be deemed 'marginal' for agricultural production, but same land could be vital for another use such as for animal pasture. Hence, the need for a clear and globally accepted definition for 'marginal' land. For this reason and in the context of this dissertation, the use of low productive agricultural land is preferred over the use of 'marginal' land.

Low productive agricultural lands, low in soil C play key role in sustainable climate change solutions through their potential for soil C sequestration and improving soil health; both of which contribute to climate change mitigation and adaptation (Bossio et al., 2020; Griscom et al., 2020).

The potential of soils to sequester C, and the amount of C a soil is capable of sequestering, is influenced by how much C is already contained in that soil, *i.e.* inherent SOC (Chung et al., 2010). There is a limit of a soil's capacity to sequester C. For instance, Chung et al (2010) observed that soils that were already high in C did not sequester any additional C when they were subjected to a broad range of C inputs. On the contrary, SOC sequestration in soil with an inherently low C content such as low productive agricultural lands were able to store large quantities of C once rehabilitated with perennial vegetation (Lal, 2003). After 9 years of establishing bioenergy crops on low productive agricultural land in southern Ontario, Bazrgar et al. (2020) observed 11.8, 11.7, 10.3, and 7.7, Mg ha^{-1} SOC gains to a 30 cm depth in switchgrass, willow, poplar, and miscanthus, respectively. Coleman et al. (2018) also observed 1.16 Mg C ha^{-1} yr^{-1} SOC increase in an 8-year-old short rotation bioenergy woody crops on low productive agricultural land in southern Ontario. A study in Europe (McCalmont et al., 2017) established that growing miscanthus on low C former agricultural land resulted in 0.7–2.2 Mg C ha^{-1} yr^{-1} SOC gains.

Evidence of SOC gains from the results above indicate the enamours potential low productive agricultural lands have and the crucial role they can play in climate change mitigation through soil C storage when grown with perennial bioenergy crops. Moreover, given that soils in low productive agricultural lands are farther from their C saturation level relative to prime agricultural lands (Georgiou et al., 2022; Lal, 2018), converting low productive agricultural lands to perennial bioenergy crops would likely yield greater climate benefits through gains in soil C stocks and sequestration than when fertile lands are used.

1.3 Bioenergy Crop Production

Bioenergy crop production refers to the growing of crops solely for the purpose of using their biomass yields for energy (Röder & Welfle, 2019). Bioenergy crops include fast-growing

herbaceous or woody crop species (Agostini et al., 2015). These crops are characterized by having high energy potentials, and the capacity to produce large volumes of biomass on low productive agricultural soils (Lemus & Lal, 2005). Plant produces biomass by converting CO_2 from the atmosphere and water from the environment into sugars, starch and cellulose using energy from sunlight through the process called photosynthesis (Ness & Moghtaderi, 2007). The energy in the sunlight is stored as chemical energy in the plant's biomass which can then be converted into useable forms of energy such as electricity, heat, and transportation fuels (Ness & Moghtaderi, 2007; Perea-Moreno et al., 2019). Depending on the biomass source, bioenergy crop can be classified as either first generation, second generation or third generation crop (Figure 3.1) (Lutes et al., 2016; Nanda et al., 2018; Perea-Moreno et al., 2019).

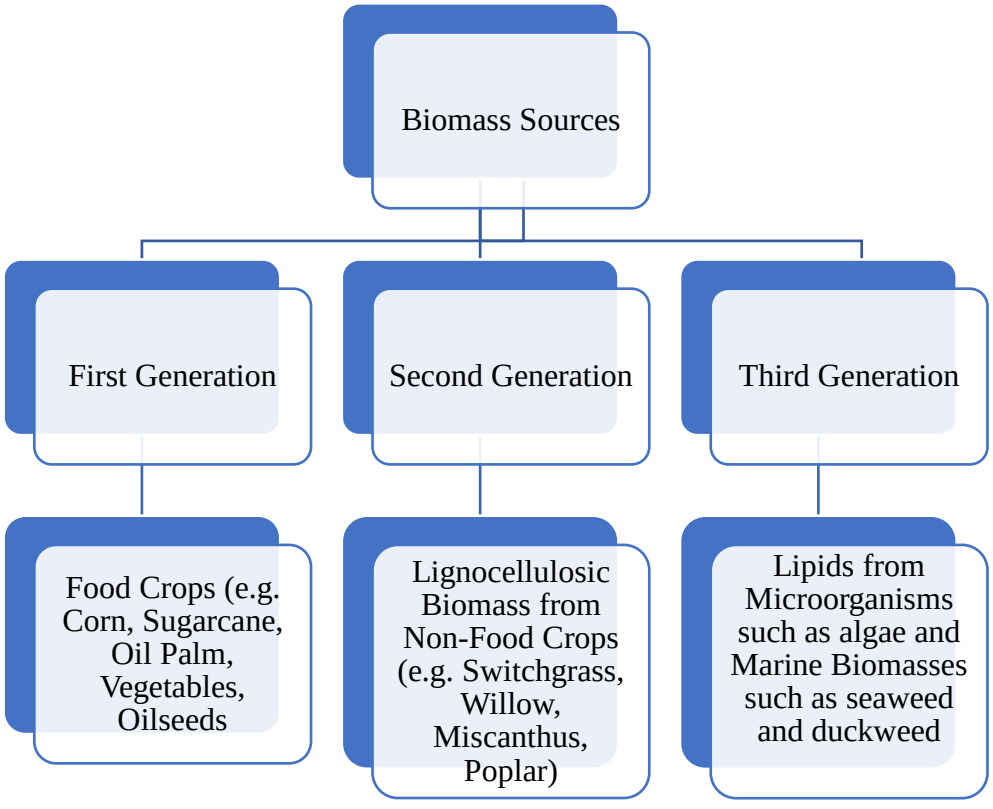


Figure 1.1: A schematic overview of the different sources of biomass for bioenergy production.

First-generation bioenergy crops are food crops grown on prime agricultural lands purposely for using the sugar, starch or oil in their seeds, grains, and other edible parts for energy production (Kindberg, 2010). First-generation bioenergy crops such as corn, wheat, sorghum, canola, soybeans are annual crops whose production are characterised by intensive soil tillage, large amount of fertilizer, pesticides, and herbicides inputs (Fess et al., 2018). Also, these crops (first-generation bioenergy crops) are usually grown on large scale in monoculture systems with heavy and frequent machinery use (Fess et al., 2018).

The second-generation bioenergy crops are non-food crops purposely grown for using their cellulose (a primary component in their cell walls) for energy (Nanda et al., 2018). The second-generation bioenergy crops, otherwise referred to as 'lignocellulosic crops' include perennial short rotation woody and herbaceous crops such as switchgrass, miscanthus, willow, poplar (Kindberg, 2010; Lutes, 2016). These crops are low inputs and low maintenance crops characterized by long-term land occupation, continuous biomass production and residue addition to the soil, well-developed root systems and little soil disturbance (Chimento et al., 2016; Lemus & Lal, 2005). Unlike the starch-based feedstocks from the first-generation bioenergy crops that can be directly used in biorefineries for fuel production, the second-generation bioenergy crops require a series of pretreatment to recover the fermentable sugars (Nanda et al., 2018). These required additional steps in pretreatment can result in additional operational costs in the utilization of feedstock from second-generation bioenergy crops compared to the first-generation crops.

The third-generation biomasses include sources from marine and microbial biomass (Nanda et al., 2018). These include agal biomasses (microalgae and macroalgae) such as diatoms and kelp and marine biomasses such as hyacinth, caltrop, duckweed, and salvinia (Nanda et al., 2018). These aquatic biomasses have been studied to be good candidates for biofuel production,

especially, biodiesel (Saad et al., 2019). Whereas first- and second-generation bioenergy crops require arable and big land area along with other resources for production, third-generation biomasses such as microalgae do not require arable or big land area and have faster growth rate (Maliha & Abu-Hijleh, 2023; Mat Aron et al., 2020). Moreover, algal biomasses have higher oil contents than oil crops for biodiesel production, and higher biomass production capacity than terrestrial plants (Saad et al., 2019; Maliha & Abu-Hijleh, 2023).

Even though, growing biomass purposely for energy is considered as an important component of the transition from a fossil fuel-based economy to renewables which can offer sustainable energy alternative (Clifton-Brown et al., 2019), production of these crops could cause other sustainability challenges such as food security and other environmental issues (German et al., 2011; Searchinger & Heimlich, 2015). The nature and the magnitude of these impacts, however, depends on the biomass feedstock type, the context of the feedstock production, and the agricultural practices adopted during the feedstock production (Gasparatos et al., 2013; German et al., 2011; Norgrove, 2010). Therefore, to realize the full potential benefits associated with bioenergy, it is crucial to assess how bioenergy crop production impact food security and cause other environmental issues to design appropriate production techniques needed to avert these associated challenges.

1.4 The Fuel-Food-Environment Trilemma of Bioenergy Crop Production

While energy from biomass is considered a C neutral energy source with potential for meeting future energy demand at lower environmental cost compared to fossil fuels (Chimento et al., 2016; Perea-Moreno et al., 2019), questions continue to be asked of their long-term future in the global energy market (Searchinger & Heimlich, 2015). It is argued that for bioenergy to be considered a major part of the global energy system, bioenergy crop production would have to be

upscaled (Anderson-Teixeira et al., 2012; McCalmont et al., 2017). This will, however, come at environmental and food security costs (Reid et al., 2020; Searchinger & Heimlich, 2015), resulting in a fuel-food-environment trilemma, which need addressing to avert the sustainability challenges associated with bioenergy crop production.

1.4.1 Implications of bioenergy crop production on global food security

The impact of bioenergy crop production on global food security has been widely studied and there exists a body of literature with the consensus that growing dedicated crops for energy production, particularly, those from food crop sources (first-generation bioenergy crops) greatly compromise food security (Tilman et al., 2011; Wise, 2012; Cassidy et al., 2013; Searchinger & Heimlich, 2015). The Food and Agriculture Organization of the United Nations (FAO) defines food security as "when all people, at all times, have physical and economic access to sufficient safe and nutritious food to meet their dietary needs and food preferences for a healthy and active life" (Norgrove, 2010).

In major bioenergy production countries, crops such as corn, sugarcane, soybeans, oil palm, canola and other oilseeds and cereals have largely been used for bioenergy (Davis et al., 2012; Wise, 2012; Pool, 2013; Koizumi, 2015). This is despite the great deal of hunger which continues to threaten global population, especially low-income people around the world (FAO, 2018). According to the FAO (2018), there are over 800 million people globally who are faced with hunger in every day of their lives. In addition to this global hunger is the existence of enormous pressure to increase food production to meet the demands of a growing population. Using 2010 as the reference year, van Dijk et al. (2021) estimates that food production would have to increase by 45% to 56% to meet the food demand of a projected 9.15 billion population by 2050. Alexandratos & Bruinsma (2012) projected 60% increase in global food crop demand by the year 2050 using

2005/2007 as the base year. Hence, with the emergence of the bioenergy industry which will cause more food crops and agricultural lands to be diverted into bioenergy production, less crops will be available for household consumption exacerbating hunger (Pool, 2013). Cassidy et al. (2013) observed that together, the USA and Brazil, respectively, dedicated over 460 million tonnes of maize and sugarcane to biofuel production in 2010, which represented 6% of global crop production by mass.

According to the FAO (2008), diverting crops, land, water, and other productive resources from food production towards bioenergy crop production affects availability, accessibility, stability, and utilization of food. For instance, Cassidy et al. (2013) indicated that the amount of human-edible calories that was used for bioenergy production increased fourfold between 2000 and 2010. This increased diversion of crop and productive resources into bioenergy production has tripled the frequency of global food crisis since 2005 (Pool, 2013). Wise (2012) reported that 20–40% of global food price increases in 2007-2008 was due to bioenergy crop production. Again, analyses by Thompson et al. (2016) indicated that a one-billion-gallon expansion of USA corn ethanol mandate, representing ~370 million bushels of corn can cause the average price of corn to rise by US$ 0.27 per bushel. Searchinger & Heimlich (2015) also explained that in a business-as-usual scenario where fuels are produced from food crops and/or from agricultural lands, meeting 10% of the world's transportation energy needs from bioenergy by 2050 will increase the world's food crop calorie gap between 2006 and 2050 from 70% to 90%.

Government policies and regulations on bioenergy production have been blamed for the negative impact of bioenergy crop production on food security (Wise, 2012; Searchinger & Heimlich, 2015; Renzaho et al., 2017). For example, Wise (2012) reported that about 40% of USA produced corn goes into ethanol production. This increase in corn-based ethanol production in the

USA has been attributed to the government's subsidies and incentives towards the production and consumption of corn-based ethanol (Wise, 2012). Therefore, as incentives to grow bioenergy crops increases, less acres will be committed to the production of other food crops, leading to short supplies in food commodities and a resultant increase in food prices (Searchinger & Heimlich, 2015). This calls for the need for government policies on biofuels to be strategic, as incentivising bioenergy crop production without proper policy implementations might lead to more acres being committed to growing bioenergy crops at the expense of food crops, resulting in food insecurity.

1.4.2 Environmental implications of bioenergy crop production

1.4.2.1 Impact on land-use change

Production of bioenergy crops can result in various environmental consequences ranging from degradation of natural vegetation (Don et al., 2012; Pool, 2013; Jaafar et al., 2020), net GHG emissions (Searchinger et al., 2008; McGowan et al., 2018a; Searchinger et al., 2018; Jin et al., 2019), pollution and depletion of water resources (Gasparatos et al., 2013; Pool, 2013; Filoso et al., 2015), and biodiversity loss (Pool, 2013; Correa et al., 2017). These environmental impacts mostly result from biomass production from first-generation bioenergy crops owing to production methods and the high input requirements in producing these crops (Norgrove, 2010; Searchinger & Heimlich, 2015; Fess et al., 2018).

Upscaling bioenergy crop production to meet the bioenergy mandates or targets set by countries can lead to direct and indirect land-use change with associated increase in GHG emissions (Searchinger et al., 2008). Direct land-use change from bioenergy crop production occurs when bioenergy crops replace a different land-use such as a land previously grown to food crops or natural vegetations (e.g., forests and grasslands) (Koh and Ghazoul, 2008). On the other hand, indirect land-use change occurs when bioenergy is grown on an existing land which causes

new land to be used to compensate for the land lost to the bioenergy crop (Koh and Ghazoul, 2008). Both direct and indirect land-use change can occur inside or outside the bioenergy producing and consuming country, which Don et al. (2012) classified as 'internal' and 'external' land-use change (Figure 3.2). The external land-use change occurs when bioenergy feedstock is imported, and the land-use change resulting from the bioenergy crop takes place somewhere outside the bioenergy consuming country (producer) (Don et al., 2012). Conversely, if the land-use change to the bioenergy crop occurs in the bioenergy consuming country (consumer), it is referred to as internal land-use change (Don et al., 2012).

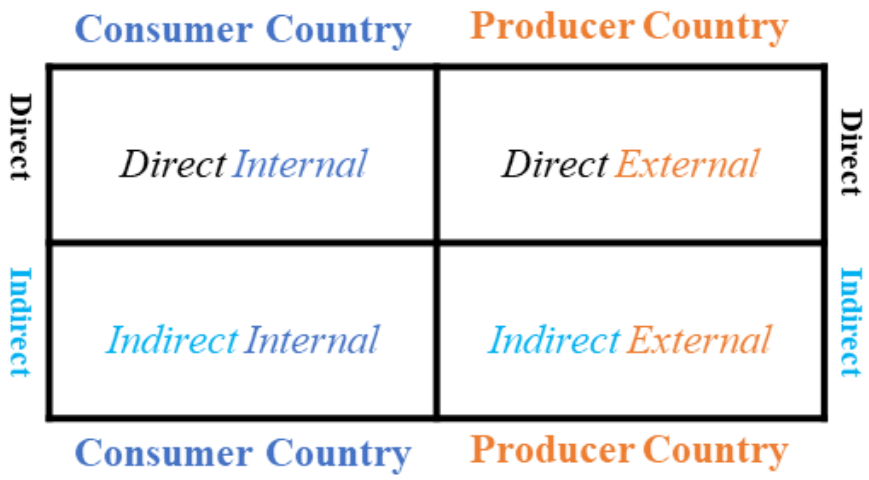


Figure 1.2: Schematic diagram depicting the differences in land-use changes associated with bioenergy crop production.

Deforestation of high value ecosystem vegetations in developing countries such as tropical primary forests can be caused by both internal and external land-use change from biofuel demands and consumption from well developed countries. Searchinger et al. (2008) explained that increased ethanol corn production in the USA and a resultant decrease in soybean acres could trigger higher soybean prices, which would accelerate the cultivation of Brazilian rainforest. It is also, estimated

that biofuels demand in Europe can cause ~ 4 to 7 million ha of indirect external land-use change mainly in countries like Brazil and India (Bowyer, 2010). Again, the increased demand for palm oil for biodiesel production from importer countries has led to major deforestation problems and increased peatlands conversion to oil palm plantations in Malaysia (Jaafar et al., 2020). Pool (2013) estimated that about 15% of Malaysia's total land area has been transformed into oil palm plantations. Jaafar et al (2020) also observed that from 1990 to 2018, the palm oil industry was responsible for the loss of ~13% and 16% forest cover, and 21% and 19% peatlands in Sarawak and Sabah, respectively. Sarawak, which occupies ~38% of the total land area of Malaysia and Sabah are the two largest oil palm producing regions in the country (Jaafar et al., 2020). These land-use changes from bioenergy crop production additionally, contribute to GHG emissions.

1.4.2.2 Impact on greenhouse gas emissions

Changes in land-use can impact GHG emissions in a variety of ways – such as changes in soil C stocks and vegetation, changes in CO_2 assimilation, and changes in N_2O emissions from fertilizer use (Hughes & Qureshi, 2014). Searchinger et al. (2008) explained that but for bioenergy, the amount of land cultivated for crop production will reflect the demand for food and fibre. This means that producing fuel from biomass will require the clearing of more forests and grasslands, which will release much of the C contained in the plants and soils to the atmosphere (Searchinger et al., 2008). The implications of these are that converting forests or grasslands to biofuel crops will rather result in a net increase in GHGs as the cleared vegetation will no longer exist to sequester the C it did (Pool, 2013).

Fargione et al. (2008) argued that converting C-rich habitats such as forests, peatlands, savannas, or grasslands to food crop-based biofuels create a "biofuel C debt". For instance, converting Malaysian or Indonesian tropical rainforest and peatland to oil palm plantation; the

Brazilian Amazon and Cerrado to soybean and sugarcane; and the US central grassland to corn will result in C debts of between 33 and 3003 Mg CO_2 ha^{-1}, which could take between 17 and 423 years to repay (Fargione et al., 2008). Searchinger & Heimlich (2015) indicated that the only way converting a land to growing bioenergy crops will yield a positive outcome in offsetting GHG emissions is if more CO_2 is taken up by the bioenergy crop than was taken up by whatever was growing on that land before the bioenergy crop.

Fertilizer management such as N fertilizer in bioenergy crop production is another key source of GHG emissions, particularly, N_2O (Behnke et al., 2012; Jin et al., 2019). Gauder et al. (2012) found that applying 80 kg ha^{-1} N to miscanthus in Germany increased mean N_2O fluxes to 4.3 μg N m^{-2} hr^{-1}, compared with –1.0 μg N m^{-2} hr^{-1} in the unfertilized miscanthus plots. Kavdir et al. (2008) also measured significant soil N_2O emissions after N fertilization of willow. Wile et al. (2014) observed that fertilization of switchgrass with 120 kg N ha^{-1} in Nova Scotia, Canada, increased 2-year cumulative N_2O emissions by 73% compared to the unfertilized crop. In southern Canada, Lutes et al. (2016) measured 22 to 26 μg N_2O-N m^{-2} hr^{-1} mean N_2O emissions from two willow clones [*Salix miyabeana* (SX67), *S. dasyclados* (SV1)] with N fertilization compared to 16 to 17 μg N_2O-N m^{-2} hr^{-1} in the unfertilized crops.

A study by Don et al. (2012) on GHG balance in bioenergy production in Europe observed that soil N_2O emissions on the average, can account for 27% of total GHG emissions from food crop-based biofuels, with contributions ranging between 5 and 80%. Kim & Dale (2009) also observed N_2O emissions from corn-based ethanol accounted for 11–37% of the total GHG emissions whereas contribution from soybean-based biodiesel was 13–57%. Moreover, Yang et al. (2021) found that the global mean total N_2O emission from sugarcane production is 2.26 kg N_2O-N ha^{-1} yr^{-1}. Comparing N_2O, CO_2, and CH_4 emissions from miscanthus and willow (second-

generation bioenergy crops) to annual food crops of wheat and canola (first-generation bioenergy crops) in England, Drewer et al. (2012) observed that whilst N_2O fluxes were higher in the first-generation bioenergy crops than the second-generation crops, CO_2 fluxes did not differ between the crops. Meanwhile, both crop types were sinks for CH_4. Evidence from these results imply that GHG emissions from bioenergy crop production depends not only on N fertilization but also on the type of crop with first-generation crops associated with comparatively higher emissions than second-generation crops (Searchinger & Heimlich, 2015).

1.4.2.3 Impact on biodiversity loss and water resources

The FAO (2008) defines two pathways for biodiversity loss – (1) habitat loss following land conversion, and (2) loss of agrobiodiversity induced by intensification on croplands, in the form of crop genetic uniformity. The first pathway of biodiversity loss involves the loss of habitat when natural ecosystems such as forests and grasslands are converted to bioenergy crop, causing a loss of wild biodiversity in these areas (FAO, 2008). The second pathway for biodiversity loss (agrobiodiversity loss) occurs when bioenergy crops are grown in large-scale monocropping systems, which increases the vulnerability of these crops to new pests and diseases (FAO, 2008). Using the DPSIR (driver-pressure-state-impact-response) framework in analyzing bioenergy crop production and biodiversity loss, Correa et al. (2017) reported that land-use change, and land-use intensification are the main pressures causing biodiversity loss in bioenergy crop production. This is because land-use change such as converting biodiverse and native ecosystems to bioenergy crops and the increased intensification of these crops in monoculture systems result in habitat loss and degradation as well as a decrease in species richness and abundance (Correa et al., 2017).

Koh (2007) investigated the likely biodiversity and habitat losses that may occur from a future increase in biodiesel production to meet a projected 276.7 million t yr^{-1} global demand by

the year 2050. To achieve this future biodiesel demand, Koh (2007) estimated that cultivated areas for biodiesel feedstocks (*e.g.,* soybeans, canola, and oil palm) will increase. However, the agricultural expansion of soybeans and oil palm would occur mostly in developing countries in the tropics such as Brazil, Indonesia, Malaysia, and Nigeria, which also happen to be biodiversity hotspots (Koh, 2007). Therefore, without any proper mitigation guidelines, Koh (2007) argues that any future intensification of these energy crops in these areas will further threaten the high concentrations of globally endemic species in these biodiversity hotspots. Landis et al. (2018) also observed in bioenergy cropping systems in North Central USA that the more diverse bioenergy crops had increased abundance and diversity of arthropods, bee species richness, and predatory insects which increased pest suppression and pollination in the diverse cropping system compared to the monoculture system, indicating the great impact producing crops for energy can have on biodiversity loss.

Increasing bioenergy production is also projected to result in greater water use due to the high amount of water required in producing energy from biomass (Gasparatos et al., 2013). In the bioenergy production process, water is required both in the development and the processing stages of the feedstocks into energy (Pool, 2013). The development stage constitutes the agriculture phase where the growth of the crop and its water need is supplied by either natural precipitation or irrigation (from surface and/or groundwater) (Filoso et al., 2015). Water use at the processing stage, also termed as the "industrial phase" includes water used for washing feedstock to remove soil particles and small debris, fermentation, and distillation – all three of which could account for 90% of the water consumed in sugarcane ethanol production mills (Filoso et al., 2015).

Globally, biofuels account for ~44 km^3, or 2% of all irrigation water withdrawals and about 100 km^3, or 1% of all water transpired by crops (de Fraiture et al., 2008). Further, ~2,500 L water

is needed on the average to produce 1 L of liquid biofuel, which is the same amount needed to produce food for one person for one day (WWAP, 2009). The USA National Research Council (NRC) (2008) also explained that it will require an equivalent amount of the water supply for a town of about 5,000 people to produce approximately, 100 million gallons of ethanol per year. This increased water appropriation of bioenergy production will add to the already existing pressure on scarce water resources. For instance, Pool (2013) explained that the water table of the aquifers of the Ogallala (or High Plains) regions has declined over 100 feet since about the 1940s. Meanwhile, the Ogallala aquifer of the Nebraska region will have to provide about 2,000 gallons of water per bushel of corn produced, thereby, further drawing down the aquifer (Pool, 2013).

Additionally, the use of agrochemicals, fertilizers, and industrial effluent from biofuel production impact water quality by polluting water bodies (Gasparatos et al., 2013). For instance, water pollution across the state of São Paulo in Brazil and in parts of Southeast Asia have been attributed to increased sugarcane and palm oil production, resulting in fertilizer, pesticide, and effluent from sugarcane and palm oil runoff into water bodies (Gasparatos et al., 2013). Filoso et al. (2015) explained that vinasse, which is the effluent generated in sugarcane ethanol production could be a major cause of eutrophication in water bodies due to the high concentration of potassium and N contained in this liquid waste. As much as 20 L of vinasse is generated for each liter of ethanol produced (Filoso et al., 2015), indicating the potential for water quality contamination from improper disposal of this effluent. Nobre and Nobre (2011) also explained that ethanol corn production has resulted in N and phosphorus loading in the Mississippi River, contributing to the "dead zones" in the Gulf of Mexico. Whereas the use of irrigation to supply crop-water need might deplete groundwater source, the use of pesticides and fertilizers in growing biofuel crops end up polluting waterbodies, hence, affecting both the quantity and quality of water.

1.5 Growing Perennial Bioenergy Crops on Low Productive Agricultural Lands: A Panacea to the Fuel-Food-Environment Trilemma?

At a time when debate over the sustainability of bioenergy crop production continues to linger on due to the food security and environmental challenges discussed above, growing perennial bioenergy crops (second-generation) on low productive agricultural lands continue to gain widespread attention (Clifton-Brown et al., 2017; Lewandowski, 2015). This is due to the elimination of the food versus fuel debate (Pool, 2013). Moreover, the capability of these crops to grow and rejuvenate low productive agricultural lands have been touted (Lemus & Lal, 2005; Blanco-Canqui, 2016; Chimento et al., 2016. As such, managing perennial bioenergy crops on low productive agricultural land is seen as a win-win-win scenario of producing sustainable energy that comes with numerous environmental benefits and eliminates the food security threat associated with producing bioenergy crops (Mangold et al., 2019). Potential low productive agricultural lands capable of growing perennial bioenergy crops include flood-prone soils, highly erodible lands, reclaimed mine soils, compaction prone soils, sloping soils, drought prone soils, acid and saline soils, urban marginal soils, sandy soils, and contaminated soils (Figure 3.3) (Blanco-Canqui, 2016). Converting these lands to perennial bioenergy crops can yield environmental benefits such as improved soil health, biological diversity, soil C sequestration and reduced GHG emissions.

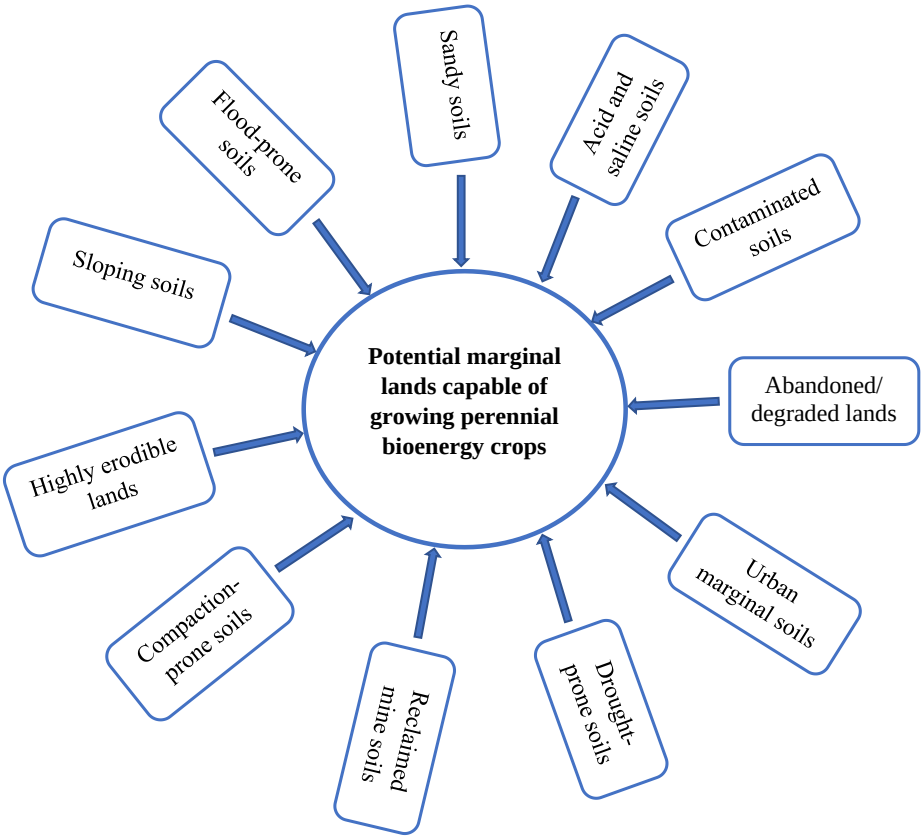


Figure 1.3: Potential low productive agricultural lands that have been studied to have the capability of growing perennial bioenergy crops (adopted from Blanco-Canqui 2016).

1.5.1 Biological diversity and soil health improvement

Absence of tillage operations and enhanced organic matter addition in perennial bioenergy crops stimulate biological diversity and activities which can promote soil productivity of low productive agricultural lands (Bourgeois et al., 2015; Mafa-Attoye et al., 2020; Sprunger et al., 2020). For instance, arbuscular mycorrhizal fungi (AMF), which promotes soil productivity by improving soil aggregation, nutrient, and water availability for plants uptake (Mafa-Attoye et al., 2020; Bennett et al., 2020) can be reduced through tillage operations (de la Cruz-Ortiz et al., 2020; Nyamwange et al., 2018). Jesus et al. (2016) observed higher AMF biomass in switchgrass than

agricultural soils due to the absence of soil tillage and potentially, more extensive root network in the switchgrass. In metal contaminated soils in France, enhanced organic matter input, and the absence of soil tillage and pesticide use in miscanthus, and switchgrass stimulated higher abundance and diversity of soil collembolans (Chauvat et al., 2014), densities and diversity of soil invertebrates (Hedde et al., 2013), and fungal and bacterial diversity (Bourgeois et al., 2015) in the perennial crops compared to annual cropping systems. Felten & Emmerling (2012) also observed a 15-year-old miscanthus plantation in Germany had 70 m^{-2} more earthworms and 2 more different earthworm species than a corn monocrop farm due to extensive litter cover at ground level under miscanthus compared to the annual corn crop. Given the important role the soil biological community plays in soil fertility and productivity (McTavish & Murphy, 2022), their increase activities and diversities in perennial bioenergy cropping systems can help improve soil productivity in low productive agricultural lands when grown with these bioenergy crops.

Aside higher soil biological diversity and activities, higher bird diversity and abundance have been observed in perennial bioenergy crops compared to annual crops due to habitat structure and greater availability of diverse food supply induced by crop canopies (Robertson et al., 2011). Bellamy et al (2009) observed the provision of winter shelter and nesting space in miscanthus stands in England resulted in the presence of 24 bird species in the miscanthus compared to 11 species in wheat fields. Robertson et al. (2011) also observed a total of 35 birds comprising 22 different species in switchgrass compared to 29 birds of 6 different species in corn fields in southern Michigan, USA. Again, Bennett et al. (2014) estimated that converting annual crops on low productive agricultural soil to perennial grasses could increase bee abundance from 0 to 600 % and bee diversity between 0 and 53%. These findings imply that growing perennial bioenergy crops on low productive agricultural lands could be very important in restoring and enhancing

biodiversity on these lands whiles meeting our energy demands without compromising food security.

1.5.2 Improvement in water resources

In perennial bioenergy crop systems, there is a reduced risk of surface and ground water contamination from fertilizer and other agrochemicals. This is due to the efficacy of the deep and extensive roots of perennial crops to exploit and recycle nutrients as well as supress weed growth thereby reducing their requirements for fertilizers and herbicides (Blanco-Canqui 2010; McCalmont et al., 2017). von Cossel et al. (2020) indicated that N fertilizer requirements of miscanthus could be as low as 52 kg N ha^{-1} yr^{-1}, which is one-third that of corn (150 kg N ha^{-1} yr^{-1}). This low N fertilizer requirements of miscanthus reduces the risk of ground water contamination from NO_3^- leaching in perennial bioenergy crops compared to annual crops such as corn. For instance, McIsaac et al. (2010) observed significantly lower NO_3^- leaching rates under miscanthus (3.0 kg N ha^{-1} yr^{-1}) and switchgrass (1.4 kg N ha^{-1} yr^{-1}) than in annual corn/soybean rotation (40.4 kg N ha^{-1} yr^{-1}). Additionally, perennial grasses such as switchgrass and miscanthus are very effective in reducing soil erosion and sediment transport in runoff water (Blanco-Canqui 2010; Samson et al. 2016; Withers et al. 2016). With soil erosion and run-off water being some of the major means through which phosphorus and nutrients from agricultural fields are deposited in water bodies (Reid et al., 2018), the ability of these crops to prevent soil erosion and surface runoff will prevent phosphorus and nutrient loading in water bodies, mitigating the water pollution threat posed by growing bioenergy crops.

1.5.3 Reduced greenhouse gases emissions and soil organic carbon stabilization in perennial bioenergy crops

A key advantage of growing perennial bioenergy crops on low productive agricultural lands are their ability to reduce GHG emissions and increase SOC sequestration (Koh & Ghazoul, 2008; Chimento et al., 2016). Minimal N fertilizer requirements of perennial bioenergy crops (Chimento et al., 2016; von Cossel et al., 2020) coupled with their ability to exploit and recycle nutrients in their deep and extensive root systems (Thevathasan et al., 2012) reduces the potential for N_2O emissions in these crops relative to annual row crops. Oates et al. (2016) measured 142% higher cumulative N_2O emissions over three years from annual cropping system compared to perennial bioenergy crops of switchgrass, miscanthus, poplar, and prairie mixed grasses in North Central USA. Drewer et al. (2012) observed 0.2 μg m^{-2} hr^{-1} and 4 μg m^{-2} hr^{-1} average N_2O-N fluxes in willow and miscanthus, respectively, compared to 8 μg m^{-2} hr^{-1} wheat and 32 μg m^{-2} hr^{-1} in canola in the UK. Smith et al. (2013) also found 3-year cumulative N_2O-N emissions were four times higher in annual row crops than switchgrass and miscanthus, indicating the potential of perennial bioenergy crops to reduce non-CO_2 emissions.

Moreover, establishing perennial bioenergy crops on low productive agricultural lands enhances soil C storage. Heaton et al. (2004) estimates 0.5 Mg ha^{-1} yr^{-1} SOC contribution by miscanthus on low productive agricultural lands. Don et al. (2012) also observed that conversion from annual cropland to perennial crops yielded SOC gains of 0.44 Mg soil C ha^{-1} yr^{-1} in short rotation coppice willows and poplar and 0.66 Mg soil C ha^{-1} yr^{-1} in miscanthus. More references (*e.g.*, McCalmont et al., 2017; Coleman et al., 2018; Bazrgar et al., 2020) are provided in *Section 1.2.2* in this chapter on SOC gains from different perennial bioenergy crops on low productive agricultural land in southern Canada. Whiles these studies revealed gains in soil C, Graham et al.

(2019) did not find significantly higher SOC stocks in switchgrass and miscanthus compared to an agricultural site and attributed this lack of SOC gains to frequent biomass harvest in the bioenergy crops. This goes to show that the SOC storage outcome of perennial bioenergy crops may not always be positive and could be affected by site and management practices (Amichev et al., 2014).

Not only do perennial bioenergy crops contribute to higher C accumulation, but they also enhance the stability of the contributed C through the stabilization of soil organic matter (Lemus & Lal, 2005; Blanco-Canqui, 2010; Chimento et al., 2016). Central to soil organic matter stabilization are soil aggregates which are formed through the association of mineral particles with organic and/or inorganic binding agents (Six et al., 2002). Depending on diameter, soil aggregates can be classified as macroaggregates (250-2000 μm diameter), microaggregates (53-250 μm diameter), or silt and clay-sized fractions (<53 μm) (Figure 3.4) (Tisdall & Oades, 1982). Whereas SOC associated with macroaggregates are considered less stable, those associated with microaggregates, and the silt and clay-sized fractions are more stable and represent a long-term C store (Nair et al., 2010; Torres-Sallan et al., 2017). Moreover, the age, nature and size of C differs with the aggregate classes in which the C is located (Nair et al., 2010). For instance, C in the macroaggregates represent recently deposited C in the soil with those in microaggregates and the silt and clay-sized fractions representing old and humified C pools (Nair et al., 2010).

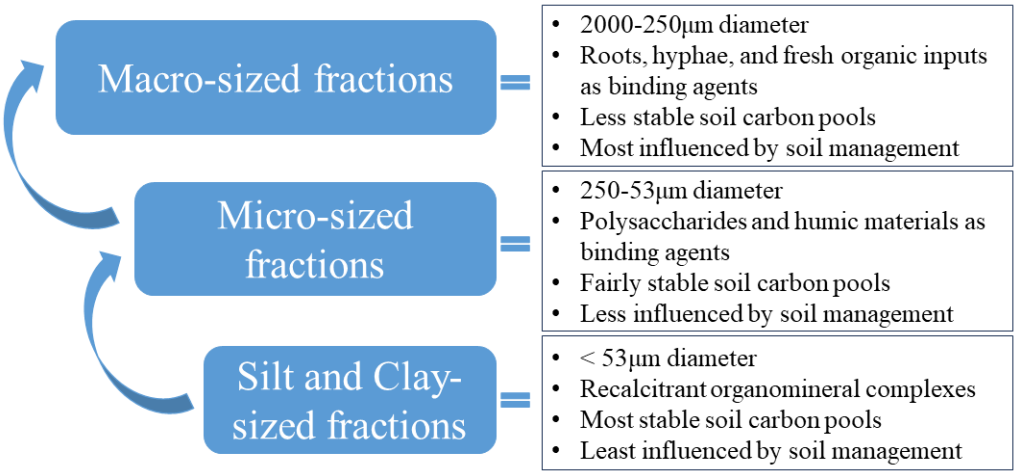


Figure 1.4: A schematic overview of the soil aggregate hierarchy model.

According to the aggregate hierarchy model by Tisdall & Oades (1982), in soils where the major binding agents for aggregate formation are organic matter such as in temperate soils, SOC is expected to be higher in macroaggregates than in microaggregates and the silt and clay-sized fractions. This may, however, depend on the land use type as Baah-Acheamfour et al. (2014) found across sites of three agroforestry systems in central Alberta – Canada that 23.1% of the SOC to a 10-cm depth was contained in the macro-sized aggregates with 28.5% and 48.4% in the micro-sized and silt and clay-sized aggregates, respectively. The higher SOC content in the microaggregates and silt and clay-sized fractions compared to the macroaggregates was attributed to slower turnover of macroaggregates due to decreased soil disturbance. This reduced turnover of the macroaggregates may have allowed for the gradual formation of fine occluded organic matter within the silt and clay-sized fractions and the creation of more stable microaggregates from microbial products over time (Baah-Acheamfour et al., 2014).

Continuous organic matter input and reduced soil disturbance in perennial bioenergy crops can potentially enhance the soil organic matter stability through improved soil aggregation and

reduced organic matter turnover contributing to long-term C storage (Lemus & Lal, 2005; Blanco-Canqui, 2010; Chimento et al., 2016). Despite this, SOC in different soil-sized fractions under perennial bioenergy crops have seldom been studied and currently, there is a gap in research data on SOC associated with different soil-sized fractions under perennial bioenergy crops. Moreover, it is important to trace the source of the C in the different soil-sized fractions to understand the contribution of different perennial bioenergy crops to SOC in different soil-sized fractions. This will help elucidate the impact of these crops in long-term carbon sequestration and climate change mitigation.

1.6 Stable Isotopes as Tool for Soil Carbon and Nitrogen Study – Applicability in Perennial Bioenergy Crop Systems

Soil ^{13}C and ^{15}N natural abundance stable isotope techniques are an innovative approach to understanding soil C and N dynamics in managed and natural ecosystems (Oelbermann et al., 2006; Oelbermann & Voroney, 2007; Denef et al., 2013; Park et al., 2023). For instance, ^{15}N natural abundance expressed as the difference in $^{15}N/^{14}N$ ratios relative to that in atmospheric N_2 in per mil, ‰ ($\delta^{15}N$) has been used to study N cycling and transformation in terrestrial ecosystems (Scheer & Rütting, 2023; Chen et al., 2024). Plants can acquire N from a number of different sources, each of which can have a unique ^{15}N signature (Craine et al., 2009). The amount of ^{15}N in plant biomass can be impacted by the mean annual temperature and precipitation, availability, source, and the form of N such as NH_4^+ and NO_3^- (Craine et al., 2009; Sheng et al., 2014). Generally, ^{15}N in NH_4^+ is more enriched than that in NO_3^-, as isotope fractionation during nitrification depletes ^{15}N in NO_3^- (Sheng et al., 2014; R. Wang et al., 2021; Chen et al., 2024). In view of this, systems with increased biomass uptake of soil NO_3^- relative to NH_4^+ result in lower biomass $\delta^{15}N$ values (Chen et al., 2024). In a global study, Craine et al. (2018) observed foliar $\delta^{15}N$ values increased with increased N availability and concentrations of foliar N. This contrasted with

findings by Chen et al. (2024) who observed lower $\delta^{15}N$ values with increasing N concentrations in leaves and roots in shrubs and grasses. Chen et al. (2024) linked the lower $\delta^{15}N$ values to increase uptake of the much ^{15}N-depleted NO_3^- pools relative to ^{15}N enriched NH_4^+.

Association of mycorrhizal fungi with plants can impact the amount of ^{15}N in plant biomass (Craine et al., 2015). In a review of studies, Craine et al. (2009) found that plants with mycorrhizal fungi were depleted in foliar $\delta^{15}N$ by 2‰ to 5.9‰ compared to nonmycorrhizal plants. Depletion in foliar $\delta^{15}N$ was attributed to the supply of ^{15}N-depleted N to plants by the mycorrhizal fungi (Craine et al., 2009). Additionally, atmospheric deposition of N can impact amount of ^{15}N in plant biomass (Craine et al., 2015). Sheng et al. (2014) explained that ammonia (NH_3) volatilisation is a highly fractionating process which produces much ^{15}N-depleted NH_3. Because of this the $\delta^{15}N$ values of deposited NH_4^+ are usually negative (Sheng et al., 2014). Xiao & Liu (2002) reported mean $\delta^{15}N$ values of –2.1‰ and –5.0‰ in wet deposited NO_3^- and NH_4^+, respectively, in seven different sites around the world. Hence, in N-limiting terrestrial environments where atmospheric deposition of N contributes majorly to biomass N source, lower $\delta^{15}N$ values in plant biomass may be expected.

Compared to mineral soils, Sheng et al. (2014) found biomass from matured forest ecosystems in eastern China were significantly depleted in ^{15}N. In the soil, the preferential loss of the lighter ^{14}N through processes such as leaching, volatilisation, nitrification and denitrification leaves the soil enriched in ^{15}N (higher $\delta^{15}N$ values) (Craine et al., 2015; Wang et al., 2021b). Because of this discrimination against ^{15}N during these soil processes, the N taken up by plants may usually constitute ^{15}N-depleted N (Chen et al., 2024). The increased uptake of ^{15}N-depeleted N results in depletion of ^{15}N in plant biomass (lower $\delta^{15}N$ values) (Chen et al., 2024). Sheng et al. (2014) found soil $\delta^{15}N$ values increased with soil depth and attributed this observation to inputs of

biomass litter containing ^{15}N-depleted N to surface soils which may have resulted in lower $\delta^{15}N$ signatures in surface soils compared to deeper soil depths. Furthermore, discrimination against ^{15}N during mineralisation and the possible loss of ^{15}N-depleted nitrogen from soils due to root uptake, nitrate leaching, and denitrification was explained to have contributed to the enrichment of ^{15}N in deeper soils compared to surface soils (Sheng et al., 2014).

Similar to the ^{15}N natural abundance technique, $\delta^{13}C$ signatures of SOC are powerful indicators and tracers of C fluxes through soils and of transformation processes within soils (Krüger et al., 2024). The extent to which plants discriminate against ^{13}C during photosynthesis depends on their photosynthetic pathways (Chalk et al., 2021). The photosynthetic pathway for C_3 plants which goes through the Calvin cycle reduces CO_2 to phosphoglycerate, a 3-carbon compound, via the enzyme ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCO) (Wang et al., 2012; Chalk et al., 2021). This process discriminates against $^{13}CO_2$ resulting in C_3 plants to have lower $\delta^{13}C$ values ranging from –32‰ to –22‰, with an average of –27‰ (Chalk et al., 2021). The C_4 photosynthetic pathway uses the Hatch-Slack pathway C_4 where CO_2 is reduced to aspartic or malic acids, both 4-carbon compounds, via the enzyme phosphophenyl pyruvate carboxylase (Wang et al., 2012; Chalk et al., 2021). The C_4 photosynthetic pathway discriminates against $^{13}CO_2$ to a lesser extent than the C_3 pathway which causes C_4 plants to have higher $\delta^{13}C$ than C_3 plants with values ranging from –17‰ to –9‰, and an average value of –13‰ (Chalk et al., 2021). For example, in an alley cropping system in southern Canada, Oelbermann & Voroney (2007) calculated $\delta^{13}C$ values of –28.66‰, –28.23‰, and –29.57‰ in C_3 vegetations of hybrid poplar, soybean, and wheat, respectively, compared to –11.83‰ in C_4 corn.

Since the isotopic composition of soil organic matter closely resembles that of the vegetation from which it was derived (Chalk et al., 2021), the ^{13}C natural abundance technique has

been successfully used to study shift in ^{13}C abundance in SOC under different land-use systems where a mixture of C_3 and C_4 plants are grown on the same land area or where there had been a switch in vegetation from C_3 to C_4 or vice versa (Berhongaray et al., 2019). Within a given land management system, the proportions of C_3- and C_4-derived C are calculated using a two-end-member mixing models according to Balesdent et al. (1987).

$$f_{C3} = \frac{\delta^{13}C_{SOM} - \delta^{13}C_{C4}}{\delta^{13}C_{C3} - \delta^{13}C_{C4}} \quad (1.1)$$

where $\delta^{13}C_{SOM}$ is the $\delta^{13}C$ of soil from a mixture of C_4 and C_3 sources, $\delta^{13}C_{C4}$ is the mean $\delta^{13}C$ for the C_4 residue, and $\delta^{13}C_{C3}$ is the $\delta^{13}C$ of the initial soil or similar soil from a C_3 vegetation.

The fraction of C_4-C (f_{C4}) can be calculated using the following equation:

$$f_{C4} = 1 - f_{C3} \quad (1.2)$$

The ^{13}C natural abundance technique has been successfully used to calculate the proportion of carbon contributions from C_3 and C_4 sources in simple and complex land management systems such as in annual croplands (Denef et al., 2013; Bichel et al., 2016), natural vegetation (Denef et al., 2013), agroforestry systems (Oelbermann et al., 2006; Oelbermann & Voroney, 2007), and perennial bioenergy cropping systems (Dondini et al., 2009; Collins et al., 2020). This indicates the suitability of the ^{13}C natural abundance technique in SOC studies in bioenergy crop systems to elucidate how different perennial bioenergy crops can contribute to soil C storage when grown on low productive agricultural lands.

1.7 Winter Freeze-Thaw and GHG Emissions – Impact of Perennial Cropping Systems

Carbon and N transformations in the soil are microbially mediated and regulated by a variety of abiotic and biotic factors (Congreves et al., 2018; Risk et al., 2013; Zhang et al., 2011). Among the abiotic factors is temperature fluctuations, which causes freeze-thaw cycles in cool

temperate regions in winter seasons (Zhang et al., 2011). Freeze-thaw processes affect the soil's microbes, soil aggregates, SOC and N pools, which in turn influences freeze-thaw related N_2O and CO_2 emissions (Libby et al., 2020). Freeze-thaw events can account for up to 70% and 50% of annual N_2O and CO_2 emissions, respectively (Kurganova et al., 2007; Hung et al., 2021). Wagner-riddle et al. (2007) observed N_2O emissions from winter freeze-thaw accounted for 30% to 90% of total annual N_2O emissions in southern Canada over a 5-year period. This indicates the crucial contribution of freeze-thaw-induced emissions to annual GHG budgets. The extent of freeze-thaw-related N_2O and CO_2 emissions can vary with land-use type (Lutes et al., 2019; Wagner-Riddle et al., 2017), vegetation cover (Ferchaud et al., 2020; Lovett et al., 2002), severity and number of repeated freeze-thaw cycles (Grogan et al., 2004; Zhang et al., 2011).

Freezing and thawing increases the amount of labile C and soil N through mortality of microbial cells and fine roots, and the breakdown of soil aggregates making previously inaccessible substrates available for mineralization (Chai et al., 2014; Congreves et al., 2018; Libby et al., 2020; Risk et al., 2013). The mineralization of these labile C and soil N pools results in increased NH_4^+ and NO_3^- concentrations causing an increased production and emission of N_2O and CO_2 (Green et al., 2022; Pi et al., 2021; Wagner-Riddle et al., 2017). Chai et al. (2014) observed reduction in water stable soil aggregates which resulted in depletion of soil C and N pools. Congreves et al. (2018) noted that soil aggregate disruption following freeze-thaw was one of the main sources of labile soil C and N pools used by the soil microbial community for N_2O production and emission during soil freeze-thaw processes.

Compared to annual row crops, perennial systems such as perennial bioenergy crops have greater capacity to reduce GHG emissions during soil freeze-thaw. Continued presence of perennial crops on land during winter seasons enhances snow capture (Hayashi, 2013). The

captured snow provides cover to the soil insulating the soil from increased freezing depth and intensity (Libby et al., 2020). Similar to soil insulation provided from snow cover, increased organic matter input in perennial cropping systems can insulate soil against severe freezing and a consequent freeze-thaw-induced emissions. Wagner-Riddle et al. (2007) studied that the insulating effects of snow cover and crop residue during winter accounted for 80% of the total N_2O emission reduction over 5 years under no-tillage systems in southern Canada. Kurganova et al. (2007) also observed a higher freezing rate in agricultural soils compared to forest soils due to higher organic matter content, which provided insulation against freezing in the forest soil. Moreover, increased nutrient uptake by the continued presence of growing biomass in perennial systems (Thevathasan et al., 2012) reduces soil available N for loss as N_2O. Wagner-Riddle et al. (2017) found lower cumulative winter N_2O emissions in perennial crops compared to annual crops due to higher residual N content in the croplands. These observations imply that with projected future climate scenarios suggesting frequent freeze-thaw events in cold temperate regions (Kurganova et al., 2007; Wagner-Riddle et al., 2017; Rezanezhad et al., 2023), growing perennial bioenergy crops may present a greater capacity to reduce freeze-thaw-induced emissions contributing to climate change mitigation. This makes it important for the assessment of not just the C storage potential of perennial bioenergy crops, but also GHG emissions and as influenced by abiotic processes such as soil freeze-thaw. This will help provide comprehensive understanding of the climate benefits associated with growing different perennial bioenergy crops on low productive agricultural lands.

1.8 Research Needs

Currently, there is a gap in research on SOC stocks in soil-sized fractions under different perennial bioenergy crops. This is despite the key role soil-sized fractions play in soil C stabilization and long-term SOC storage (Nair et al., 2010; Torres-Sallan et al., 2017). Also, studies

comparing the proportion of C contribution from different perennial bioenergy crops to different soil-sized fractions using ^{13}C natural abundance isotope technique are lacking. Additionally, whereas studies have quantified and compared GHG emissions in fertilized perennial bioenergy crops to annual row crop sites (*e.g.*, Abraha et al., 2018; Davis et al., 2012; Jin et al., 2019; McGowan et al., 2018b; Oates et al., 2016), those comparing GHG emissions in unfertilized perennial bioenergy crops to a successional site are absent. This is despite the argument that leaving low productive agricultural lands to secondary regrowth vegetation could provide greater climate benefits compared to converting low productive agricultural land to perennial bioenergy crops (Searchinger & Heimlich, 2015). Moreover, with soil freeze-thaw capable of contributing >50% of annual N_2O and CO_2 emissions coupled with the projected increase in soil freeze-thaw events with a changing climate (Kurganova et al., 2007; Wagner-Riddle et al., 2017), understanding how different perennial bioenergy crops influence N_2O and CO_2 emissions under freeze-thaw conditions is crucial to assessing the climate benefits of different perennial bioenergy crops. Understanding the soil organic carbon (SOC) content in various soil-sized fractions, alongside N_2O and CO_2 emissions as influenced by miscanthus, switchgrass, and willow compared to a successional site, will offer crucial insights into the climate change mitigation benefits of unfertilized perennial bioenergy crops on low-productivity agricultural land.

1.8.1 Specific research objectives of the current study

To address the above-mentioned research needs, the specific research objectives of the current study were:

- ✓ To quantify the N_2O and CO_2 emissions in perennial bioenergy crops of miscanthus, switchgrass, willow, and a natural regrowth (successional) site over two growing seasons.

- ✓ To assess the dominant soil factors influencing N_2O and CO_2 emissions over the two growing seasons.
- ✓ To measure and compare water stable soil aggregates before- and after soil freeze-thaw in perennial bioenergy crops and the successional site and assess their contributions to N_2O and CO_2 emissions.
- ✓ To determine and compare how repeated freeze-thaw cycles impact the C and N cycling and N_2O and CO_2 emissions in the perennial bioenergy crops and successional site.
- ✓ To assess the dominant soil and soil porewater properties that influence N_2O and CO_2 emissions following soil freeze-thaw in perennial bioenergy crops and successional site.
- ✓ To quantify SOC and total N contents in whole and soil-sized fractions under different perennial bioenergy crops.
- ✓ To quantify C contributions from miscanthus, switchgrass and willow to different soil-sized fractions using the ^{13}C natural abundance technique.
- ✓ To predict and compare long-term SOC dynamics in different perennial bioenergy crops to a secondary growth successional site and a row crop agroecosystem using the Century model.

1.8.2 Hypotheses

The specific hypotheses of this study were:

- ✓ Perennial bioenergy crops and successional site would influence soil moisture, temperature, EC, and available N differently, resulting in varied responses to N_2O and CO_2 emissions under field conditions during growing seasons.
- ✓ Presence of diverse plant species in the successional site would promote increased microbial activities leading to higher CO_2 emissions compared to the monoculture systems

in the perennial bioenergy crops during growing season under field and freeze-thaw conditions in the laboratory.

- ✓ Soil parameters and resultant N_2O and CO_2 emissions would differ between years due to potential differences in environmental conditions between two growing seasons.
- ✓ Efficient N cycling in the perennial bioenergy crops due to their extensive root network compared to the successional site would reduce the amount of residual soil inorganic N available for loss as N_2O in the bioenergy crops.
- ✓ Improved root network in perennial bioenergy crops would enhance water stable soil aggregates in the bioenergy crops similar to the successional site minimizing freeze-thaw-induced soil aggregate disruptions and related N_2O and CO_2 emissions.
- ✓ Higher soil C/N ratios from perennial bioenergy crops (monocrops) than successional site with diverse plant species would promote lower N_2O and CO_2 emissions in perennial bioenergy crops compared to the successional site.
- ✓ Despite frequent biomass harvest and the absence of fertilization, perennial bioenergy crops would increase SOC contents after 12 years of land occupation compared to the baseline SOC content in 2009 (before the bioenergy crops cultivation).
- ✓ Perennial bioenergy crops would have a similar effect on enhancing SOC pools and macro-sized soil fractions comparable to the successional site.
- ✓ SOC content distribution in perennial bioenergy crops would follow the hierarchical model proposed by Tisdall & Oades (1982), where SOC would be highest in the macro-sized fractions, followed by micro-sized fractions, with the silt + clay-sized fractions containing the least.

- ✓ The $\delta^{13}C$ signatures of SOC in the soil-sized fractions would reflect the vegetation composition of the bioenergy crops, with the miscanthus and switchgrass showing C_4-dominant $\delta^{13}C$ signatures and C_3-dominant $\delta^{13}C$ signatures under the willow.
- ✓ Reduced soil disturbance in the perennial bioenergy crops would enhance slow and passive SOC fractions, representing the more stabilized and long-lived soil C pools relative to the much-disturbed soil in the row crop system.

1.9 Study Site

The research was conducted at the University of Guelph Agroforestry Research Station in Guelph, Ontario (Latitude 43º 32' 28" N, longitude 80º 12' 32" W) (Figure 1.5). The site is 346 m above sea level with an average annual precipitation of 923 mm and mean annual temperature of 6.5 ºC (Mann, 2012). The soil is classified as a gray-brown Luvisol with a fine sandy loam texture (56% sand, 34% silt and 10% clay). This land falls under class 4 in the Canadian Land Inventory classification (Marsal et al., 2016). Since lands classified as class 4 or lower are considered marginal (low productive) agricultural lands, these lands are less suitable for food crops (Marsal et al., 2016). Prior to the establishment of the perennial bioenergy crops, the site was under corn, bean (*Glycine max* L.) and wheat (*Triticum vulgare* L.) rotation for about 25 years until 2008. Since row crop yields were very low, the area was converted to a perennial bioenergy biomass research site in 2009 (Marsal et al., 2016).

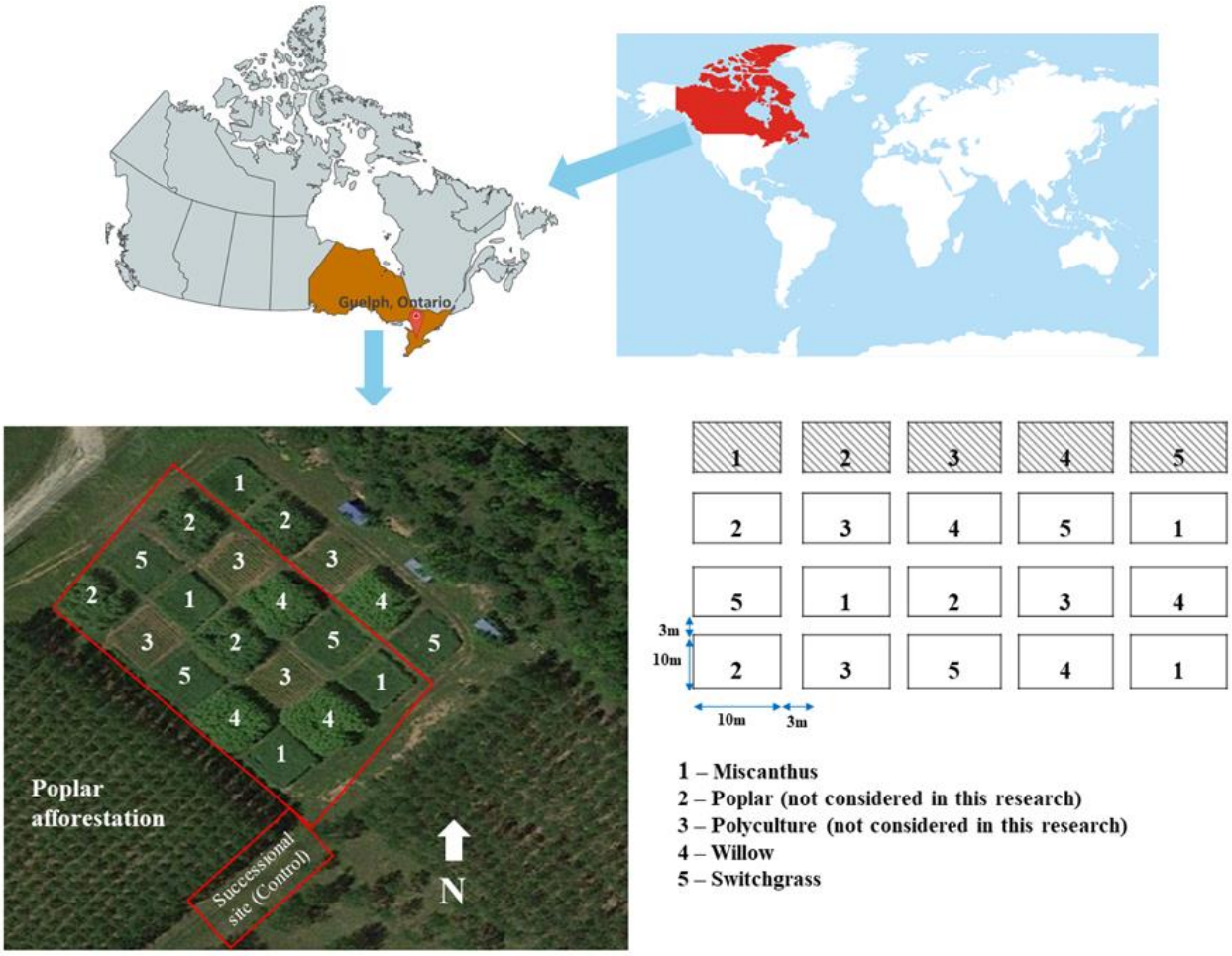


Figure 1.5: Map of Canada showing the research location (Guelph) in Ontario and the experimental layout of the bioenergy crops at the University of Guelph Agroforestry Research Station in southern Canada.

1.9.1 Perennial bioenergy crop establishment

Five treatments of i) willow (*Salix miyabeana* clone SX67), ii) poplar (*Populus spp*. clone 2293-19), iii) switchgrass (*Panicum virgatum* (cave-in-rock)), iv) *Miscanthus giganteus* (Nagara), and v) herbacous polyculture in a 25:25:25:25 mix composed of [indiangrass (*Sorghastrum nutans*); big bluestem (*Andropogon gerardi*); little bluestem (*Schizachyrium scoparium*); and switchgrass (*Panicum virgatum*)] were established in 2009 using a randomized complete block

design with 4 replications (Mann, 2012). Each of the treatment replicates consisted of a 10 m x 10 m plot separated from each other by 3 m boarders (Figure 1.5).

Prior to the bioenergy crop planting, the site was rototilled to a 15 cm depth. Willow and poplar were planted at 15000 stools/ha, miscanthus at 18000 plants/ha, and the switchgrass and polyculture were seed broadcast at 45 kg ha^{-1} (Mann, 2012). Prior to 2017, the bioenergy crops received 60 kg N ha^{-1} according to information provided by the site manager. However, all treatment replicates did not receive fertilization since 2017. The treatment with the polyculture did not respond well due to weed infestation, and poplar was not responding well after coppicing (based on field observations and conversations with the site manager), hence, only the willow, switchgrass, and miscanthus were considered for this study. Additionally, a natural vegetation site located adjacent to the bioenergy crops site, which was under the same management system as the site with the bioenergy crops prior to their establishment, was included in this research as a control (hereafter referred to as successional site) (Figure 1.6). Vegetation on the successional site consisted of a mixture of annual and perennial plants which included quackgrass (*Elymus repens* L.), perennial ryegrass (*Lolium perenne* L.), lamb's quarters (*Chenopodium album* L.), pigweed (*Amaranthus* spp.), chickweed (*Stellaria media* L.), Canada goldenrod (*Solidago canadensis* L.), red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), common bird's-foot trefoil (*Lotus corniculatus* L.), prairie coneflower (*Ratibida pinnata* L.), alfalfa (*Medicago sativa* L.), cow vetch (*Vicia cracca* L.), black medick (*Medicago lupulina* L.), common dandelion (*Taraxacum officinale* L.), common buckthorn (*Rhamnus cathartica* L.), common plantain (*Plantago major* L.), and wild carrot (*Daucus carota* L.). For this research, three blocks representing three replicates in each of miscanthus, switchgrass, and willow (Figure 1.6) and three subplots, representing three replicates in the successional site were selected for the study. The most

recent biomass harvest of miscanthus and switchgrass occurred in April 2020 and that for willow occurred in the Fall in 2018.

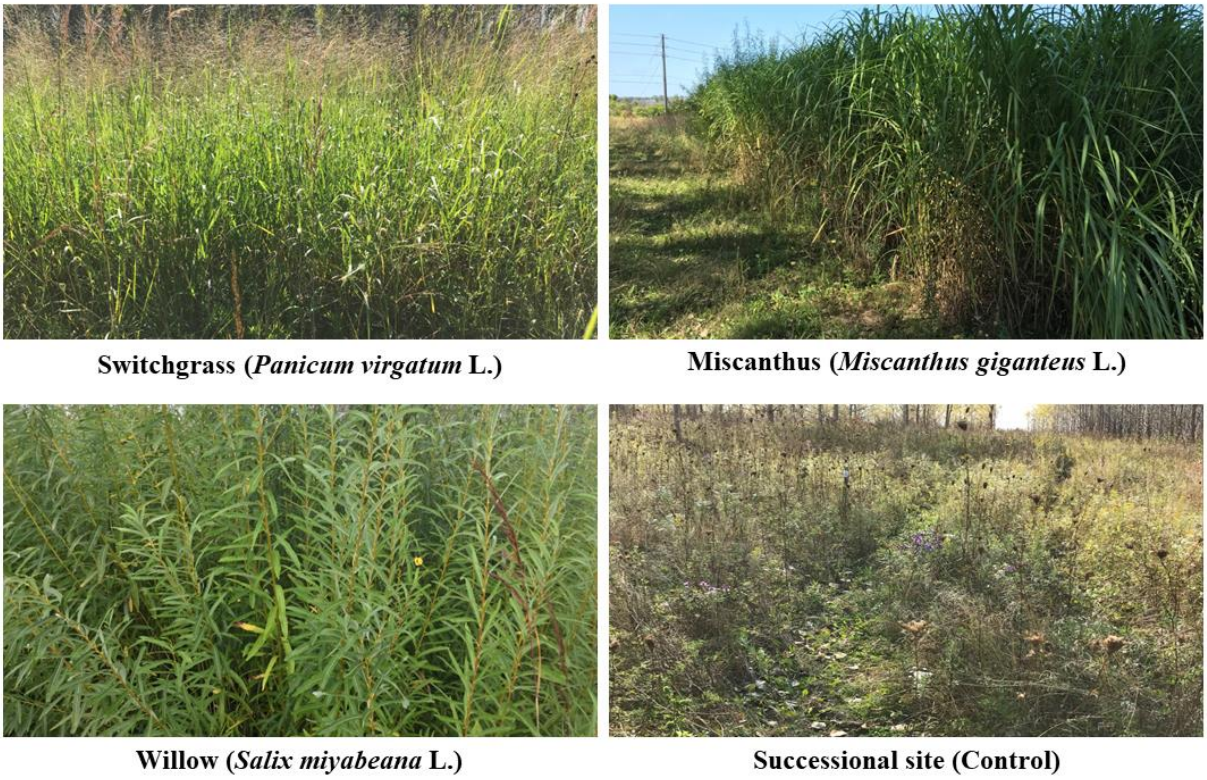


Figure 1.6: The perennial bioenergy crops and successional site at the University of Guelph Agroforestry Research Station in southern Canada.

CHAPTER 2

2.0 Comparing greenhouse gas emissions in unfertilized perennial bioenergy crops on low productive agricultural land in southern Ontario over two growing seasons

2.1 Introduction

Price volatility and uncertainty regarding the future of fossil fuels to meet global energy demands, along with concerns about GHG emissions associated with this energy source, have catalyzed the rapid development of renewable energy options (Khan et al., 2019; Acosta-Michlik et al., 2011; Lemus & Lal, 2005). Chief among these alternatives is bioenergy, which is widely regarded as a C-neutral energy source (Perea-Moreno et al., 2019; Chimento et al., 2016). This is because the CO_2 emitted during combustion is offset by the C absorbed during plant growth (Perea-Moreno et al., 2019). Despite this, questions continue to be asked about the C neutrality of bioenergy (Searchinger & Heimlich, 2015; Pool, 2013).

Debate around the C neutrality of bioenergy stems from the multitude of sources of bioenergy feedstocks and the different methods used to produce these feedstocks (Gasparatos et al., 2013; Blanco-Canqui, 2010; Norgrove, 2010). For instance, the use of N-based fertilizers in growing crops such as corn, canola, and soybean, which are termed first-generation bioenergy crops, not only leads to food security challenges (Searchinger & Heimlich, 2015; Pool, 2013) but also contributes significantly to GHG emissions such as N_2O (Crutzen et al., 2008). In view of this, perennial bioenergy crops or bioenergy production from second-generation crops have received wider recognition for their ability to yield high biomass on low productive agricultural lands (Marsal et al., 2016; Lewandowski, 2015), eliminate competition with food crops for land occupation (Agostini et al., 2015; German et al., 2011; Norgrove, 2010), and offer climate benefits through reduced GHG emissions (Abraha et al., 2019; Koh & Ghazoul, 2008).

The potential of perennial bioenergy crops to reduce GHG emissions compared to annual crop systems is attributed to the reduced use of N fertilizer in perennial bioenergy, which reduces the risk of N losses as N_2O (Wagner-Riddle et al., 2017; Chimento et al., 2016; Don et al., 2012). Additionally, the extensive root network in perennial bioenergy crops enhances N uptake, reducing soil N available for loss as N_2O (Thevathasan et al., 2012). Jin et al. (2019) observed that the net GHG outcome of no-till corn systems for biofuel ranged from 0.3 to 1.6 Mg CO_2-eq ha^{-1} yr^{-1} compared to −3.1 to 1.1 Mg CO_2 eq ha^{-1} yr^{-1} in switchgrass. Oates et al. (2016) also found that cumulative three-year N_2O emissions from annual biofuel cropping systems were 142% higher than from perennial systems. While these results indicate the potential of perennial bioenergy crops to reduce GHG emissions compared to annual systems, differences in management practices (Lutes et al., 2019; Ferchaud et al., 2020), biomass species (Osei et al., 2024; McGowan et al., 2018b; Lutes et al., 2016), and soil and environmental conditions (Osei et al., 2024; Oates et al., 2016) can impact GHG emissions differently. For example, Gauder et al. (2012) measured –1.0 μg N m^{-2} hr^{-1} in unfertilized miscanthus compared to 4.3 μg N m^{-2} hr^{-1} in miscanthus fertilized with 80 kg ha^{-1} of N. McGowan et al. (2018a) also observed two-year cumulative N_2O emissions of 4.27 kg N_2O–N ha^{-1} yr^{-1} in miscanthus compared to 6.66 kg N_2O–N ha^{-1} yr^{-1} in switchgrass. These studies highlight the impact differences in management and crop species can have on N_2O emissions in perennial bioenergy crops.

Additionally, the capability of perennial bioenergy crops to yield greater climate benefits on low productive agricultural lands compared to natural regrowth vegetation on these low productive lands has been debated (Pool, 2013). Searchinger & Heimlich (2015) argued that low productive agricultural lands, when left abandoned, can undergo natural succession with regrowth vegetation that provides climate benefits, hence, questioning the basis of using these lands to grow

perennial bioenergy crops. To understand the climate benefits of converting low productive agricultural lands to perennial bioenergy crops compared to natural regrowth vegetation, well-replicated long-term studies are required (Sartori et al., 2006). This will help provide an accurate assessment of the environmental footprint of different perennial bioenergy crop species compared to leaving the land to undergo its natural succession.

Currently, studies comparing GHG emissions in different perennial bioenergy crop species on low productive agricultural lands to natural regrowth sites are lacking. Hence, this study quantified the N_2O and CO_2 emissions in perennial bioenergy crops of miscanthus, switchgrass, willow, and a natural regrowth (successional) site over two growing seasons to understand the GHG implications of these crops compared to the successional site. Owing to differences in crop phenology and vegetation cover, the study hypothesized that the perennial bioenergy crops and successional site would influence soil moisture, temperature, EC, and available N differently, resulting in varied responses to N_2O and CO_2 emissions. For instance, it is assumed that the presence of diverse plant species in the successional site would promote increased microbial activities leading to higher CO_2 emissions compared to the monoculture systems in the perennial bioenergy crops. It is again hypothesized that soil parameters and resultant N_2O and CO_2 emissions would differ between years due to potential differences in environmental conditions between the two years.

2.2 Materials and Methods

The study took place at the University of Guelph Agroforestry Research Station in Guelph, Ontario, Canada (cf. Chapter 1, Section 1.9).

2.2.1 Measurement of greenhouse gas emissions from the field

Greenhouse gases were sampled as described by Lutes et al. (2016), who also worked in an adjacent willow bioenergy plantation. Two chambers (25 cm height, 10 cm diameter) per treatment (bioenergy crop) replicate and three chambers in a control site (site with no crops) were installed to a 10 cm depth leaving a headspace of 15 cm (Lutes et al., 2016). Each chamber consisted of a fixed anchor and a detachable cap constructed from white PVC pipe. Chamber caps were covered with reflective insulation and consisted of a 1-cm-diameter central opening fitted with rubber septa for gas sampling and a 10-cm-long (9 mm inner diameter) vent tube to prevent pressure perturbations during gas sample collection (Lutes et al., 2016; Parkin & Venterea, 2010). Chamber caps were removed after each sampling event, and soil was exposed to air to maintain equilibrium (Baskerville et al., 2021; Parkin & Venterea, 2010).

Prior to gas sampling, all vegetation growing within the chamber was carefully removed to minimize CO_2 captured from plant and root respiration (Parkin & Venterea, 2010). Chambers were closed and gas was sampled at t = 0, 15 and 30 minutes (Lutes et al., 2016). Samples were stored in evacuated 3 ml LabCo vials (LabCo Ltd., High Wycombe, UK) and analyzed on a gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA) for CO_2 and N_2O. Measurement for CH_4 were not considered for this study, as upland soils are generally considered as sinks rather than sources of CH_4 emissions (Oertel et al., 2016). Bi-weekly measurements were taken over a 2-year period (2020 to 2022) from beginning of May to end of October for each year to quantify temporal variation of GHG emissions. Immediately following the gas sampling, measurements were taken for soil moisture, EC, and temperature using a WET-2 sensor to a 10 cm depth. Soil samples were also collected to 10 cm depth after each gas sampling event and brought to the lab

for analysis for ammonium and nitrate. To minimize diurnal variation bias, gas sampling in the field was always carried out between 10 am and 3 pm.

Gas fluxes were calculated with the HMR model in R using the chamber volume, surface area of the chamber base, and rate of change in chamber gas concentration (equation 2.1) (Pedersen et al., 2010).

$$C(t) = \varphi + f0\ \frac{\exp(-kt)}{(-kh)} \qquad (2.1)$$

The *C(t)* denotes the chamber concentration at time *t*, φ denotes the chamber equilibrium concentration, *f0* denotes the initial flux to be estimated, $k > 0$ is a non-linearity parameter, and h = V/A denotes the effective chamber height, i.e., the chamber volume divided by the cross-sectional area.

Prior to N_2O emission calculation in R, the chamber gas concentrations were converted from volumetric concentrations to mass per volume using the ideal gas law and chamber air temperature equation (2.2).

$$C_m = \frac{(C_v \times M \times P)}{(R \times T)} \qquad (2.2)$$

Where C_m is the mass/volume concentration of gas (μg L^{-1}); C_v is the volumetric concentration of N_2O (μl N_2O L^{-1}); M is the molecular weight of N in N_2O (28 μg N μmol^{-1} N_2O); P is the atmospheric pressure at 1 atm; T is the temperature of the chamber headspace (K) at the time of gas sampling; and R is the universal gas constant (0.082 L atm mol^{-1} K^{-1}).

The N_2O emissions from each sampling day was used to calculate the cumulative N_2O emissions over the year (growing season) using the trapezoidal integration method. The trapezoidal integration of daily fluxes assumes that the measured N_2O emissions represented the mean daily

emissions, and that the mean daily emissions changed linearly between measurements (Equation 2.3) (Lutes et al., 2019).

$$Cumulative\ N_2O\ emissions = \sum \Delta day.\left(\frac{fday1+fday2}{2}\right) \qquad (2.3)$$

Where *Δ day* is the number of days between sampling intervals and *f day* represents the average quantified daily emission (in μg N_2O-N m^{-2} day^{-1}) on each respective sampling day.

Overall (N_2O + CO_2) average combined emissions in CO_2-eq for the two growing seasons (Mg CO_2-eq ha^{-1} 6-$month^{-1}$) for each land use were calculated using 273 global warming potential of N_2O (Forster et al., 2021).

2.2.2 Soil analyses for ammonium and nitrate

Following every gas sampling event on the field, sampled soils for NH_4^+ and NO_3^- analyses were brought to the laboratory, air-dried, and sieved to 2 mm before extraction for NH_4^+ and NO_3^-. For soil extraction for NH_4^+ and NO_3^-, 5 g of the air-dried sieved soil was mixed with 25 mL of 2.0 M potassium chloride (KCl). The mixture was shaken on a reciprocating shaker for 15 minutes at 180 rpm and filtered using Whatman 42 filter paper. Filtered soil extracts were analyzed on a Shimadzu 1800 UV–Vis Spectrophotometer (Shimadzu Corp., Kyoto, Japan) at wavelengths 650 nm for ammonium and 540 nm for nitrate (Maynard & Kalra, 1993).

2.2.3 Statistical analysis

Data processing and analysis were carried out using SPSS (IBM Corp.). A repeated measure analysis of variance (ANOVA) was used to quantify differences between GHG emissions, NH_4^+, NO_3^-, soil moisture, soil temperature, and EC from the different treatments over the two growing seasons. Prior to the ANOVA, data was assessed for homogeneity of variance (Levene's) and normality (Shapiro-Wilk Test) (Steel et al., 1997). The CO_2 fluxes, cumulative N_2O and CO_2

emissions data were found to have violated the assumption of normality, hence, were log-transformed. The log-transformed data were reassessed for normality and were found to have normal distributions. Univariate ANOVA analysis was used to compare the effect of land use type and growing season on N_2O-N and CO_2-C emissions, soil NH_4^+ and NO_3^-, soil temperature, moisture, and EC. The Tukey's HSD test was used as a multiple comparison test to determine significantly different main effects, while significantly different simple effects were determined using Fisher's Least Significant Difference (LSD). All type I error for all analyses were set at $p<0.05$. The relationship between cumulative emissions and soil parameters was assessed using Pearson correlation coefficients (r).

2.3 Results

2.3.1 Soil parameters

Averaged over 6 months, representing a growing season (year), soil parameters of NH_4^+, NO_3^-, moisture, temperature, and EC varied among land use types and between years (Figure 2.1; Table 2.1). Soil NH_4^+ did not show significant differences among land use types in both years but was significantly higher ($p<0.05$) in 2020 compared to 2021. Similarly, NO_3^- contents in 2020 were significantly higher ($p<0.05$) in all the land use types than in 2021. Notably, differences in soil NO_3^- between land use types were observed only in 2020, with the successional site and miscanthus exhibiting significantly higher NO_3^- contents than willow. The elevated levels of NH_4^+ and NO_3^- in 2020, as opposed to 2021, resulted in higher inorganic N contents in 2020 compared to 2021 (Figure A1.1).

Significant differences ($p<0.05$) in soil moisture were observed between land use types in 2020, but no differences were found in 2021 (Figure 2.1; Table 2.1). In 2020, soil moisture was

significantly higher ($p<0.05$) in miscanthus and switchgrass compared to willow and the successional site. Between the 2020 and 2021 growing seasons, soil moisture in willow and the successional site was significantly higher in 2021 than in 2020, whereas the reverse was observed in switchgrass. Soil moisture in miscanthus remained unchanged between the two years.

Soil temperature was significantly higher ($p<0.05$) during the 2020 growing season than in 2021 for all land use types (Figure 2.1; Table 2.1). Except for switchgrass, soil temperature was significantly higher in the successional site than in the bioenergy crops in both 2020 and 2021. Between switchgrass and the successional site, soil temperature did not differ significantly ($p>0.05$) in both years. Soil temperature in the bioenergy crops was the same in 2020, but in 2021, switchgrass had a higher soil temperature than miscanthus and willow. Although EC did not differ between 2020 and 2021, EC in the successional site was significantly higher in both years than the bioenergy crops, except for 2020, where EC in the successional site was similar to switchgrass and willow (Figure 2.1; Table 2.1).

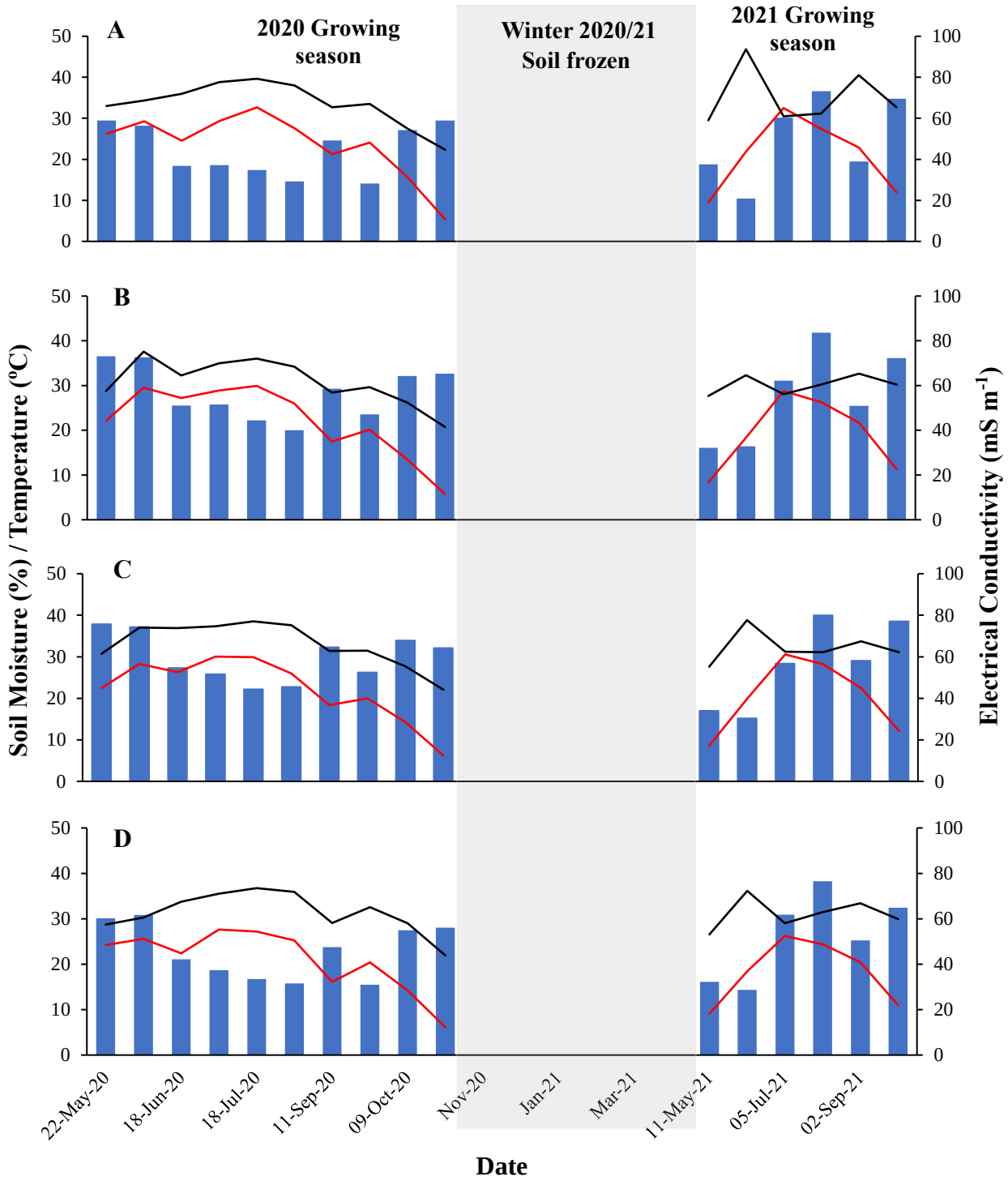


Figure 2.1: Soil moisture (blue bars), soil temperature (red line), and soil electrical conductivity (black line) in the (A) successional site, (B) miscanthus, (C) switchgrass, and (D) willow during the 2020 and 2021 growing seasons at the University of Guelph Agroforestry Research Station in southern Canada.

Table 2.1: Mean N_2O (μg N_2O-N m^{-2} hr^{-1}) and CO_2 (mg CO_2-C m^{-2} hr^{-1}) fluxes, soil NH_4^+ (mg kg^{-1}), NO_3^- (mg kg^{-1}), EC (mS m^{-1}), moisture (%), and temperature (°C) measured in the perennial bioenergy crops and successional site during the 2020 and 2021 growing seasons at the University of Guelph Agroforestry Research Station in southern Canada.

	Successional site	Miscanthus	Switchgrass	Willow
	2020 Growing season			
Mean N_2O-N	$0.007 \pm 0.001^{A,a}$	$0.007 \pm 0.006^{A,a}$	$0.013 \pm 0.001^{A,a}$	$0.016 \pm 0.006^{A,a}$
Mean CO_2-C	$0.084 \pm 0.004^{B,b}$	$0.124 \pm 0.021^{A,a}$	$0.123 \pm 0.009^{A,a}$	$0.118 \pm 0.007^{A,ab}$
NH_4^+	$5.076 \pm 0.213^{A,a}$	$4.577 \pm 0.104^{A,a}$	$4.569 \pm 0.115^{A,a}$	$4.468 \pm 0.051^{A,a}$
NO_3^-	$12.246 \pm 0.427^{A,a}$	$12.328 \pm 0.775^{A,a}$	$10.837 \pm 0.944^{A,ab}$	$9.828 \pm 0.785^{A,b}$
EC	$67.167 \pm 1.452^{A,a}$	$61.783 \pm 2.631^{A,b}$	$66.167 \pm 1.619^{A,ab}$	$62.700 \pm 0.629^{A,ab}$
Soil moisture	$22.177 \pm 0.284^{B,b}$	$28.415 \pm 1.006^{A,a}$	$29.748 \pm 2.380^{A,a}$	$22.802 \pm 1.296^{B,b}$
Soil temperature	$23.588 \pm 0.423^{A,a}$	$22.052 \pm 0.555^{A,b}$	$22.247 \pm 0.399^{A,ab}$	$20.884 \pm 0.257^{A,b}$
	2021 Growing season			
Mean N_2O-N	$0.009 \pm 0.005^{A,a}$	$0.005 \pm 0.005^{A,a}$	$0.010 \pm 0.006^{A,a}$	$0.010 \pm 0.005^{A,a}$
Mean CO_2-C	$0.133 \pm 0.011^{A,a}$	$0.097 \pm 0.003^{A,a}$	$0.114 \pm 0.026^{A,a}$	$0.062 \pm 0.004^{B,b}$
NH_4^+	$3.275 \pm 0.027^{B,a}$	$3.350 \pm 0.024^{B,a}$	$3.247 \pm 0.050^{B,a}$	$3.262 \pm 0.075^{B,a}$
NO_3^-	$9.415 \pm 1.296^{B,a}$	$8.423 \pm 1.480^{B,a}$	$6.049 \pm 0.448^{B,a}$	$7.564 \pm 0.222^{A,a}$
EC	$70.389 \pm 1.060^{A,a}$	$60.417 \pm 0.839^{A,b}$	$64.528 \pm 2.342^{A,b}$	$62.194 \pm 0.755^{A,b}$
Soil moisture	$25.039 \pm 0.278^{A,a}$	$27.842 \pm 0.361^{A,a}$	$28.003 \pm 2.356^{B,a}$	$26.208 \pm 1.069^{A,a}$
Soil temperature	$21.017 \pm 0.123^{B,a}$	$19.133 \pm 0.199^{B,b}$	$20.311 \pm 0.624^{B,a}$	$18.264 \pm 0.093^{B,b}$

Means followed by the same lowercase letter, comparing differences among land use type for a given year are not significantly different (p>0.05) and means followed by the same uppercase letter, comparing differences between years for a given land use type are not significantly different (p>0.05) according to Fisher's least significant difference (LSD).

2.3.2 Nitrous oxide and carbon dioxide emissions

Mean N_2O fluxes during the two growing seasons in the successional site, miscanthus, switchgrass, and willow ranged from –0.008 to 0.037 µg N_2O-N m^{-2} hr^{-1}, –0.020 to 0.034 µg N_2O-N m^{-2} hr^{-1}, –0.020 to 0.034 µg N_2O-N m^{-2} hr^{-1}, and –0.022 to 0.087 µg N_2O-N m^{-2} hr^{-1}, respectively (Figure 2.2A). The corresponding mean CO_2 fluxes ranged from 0.033 to 0.218 mg CO_2-C m^{-2} hr^{-1}, 0.019 to 0.260 mg CO_2-C m^{-2} hr^{-1}, 0.023 to 0.222 mg CO_2-C m^{-2} hr^{-1} and 0.010 to 0.266 mg CO_2-C m^{-2} hr^{-1} in the successional site, miscanthus, switchgrass, and willow, respectively (Figure 2.2B).

Mean N_2O fluxes showed no significant differences between land use types or years (Table 2.1). However, mean CO_2 fluxes were significantly higher ($p<0.05$) in miscanthus and switchgrass compared to the successional site in 2020, and in the successional site, miscanthus, and switchgrass compared to willow in 2021 (Table 2.1). Over the two years, mean CO_2 fluxes were significantly higher ($p<0.05$) in the successional site in 2020 than in 2021, with willow showing significantly higher CO_2 fluxes in 2021 than in 2020.

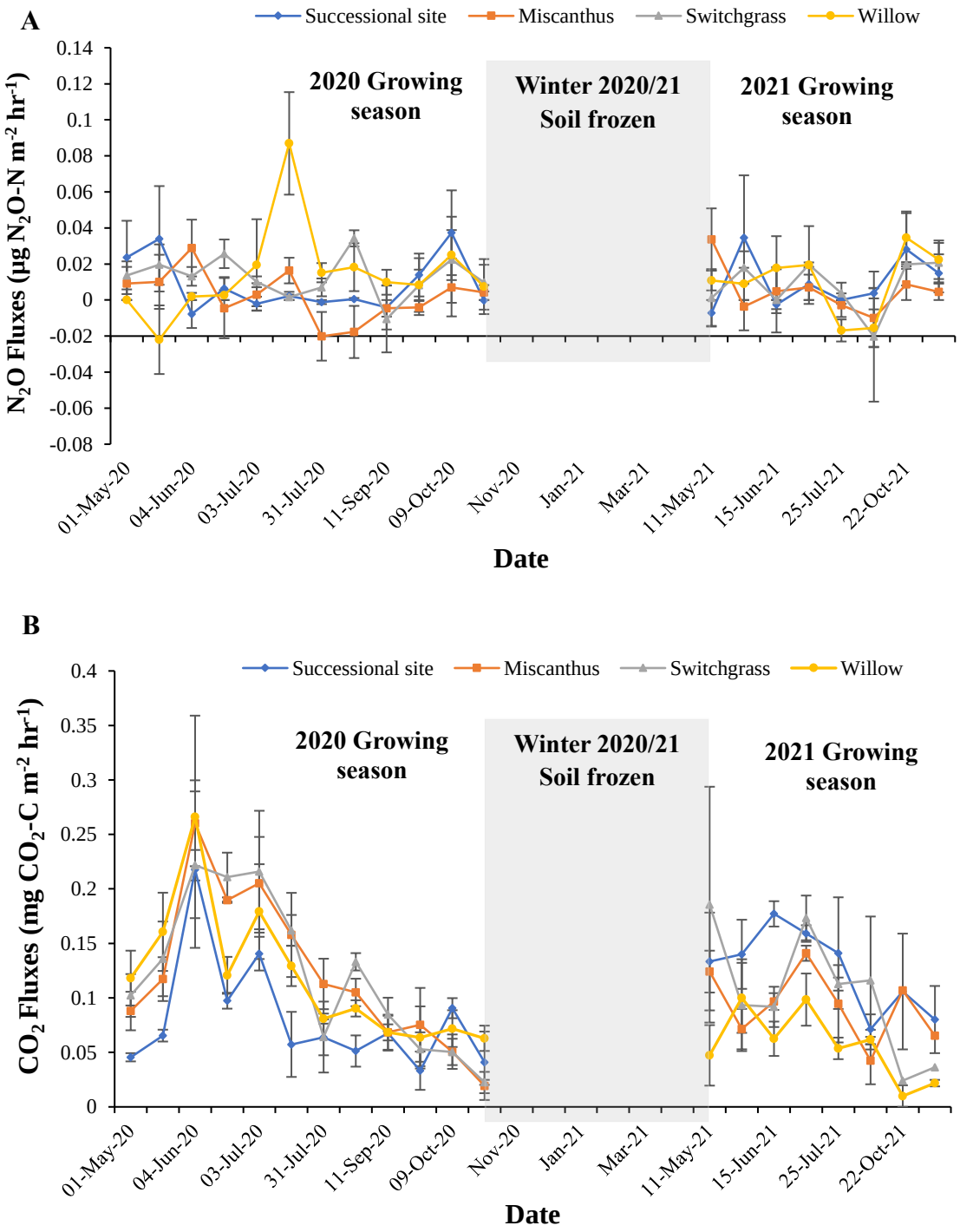


Figure 2.2: Mean (A) N_2O (µg N_2O-N m^{-2} hr^{-1}) and (B) CO_2 (mg CO_2-C m^{-2} hr^{-1}) fluxes in the successional site and perennial bioenergy crops for the 2020 and 2021 growing seasons at the University of Guelph Agroforestry Research Station in southern Canada.

Cumulative N_2O emissions ranged from 48.04 µg N_2O-N m^{-2} (successional site) to 93.66 µg N_2O-N m^{-2} (willow) in 2020 and from 38.12 µg N_2O-N m^{-2} (miscanthus) to 86.23 µg N_2O-N m^{-2} (switchgrass) in 2021 (Figure 2.3A). Like the mean N_2O fluxes, cumulative N_2O emissions did not show any significant differences between land use types or years (Figure 2.3A). The 2-year total cumulative N_2O emissions in the successional site, miscanthus, willow, and switchgrass were 93.75 µg N_2O-N m^{-2}, 124.26 µg N_2O-N m^{-2}, 148.94 µg N_2O-N m^{-2}, and 174.50 µg N_2O-N m^{-2}, respectively, and was significantly higher ($p<0.05$) in switchgrass than the successional site (Figure 2.3A). Of these total cumulative emissions, 51% in the successional site and switchgrass occurred in 2020, while 63% and 69% in willow and miscanthus, respectively, occurred in the same year.

Cumulative CO_2 emissions ranged from 354.88 mg CO_2-C m^{-2} (successional site) to 534.18 mg CO_2-C m^{-2} (switchgrass) in 2020 and from 251.35 mg CO_2-C m^{-2} (willow) to 540.19 mg CO_2-C m^{-2} (successional site) in 2021 (Figure 2.3B). Cumulative CO_2 was significantly higher in all the bioenergy crops than the successional site in 2020. In 2021, willow had significantly lower ($p<0.05$) cumulative CO_2 than the other bioenergy crops and the successional site. The total cumulative CO_2 emissions for the two years were 760.68 mg CO_2-C m^{-2}, 895.08 mg CO_2-C m^{-2}, 920.36 mg CO_2-C m^{-2}, and 1009.04 mg CO_2-C m^{-2} in willow, the successional site, miscanthus and switchgrass, respectively (Figure 2.3B). Approximately, 40%, 53%, 57%, and 67% of the total cumulative CO_2 emissions in the successional site, switchgrass, miscanthus, and willow, respectively, occurred in 2020.

The overall growing season average cumulative emissions (Mg CO_2-eq ha^{-1} 6-$month^{-1}$) were 4.96, 6.15, 6.82, and 8.18 in the successional site, miscanthus, willow, and switchgrass, respectively (Figure 2.4). Whereas overall cumulative emissions in the switchgrass were

significantly higher (*p=0.044*) than the successional site, overall cumulative emissions in miscanthus and willow did not differ significantly from the successional site and switchgrass.

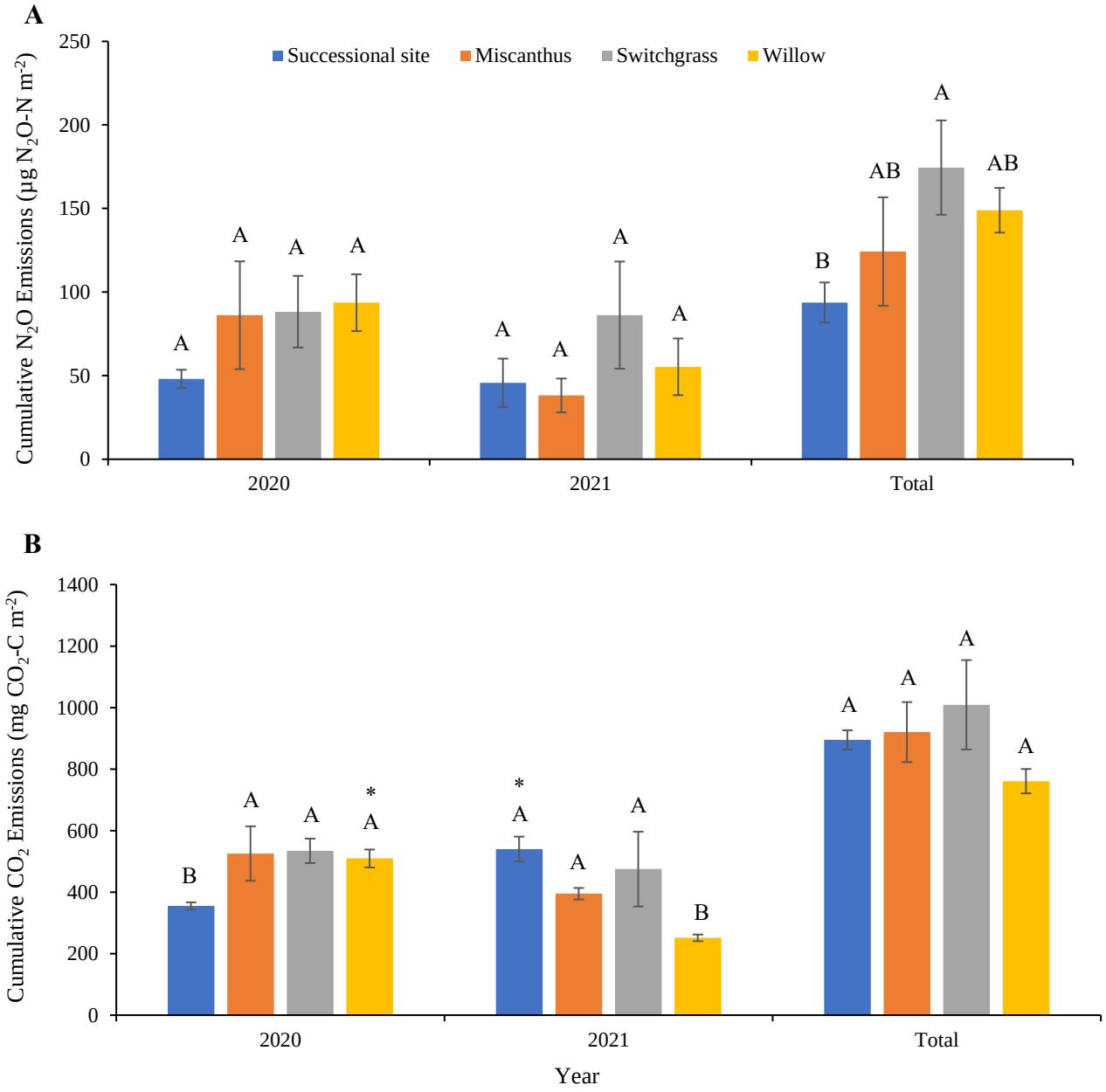


Figure 2.3: Cumulative N_2O-N (µg N_2O-N m^{-2}) and CO_2-C emissions (mg CO_2-C m^{-2}) measured for the 2020 and 2021 growing seasons in the successional site and perennial bioenergy crops at the University of Guelph Agroforestry Research Station in southern Canada. Bars represent standard error of cumulative means ($n = 3$). Error bars with same uppercase letters show cumulative means are not significantly different ($p>0.05$) according to Fisher's least significant difference (LSD). * indicates cumulative CO_2 means in willow and successional site are significantly different ($p<0.05$) between years.

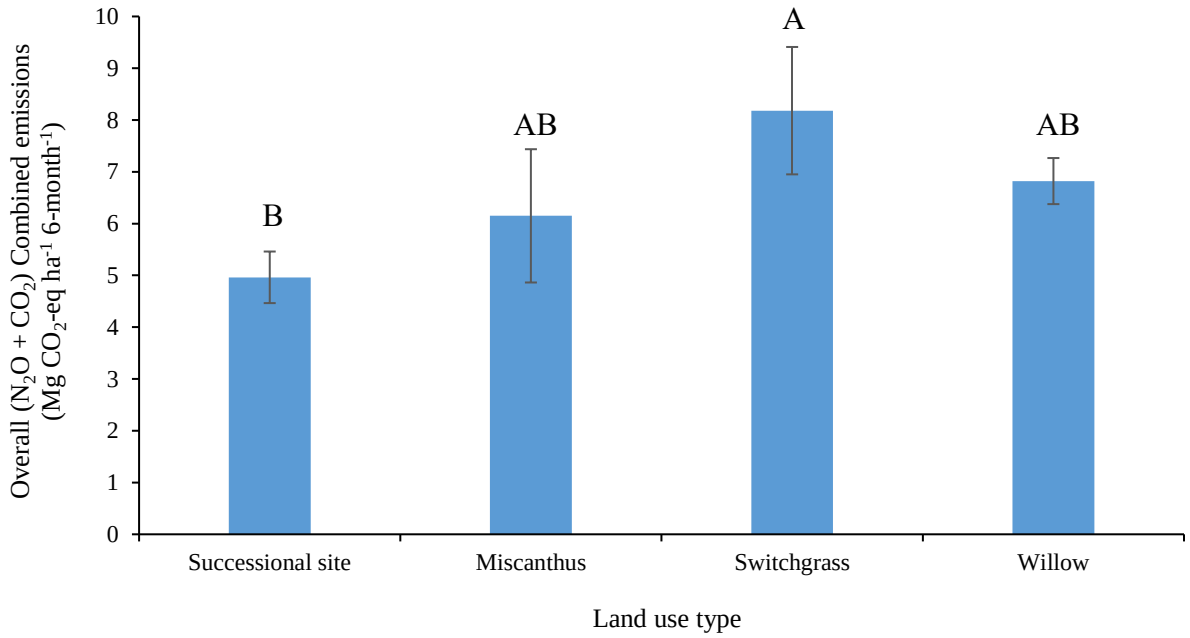


Figure 2.4: Overall average combined emissions (Mg CO_2-eq ha^{-1} 6-$month^{-1}$) for the 2020 and 2021 growing seasons in the successional site and perennial bioenergy crops at the University of Guelph Agroforestry Research Station in southern Canada. Bars represent standard error of cumulative average means (n = 3). Error bars with same uppercase letters show cumulative average means are not significantly different (*p>0.05*) according to Fisher's least significant difference (LSD).

2.3.3 Relationship between soil parameters and greenhouse gas fluxes

The relationship between soil parameters and GHG fluxes for both growing seasons were first analysed for each individual land use type (Table A1.2–A1.5). However, since the individual land use types showed similar results to the combined land uses (Table 2.2), the relationship between soil parameters and GHG fluxes for the combined land uses was considered. No significant relationship was found between N_2O and any of the soil parameters in 2020 and 2021 (Table 2.2). In 2020, CO_2 fluxes showed significant positive relationships with soil temperature, EC, and soil NO_3^- concentration, and a significant negative relationship with soil NH_4^+

concentration. In 2021, however, no significant relationship was observed between CO_2 fluxes and any of the soil parameters.

Table 2.2: Pearson correlation coefficient between measured emissions and soil parameters during the 2020 and 2021 growing seasons from the successional site and perennial bioenergy crops at the University of Guelph Agroforestry Research Station in southern Canada.

Variables	N_2O ($\mu g\ m^{-2}$)	CO_2 ($mg\ m^{-2}$)	Temperature (°C)	Moisture (%)	EC ($mS\ m^{-1}$)	NH_4^+ ($mg\ kg^{-1}$)	NO_3^- ($mg\ kg^{-1}$)
	---------- **2020** ----------						
N_2O	1						
CO_2	-0.039	1					
Temperature	-0.112	0.634***	1				
Moisture	0.043	0.193	-0.361**	1			
EC	-0.049	0.485***	0.931***	-0.496***	1		
NH_4^+	-0.073	-0.363**	-0.121	-0.037	-0.082	1	
NO_3^-	-0.069	0.544***	0.692***	-0.048	0.676***	-0.191	1
	---------- **2021** ----------						
N_2O	1						
CO_2	-0.023	1					
Temperature	-0.265	0.278	1				
Moisture	-0.179	-0.152	0.345	1			
EC	0.135	-0.024	0.208	-0.391	1		
NH_4^+	0.258	-0.058	-0.828***	-0.037	-0.540***	1	
NO_3^-	0.112	-0.279	-0.336	0.210	0.026	0.320	1

*p<0.05; **p<0.01; ***p<0.001

2.4 Discussion

2.4.1 Influence of the different perennial bioenergy crops on soil parameters

Differences in soil moisture, temperature, EC, and available N among land uses and between the two years confirmed the hypothesis that differences in crop phenology and vegetation from the different bioenergy crops and successional site would influence soil parameters differently and that these parameters would differ between years. The higher soil moisture content in the miscanthus and switchgrass in 2020 may have been due to the miscanthus and switchgrass biomass harvest before the onset of the 2020 growing season. Late winter or early spring harvest of herbaceous bioenergy crops such as miscanthus and switchgrass is often recommended over early fall harvest (Iqbal et al., 2017; Varnero et al., 2018). This is due to the observation that late harvest improves the combustion quality of biomass feedstock (Meehan et al., 2013). A disadvantage of late winter or early spring harvest is the loss of senescent leaves in winter which can form a mulch on the surface of the soil (Peyrard et al., 2017). Abraha et al. (2015) observed that, the absence of live biomass and the presence of dead biomass which provided shade to the soil surface reduced evapotranspiration and soil water loss in switchgrass and mixed-prairie grasses. It is, therefore, possible that the late spring biomass harvest in the miscanthus and switchgrass and mulch from senescent leaves and litter cover on the soil surface were enough to substantially reduce soil moisture loss through evapotranspiration. This may explain the higher soil moisture content in the miscanthus and switchgrass compared to the undisturbed vegetation in the successional site and willow in 2020 where increased evapotranspiration and soil water uptake by the growing biomass may have caused a decline in soil moisture.

While potential higher soil moisture in the successional site and willow in 2021 than 2020 could have resulted from differences in precipitation amount between the years, it is also possible that the higher soil temperature observed in 2020 compared to 2021 may have equally played a role in reducing soil moisture content in the successional site and willow in 2020. Increase in soil temperature increases soil water loss through evapotranspiration (Brzostek et al., 2012; Onwuka & Mang, 2018; Wang et al., 2022a). Consequently, the higher soil temperatures in 2020 may have increased evapotranspiration in the successional site and willow which resulted in soil moisture loss in the successional site and willow in 2020 compared to 2021.

Lower soil temperature in the miscanthus and willow than the successional site over the two years may be due to increased shade from leaf canopy due to higher biomass production from the willow and miscanthus compared to the successional site and switchgrass. Compared to switchgrass, miscanthus and willow have bigger crown size and plant height (Marsal et al., 2016; Oliveira et al., 2017). These features can be crucial in giving miscanthus and willow the edge over switchgrass in terms of canopy closure and, consequentially, microclimate modification under their canopies. He et al. (2022) in a study found that growing miscanthus on low productive agricultural land can cool the USA's Midwest Heartland summer by up to 1°C. This cooling effect from miscanthus was mainly caused by the increased duration and size of miscanthus leaf canopy compared to the existing vegetations on the low productive agricultural land. Hence, the lower soil temperature in the miscanthus and willow than the successional site in both years from this study is indicative of the potential these bioenergy crops may have in regulating soil temperature relative to a successional site of regrowth secondary vegetation. This attribute could be very crucial with future climate scenarios pointing to increased soil warming in temperate regions such as in this study area.

As higher soil EC can be indicative of higher nutrient availability in soil solution (Smith & Doran, 1997), higher EC values in the successional site than the bioenergy crops, especially, in 2021 may mean potential higher nutrient availability in the successional site than the bioenergy crops. Higher soil nutrient availability in the successional site than the bioenergy crops can be expected when it is considered that extensive root network in the bioenergy crops can enhance nutrient uptake and removal from the soil solution compared to the successional site (Thevathasan et al., 2012). Moreover, frequent biomass harvest of the bioenergy crops without nutrient replacement can deplete nutrients in the soil solution (Fixen, 2007), causing lower EC in the soils under the bioenergy crops compared to the successional site.

Except for the significantly lower soil NO_3^- in the willow than the miscanthus and successional site in 2020, no significant differences in soil NH_4^+ and NO_3^- between the bioenergy crops and successional site were observed in both years. Higher NO_3^- in the successional site than willow may be due to increased potential of organic matter mineralization and reduced uptake of available N in the successional site relative to the willow. Soils with diverse litter inputs promote increased organic matter decomposition which can result in higher soil available N (Beugnon et al., 2021; Cong et al., 2014; Cong et al., 2015a; Truong & Marschner, 2018; Zhang et al., 2023). Unlike the willow monocrop, the presence of diverse plant species on the successional site may have stimulated increased organic matter decomposition resulting in higher NO_3^- in the successional site than the willow. The lack of observable differences between NH_4^+ and NO_3^- in the miscanthus and switchgrass compared to the successional site may have been due to reduced soil N uptake and removal in the harvested switchgrass and miscanthus plots in the early part of the 2020 growing season. Ameen et al. (2019) observed higher N return to the soil in switchgrass that was harvested in fall after a killing frost. Engbers (2012) also studied that delaying switchgrass

and miscanthus harvests to spring in southern Ontario decreased soil N removal in the harvested biomass. Peyrard et al. (2017) equally observed higher accumulation of NO_3^- in soil of late winter harvest miscanthus compared to early fall harvest. A potential reduction in soil available N uptake and removal in switchgrass and miscanthus at the onset of the 2020 growing season may equally explain the higher NO_3^- in the miscanthus than willow in 2020 and higher NH_4^+ and NO_3^- in both switchgrass and miscanthus in 2020 than in 2021.

Also, while the higher NH_4^+ and NO_3^- in the herbaceous bioenergy crops and successional site in 2020 than 2021 may have been influenced by decline in soil available N owing to N losses and possible N immobilization by microbial and vegetation in 2021, higher soil temperature in 2020 than 2021 may have equally been a contributing factor to this observation. Increased soil temperature can accelerate mineralization and nitrification of soil organic matter increasing soil available N (Brzostek et al., 2012; Onwuka & Mang, 2018). A meta-analysis by Dai et al. (2020) revealed that elevated temperature decreased soil microbial biomass N and increased N mineralization rates. It is, therefore, possible that increased soil warming in 2020 relative to 2021 may have resulted in increased organic matter mineralization making available higher NH_4^+ and NO_3^- in the soil. This may explain the higher inorganic N in 2020 than 2021.

2.4.2 Nitrous oxide and carbon dioxide emissions

Although, the lack of significant differences in mean N_2O fluxes among land use types and between years did not support the hypothesis of this study that varied responses of N_2O would be observed between years and among land use types, the significantly higher total cumulative N_2O emissions in switchgrass than successional site over the two growing seasons was consistent with this hypothesis. Additionally, whereas mean CO_2 fluxes differed among land use types and between years in the successional site and willow which supported the hypothesis that CO_2 would

differ between years and among land use types, significantly higher mean CO_2 fluxes in bioenergy crops in 2020 than successional site and the lack of significant difference in total cumulative CO_2 emissions over the two growing seasons rejected the hypothesis that potential increased soil microbial activities in the successional site with diverse plant species would result in higher CO_2 emissions compared to the monoculture stands of the bioenergy crops.

Mean N_2O fluxes reported for the willow in this study (0.016 and 0.010 μg N_2O-N m^{-2} hr^{-1} in 2020 and 2021, respectively) are much lower than the 9.5–20.2 μg N_2O-N m^{-2} hr^{-1} from the same site in 2014 by Lutes et al. (2019) but closer to the 0.2 μg N_2O-N m^{-2} hr^{-1} reported in England by Drewer et al. (2012). Gauder et al. (2012) on the other hand found much lower mean N_2O fluxes (-0.4 μg N m^{-2} hr^{-1}) in unfertilized willow in southwest Germany. Drewer et al. (2012) in the same study measured mean N_2O fluxes of 3.7 μg N_2O-N m^{-2} hr^{-1} from unfertilized miscanthus which is higher than the 0.007 and 0.005 μg N_2O-N m^{-2} hr^{-1} reported for the miscanthus in 2020 and 2021, respectively, in this study. The results from this study are, however, higher than the –1.0 μg N m^{-2} hr^{-1} reported by Gauder et al. (2012) in unfertilized miscanthus plots in Germany.

The much lower mean N_2O fluxes in this present study compared to the other studies (Drewer et al., 2012; Lutes et al., 2019) from similar environments could be due to differences in management systems, environmental, and edaphic factors such as soil available N contents, moisture, and temperature (Gauder et al., 2012; Lutes et al., 2016; Ferchaud et al., 2020). Among these factors, soil available N can be the most important component driving N_2O emissions due to its role in nitrification and denitrification (Risk et al., 2013; Congreves et al., 2018). In the study by Lutes et al. (2019), soil NH_4^+ and NO_3^- in the unfertilized willow were 2130 mg kg^{-1} and 3470 mg kg^{-1}, respectively. These soil NH_4^+ and NO_3^- contents were 477% and 353% more than was

measured from the same site in this study in 2020 and 653% and 459% more than was measured in 2021.

Evidence from studies (*e.g.,* Kavdir et al., 2008; Gauder et al., 2012) showed increased soil N_2O emissions in perennial bioenergy crops with increased soil available N. For instance, the study by Gauder et al. (2012) in southwest Germany found that applying 80 kg ha^{-1} N to miscanthus increased mean N_2O fluxes to 4.3 μg N m^{-2} hr^{-1}, compared with –1.0 μg N m^{-2} hr^{-1} in the unfertilized miscanthus plots. Again, Kavdir et al. (2008) measured significant soil N_2O emissions after N fertilization of willow while Wile et al. (2014) also observed that switchgrass fertilized with 120 kg N ha^{-1} resulted in 553 g N_2O-N ha^{-1} cumulative N_2O emissions averaged over two years compared to 319 g N_2O-N ha^{-1} in the unfertilized crops. In well-aerated soils, such as the sandy loam soil in this study, most of the N_2O emissions would be derived from nitrification rather than denitrification (Kavdir et al., 2008; Lutes et al., 2016). Lutes et al. (2016) observed in the willow from the study site that soil available N reserves were low when N_2O production peaked and explained that nitrification was the key in driving N_2O emissions. Increased competition for N in low N soils by the soil microbial community and plant can reduce the amount of N available for loss as N_2O (Kuzyakov & Xu, 2013). It is, therefore, not surprising that the lack of any significant differences in soil available N between the different bioenergy crops and successional site in both years reflected in the lack of any observable differences in the mean N_2O fluxes and cumulative N_2O emissions. The only significant difference in soil available N was in 2020 when the willow had statistically lower soil NO_3^- than the successional site and miscanthus. This difference in soil NO_3^- was not enough to cause any significant difference in N_2O fluxes between these land uses.

Higher soil NH_4^+ and NO_3^- in 2020 than 2021 in all the bioenergy crops and successional site may also explain why >50% of the combined total cumulative N_2O emissions occurred in 2020. Despite the lack of significant differences in mean N_2O fluxes and cumulative emissions among the land uses and between years, the combined total cumulative emissions over the two years were only significantly higher in the switchgrass than the successional site. The higher total cumulative emissions in the switchgrass than successional site may have been due to other soil factors such as soil microbial community induced by differences in vegetation cover (Bahulikar et al., 2014; Chen et al., 2019; Gushgari-Doyle et al., 2021; Lange et al., 2015; Mafa-Attoye et al., 2020). Further studies will be required to comprehensively understand what may have caused this observed increased in the total cumulative N_2O emissions in the switchgrass relative to the successional site in this study.

Growing season soil CO_2-C emissions are partly governed by soil temperature and increased microbial activity (Lutes et al., 2006). The main drivers of CO_2 emissions from soil respiration include soil moisture, temperature, availability of soil nutrient such as C and available N (Hursh et al., 2017; Li et al., 2020; Oertel et al., 2016). Contrary to the assumption that CO_2 emissions would be higher in the successional site than bioenergy crops, the significantly higher CO_2 fluxes in miscanthus and switchgrass than the successional site in 2020 and higher CO_2 fluxes in the successional site, miscanthus, and switchgrass compared to willow in 2021 cannot solely be attributed to microbial activity driven by optimal soil conditions and available soil nutrients. This is because differences in soil available N, moisture, and temperature did not reflect in differences in mean CO_2 fluxes in both years. If these soil factors were solely responsible for CO_2 emissions, higher soil temperature, NH_4^+ and NO_3^- in 2020 than 2021 would have corresponded with higher mean CO_2 fluxes and cumulative CO_2 emissions in 2020 than 2021. For instance, the herbaceous

bioenergy crops had significantly higher mean CO_2 fluxes than the successional site in 2020, but mean soil NH_4^+ and NO_3^- did not differ significantly between the herbaceous crops and the successional site in 2020. Additionally, the higher mean CO_2 fluxes in the successional site and herbaceous bioenergy crops than the willow in 2021 did not reflect in higher soil NH_4^+, NO_3^-, moisture, and temperature. Therefore, it can be argued that most of the CO_2 may have been from root contributions rather than microbial respiration. This is because CO_2 from soil respiration represents CO_2 from microbial (heterotrophic) and live root (autotrophic) respiration (Zhou et al., 2014), with root respiration capable of contributing more than 50% of the total soil CO_2 emissions (Oertel et al., 2016). Hanson et al. (2000) determined that root contribution may even represent up to 95% of the total soil respired CO_2 depending on season and vegetation type, with root contributions commonly higher in the growing than non growing seasons.

Unlike microbial respiration of soil CO_2 which is dependent on soil C (Laganière et al., 2012; Lutes et al., 2016), the fraction of total soil respiration derived from live roots is independent of soil C pools (Hanson et al., 2000). Instead, root respiration is impacted by the amount of assimilated C belowground (Hopkins et al., 2013). Belowground C assimilation in roots and rhizomes can be impacted by aboveground biomass disturbances such as harvest operations (Don et al., 2012; Laihonen et al., 2022; Lemus & Lal, 2005; Xu et al., 2022). In perennial bioenergy crops, frequent aboveground biomass harvest can result in increased belowground biomass C (Pugliese et al., 2019; Xu et al., 2022), which can stimulate increased CO_2 emissions from root respirations (Hopkins et al., 2013). This potential increase in root respiration from disturbed aboveground biomasses may explain the higher mean CO_2 fluxes and cumulative CO_2 in the herbaceous bioenergy crops in 2020 and 2021 than the successional site and willow, respectively,

as the biomass harvest in the herbaceous crops as indicated earlier may have resulted in increased assimilation of C belowground.

Despite the variation in cumulative CO_2 with land use type and year, there was no difference in the combined total cumulative CO_2 emissions over the two growing seasons. Also, as observed from the overall combined emissions, there was a lack of significant difference in the overall combined emissions in miscanthus and willow compared to the successional site. Meanwhile, the overall combined emissions were significantly higher in the switchgrass compared to the successional site. This implies that converting low productive agricultural lands to miscanthus and willow may not be at the expense of environmental degradation, unlike the switchgrass which may induce higher combined emissions aided by their higher N_2O emissions.

2.4.3 Relationship between greenhouse gas fluxes and soil parameters

The significant positive correlation between CO_2 and soil temperature, EC, and soil NO_3^- concentration in 2020 may stem from the influence of soil available N on the soil microbial community and activities. The positive and negative correlation between CO_2 and NO_3^- and NH_4^+ implies that while NO_3^- accumulated with increased CO_2, NH_4^+ declined with CO_2 emissions. As increase in NO_3^- and decrease in NH_4^+ in soil indicates an increase in nitrification activities (Zheng et al., 2021), it could be assumed that CO_2 emissions occurred with increased nitrification activities. Increased nitrification process may have also occurred with increased soil temperature (Dai et al., 2020), for which reason temperature showed a significant positive correlation with NO_3^-. Higher soil NO_3^- from nitrification have been observed with increased soil temperature (Hu et al., 2023; Lang et al., 2010; Wang et al., 2017). Hence, it is possible that the heterotrophic component of CO_2 produced from these well-aerated and low N soils may have mostly originated from the respiration of the nitrifier community in these soils.

Also, since the increased presence of NO_3^- in the soil can result in higher soil EC (Adviento-Borbe et al., 2006), respired CO_2 from the soil nitrifier community and a resultant increase in soil NO_3^- content may explain the significant positive relationship between CO_2 and EC in this study. For instance, Smith & Doran (1997) found significant positive correlations between EC and NO_3^- for fall and summer measurements in a winter wheat–dry pea rotation. On the contrary, since it is assumed in this study that most of the CO_2 may have originated from autotrophic rather than heterotrophic respiration, increased N uptake by live roots from the study site aided by a warming soil temperature may have corresponded with higher CO_2 emissions in 2020.

The absence of significant relationship between N_2O and any of the soil parameters in both years could be due to low soil available N content. As soil available N controls N_2O emissions in soils (Congreves et al., 2018; Risk et al., 2013; van Groenigen et al., 2010), the lower soil available N in this study may have curtailed N_2O emissions even if environmental and soil conditions were conducive for N_2O production and emission. It would, therefore, be reasonable not to expect a greater impact of these soil factors such as moisture and temperature on N_2O emissions, eliminating any possible relationship between N_2O emissions and the soil parameters considered in this present study.

2.5 Conclusions

The 2-year total cumulative N_2O emissions ranged from 93.75 μg N_2O-N m^{-2} to 174.50 μg N_2O-N m^{-2} and was significantly higher in switchgrass than the successional site. Of the 2-year total cumulative N_2O emissions, 51% in the successional site and switchgrass occurred in 2020, while 63% and 69% in willow and miscanthus, respectively, occurred in the same year. The 2-year

cumulative CO_2 emissions on the other hand ranged from 760.68 mg CO_2-C m^{-2} to 1009.04 mg CO_2-C m^{-2} and was not significantly different among land use type. The significantly higher cumulative CO_2 in all the bioenergy crops compared to the successional site in 2020 and the lack of any significant difference in the 2-year total cumulative CO_2 among all the land use types did not support the hypothesis that CO_2 emissions would be higher in the successional site due to potential higher microbial activities arising from diverse plant species in the successional site than the monoculture systems in the bioenergy crops. Although, soil parameters such as temperature and available N were favourable for higher CO_2 emissions in 2020 than 2021, the absence of any remarkable differences in CO_2 emissions between both years suggest that majority of the CO_2 emitted may have been contributed by root respiration rather than from soil microbial respiration. Moreover, the overall combined emissions which ranged from 4.96 Mg CO_2-eq ha^{-1} 6-$month^{-1}$ to 8.18 Mg CO_2-eq ha^{-1} 6-$month^{-1}$ were significantly higher in switchgrass compared to the successional site. The implication of this is that, unlike miscanthus and willow, converting low productive agricultural land to switchgrass may result in an overall higher emission with negative implications on the global climate.

CHAPTER 3

3.0 Impact of freeze-thaw cycles on greenhouse gas emissions in low productive agricultural land under different perennial bioenergy crops

This chapter is an adaptation of the following published article: Osei, A. K., Rezanezhad, F., and Oelbermann, M. (2024). Impact of freeze-thaw cycles on greenhouse gas emissions in marginally productive agricultural land under different perennial bioenergy crops. J. Environ. Manage. 357, 120739. https://doi.org/10.1016/j.jenvman.2024.120739

3.1 Introduction

Soil freeze-thaw is a common phenomenon in cold temperate regions that contributes significantly to annual greenhouse gas (GHG) budgets (Kurganova et al., 2007; Pi et al., 2021; Wagner-Riddle et al., 2017; Wang et al., 2021a). For instance, freeze-thaw events can account for up to 70% and 50% of annual nitrous oxide (N_2O) and carbon dioxide (CO_2) production, respectively (Hung et al., 2021; Kurganova et al., 2007). Since ~66 million km^2, representing 52.5% of global land area, experiences seasonal soil freezing (Kim et al., 2010), the contribution of winter induced-GHG emissions to climate warming cannot be overstated. Moreover, increased climate variability projected from global warming scenarios suggests that freeze-thaw events will become more frequent in temperate latitudes, resulting in greater freeze-thaw-induced emissions (Kurganova et al., 2007; Pi et al., 2021; Rezanezhad et al., 2023).

Soil freezing and thawing increases the amount of labile C and soil N through the mortality of microbial cells and fine roots and the breakdown of soil aggregates making previously inaccessible substrates available for mineralization (Chai et al., 2014; Congreves et al., 2018; Libby et al., 2020; Risk et al., 2013). The increased availability of labile C and N provide energy sources for microbial production of N_2O and CO_2 during soil freeze-thaw (Green et al., 2022; Kurganova et al., 2007; Pi et al., 2021; Wagner-Riddle et al., 2017). The extent of freeze-thaw-

related N_2O and CO_2 emissions may, however, differ with management systems (Lutes et al., 2019; Wagner-Riddle et al., 2017) and vegetation type (Ferchaud et al., 2020; Lovett et al., 2002). For instance, Kurganova et al. (2007) observed a higher freezing rate in agricultural soils compared to forest soils due to higher organic matter content, which provided insulation against freezing in the forest soil. Wagner-Riddle et al. (2017) also found lower cumulative winter N_2O emissions in perennial crops compared to annual crops due to higher residual N content in the croplands.

Converting low productive agricultural lands to perennial bioenergy crops is estimated to provide climate benefits through reduced GHG emissions (Chimento et al., 2016; Koh & Ghazoul, 2008). Achieving GHG emission reduction will require perennial bioenergy crops on low productive agricultural lands to reduce freeze-thaw related N_2O and CO_2 emissions compared to leaving unproductive agricultural lands as abandoned lands with natural regrowth vegetation. The capability of perennial bioenergy crops to reduce N_2O and CO_2 emissions under freeze-thaw conditions may, however, depend on the crop species. This is due to the great influence vegetation cover and litter chemistry has on soil C and N cycling (Guo et al., 2020). For instance, the composition of dissolved organic C (DOC) which stimulates microbial production of N_2O and CO_2 during soil freeze-thaw differs with plant species and litter chemistry such as C/N ratio (Guo et al., 2020; Wu et al., 2020a). Guo et al. (2020) found higher DOC concentrations in temperate broadleaf vegetation than coniferous species and attributed this finding to difference in litter chemistry. Christopher et al. (2008) also observed higher net N mineralization and nitrification in temperate deciduous forest floor with lower C/N ratio than a mixed deciduous and coniferous forest floor with higher C/N ratio. Aside C and N cycling, difference in litter chemistry and vegetation species composition can also have a profound influence on the response of soil N_2O and CO_2 producing microbes to freeze-thaw perturbations (Lovett et al., 2002; Song et al., 2017; Watanabe et al.,

2019). This implies that understanding how different vegetation species respond to freeze-thaw in C and N cycling leading to N_2O and CO_2 emissions is crucial to elucidating their contribution to controlling freeze-thaw-induced GHG emissions.

While several field and laboratory studies have examined the impact of freeze-thaw on soil GHG emissions in natural and managed terrestrial ecosystems (e.g., Badewa et al., 2022; Kurganova et al., 2007; Wang et al., 2021a; Wu et al., 2020a; Wu et al., 2020b), research on the effects of soil freeze-thaw on soil C and N dynamics, as well as GHG emissions, in different perennial bioenergy crops species, particularly those grown on low productive agricultural lands without fertilization, is lacking. This is despite the global attention given to growing perennial bioenergy crops on low productive agricultural lands as a land management strategy for climate change mitigation (Avagyan, 2021; Avagyan & Singh, 2019; Lewandowski, 2015). To understand how freeze-thaw would impact C and N cycling and N_2O and CO_2 emissions from soils under different perennial bioenergy crops on low productive agricultural land compared to a successional site, a 49-day freeze-thaw laboratory incubation experiment was conducted to quantify N_2O and CO_2 emissions over 7 sequential freeze-thaw cycles.

Due to differences in crop species and vegetation cover, we hypothesized that a natural regrowth (successional) site and perennial bioenergy crops would differ in C and N dynamics following soil freeze-thaw and that the bioenergy crops would effectively control N_2O and CO_2 emissions during freeze-thaw events compared to the soil from the successional site. This hypothesis was based on assumptions that: i) efficient N cycling in the perennial bioenergy crops due to their extensive root network compared to the successional site would reduce the amount of residual soil inorganic N available for loss as N_2O in the bioenergy crops; ii) despite frequent biomass harvest in the bioenergy crops, improved root network would enhance water stable soil

aggregates in the bioenergy crops similar to the successional site minimizing freeze-thaw-induced soil aggregate disruptions and related N_2O and CO_2 emissions; iii) higher soil C/N ratios from perennial bioenergy crops (monocrops) than successional site with diverse plant species would produce higher microbial N demand in the perennial bioenergy crops leaving less N available for N_2O production and emission; and iv) lower soil C/N ratio in the successional site arising from the presence of diverse plant species would promote higher CO_2 emissions compared to the perennial bioenergy crops. To examine these hypotheses, the study: 1) measured and compared water stable soil aggregates before- and after soil freeze-thaw in perennial bioenergy crops and the successional site and assessed their contributions to N_2O and CO_2 emissions; 2) determined and compared how repeated freeze-thaw cycles impacted the C and N cycling and N_2O and CO_2 emissions in the perennial bioenergy crops and the successional site; and 3) assessed the dominant soil and soil porewater properties that influenced N_2O and CO_2 emissions following soil freeze-thaw in the perennial bioenergy crops and the successional site.

3.2 Materials and Methods

The study took place at the University of Guelph Agroforestry Research Station in Guelph, Ontario, Canada (cf. Chapter 1, Section 1.9).

3.2.1. Soil sampling and preparation

In April 2022, three soil samples were collected randomly from each of the three treatment replicates to a 20 cm depth using a garden shovel. Soil samples were brought to the laboratory, air dried and sieved to 2 mm. For each treatment, soil samples from the three replicates were bulked together and homogenized. Gravimetric water content was determined for the air-dried homogenized soil samples for each treatment by oven drying at 105°C for 48 hours. At the time

of soil sampling, a soil bulk density ring with a 5 cm inner diameter and 5 cm height was used to collect soil samples for bulk density determination. Three bulk density samples were collected per treatment replicate. For each treatment, bulk density values for all the replicates were averaged to get a representative value for that treatment. Bulk density values for each treatment were used to calculate total soil porosity using a particle size density of 2.65 g cm^{-3}. Moisture for the air-dried homogenized soil samples were adjusted to 60% water-filled pore space (WFPS) using the calculated soil porosity values and residual water measured in the air-dried soil samples. The choice of 60% WFPS was based on a 2-year field moisture data from our study site. Soil moisture adjustment was achieved using rainwater and snowmelt water samples obtained from the research site. The snow and rainwater from the field was used to minimize disturbance to the native microbial community from osmotic or pH shock.

3.2.2. Soil incubation experiment

After adjusting soil moisture to 60% WFPS, three subsamples of equal mass (equivalent to 300 g air-dried soil) from each homogenized sample per treatment were added to 1000-mL mason glass jars, filling less than half the space. A total of 12 Mason jars representing 4 treatments and 3 replications were incubated in an environmental chamber (Percival I-41NL XC9) at +5°C for one month during which N_2O and CO_2 were measured until emissions reached a constant rate. After one-month of pre-incubation, soil samples were subjected to 7 freeze-thaw cycles for 49 days (Table 3.1). A freeze-thaw cycle consisted of a series of 7 sequential temperature settings of +5, 0, −5, −10, −5, 0, and +5°C over 7 days. The choice of +5 and −10°C temperature range for our temperature settings was based on a 2-year in field soil temperature reading from the study site. For each temperature settings, the jars were incubated for 24 hours before N_2O and CO_2 measurement. Soil moisture loss during the incubation period was negligible as weight change in

jars was not detected during the incubation period. This was not surprising as the jars were incubated with their lids closed throughout the duration of the experiment.

Table 3.1: Time course of the freeze-thaw cycles, temperature, duration, and the soil incubation days under freeze-thaw over the 49-day period used to assess the greenhouse gas emissions in the perennial bioenergy crops.

Cycle	Activity	Temperature (ºC)	Duration (days)	Freeze-thaw Incubation Days	Cycle	Activity	Temperature (ºC)	Duration (days)	Freeze-thaw Incubation Days
	Pre-incubation	+5	30		4th Cycle	thawing	–10 to –5	1	25
					(cont'd)	thawing	–5 to 0	1	26
1st	freezing	+5 to 0	1	1		thawing	0 to +5	1	27
Cycle	freezing	0 to –5	1	2		freezing	+5 to 0	1	28
	freezing	–5 to –10	1	3					
	thawing	–10 to –5	1	4	5th	freezing	+5 to 0	1	29
	thawing	–5 to 0	1	5	Cycle	freezing	0 to –5	1	30
	thawing	0 to +5	1	6		freezing	–5 to –10	1	31
	freezing	+5 to 0	1	7		thawing	–10 to –5	1	32
						thawing	–5 to 0	1	33
2nd	freezing	+5 to 0	1	8		thawing	0 to +5	1	34
Cycle	freezing	0 to –5	1	9		freezing	+5 to 0	1	35
	freezing	–5 to –10	1	10					
	thawing	–10 to –5	1	11	6th	freezing	+5 to 0	1	36
	thawing	–5 to 0	1	12	Cycle	freezing	0 to –5	1	37
	thawing	0 to +5	1	13		freezing	–5 to –10	1	38
	freezing	+5 to 0	1	14		thawing	–10 to –5	1	39
						thawing	–5 to 0	1	40
3rd	freezing	+5 to 0	1	15		thawing	0 to +5	1	41
Cycle	freezing	0 to –5	1	16		freezing	+5 to 0	1	42
	freezing	–5 to –10	1	17					
	thawing	–10 to –5	1	18	7th	freezing	+5 to 0	1	43
	thawing	–5 to 0	1	19	Cycle	freezing	0 to –5	1	44
	thawing	0 to +5	1	20		freezing	–5 to –10	1	45
	freezing	+5 to 0	1	21		thawing	–10 to –5	1	46
						thawing	–5 to 0	1	47
4th	freezing	+5 to 0	1	22		thawing	0 to +5	1	48
Cycle	freezing	0 to –5	1	23		freezing	+5 to 0	1	49
	freezing	–5 to –10	1	24					

3.2.3. Gas sampling and analysis

Twenty-four hours after each temperature setting, gas from each mason jar headspace was sampled and analyzed for N_2O and CO_2 using an Agilent Gas Chromatograph (GC) 6890 equipped with a micro-electron capture detector (ECD) (Agilent Technologies Inc., Santa Clara, USA). Prior to gas sampling, headspaces of jars were fully aerated by removing lids for 5 minutes. The jars were then sealed tightly with the lids and gas from headspace sampled at times 0, 15, and 30 minutes. The flux of N_2O and CO_2 emissions were calculated according to the HMR model in R (Pedersen et al., 2010).

3.2.4. Soil analysis

The initial soil samples and the samples after the incubation experiment were analyzed for water stable aggregate, soil organic carbon (SOC), soil total N, and soil ammonium (NH_4^+) and nitrate (NO_3^-) contents. Water stable aggregates were determined following a modified procedure from Carter (2002) and Mehuys et al. (2007). Briefly, 100 g of 2 mm sieved air-dried soil samples (W1) were placed in pre-weighed aluminum tins and pre-wet by gently misting soil to ~50% field capacity to avoid slaking effect 5 minutes prior to sieving. The soil was then transferred onto a 250 μm sieve in de-ionized water and sieved by uniformly moving the sieve up and down in the water for 50 times over 2 minutes at an amplitude of 4 cm. The portion of soil remaining on the sieve (>250 μm) was washed into the aluminum tin, oven-dried at 105°C for 24 hours or until no more weight loss was observed and weight recorded as W2. To separate aggregates from coarse particles, the >250 μm oven-dried samples were mixed with 50 mL of 0.5% w/w sodium hexametaphosphate and shook on a reciprocating shaker (Heidolpj Unimax 1010 DT) at 180 rpm for 45 minutes. The mixture was then again sieved through the 250 μm sieve and the remaining

content inside the sieve representing the coarse particles were rinsed into the aluminum tins, over-dried and weight recorded as W3. Percentage water stable aggregate was then calculated as:

$$\%Water\ Stable\ Aggregate = [(W2 - W3)/(W1 - W3)] \times 100\% \qquad (3.1)$$

Soil organic carbon and total N were analyzed using an elemental analyzer (Costech 4010, Cernusco, CA, USA). Prior to the elemental analysis, soil carbonates were removed according to Dyer et al. (2012). Soil extracts for analysis of NH_4^+ and NO_3^- were prepared using 10 g of air-dried 2 mm sieved soil mixed with 50 mL of 2 M KCl. The mixture was shaken on a reciprocating shaker for 15 minutes at 180 rpm and filtered using Whatman 42 filter paper. Filtered soil extracts were analyzed on a Shimadzu 1800 UV–Vis Spectrophotometer (Shimadzu Corp., Kyoto, Japan) (Doane & Horwáth, 2003). Soil net N mineralization after soil incubation was calculated by subtracting initial soil inorganic N (NH_4^+ and NO_3^-) from incubated soil inorganic N content (Christopher et al., 2008). Soil net N nitrification was calculated by subtracting initial soil NO_3^- from incubated soil NO_3^- content (Christopher et al., 2008).

3.2.5. Porewater extraction and analysis

A portion of each soil sample at 60% WFPS before- and after the freeze-thaw incubation was centrifuged at 5,000 rpm for 30 minutes and the supernatant porewater filtered through a 0.45 μm membrane filter (polypropylene syringe filters, VWR). Filtered porewater was analyzed for pH, electrical conductivity (EC), DOC, dissolved inorganic C (DIC), total N (TN), and dissolved inorganic N (DIN). Dissolved organic N (DON) was calculated as the difference between TN and DIN. The pH and EC were measured using handheld meters (LAQUA Twin meters, model Horiba B-213). Concentrations of TN and DOC in filtered porewater samples were measured using a total organic carbon analyzer (Shimadzu TOC-LCPH/CPN). Prior to analyzing for TN and DOC, porewater samples were acidified to pH < 3 using 1 M HCl whereas non-acidified porewater

samples were analyzed for DIC using the same total carbon analyzer. All porewater analyses were performed on both the final soil samples following the incubation and on the initial soil samples before the incubation.

3.2.6. Statistical analyses

A one-way analysis of variance (ANOVA) was used to determine whether there were any statistically significant differences in N_2O-N and CO_2-C emissions along with soil and porewater properties among land use types. The student *t* test was used to quantify differences in soil and porewater properties between before- and after soil freeze-thaw. Prior to the ANOVA, data was assessed for homogeneity of variance (Levene's) and normality (Shapiro-Wilk Test) (Steel et al., 1997). Data that violated the assumption of normality were log-transformed. Where statistically significant, means were separated using Fisher's Least Significant Difference (LSD). The significance threshold for all analyses was set at $p \leq 0.05$. The relationship between GHG emissions and soil and porewater properties were determined using Pearson correlation coefficients (*r*). Strength of the correlation was determined by categorizing Pearson's *r* value from 0 to 0.39 (weak), from 0.40 to 0.59 (moderate) and from 0.60 to 1.0 (strong). All data processing and analysis were carried out using SPSS (IBM SPSS Inc.).

3.3. Results

3.3.1. Water stable aggregate

Percentage of water stable aggregates (%WSA) before freeze-thaw ranged from 45% in the successional site to 50% in willow and 17% in miscanthus to 25% in willow after the freeze-thaw incubation period (Figure 3.1). The %WSA before- and after the freeze-thaw did not differ significantly ($p>0.05$) among bioenergy crops and the successional site. However, freeze-thaw

significantly decreased %WSA for all land-use types (Figure 3.1). The %WSA for soil under miscanthus, the successional site, switchgrass, and willow were reduced by 65%, 57%, 54%, and 49%, respectively, following the freeze-thaw incubations.

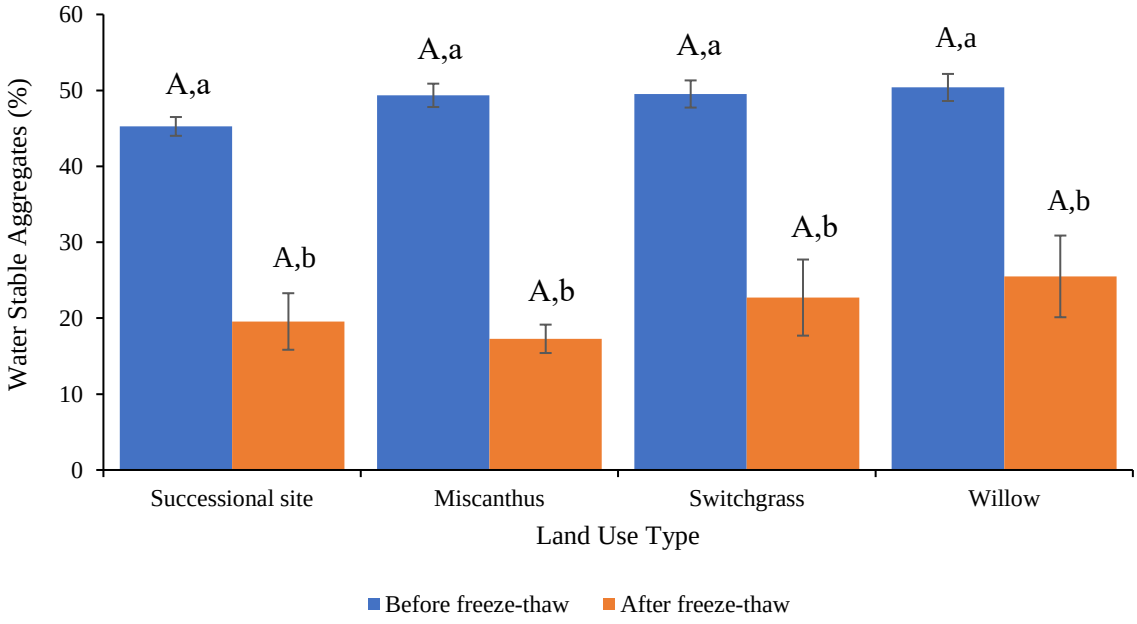


Figure 3.1: Percent water stable aggregates of soil from different perennial bioenergy crops and successional site before- and after the freeze-thaw incubation experiment. Bars represent standard error of percent water stable aggregate means (n = 3). Before- and after freeze-thaw, error bars with same uppercase letters show percent water aggregate means are not significantly different (p>0.05) among land use type and error bars with different lowercase letters show significant difference (p<0.05) in percent water stable aggregate means between before- and after freeze-thaw for the same land use type according to Fisher's least significant difference (LSD).

3.3.2. Carbon and nitrogen cycling

3.3.2.1. Successional site versus perennial bioenergy crops after freeze-thaw incubation

Soil C/N ratio before the freeze-thaw incubation ranged from 10.8 to 11.8 and was significantly lower (p<0.05) in successional site than the bioenergy crops (Table 3.2). Despite the lower soil C/N ratio, SOC was significantly higher in the successional site (20.9 g C kg^{-1}) than the bioenergy crops (17.8 to 18.5 g C kg^{-1}) before the freeze-thaw incubation but did not differ after

the freeze-thaw incubation (Table 3.2). After the freeze-thaw incubation period, a 6.2% reduction in SOC content was observed in the successional site but SOC content in the bioenergy crops remained unchanged. Except for the willow where DOC concentrations were significantly higher ($p<0.05$) than the successional site before the soil freeze-thaw (Figure 3.2B), soil NO_3^- contents, DIN concentrations, DON concentrations, and DOC concentrations were significantly higher ($p<0.05$) in soil under the successional site than the bioenergy crops before- and after the soil freeze-thaw incubation (Table 3.1; Figure 3.2A and 3.2B).

Table 3.2: Soil properties of the soil samples from the perennial bioenergy crops and successional site before- and after the 49-day period over the 7 freeze-thaw cycles at 60% water-filled pore space.

	Before freeze-thaw				**After freeze-thaw**			
	Successional site	Miscanthus	Switchgrass	Willow	Successional site	Miscanthus	Switchgrass	Willow
pH	7.49 (0.018)^{A,a}	7.28 (0.025)^{B,b}	7.13 (0.017)^{B,c}	7.29 (0.015)^{B,b}	7.43 (0.020)^{A,c}	7.76 (0.036)^{A,b}	7.51 (0.009)^{A,c}	7.89 (0.019)^{A,a}
EC (mS cm^{-1})	0.41 (0.003)^{B,c}	0.49 (0.003)^{A,b}	0.55 (0.009)^{B,a}	0.40 (0.006)^{A,c}	0.83 (0.009)^{A,b}	0.48 (0.033)^{A,c}	0.95 (0.019)^{A,a}	0.36 (0.019)^{A,d}
SOC (g kg^{-1})	20.89 (0.442)^{A,a}	17.83 (0.274)^{A,b}	18.52 (0.346)^{A,b}	17.89 (0.761)^{A,b}	19.60 (0.410)^{A,a}	18.98 (0.330)^{A,a}	18.70 (0.297)^{A,a}	18.49 (0.266)^{A,a}
Soil NH_4^+-N (mg kg^{-1})	4.43 (0.016)^{A,ab}	4.36 (0.026)^{A,ab}	4.25 (0.039)^{A,b}	4.58 (0.125)^{A,a}	4.27 (0.068)^{A,a}	4.17 (0.038)^{B,a}	4.45 (0.167)^{A,a}	4.19 (0.061)^{B,a}
Soil NO_3^--N (mg kg^{-1})	1.26 (0.067)^{B,a}	0.95 (0.046)^{B,b}	1.01 (0.009)^{B,b}	0.71 (0.025)^{B,c}	7.23 (0.028)^{A,a}	1.50 (0.167)^{A,c}	6.25 (0.191)^{A,b}	1.59 (0.091)^{A,c}
Soil C/N ratio	10.80 (0.042)^{A,b}	11.60 (0.056)^{A,a}	11.48 (0.037)^{A,a}	11.76 (0.177)^{A,a}	11.25 (0.174)^{A,b}	11.80 (0.159)^{A,a}	11.66 (0.063)^{A,ab}	11.81 (0.135)^{A,a}
Water stable aggregates (%)	45.25 (1.238)^{A,a}	49.34 (1.538)^{A,a}	49.52 (1.789)^{A,a}	50.38 (1.783)^{A,a}	19.56 (3.726)^{B,a}	17.27 (1.871)^{B,a}	22.71 (5.011)^{B,a}	25.50 (5.379)^{B,a}

Values in parentheses represent standard error of the means (n = 3). Before- and after freeze-thaw, means of soil properties with same lowercase letters are not significantly different (p>0.05) among land use type and means followed by the same uppercase letters are not significantly different (p>0.05) between before- and after freeze-thaw for the same land use type according to Fisher's least significant difference (LSD).

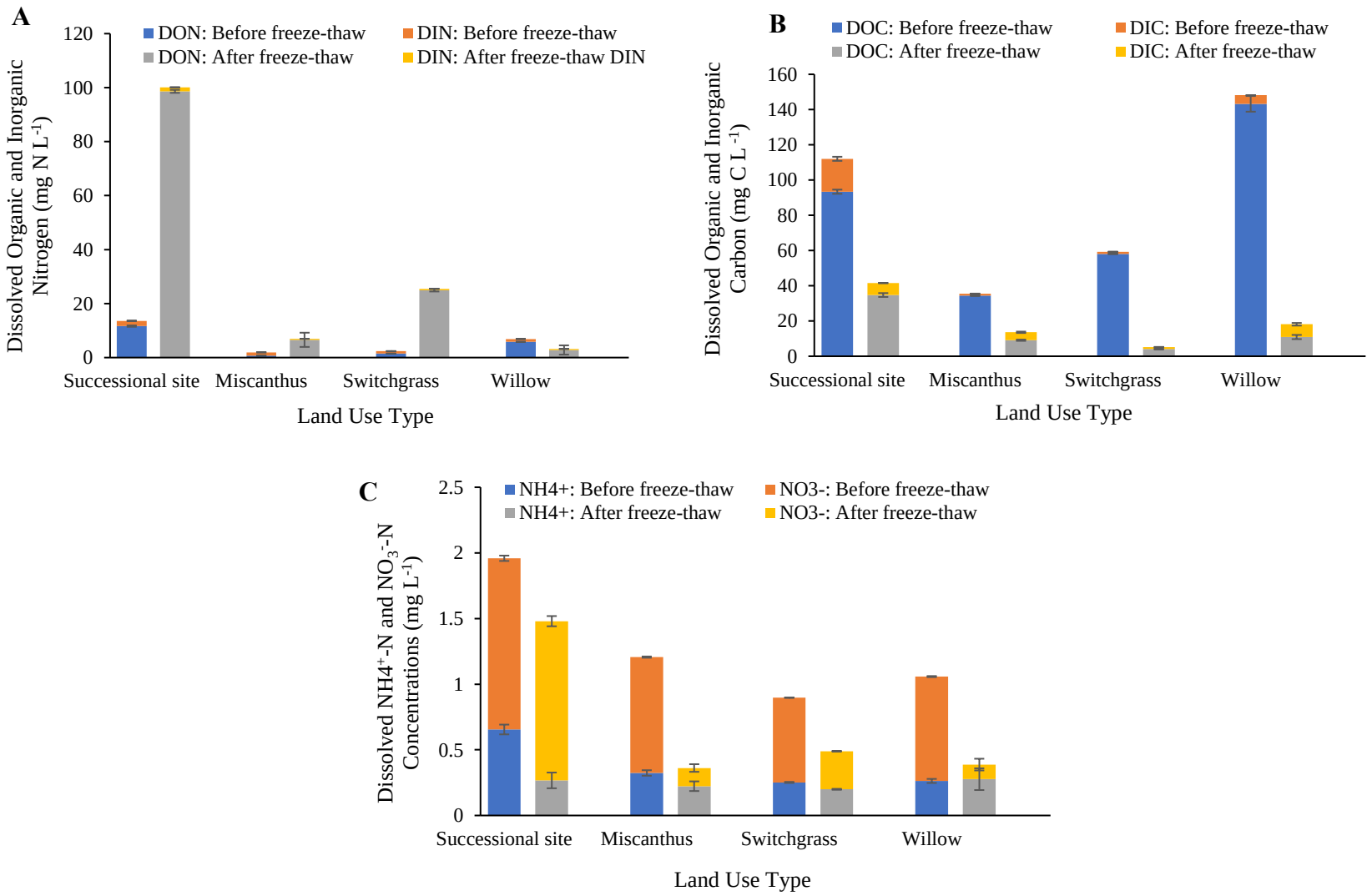


Figure 3.2: Dissolved organic and inorganic nitrogen (A), dissolved organic and inorganic carbon (B), and NH_4^+ and NO_3^- (C) concentrations in soil porewater measured before- and after the 49-day soil incubation from perennial bioenergy crops and successional site over 7 freeze-thaw cycles at 60% WFPS. Error bars represent standard error of means (n =3).

3.3.2.2. Before- and after soil freeze-thaw incubation

Freeze-thaw over the 49-day period significantly increased ($p<0.05$) soil NO_3^- contents in miscanthus, willow, successional site, and switchgrass by 57.9%, 123.9%, 473.8%, and 518.8 %, respectively. Meanwhile, soil NH_4^+ contents either remained the same in the successional site and switchgrass or was reduced significantly ($p<0.05$) in miscanthus and willow after soil freeze-thaw (Table 3.2). Except for the willow where freeze-thaw reduced DON concentration by 51.8%, freeze-thaw significantly enhanced DON concentrations in the successional site, miscanthus, and switchgrass by 746.5%, 801.4%, and 1576.5%, respectively. Freeze-thaw on the other hand significantly ($p<0.05$) reduced DIN concentrations in all land use types. After the 49 days of freeze-thaw, DIN concentrations decreased from 1.96 to 1.48 mg N L^{-1} in the successional site, 1.21 to 0.36 mg N L^{-1} in miscanthus, 0.90 to 0.49 mg N L^{-1} in switchgrass, and 1.06 to 0.39 mg N L^{-1} in willow (Figure 3.2A). Similar to DIN, freeze-thaw reduced DOC concentrations by 62.8%, 73.5%, 92.4%, and 93.0% in soil from the successional site, miscanthus, willow, and switchgrass, respectively. At the end of the freeze-thaw incubation, we measured significantly higher soil N net mineralization and nitrification in the successional site (5.80 and 5.96 mg N kg^{-1}; mineralization and nitrification, respectively) and switchgrass (5.44 and 5.24 mg N kg^{-1}) than miscanthus (0.35 and 0.55 mg N kg^{-1}) and willow (0.49 and 0.88 mg N kg^{-1}) (data not shown).

3.3.3. Nitrous oxide and carbon dioxide emissions

The highest mean N_2O and CO_2 emissions recorded over the 49-day freeze-thaw incubation were 923 µg N_2O-N m^{-2} hr^{-1} (Figure 3.3) and 127 mg CO_2-C m^{-2} hr^{-1} (Figure 3.4), respectively. Total cumulative N_2O emissions over the 49-day incubation were highest ($p<0.05$) in miscanthus (148 mg N_2O-N m^{-2}), followed by willow (80 mg N_2O-N m^{-2}), switchgrass (26 mg N_2O-N m^{-2}), and the successional site (17 mg N_2O-N m^{-2}) (Figure 3.5A). Total cumulative N_2O emissions in

switchgrass were not significantly different ($p>0.05$) from the successional site. The total cumulative CO_2 emissions over the same incubation period were highest in the successional site (25,262 mg CO_2-C m^{-2}) when compared to switchgrass (18,845 mg CO_2-C m^{-2}), miscanthus (17,302 mg CO_2-C m^{-2}), and willow (15,403 mg CO_2-C m^{-2}) (Figure 3.5B). Whereas the total cumulative CO_2 emissions were significantly higher ($p<0.05$) in the successional site than any of the bioenergy crops, total cumulative CO_2 emissions did not differ ($p>0.05$) among bioenergy crops. Over the 7 freeze-thaw cycles (49-day incubation period), about 67% (successional site), 75% (willow), and 78% (switchgrass and miscanthus) of the total N_2O emitted occurred in the first 21 days, representing the first 3 freeze-thaw cycles (Figure B1.1), whereas that of total CO_2 emitted over the same freeze-thaw cycles and days constituted 57% for miscanthus, 59% for willow, 62% for successional site, and 67% for switchgrass (Figure B1.2).

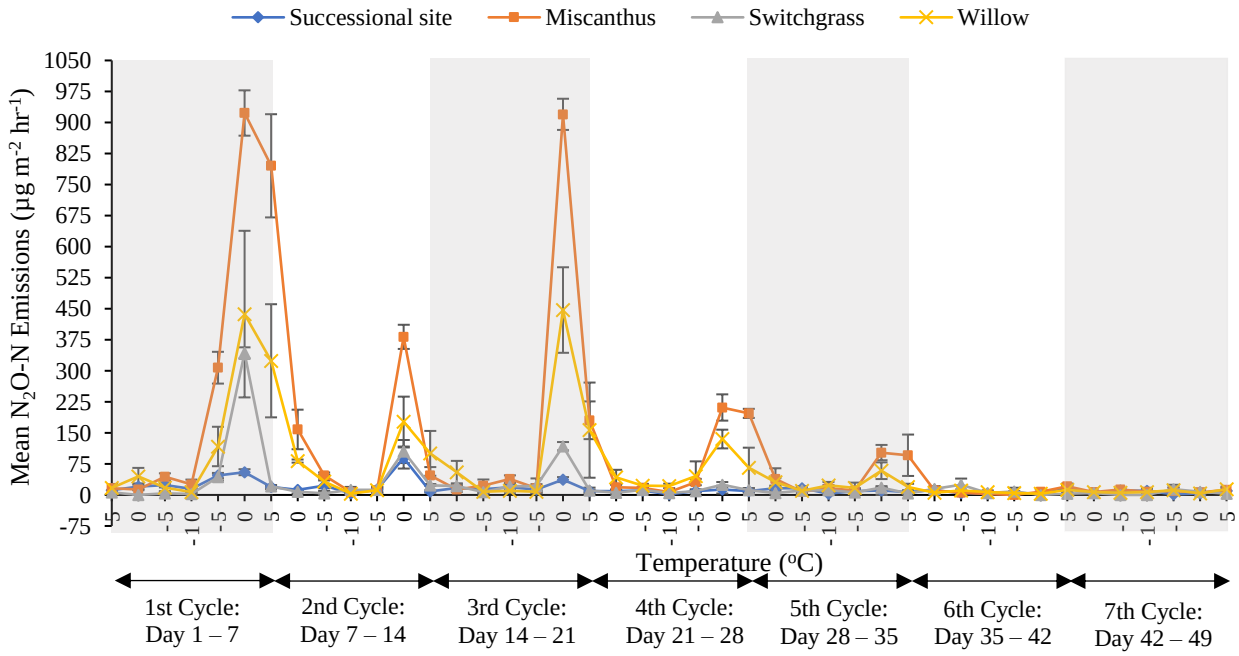


Figure 3.3: Mean N_2O-N emissions (µg m^{-2} hr^{-1}) over 7 freeze-thaw cycles of incubated soil from perennial bioenergy crops and successional site. Bars represent standard error of the means (n = 3).

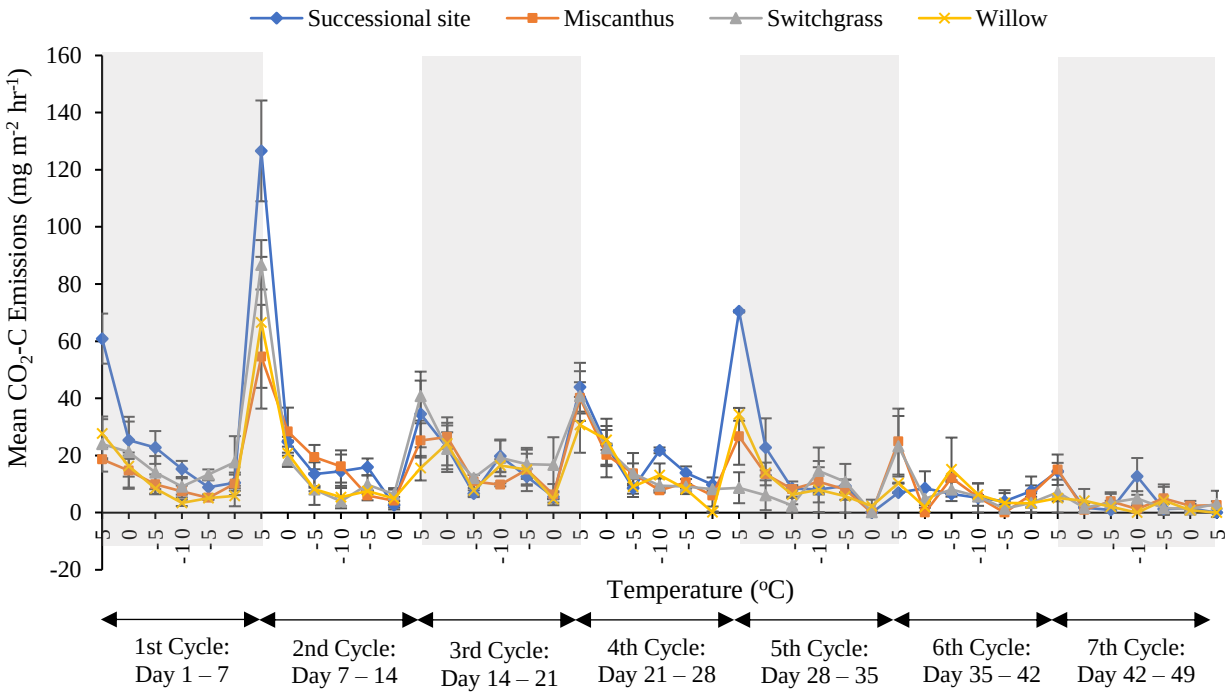


Figure 3.4: Mean CO_2-C emissions (mg m^{-2} hr^{-1}) over 7 freeze-thaw cycles of incubated soil from perennial bioenergy crops and successional site. Bars represent standard error of the means (*n* = 3).

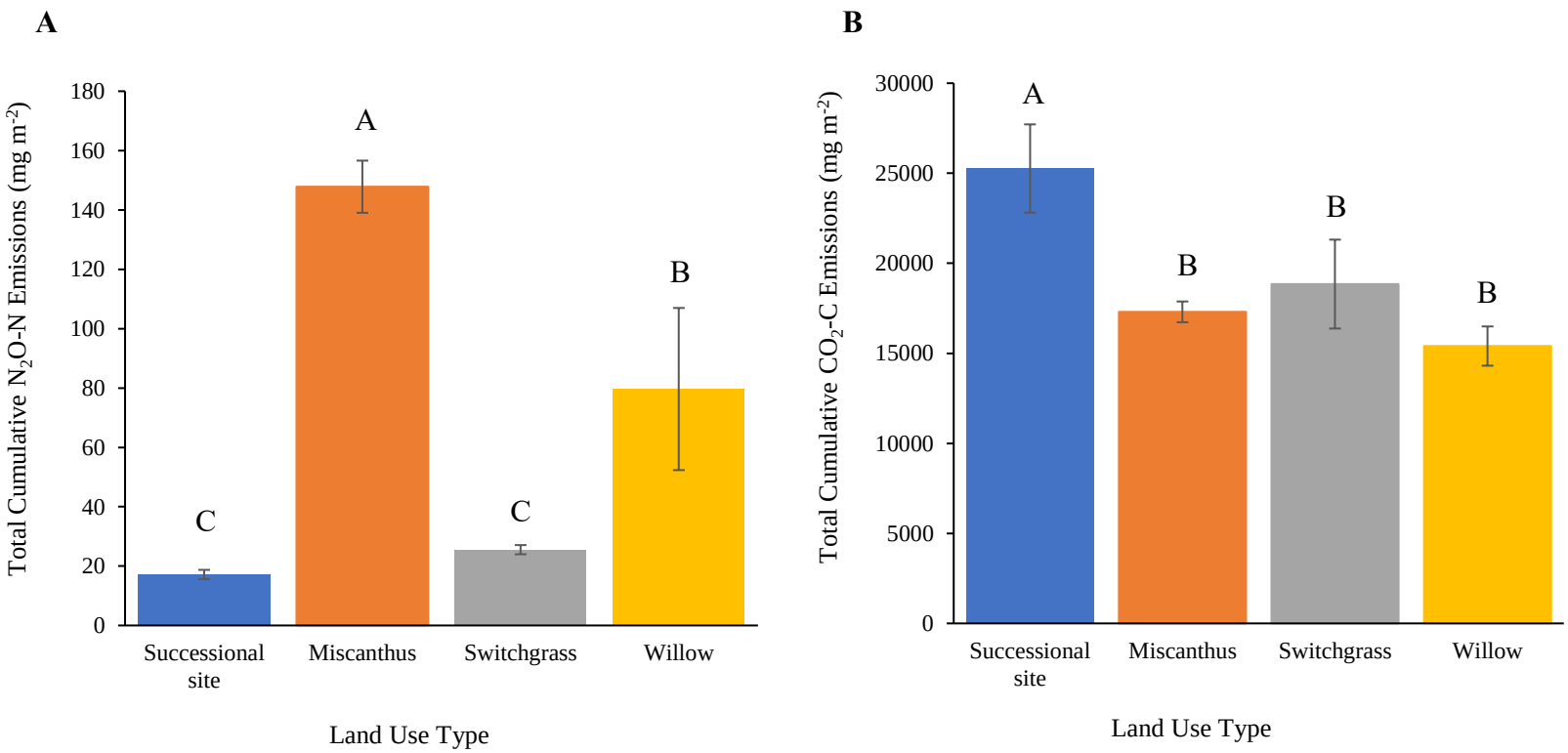


Figure 3.5: Total cumulative N_2O-N (mg m^{-2}) (A) and CO_2-C emissions (mg m^{-2}) (B) after the 49-day incubation period over 7 freeze-thaw cycles of soil from perennial bioenergy crops and successional site. Bars represent standard error of total cumulative means (n = 3). Error bars with same uppercase letters show total cumulative means are not significantly different (p>0.05) according to Fisher's least significant difference (LSD).

3.3.4. Relationship between greenhouse gas emissions and soil and porewater properties

After soil freeze-thaw, total cumulative N_2O emissions showed a strong negative relationship ($p<0.05$) with DIN, DON, soil NO_3^-, EC of the soil porewater, soil net N mineralization and nitrification. Conversely, soil C/N ratio and pH were positively correlated with N_2O emissions (Figure 3.6). Also, total cumulative CO_2 emissions were positively correlated ($p<0.05$) with DIN, DON, and soil NO_3^- after soil freeze-thaw (Figure 3.6). Soil organic C values measured after the incubation did not show any significant relationship with cumulative soil CO_2 emissions. Rather, CO_2 only correlated positively with the SOC values measured before the soil incubation (data not shown). Moreover, DOC and NH_4^+ did not show any significant correlation with either N_2O or CO_2 emissions (Figure 3.6). We found that all soil and porewater parameters that had a significant relationship with N_2O and CO_2 emissions and were strongly correlated ($p<0.05$) with soil C/N ratio.

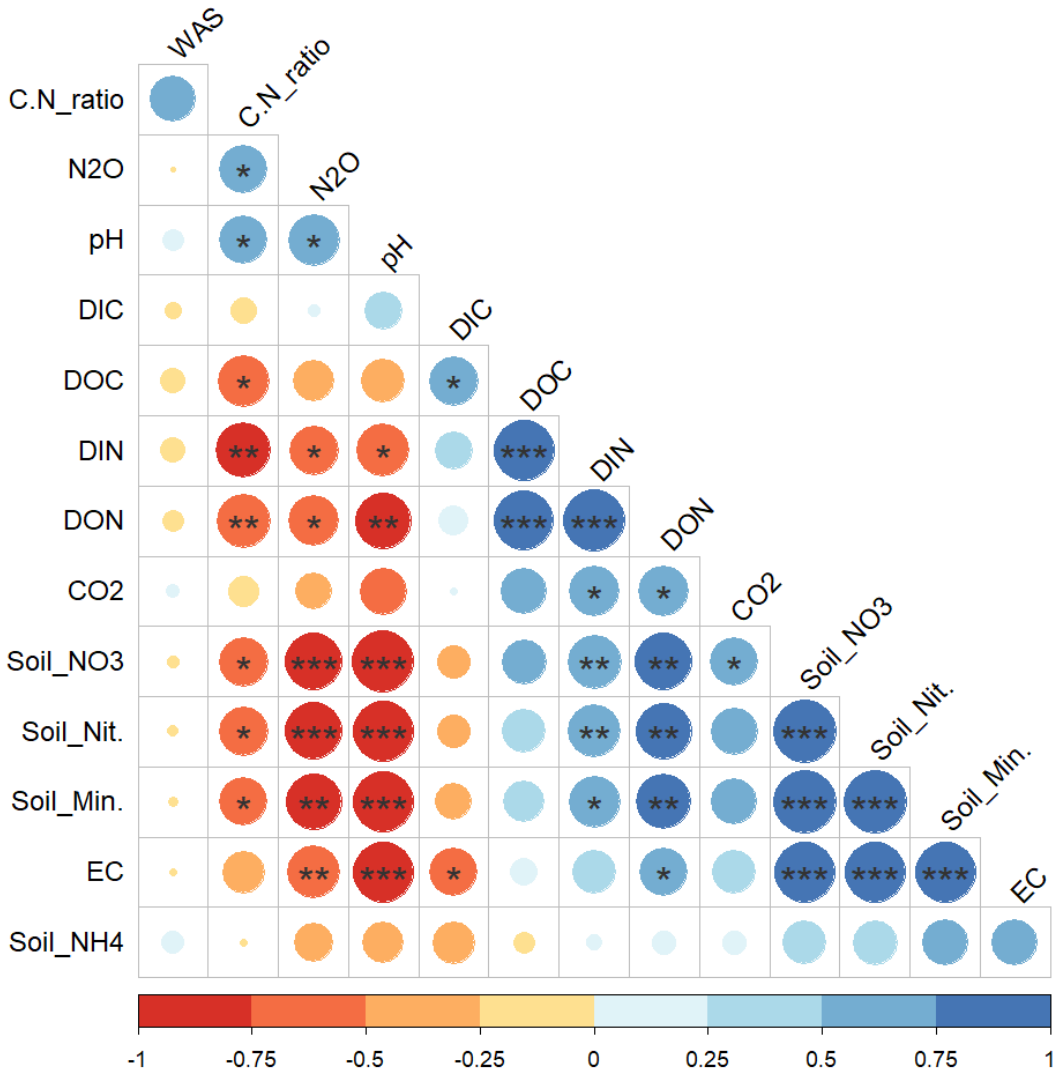


Figure 3.6: Pearson correlation coefficient between measured emissions and soil and soil porewater properties after the 49-day soil incubation period over 7 freeze-thaw cycles. C.N_ratio (soil C/N ratio), N_2O (N_2O emissions), CO_2 (CO_2 emissions), pH (pH of soil porewater), EC (electrical conductivity), DIC (dissolved inorganic carbon), DOC (dissolved organic carbon), DIN (dissolved inorganic carbon), DON (dissolved organic nitrogen), Soil_NO_3 (soil NO_3^--N), Soil_NH_4 (soil NH_4^+-N), Soil_Nit. (soil N nitrification), Soil_Min. (soil N mineralization). *p<0.05; **p<0.01; ***p<0.001.

3.4. Discussion

3.4.1. Water stable aggregates

Consistent with the hypothesis, %WSA before- and after freeze-thaw did not differ between the perennial bioenergy crops and successional site. Even though, the %WSA did not differ among the different land uses, freeze-thaw decreased water stable soil aggregates in all of the land use types. A decrease in %WSA observed in the study, following the 7 freeze-thaw cycles over the 49-day period, agreed with findings from other studies (*e.g.,* Oztas & Fayetorbay, 2003; Xiao et al., 2020) where a reduction in soil aggregates was observed with an increasing number of freeze-thaw cycles. For instance, Oztas & Fayetorbay (2003) found that increasing the number of freeze-thaw cycles from 6 to 9 over 12 to 18 days decreased soil aggregate stability whereas the opposite effect was observed when the number of freeze-thaw cycles were less than 6 (12 days). This is because as freeze-thaw cycles increase, the cohesive forces between soil particles are gradually destroyed resulting in significant reduction in soil aggregate stability (Zhang et al., 2021b). Dagesse (2013) showed that freezing alone and freeze-dry treatments resulted in greater aggregate stability in Canadian soils with varying clay and water content. However, introducing a thaw component after freezing resulted in the degradation of the soils' aggregate stability (Dagesse, 2013). Therefore, it is logical to expect that after continuous freezing and thawing of the soils for 49 days, a reduction in water stable soil aggregates would be observed.

Also, the effect on soil aggregate disruption following freeze-thaw processes depends on the soil texture, initial aggregate size, organic matter content, soil water content at the time of freezing, the number of freeze-thaw cycles and temperature of freezing (Song et al., 2017). Considering that the factors which impact soil aggregate stability during freeze-thaw were the same (albeit marginally higher SOC in successional site before freeze-thaw) across the perennial bioenergy

crops and successional site, it was not surprising that soil from these different land use types exhibited similar decreases in aggregate stability after freezing and thawing. Despite this, the magnitude of soil aggregate disruption, as indicated by the percent reduction in water stable aggregates after freezing and thawing, was least in willow (49%) and highest in miscanthus (65%). This observation may have been due to other soil processes other than the aforementioned factors. For instance, Ma et al. (2021b) observed that soil total porosity and pore structure had profound impact on soil aggregate stability following repeated freeze-thaw cycles. Moreover, the chemistry of soil water, as influenced by litter composition, may impact the rate and intensity of soil freezing (Marion, 1995). This implies that the processes governing soil aggregate stability during soil freeze-thaw may be complex which require further studies to enhance our understanding on the key soil processes that influence soil aggregate stability during soil freezing and thawing.

3.4.2. Carbon and nitrogen cycling

3.4.2.1. Successional site versus perennial bioenergy crops after freeze-thaw incubation

Lower soil C/N ratio and higher N in the successional site than the perennial bioenergy crops supported the hypothesis that the presence of diverse plant species on the successional site would promote lower soil C/N ratio than soil from the monocrop sites of the perennial bioenergy crop species. Plant species composition drive differences in litter quality which in turn influence the variability in soil C/N ratio (Cools et al., 2014; Lovett et al., 2002). Soils with diverse litter inputs promote lower soil C/N ratios and increased organic matter decomposition which can result in higher soil N (Cong et al., 2014, 2015a). Increased organic matter decomposition rates in soils with increased litter diversity are attributed to the transfer of nutrients from nutrient rich organic materials to nutrient poor materials (Cong et al., 2015a), causing changes in the composition of the decomposer community (Beugnon et al., 2021; Zhang et al., 2023), niche complementarity

within the decomposer community (Baudy et al., 2021; García-Palacios et al., 2017), and priming effects in which high-nutrient materials enhance the decomposition of low-nutrient materials (Truong & Marschner, 2018). In a 7-year study, Cong et al. (2015b) found that a cereal-legume intercrop enhanced soil C and N contents and decreased soil C/N ratio compared to their corresponding monocrops. As plant species richness increases soil net N mineralization, greater species diversity and a resultant decrease in soil C/N ratio could be expected (Cong et al., 2014). Increased N mineralization and potentially higher microbial activities (Lange et al., 2015; Truong & Marschner, 2018) in the successional site than the bioenergy crops may have contributed to the higher C and N contents in soil and soil solution as reflected in higher SOC, soil NO_3^-, DIN, DON, and DOC concentrations in the successional site than bioenergy crops. Higher C and N contents in soil and soil solution with increased plant diversity in this study are consistent with findings from other studies (*e.g.*, Cong et al., 2014; Lange et al., 2021).

Moreover, as hypothesized, perennial bioenergy crops have an extended root network which aids in effective and efficient nutrient uptake and use (Thevathasan et al., 2012). However, frequent biomass harvest of bioenergy crops without N replacement can deplete soils of their N status (Fixen, 2007). It is worth emphasizing that the bioenergy crops in this study had not received any form of fertilization for half a decade. Therefore, N removal in harvested biomass without replacement may have contributed to lower soil available N in the bioenergy crops compared to the successional site which may have reflected in the higher N contents in the successional site than the bioenergy crops.

3.4.2.2. Before- and after soil freeze-thaw incubation

Contrary to the expectations that freeze-thaw would enhance C and N contents in soil and the soil solution (porewater), lower concentrations in DOC and DIN following soil freezing and

thawing in the successional site and the bioenergy crops were observed. However, increased DON concentrations and soil inorganic N contents after the freeze-thaw incubation was consistent with the hypothesis of this study. The observation of lower DOC and DIN concentrations differed from results of a meta-analysis by Song et al. (2017) who found a 27.5% and 35.4% increase in DOC and DIN concentrations, respectively, following soil freeze-thaw events. Freezing and thawing soils induce labile organic substrate availability through soil aggregate disruption, and cell lysis and death of soil microbial biomass, enhancing mineralization and resulting in increased C and N contents (Chai et al., 2014; King et al., 2021; Libby et al., 2020). Moreover, DOC represents an available labile C source to microorganisms that is often utilized rapidly by the soil microbial community (Iqbal et al., 2010). Increasing the number of freeze-thaw cycles can deplete soils of labile substrate available for mineralization if substrate is in limited supply (Henry, 2007; Herrmann and Witter, 2002). The study by Song et al. (2017) included both field and laboratory incubation experiments. In their field experiments for instance, the continuous presence of plant biomass on the field provides regular substrate supply for microbial degradation and mineralization (Campbell et al., 2014; Song et al., 2017), resulting in increased DOC and DIN concentrations after repeated freeze-thaw cycles. Therefore, after 7 successive freeze-thaw cycles over 49 days without any additional substrate supply in this study, it was assumed that the limited available labile substrates present in the soil became depleted which may have caused a decline in the DOC and DIN concentrations after the freeze-thaw incubation.

Again, reduction in dissolved NH_4^+ and NO_3^- (DIN concentrations) associated with the soil water phase during the freeze-thaw process may have been fostered by NO_3^- losses through denitrification processes (Chantigny et al., 2017; Chen et al., 2021; Congreves et al., 2018; Müller et al., 2003; Risk et al 2013) and possible microbial immobilization of the dissolved NH_4^+ and

NO_3^- (Kirchman & Wheeler, 1998; Larsen et al., 2002; Müller et al., 2002). This assumption was based on the observation that while the dissolved NH_4^+ and NO_3^- concentrations declined after the freeze-thaw process, that of soil inorganic N was enhanced. Anoxic conditions created by the soil water phase may have provided the necessary conditions for increased denitrification activities (Bayer et al., 2015; Congreves et al., 2018). The release of both DOC and NO_3^- into soil solution can act synergistically to stimulate N_2O emissions with DOC providing the energy source for the denitrifier community (Wu et al., 2020a). This synergistic interaction between DOC and NO_3^- for N_2O production by the soil denitrifier community may have increased the depletion of both DOC and DIN concentrations following the freeze-thaw incubation period.

Although, soil inorganic N (soil NH_4^+ and soil NO_3^-) was enhanced after the incubation period, it was observed that the higher soil inorganic N, after the freeze-thaw, was due to increased soil NO_3^- rather than soil NH_4^+. The lower soil NH_4^+ relative to soil NO_3^- was attributed to soil N nitrification and a possible microbial immobilization of N in the form of NH_4^+. Due to the energy cost associated with microbial assimilation of NO_3^- (Geisseler et al., 2010), soil microbes most often have greater preference for N uptake in the form of NH_4^+ compared to NO_3^- (Christie & Wasson, 2001; Fraterrigo et al., 2011; Kirchman & Wheeler, 1998). Ma et al. (2021a) observed that 56% of total N applied was taken up in the form of NH_4^+-N compared to 40% in NO_3^--N form in temperate agricultural soil. Christie & Wasson (2001) also observed that NH_4^+ showed twice the microbial immobilization rate of NO_3^- in temperate grassland soil incubated with glucose addition as C source. The extent of soil NH_4^+ loss or soil NO_3^- increase following the soil incubation differed with bioenergy crop species and successional site. Soil NH_4^+ losses were observed in miscanthus and willow but not in switchgrass and successional site. The increase in soil NO_3^- was 7 times and 11 times higher in successional site than willow and miscanthus,

respectively, and 6 times and 10 times higher in switchgrass than willow and miscanthus, respectively. Since this study only measured soil NH_4^+ and NO_3^- before- and after the incubation period and not during the incubation period, it was assumed that the lower soil NH_4^+ and NO_3^- in the miscanthus and willow than successional site and switchgrass were indicative that loss of soil NH_4^+ and NO_3^- proceeded faster in the miscanthus and willow than the switchgrass and successional site.

It is not entirely clear what may have caused the faster depletion of soil inorganic N in the miscanthus and willow relative to the switchgrass and successional site. While this study did not evaluate the soil microbial biomass or community structure and how that may have impacted C and N dynamics during the freeze-thaw incubation, evidence from other studies showed that freeze-thaw (Németh et al., 2014; Risk et al., 2013; Song et al., 2017) and bioenergy crop species (Bahulikar et al., 2014; Gushgari-Doyle et al., 2021; Mafa-Attoye et al., 2020; Oates et al., 2016; Schrama et al., 2016) have a profound influence on soil microbial biomass and microbial community structure. This study suggests that a potential shift in the soil microbial community under the different species or vegetation may have been a major contributing factor to the varied rates at which inorganic N losses occurred in the different perennial bioenergy crop species. For instance, herbaceous species such as switchgrass and miscanthus have a greater capacity to form associations with arbuscular mycorrhizae fungi (AMF) compared to woody crops such as willow (Bahulikar et al., 2014; Gushgari-Doyle et al., 2021; Jesus et al., 2016; Mafa-Attoye et al., 2020), with switchgrass averaging higher AMF biomasses than miscanthus (Mafa-Attoye et al., 2020; Schrama et al., 2016). Also, Mafa-Attoye et al. (2020) studied soil microbial biomass and recorded higher fungi to bacteria ratio in willow than switchgrass and miscanthus from the study site. Although, the successional site was not included in the study by Mafa-Attoye et al. (2020), systems

with increased plant species diversity such as the successional site in this promote higher microbial diversity and microbial biomass compared to monoculture systems such as the bioenergy crops (Lange et al., 2015; Oelbermann et al., 2015; Truong & Marschner, 2018). Oates et al. (2016) also observed higher total microbial biomass in switchgrass than miscanthus. Evidence from these different studies point to a potential higher microbial biomass and diversity in switchgrass and the successional site than miscanthus and willow. This potentially higher microbial biomass in the successional site (Lange et al., 2015; Oelbermann et al., 2015; Truong & Marschner, 2018) and switchgrass (Mafa-Attoye et al., 2020; Oates et al., 2016; Schrama et al., 2016) may have promoted inorganic N immobilization resulting in reduced N losses as N_2O compared to the miscanthus and willow.

Also, since AMF enhances soil N uptake (Hassan et al., 2022; Teutscherova et al., 2019), higher AMF associated with switchgrass (Schrama et al., 2016) than miscanthus and willow could have enhanced N uptake reducing N losses as N_2O in switchgrass. Additionally, bacteria generally have higher N requirement than fungi which can result in increased N uptake in bacteria biomass compared to fungi (Six et al., 2006). Hence, higher fungi biomass induced by willow crops from the study site (Mafa-Attoye et al., 2020) may have reduced N uptake in the willow compared to switchgrass or successional site, resulting in increased N losses as N_2O in the willow. The N uptake in microbial biomasses may have been released back into the soil solution as DON from killed microbial biomasses due to the repeated soil freezing and thawing (Risk et al., 2013). This may explain why despite reduced DIN concentrations after the freeze-thaw incubation period, DON concentrations were enhanced. Findings from this study illustrate the need for further studies on evaluating the impact of repeated soil freeze-thaw cycles on soil microbial biomass and community

structure in different perennial bioenergy crop species and how these soil microbes may influence C and N cycling leading to N_2O and CO_2 production and emission.

3.4.3. Nitrous oxide and carbon dioxide emissions after soil freeze-thaw

Although, N_2O can be produced through nitrification and nitrifier-denitrification processes, denitrification is suggested as the dominant process responsible for N_2O production during soil freeze-thaw processes, with higher NO_3^- concentrations than NH_4^+ after soil incubation indicative of N_2O production via denitrification (Chen et al., 2021; Müller et al., 2003; Risk et al., 2013). The high NO_3^- and low NH_4^+ concentrations after soil incubation in this study confirmed that denitrification rather than nitrification may have dominated the N_2O production process. The gap in research data on N_2O and CO_2 emissions in miscanthus, switchgrass, and willow as influenced by freeze-thaw cycles made it impossible to compare results from this study to others conducted in similar systems. Higher cumulative N_2O emissions measured in miscanthus and willow, especially, during the early freeze-thaw cycles and lower dissolved and soil inorganic N measured after the 49-day incubation period can only mean that N losses through denitrification processes proceeded faster in miscanthus and willow than in switchgrass and the successional site as suggested earlier. As already explained, a possible reason for the faster depletion of inorganic N in the miscanthus and willow than the switchgrass and successional site may be due to potential increased microbial activities in the switchgrass and the successional site which may have enhanced microbial N uptake (Chen et al., 2021). This increased microbial activity in the successional site and in switchgrass, may equally explain the relatively higher total cumulative CO_2 emissions in the successional site and switchgrass than miscanthus and willow after the 49-day freeze-thaw soil incubation period. Since the incubated soils were sieved and all visible roots and plant biomasses removed prior to incubation, it is assumed that the CO_2 measured were from

soil microbial respiration and activities (Song et al., 2017). In systems with increased potential for higher microbial activities, particularly, in the successional site, CO_2 would be expected to be significantly higher than in monoculture systems with less heterogenous organic input addition and limited microbial biomass and activities such as the miscanthus and willow (Beugnon et al., 2021; Chen et al., 2019; Durán & Delgado-Baquerizo, 2020; Lange et al., 2015).

Higher N_2O and CO_2 emissions during the first three freeze-thaw cycles point to higher labile substrate availability and microbial activity in the early stages of the freeze-thaw incubation which decreased in the following freeze-thaw cycles (Feng et al., 2007; Wu et al., 2020b; Yan et al., 2016). During the early stages of freeze-thaw processes, increased microbial activities and mineralization of freeze-thaw-killed microbial biomasses result in increased labile C substrate and inorganic N availability (Herrmann & Witter, 2002; Risk et al., 2013). A higher quantity of the available labile C and N is used by the surviving microbial community to drive both N_2O and CO_2 emissions (Chantigny et al., 2016; Wu et al., 2020a). As the number of freeze-thaw cycles increase, the soil becomes depleted of these labile C and N sources which results in the reduction of N_2O and CO_2 production (Congreves et al., 2018; Feng et al., 2007; Klemedtsson et al., 2005). The diminishing N_2O and CO_2 emissions after the first three freeze-thaw cycles are consistent with findings by Feng et al. (2007) and the earlier observation of reduced DOC and DIN concentrations after the 49-day incubation period. Congreves et al. (2018) and Feng et al. (2007) explained that limited supply and depletion of labile substrate during successive soil freeze-thaw cycles could be linked to why the greatest N_2O and CO_2 emissions are observed during the first series of freeze-thaw cycles and decreased N_2O and CO_2 emissions after successive freeze-thaw cycles. The same reason is ascribed for the reduced DOC and DIN concentrations to the diminished N_2O and CO_2 emissions after the first three freeze-thaw cycles and suggest that increasing the number of freeze-

thaw cycles in this study depleted the finite labile substrate available that was required to drive soil N_2O and CO_2 production and emission.

This study did not account for how different freezing intensity and duration would impact GHG emissions in perennial bioenergy crops. Pelster et al. (2019) evaluated how freezing intensity and duration influence N_2O and CO_2 emissions in sandy loam and silty clay soils in temperate region and observed that a single freeze-thaw cycle with soil freezing at –3°C for 8 days, representing 24 freezing degree-days (FDD) and thawing at +1°C increased N_2O and CO_2 emissions. Based on this experiment where a single freeze-thaw cycle had an equivalent of 20 FDD (–5°C × 1 = 5 FDD; –10°C × 1 = 10 FDD; and –5°C × 1 = 5 FDD), and with the observation that > 66% and 56% of the total N_2O and CO_2 emissions, respectively, occurred during the first 3 freeze-thaw cycles, it could be suggested that 60 FDD (representing the first 3 freeze-thaw cycles) would potentially contribute substantially to N_2O and CO_2 emissions in perennial bioenergy crops. This implies that reducing the severity of soil freezing is crucial in reducing the freeze-thaw-related N_2O and CO_2 emissions in perennial bioenergy crops, particularly, with this study pointing to the potential of N_2O originating from microbial lysis and osmolytes which can be increased by severe soil freezing (King et al., 2021; Smith et al., 2010). Hence, adopting management strategies that enhance soil insulation against severe freezing may be important in reducing freeze-thaw-related emissions in perennial bioenergy crops.

Increasing snow cover and plant litter in perennial bioenergy crops can insulate the soil from severe freezing (Elliott & Henry, 2009; Henry, 2007; Smith et al., 2010; Zhang, 2005). This can be achieved through stubble management during biomass harvest (Schillinger & Wuest, 2021). Although, the impact of stubble management in perennial bioenergy crops on snow cover has not been studied, Schillinger & Wuest (2021) observed that maintaining wheat stubble with a 75 cm

and 25 cm height was able to increase overwinter precipitation due to enhanced snow capture and retention compared to the 8-cm-tall stubble. This ability of tall stubbles to enhance snow retention (Konstantinov et al., 2021) can be an important harvest management strategy in perennial bioenergy crops where the captured snow could provide insulation to the soil against severe freezing and related GHG emissions. This may require further studies to understand how management of stubble heights in perennial bioenergy crops influence snow capture and reduction in soil freezing intensity.

3.4.4. Predominant soil and porewater properties that impacted N_2O and CO_2 emissions after the soil freeze-thaw incubation

It was expected that soil aggregate disruption from freeze-thaw would enhance the availability of labile substrate to drive N_2O and CO_2 emissions (Chai et al., 2014). However, no significant relationship was observed between %WSA and N_2O or CO_2 emissions after freeze-thaw. Neither was there a significant relationship between %WSA and any of the C and N contents in both soil and soil solution. This may indicate that the major source of organic substrate which drove N_2O and CO_2 emissions may have originated from cellular materials released from death and lysis of microbial biomass (Congreves et al., 2018; Hermann & Witter 2002) rather than from aggregate disruption (Chai et al., 2014). This observation in this study is consistent with findings from King et al. (2021) who observed in a soil freeze-thaw incubation study that majority of the substrate that drove N_2O emissions originated from microbial osmolytes rather than from soil aggregate disruption.

Also, a negative relationship between N_2O emissions and soil NO_3^-, DIN and DON concentrations, soil N mineralization and nitrification, and EC of the soil porewater were observed after soil freeze-thaw. This negative relationship implied that higher N_2O emissions corresponded with lower soil and dissolved N after the soil incubation period. Increased soil mineralization and

nitrification increase NO_3^- production which is a major precursor for N_2O production by the denitrifier community in the soil (Risk et al., 2013). With the soils in this study having a limited supply of N, increased N mineralization, and nitrification with subsequent N losses as N_2O would have led to the depletion of the soil of its N content. This may explain why N_2O emissions stabilized and were near zero after the 5th freeze-thaw cycle (35 days) in this study as the elevated N_2O emissions during the initial freeze-thaw cycles had depleted the soil N content. Additionally, the presence of NH_4^+ and NO_3^- can increase the soil EC value (Zhao et al., 2016). This means that the depletion of these soil nutrient sources may have resulted in lower EC values which also corresponded with N_2O emissions (Gil et al., 2008; Zhang & Wienhold, 2002).

Soil C/N ratio and pH of the soil porewater positively correlated with soil N_2O emissions after the freeze-thaw process. The presence of NH_4^+ can cause a reduction in soil pH (Yu et al., 2018; Zhao et al., 2016). The depletion of NH_4^+ concentration would be expected to cause a slight increase in soil pH which also favours denitrifier activities (Sun et al., 2012). Moreover, depletion of soil N would result in higher soil C/N ratio (Vesterdal et al., 2008). Hence, both soil pH and soil C/N ratio after freeze-thaw would have had a positive significant relationship with N_2O production and emission.

On the other hand, CO_2 emissions responded positively to DIN, DON, and soil NO_3- after freeze-thaw. Increasing soil N content generally leads to higher soil CO_2 emissions by stimulating microbial biomass and soil respiration (Oertel et al., 2016; Peng et al., 2011). Wang et al. (2010) observed that N fertilization of soils resulted in increased consumption of DOC leading to higher CO_2 emissions. The use of N as energy source by the soil microbial community during the freeze-thaw process and a resultant increase in microbial activity and CO_2 production could be linked to the positive correlation between CO_2 emissions and DIN, DON, and soil NO_3- contents.

Also, the soil C/N ratio significantly correlated with soil C and N contents as well as soil N net mineralization and nitrification. Considering that these were the same properties which significantly correlated with N_2O and CO_2 emissions, it implies that soil C/N ratio can be very important in controlling GHG emissions during soil freeze-thaw processes. This is not surprising because the metabolisms of microorganisms which may largely control GHG emissions in freezing and thawing soils are influenced by litter composition such as C/N ratios (Song et al., 2017).

3.5. Conclusions

In this study, it was found that the percentage of water stable aggregates (%WSA) did not differ significantly among perennial bioenergy crops and the successional site before- and after freeze-thaw cycles. Soil freeze-thaw decreased %WSA in miscanthus, the successional site, switchgrass, and willow by 65%, 57%, 54%, and 49%, respectively. This decrease in %WSA after soil freeze-thaw did not have any significant impact on N_2O and CO_2 emissions, suggesting that the N_2O and CO_2 emitted during the freeze-thaw process may have originated mostly from cellular materials released from the lysis and death of microbial biomass rather than from aggregate disruption. After repeated freeze-thaw cycles, a decline in DOC and DIN concentrations was observed while DON and soil NO_3^- contents were enhanced. Whereas soil C/N ratio and pH of the soil porewater positively correlated with soil N_2O emissions, a negative relationship was observed between N_2O emissions and soil NO_3^-, DIN, DON, soil N mineralization, soil N nitrification, and EC of the soil porewater after the soil freeze-thaw. Meanwhile, CO_2 emissions responded positively to DIN, DON, and soil NO_3^- after freeze-thaw. Overall, soil C/N ratio significantly correlated with C and N contents that were associated with N_2O and CO_2 emissions indicating the importance of soil C/N ratio in controlling GHG emissions during soil freeze-thaw processes. The total cumulative N_2O emissions measured over the 49-day incubation ranged from 148 mg N_2O-

N m^{-2} to 17 mg N_2O-N m^{-2} and were highest in miscanthus followed by willow, switchgrass, and the successional site. Total cumulative CO_2 emission on the other hand was highest in the successional site than any of the bioenergy crops and ranged from 25,262 mg CO_2-C m^{-2} to 15,403 mg CO_2-C m^{-2} after the 49 days. More than 56% of N_2O and 66% of CO_2 emitted over the 49-day incubation occurred in the first 21 days representing the first 3 freeze-thaw cycles. Total cumulative CO_2 emissions did not differ ($p > 0.05$) among bioenergy crop species. Higher CO_2 emissions and lower soil C/N ratio in the successional site than the bioenergy crops supported our hypothesis that the perennial bioenergy crops would effectively control GHG emissions such as CO_2 under freeze-thaw conditions than a successional site. However, higher N_2O in the miscanthus and willow than the successional site counteracted this suggestion and rather pointed to a higher potential GHG emissions in perennial bioenergy crops in the form of N_2O. The higher N_2O emissions in miscanthus and willow were attributed to accelerated N losses as N_2O via denitrification processes due to possible lower microbial N immobilization compared to the switchgrass and the successional site.

CHAPTER 4

4.0 Soil organic carbon storage of different soil-size fractions in perennial bioenergy crops on low productive agricultural land in southern Canada

4.1 Introduction

In an era where the adoption of land management strategies capable of enhancing soil C storage is taking center stage for their role in addressing the challenges posed by climate change (Bossio et al., 2020; Griscom et al., 2020; Kabisch et al., 2016), the cultivation of perennial bioenergy crops on low productive lands has attracted significant global interest (Lewandowski, 2015; Clifton-Brown et al., 2017; Jia et al., 2019; Perea-Moreno et al., 2019). This interest in such land management approach stems from the understanding that soils inherently low in C, such as low productive agricultural lands, have a greater capacity for long-term C storage compared to fertile agricultural lands (Coleman et al., 2004; Dondini et al., 2009; Vijayakumar et al., 2023). Furthermore, the ability of bioenergy crops to thrive and produce substantial biomass on low productive lands with less input requirements makes them excellent candidates for continued and long-term soil C storage (Blanco-Canqui 2016; Lemus & Lal, 2005; Marsal et al., 2016). Consequently, numerous studies have assessed the soil C storage and sequestration potentials of different perennial bioenergy crops under various management systems on low productive agricultural lands (Bazrgar et al., 2020; Coleman et al., 2018; McCalmont et al., 2017; Vijayakumar et al., 2023).

While knowledge on the C storage potential of different bioenergy crops is important, understanding the processes that influence their long-term storage is crucial for appreciating their climate benefits. For example, the mean residence time of C in soil, a crucial factor in long-term soil C storage, is influenced by processes such as the nature of soil-sized fractions it is associated

with (Six et al., 2002). Depending on diameter, soil-sized fractions can be classified as macro- (250-2000 μm diameter), micro- (53-250 μm diameter), and silt + clay-sized fractions (<53 μm) (Tisdall & Oades, 1982). Whereas C associated with the silt + clay- sized fractions is deemed the most stable, macro-sized-soil C is considered the least stable with micro-sized-associated C being intermediate (Nair et al., 2010; Six et al. 2002). Despite this understanding, most soil C studies in perennial bioenergy crops on low productive agricultural lands have primarily focused on whole soil, with less emphasis on C in different soil-sized fractions.

Moreover, the soil C storage benefits of converting low productive agricultural lands to perennial bioenergy crops compared to allowing natural succession has been questioned (Pool, 2013). Searchinger & Heimlich (2015) contended that low-productivity agricultural lands, if left to natural succession, can undergo regrowth vegetation, contributing to C storage. This argument gains significance when it is considered that frequent biomass harvest in perennial bioenergy crops may result in reduced biomass return to the soil as organic C. Graham et al. (2019) determined that SOC stocks did not differ significantly in switchgrass and miscanthus compared to a corn-soybean-wheat rotation row crop sites in southern Ontario. This was attributed to frequent biomass harvest and lower biomass inputs to the soil (Graham et al., 2019). Also, Vijayakumar et al. (2023) found that after 11 years of switchgrass and miscanthus cultivation on low productive agricultural land in southern Canada, SOC in the switchgrass (88.5 Mg C ha^{-1}) and miscanthus (87.9 Mg C ha^{-1}) did not differ significantly from a corn-soybeans rotation row crop site (84.8 Mg C ha^{-1}). Whereas these studies did not observe significant increases in SOC after bioenergy crop cultivation, other studies have reported gains (Bazrgar et al., 2020; Coleman et al., 2018; Don et al., 2012) and even losses (McCalmont et al., 2017) in SOC after perennial bioenergy cultivation. These varied responses of perennial bioenergy crops to SOC stocks implies that the potential C contribution

from perennial bioenergy crops would depend on site conditions and crop type, along with management practices (Lemus & Lal, 2005; Vijayakumar et al., 2023). To accurately determine the potential of perennial bioenergy crops for long-term C storage, there is the need to know the fraction of actual C contribution from the bioenergy crop and the fate of the contributed C.

Isotopic labelling of C provides the capability for tracing newly contributed C into soil organic matter pools (Oelbermann & Voroney, 2007; Bichel et al., 2016; Chalk et al., 2021). Although, the ^{13}C natural abundance technique has been used to quantify C contributions from perennial bioenergy crops in whole soils (Collins et al., 2020; Dondini et al., 2009; Xu et al., 2022), this technique has been applied only to a limited extend in fractionated soils from different perennial bioenergy crops. Hence, this study used the ^{13}C natural abundance technique to quantify C contributions from miscanthus, switchgrass and willow to different soil-sized fractions to understand how these different bioenergy crops contribute C to long-term pools. This knowledge will be important in elucidating the impact of these crops to long-term soil C storage and to help understand which species have the greatest capacity to contribute to long-term C storage. The study hypothesized that despite frequent biomass harvest and the absence of fertilization, the bioenergy crops would increase SOC contents after 12 years of land occupation compared to the baseline SOC content in 2009 (before the bioenergy crops cultivation). Additionally, the bioenergy crops would have a similar effect on enhancing SOC pools and macro-sized soil fractions comparable to the successional site. Moreover, due to reduced soil disturbance, SOC content distribution would be expected to follow the hierarchical model proposed by Tisdall & Oades (1982), where SOC would be highest in the macro-sized fractions, followed by micro-sized fractions, with the silt + clay-sized fractions containing the least. It is further proposed that the $\delta^{13}C$ signatures of SOC in the soil-sized fractions would reflect the vegetation composition of the bioenergy crops, with the

miscanthus and switchgrass showing C_4-dominant $\delta^{13}C$ signatures and C_3-dominant $\delta^{13}C$ signatures under the willow.

4.2 Materials and Methods

The study took place at the University of Guelph Agroforestry Research Station in Guelph, Ontario, Canada (cf. Chapter 1, Section 1.9).

4.2.1 Soil sampling

In May 2021, soil samples were extracted randomly from three locations in each treatment replicate at 0–10, 10–20 and 20–30 cm depths (Figure 4.1). For each replicate and depth class, soil samples from the three locations in each replicate were bulked together to get a composite sample for that treatment replicate and soil depth. The soil samples were brought to the laboratory and air-dried.

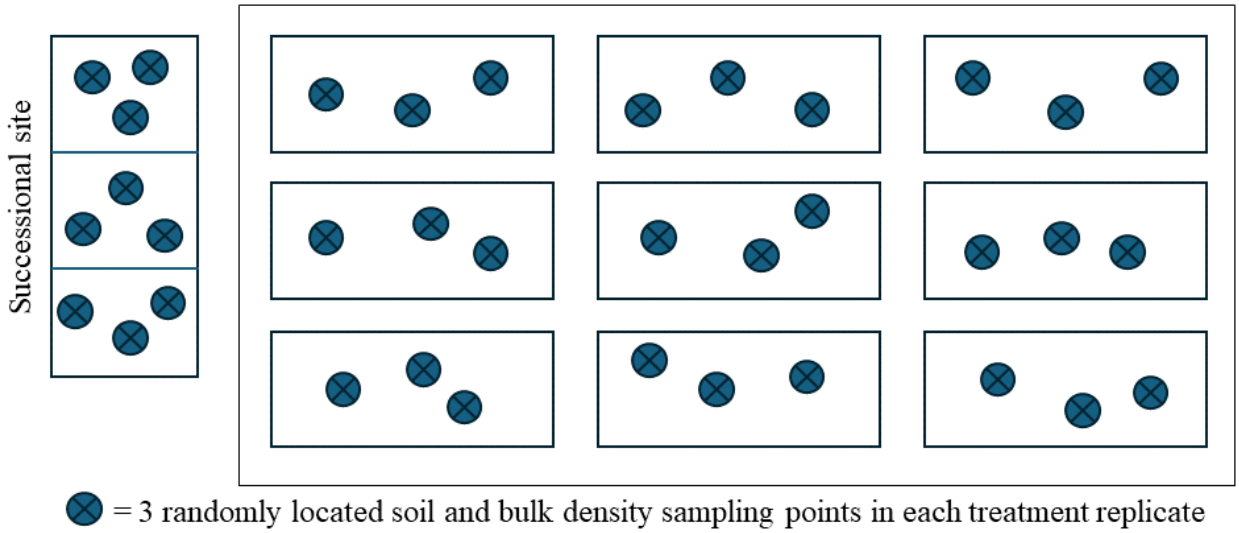


Figure 4.1: A schematic diagram showing the three soil and bulk density sampling points within each treatment replicate in the bioenergy crops and successional site at the University of Guelph Agroforestry Research Station in southern Canada. Note: Points do not show exact sampling location.

At the time of soil sampling, soil bulk density samples were also collected at each sampling site and depth using a bulk density ring with a 5 cm inner diameter and 5 cm height. The soil inside each bulk density ring was oven-dried at 105°C for 48 hours. Bulk density values were obtained by dividing the dry weight of the soil by the inner volume of the ring and corrected for roots and rock materials as described by Vijayakumar et al. (2023). For each replicate and depth, the three bulk density values from that replicate were averaged to get a representative bulk density value for the treatment replicate and depth.

4.2.2 Soil fractionation into soil-sized fractions

Sub-samples of the air-dried soil from each of the treatment replicates for each depth (0–10, 10–20 and 20–30 cm) were physically fractionated into three soil-size classes (250-2000 µm, 53-250 µm, and <53 µm) according to a dry-sieving procedure by Blaud et al. (2017). According to this procedure, the 2 mm (2000 µm) sieved soils were air-dried to a gravimetric water content of ~8%. The air-drying was required to obtain the soil fraction < 53 µm. A 100 g of the air-dried soil were put on top of two series of sieves (250 and 53 µm) and shaken at 200 rpm for 3 minutes using a 4325 Humboldt motorized sieve shaker. Soil retained on the 250 and 53 µm sieves were considered as macro-sized fractions (250-2000 µm), and micro-sized fractions (53-250 µm), respectively. The soil collected in the pan under the 53 µm sieve were the silt + clay-sized fractions (<53 µm). The different soil-sized fractions and whole soil for each depth class from each treatment replicate were analyzed for SOC, total N, and stable C and N isotopes.

4.2.3 Soil organic carbon, total nitrogen, and stable carbon and nitrogen isotopes analyses

The sieved and air-dried whole and fractionated soil for each depth class were analyzed for SOC and total N on a 4010 Elemental Analyzer (Costech Instruments, Italy). Prior to SOC and total N determination, carbonates were removed by washing soil with 50 mL of 0.5 M HCl

according to Dyer et al. (2012). Acid was removed from the acid-washed soils by washing the soil samples with deionized Ultrapure water for four days. After acid removal, the soil samples were dried at 40ºC for 2 days and ground in a ball mill to fine powder. The powdered samples were then packed in tin capsules before further analysis in an elemental analyzer (Costech ECS 4010). Soil bulk density values were used to convert the SOC and total N concentrations (%) to SOC and total N stocks (g C m^{-2}) (Oelbermann et al., 2006).

For the stable C and N isotope analyses, subsamples of the acid washed soil for the different soil-sized fractions from each treatment replicate at 0–20 cm and 20–30 cm soil depths were ground with a Retsch (Model ZM1) ball mill until the soil reached a talcum powder-like consistency (Oelbermann et al., 2006). In addition, biomass from the bioenergy crops and successional site were ground to a fine powder using both Kinematica Polymix (Model PX-MFC 90 D) grinding mill and Retsch (Model ZM1) ball mill. The ground soil and biomass samples were weighed into tin capsules (D1008, Elemental Microanalysis Ltd.) and analyzed with a 4010 Elemental Analyzer (Costech Instruments, Italy) coupled to a Delta Plus XP (Thermo-Finnigan, Germany) Continuous Flow Isotope Ratio Mass Spectrometer (CFIRMS) at the Environmental Isotope Laboratory at the University of Waterloo. Soil from a nearby undisturbed forest site from the Guelph Arboretum were used as reference samples for stable C isotope analyses. The stable isotope ratio of C was expressed as $\delta^{13}C$ per mil (‰) units relative to the Pee Dee Belemnite standard and the isotopic ratio of N was expressed as $\delta^{15}N$‰ units relative to the atmosphere.

The fraction of C originating from the switchgrass and miscanthus (f_{C4}) in SOC were calculated using a two-end-member mixing models according to Balesdent et al. (1987).

$$f_{C4} = \frac{\delta^{13}C_{SOM} - \delta^{13}C_{C3}}{\delta^{13}C_{C4} - \delta^{13}C_{C3}} \qquad (4.1)$$

where $\delta^{13}C_{SOM}$ is the $\delta^{13}C$ of soil from a mixture of C_4 and C_3 sources, $\delta^{13}C_{C4}$ is the mean $\delta^{13}C$ for the C_4 residue, and $\delta^{13}C_{C3}$ is the $\delta^{13}C$ of a reference soil from a nearby undisturbed forest site from the Guelph Arboretum with C_3 vegetation. The fraction of C_3-C (f_{C3}) was also calculated as

$$f_{C3} = 1 - f_{C4} \quad (4.2)$$

Considering the site prior to growing the miscanthus and switchgrass was under corn-wheat-soybean rotation for more than two decades, it was expected that a portion of the f_{C4} calculated for the miscanthus and switchgrass would have come from the corn. To account for this, the f_{C4} was calculated for the successional site which was under the same management system with the corn being the only C_4 crop cultivated on this site. This was possible as none of the grasses that were observed growing on the successional site were a C_4 grass. The f_{C4} from the miscanthus and switchgrass only was calculated as the difference between f_{C4} from the miscanthus and switchgrass and f_{C4} from the successional site using equation 4.3. The mean $\delta^{13}C$ for the C_4 corn residue that was used for the f_{C4} calculation in the successional site was 11.83‰ as determined by Oelbermann & Voroney (2007) from the same study area.

$$f_{C4\ (miscanthus\ or\ Switchgrass\ only)} = f_{C4total} - f_{C4successional\ site} \quad (4.3)$$

where $f_{C4total}$ is the fraction of C_4-C measured in the miscanthus or switchgrass site and $f_{C4successional\ site}$ is the C_4-C measured in the successional site.

Calculating for the fraction of C originating from the willow (f_{C3}) in SOC was challenging because the site was formerly under a C_3 vegetation before conversion to annual cropland where wheat and soybean which are also C_3 crops were grown prior to the willow crop. Owing to this and the absence of a C_4 reference site or initial soil samples before conversion to willow, it was assumed that the average C_3-C in the miscanthus, switchgrass, would represent C_3 from the old

vegetation prior to the willow crop establishment. Hence, f_{C3} from willow was calculated as the difference between f_{C3} from willow site and the average f_{C3} from the miscanthus and switchgrass using the equation below.

$$f_{C3\ (willow\ only)} = f_{C3total} - f_{C3average} \quad (4.4)$$

where $f_{C3total}$ is the fraction of C_3-C measured in the willow site and $f_{C3average}$ is the average of C_3-C measured in the miscanthus, and switchgrass.

4.2.4 Statistical analysis

Data processing and analysis were carried out using SPSS (IBM Corp.). A factorial analysis of variance (ANOVA) was used to determine the effect of land use type (bioenergy crop and successional site), soil depth, and their interactions on soil bulk density, SOC, total N, and C/N ratios in whole soil whereas the effect of land use type, soil depth, soil-sized fractions and their interactions on $\delta^{13}C$, $\delta^{15}N$, SOC, and total N were determined in the fractionated soil (Table C1.1). The Tukey's HSD test was used as a multiple comparison test to determine significantly different main effects, and Fisher's Least Significant Difference (LSD) was used to determine significantly different simple effects. Prior to the ANOVA, data was assessed for homogeneity of variance (Levene's) and normality (Shapiro-Wilk Test) (Steel et al., 1997). Soil-sized fractions, SOC and total N in soil-sized fractions were found to have violated the assumption of normality, hence, were log-transformed. The log-transformed data were reassessed for normality and were found to have normal distributions. Data that violated the assumption of normality was log-transformed. Where statistically significant, means were separated using the Fisher's Least Significant Difference (LSD). All type I error for all analyses were set at $p<0.05$.

4.3 Results

4.3.1 Bulk density, soil organic carbon and nitrogen contents

Soil bulk density from the bioenergy crops and successional site across the three depth classes ranged from 1.08 to 1.34 g cm^{-3} (Table 4.1). Although the bioenergy crops had lower bulk density values throughout the entire soil depth compared to the successional site, these bulk density values were not statistically different ($p>0.05$), except for the 10–20 cm depth where successional site had significantly higher ($p<0.05$) bulk density than the willow.

Soil organic carbon concentrations were not significantly different ($p>0.05$) between land use type throughout the three depth classes. However, for all the land use types, SOC concentrations were significantly higher ($p<0.05$) in the 0–10 cm depth compared with the 20–30 cm depth. Soil organic C concentration in the 10–20 cm depth were neither significantly higher than the 20–30 cm depth nor lower than the 0–10 cm depth. As expected, SOC stocks in the whole soil decreased with depth in all land use types but this decrease was only significant ($p<0.05$) in switchgrass and willow where SOC stocks in the 0–10 cm depths of the switchgrass and willow were significantly higher ($p<0.05$) than their corresponding stocks in the 20–30 cm depths (Figure 4.2). Between land use types, SOC stocks were significantly higher ($p<0.05$) in the successional site than the switchgrass and willow in the 10–20 cm and 20–30 cm depths (Figure 4.2). Similarly, the combined total SOC stocks for the entire 30 cm depth were significantly higher ($p<0.05$) in the successional site than the switchgrass and willow. Throughout the entire depth, SOC stocks in the miscanthus did not differ ($p>0.05$) from the successional site nor the switchgrass and willow (Figure 4.2).

The total soil N followed the same trend as SOC concentrations where total N was only significantly higher ($p<0.05$) in the 0–10 cm depth than the 20–30 cm depth in all the land use

types (Table 4.1). None of the land use types had significantly higher ($p<0.05$) total N at any of the depth classes. Also, apart from the successional site where soil C/N ratio was not affected by depth, soil C/N ratio in the bioenergy crops reduced significantly ($p<0.05$) with depth. Lower soil C/N ratios were observed in the 0–10 cm depths in the two herbaceous bioenergy crops (miscanthus and switchgrass) than the willow (Table 4.1).

Table 4.1: Soil bulk density, organic carbon and total nitrogen concentrations, and C/N ratio in the different soil depth under the different land use types at the University of Guelph Agroforestry Research Station in southern Canada.

	Bulk density (g cm^{-3})			SOC concentration (%)		
Depth (cm)	0–10	10–20	20–30	0–10	10–20	20–30
Successional site	$1.19 \pm 0.04^{A,a}$	$1.34 \pm 0.02^{A,a}$	$1.34 \pm 0.03^{A,a}$	$2.05 \pm 0.05^{A,a}$	$1.76 \pm 0.08^{AB,a}$	$1.62 \pm 0.07^{B,a}$
Miscanthus	$1.08 \pm 0.08^{A,a}$	$1.19 \pm 0.07^{A,ab}$	$1.22 \pm 0.04^{A,a}$	$1.90 \pm 0.12^{A,a}$	$1.61 \pm 0.14^{AB,a}$	$1.46 \pm 0.20^{B,a}$
Switchgrass	$1.19 \pm 0.06^{A,a}$	$1.20 \pm 0.05^{A,ab}$	$1.30 \pm 0.02^{A,a}$	$1.88 \pm 0.19^{A,a}$	$1.53 \pm 0.07^{AB,a}$	$1.28 \pm 0.09^{B,a}$
Willow	$1.11 \pm 0.07^{A,a}$	$1.16 \pm 0.10^{A,b}$	$1.17 \pm 0.06^{A,a}$	$1.96 \pm 0.07^{A,a}$	$1.62 \pm 0.06^{AB,a}$	$1.39 \pm 0.18^{B,a}$

	Total soil N concentration (%)			C/N Ratio		
Depth (cm)	0–10	10–20	20–30	0–10	10–20	20–30
Successional site	$0.20 \pm 0.01^{A,a}$	$0.18 \pm 0.01^{AB,a}$	$0.17 \pm 0.01^{B,a}$	$10.28 \pm 0.15^{A,ab}$	$9.91 \pm 0.12^{A,a}$	$9.82 \pm 0.15^{A,a}$
Miscanthus	$0.19 \pm 0.01^{A,a}$	$0.16 \pm 0.01^{AB,a}$	$0.15 \pm 0.02^{B,a}$	$10.18 \pm 0.15^{A,b}$	$9.90 \pm 0.15^{AB,a}$	$9.62 \pm 0.22^{B,a}$
Switchgrass	$0.18 \pm 0.01^{A,a}$	$0.16 \pm 0.01^{AB,a}$	$0.13 \pm 0.01^{B,a}$	$10.18 \pm 0.33^{A,b}$	$9.63 \pm 0.01^{B,a}$	$9.70 \pm 0.15^{AB,a}$
Willow	$0.18 \pm 0.01^{A,a}$	$0.16 \pm 0.01^{AB,a}$	$0.15 \pm 0.02^{B,a}$	$10.71 \pm 0.17^{A,a}$	$10.00 \pm 0.14^{B,a}$	$9.54 \pm 0.21^{B,a}$

Means followed by the same lowercase letter, comparing differences among land use type for a given depth are not significantly different ($p>0.05$) and means followed by the same uppercase letter, comparing differences between depths for a given land use type are not significantly different ($p>0.05$) according to Fisher's least significant difference (LSD).

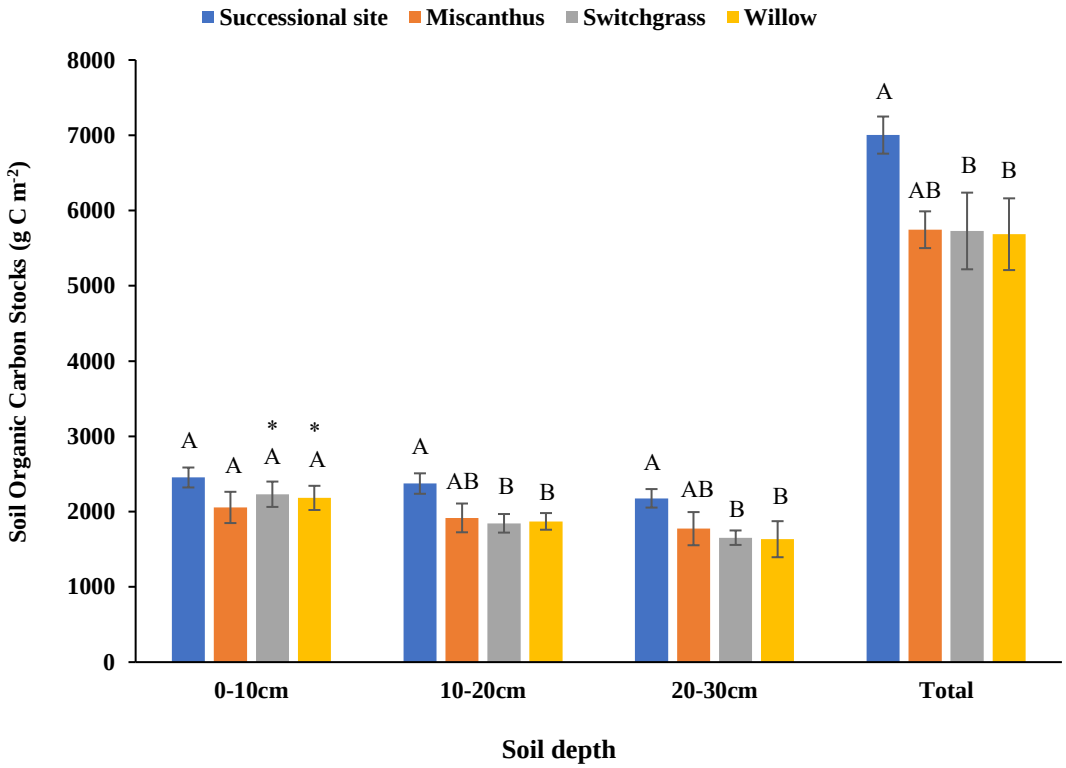


Figure 4.2: Soil organic carbon stocks in whole soil to 30 cm depth in perennial bioenergy crops and a successional site on low productive agricultural land at the University of Guelph Agroforestry Research Station in southern Canada. Bars represent standard error of means (n = 3). Error bars with same uppercase letters show means are not significantly different ($p>0.05$) according to Fisher's least significant difference (LSD). * indicates SOC stocks in switchgrass and willow are significantly higher ($p<0.05$) in 0–10 cm depth than 10–20 and 20–30 cm depths

4.3.2 Distribution of soil-sized fractions

Average mass recovery after soil fractionation was 99.2 ± 0.07% of the initial air-dried soil mass and was similar in all samples. The percentage (%) mass distribution of the soil-sized fractions for all the land use types at each depth was dominated by the macro-sized fractions followed by the micro-sized fractions with the silt + clay being the least (Figure 4.3). Throughout the entire 0–30 cm depth for all the land use types, more than 70% of soil fractions were contained in the macro-sized fractions with the micro-sized and silt + clay fractions constituting about 24%

and less than 10%, respectively (Figure 4.3). Significant difference ($p<0.05$) in % mass distribution of soil fractions was only observed at 10–20 cm depth where micro-sized and silt + clay fractions were higher ($p<0.05$) in the successional site than miscanthus. At the 10–20 cm depth, switchgrass and willow had significantly higher ($p<0.05$) micro-sized fractions than the miscanthus.

Also, aside miscanthus which showed significant difference ($p<0.05$) in % mass distribution of micro-sized and silt + clay fractions with depth, none of the other land uses showed significant difference in soil-sized fractions distribution with depth. At 20–30 cm depth, miscanthus had 30% and 61% more micro-sized fractions than at 0–10 cm and 10–20 cm, respectively. Similarly, silt + clay fractions were 39% and 60% more in the 20–30 cm depth than 0–10 cm and 10–20 cm depths, respectively (Figure 4.3).

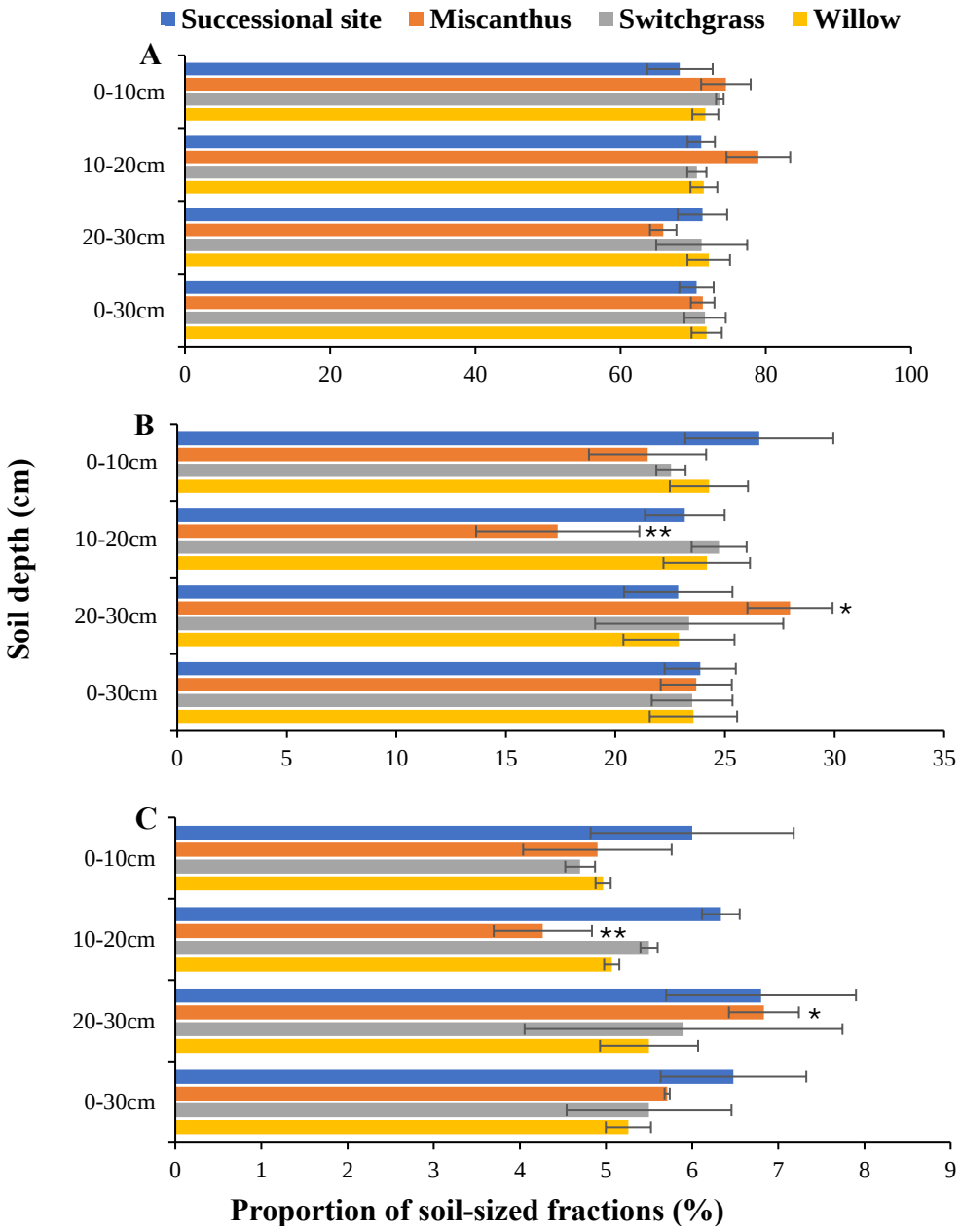


Figure 4.3: Percentage (%) mass distribution of macro-sized (A), micro-sized (B), and silt + clay-sized (C) soil fractions in 0–10, 10–20, 20–30, and the entire 0–30 cm depth in perennial bioenergy crops and a successional site on a low productive agricultural land at the University of Guelph Agroforestry Research Station in southern Canada. Bars represent standard error of means ($n = 3$). ** indicates micro-sized fractions in miscanthus at 10–20 cm depth is significantly lower ($p<0.05$) than successional site, switchgrass, and willow; and silt + clay-sized fractions in miscanthus at 10–20 cm depth is significantly lower ($p<0.05$) than successional site. * indicates silt + clay in miscanthus is significantly higher ($p<0.05$) at 20–30 cm than 0–10 and 10–20 cm depths; and micro-sized fractions in miscanthus is significantly higher ($p<0.05$) at 20–30 cm than 10–20 cm depth according to Fisher's least significant difference (LSD).

4.3.3 Distribution of soil organic carbon and total nitrogen in soil-sized fractions

Soil organic carbon stocks in all the land use types were highest ($p<0.05$) in the macro-sized fractions followed by the micro-sized and silt + clay fractions (Table 4.2). Whereas SOC in the combined 0–30cm depth was not significantly different ($p>0.05$) between land use type in the macro-sized fraction, the successional site had significantly higher ($p<0.05$) SOC stocks in micro-sized and silt + clay fractions at each depth class and in the entire 0–30 cm depth than the bioenergy crops.

Among the bioenergy crops, miscanthus had significantly higher ($p<0.05$) SOC in macro-sized fraction in 20–30cm depth than switchgrass and willow. In the micro-sized fractions SOC stocks were higher in switchgrass and willow in the 10–20 cm depth than miscanthus but the opposite was observed at 20–30 cm depth, where miscanthus had higher SOC stocks in micro-sized fractions than switchgrass and willow. SOC in silt + clay fractions in the bioenergy crops followed the same trend as observed in the micro-sized fractions. While switchgrass and willow had higher SOC in silt + clay at 10–20 cm than miscanthus, it was miscanthus at 20–30 cm that had higher SOC in silt + clay compared to switchgrass and willow.

Analyses of SOC distribution by depth for each land use type showed that while SOC in the three soil-sized fractions under the successional site did not differ with depth, miscanthus had 62% and 114% higher SOC in silt + clay at 20–30 cm than 0–10 cm and 10–20 cm depths, respectively. In the switchgrass and willow, only the macro-sized-associated SOC were significantly higher ($p<0.05$) in 0–10 cm than in 20–30 cm depth.

Table 4.2: Soil organic carbon stocks in the different soil-sized fractions in perennial bioenergy crops and a successional site on a low productive agricultural land at the University of Guelph Agroforestry Research Station in southern Canada.

Depth (cm)		Successional site	Miscanthus	Switchgrass	Willow
		SOC stocks (g C m^{-2})			
0–10	Macro-sized fractions	$1675.67 \pm 216.79^{A,a}$	$1361.03 \pm 78.06^{A,a}$	$1642.14 \pm 145.88^{A,a}$	$1551.21 \pm 83.52^{A,a}$
	Micro-sized fractions	$601.94 \pm 4.93^{A,a}$	$345.41 \pm 48.15^{B,b}$	$436.07 \pm 23.27^{A,ab}$	$442.86 \pm 47.15^{A,ab}$
	Silt + Clay-sized fractions	$151.74 \pm 21.80^{A,a}$	$108.32 \pm 23.56^{B,b}$	$118.56 \pm 9.86^{A,ab}$	$114.85 \pm 7.31^{A,ab}$
10–20	Macro-sized fractions	$1693.58 \pm 54.38^{A,a}$	$1304.34 \pm 186.73^{A,a}$	$1294.92 \pm 105.36^{AB,a}$	$1313.83 \pm 73.07^{AB,a}$
	Micro-sized fractions	$510.46 \pm 50.00^{A,a}$	$251.77 \pm 25.34^{B,b}$	$404.63 \pm 36.39^{A,a}$	$406.27 \pm 62.42^{A,a}$
	Silt + Clay-sized fractions	$185.77 \pm 8.17^{A,a}$	$82.11 \pm 14.12^{B,c}$	$126.398 \pm 7.74^{A,b}$	$113.26 \pm 8.65^{A,b}$
20–30	Macro-sized fractions	$1565.95 \pm 131.58^{A,ab}$	$1628.66 \pm 180.71^{A,a}$	$1137.70 \pm 113.49^{B,bc}$	$1082.72 \pm 130.13^{B,c}$
	Micro-sized fractions	$464.60 \pm 35.64^{A,ab}$	$588.25 \pm 56.40^{A,a}$	$338.67 \pm 56.98^{A,c}$	$347.78 \pm 81.97^{A,bc}$
	Silt + Clay-sized fractions	$195.83 \pm 26.85^{A,a}$	$175.97 \pm 11.59^{A,a}$	$120.48 \pm 27.21^{A,b}$	$115.59 \pm 20.01^{A,b}$
0–30	Macro-sized fractions	4935.20 ± 389.64^{a}	4294.03 ± 283.20^{a}	4074.77 ± 335.27^{a}	3947.76 ± 167.15^{a}
	Micro-sized fractions	1577.00 ± 25.15^{a}	1185.43 ± 127.13^{b}	1179.37 ± 91.86^{b}	1196.91 ± 191.53^{b}
	Silt + Clay-sized fractions	533.33 ± 43.11^{a}	366.40 ± 36.45^{b}	365.44 ± 26.22^{b}	343.70 ± 32.49^{b}

Means followed by the same lowercase letter, comparing differences in the same soil-sized fraction among land use type for a given depth are not significantly different ($p>0.05$) and means followed by the same uppercase letter, comparing differences in the same soil-sized fraction between depths for a given land use type are not significantly different ($p>0.05$) according to Fisher's least significant difference (LSD).

The total N distribution in soil-sized fractions followed the same trend as the SOC with the macro-sized fractions associated with the highest total N, followed by the micro-sized with the silt + clay fractions containing the least (Table 4.3). Like the SOC, total N in macro-sized fractions were not different ($p>0.05$) between land use type. Rather, the successional site had higher total N in micro-sized and silt + clay fractions than the bioenergy crops, particularly, miscanthus and switchgrass. Total N in the three sized fractions did not differ significantly ($p>0.05$) with depth under the successional site and willow. However, miscanthus had significantly higher ($p<0.05$) total N in micro-sized and silt + clay in 20–30 cm depth than the upper 20cm depth. Meanwhile, significantly higher ($p<0.05$) total N was observed in switchgrass in 10–20 cm than 0–10 cm and 20–30 cm depths.

Table 4.3: Total soil nitrogen stocks in the different soil-sized fractions in perennial bioenergy crops and a successional site on a low productive agricultural land at the University of Guelph Agroforestry Research Station in southern Canada.

Depth (cm)		Successional site	Miscanthus	Switchgrass	Willow
		Total nitrogen (g N m^{-2})			
0–10	Macro-sized fractions	$157.26 \pm 19.02^{A,a}$	$130.49 \pm 9.798^{A,a}$	$153.59 \pm 12.633^{A,a}$	$138.03 \pm 5.780^{A,a}$
	Micro-sized fractions	$51.05 \pm 1.218^{A,a}$	$28.50 \pm 4.785^{B,b}$	$38.83 \pm 2.429^{A,ab}$	$38.55 \pm 5.022^{A,ab}$
	Silt + Clay-sized fractions	$13.37 \pm 1.406^{A,a}$	$9.93 \pm 2.001^{B,a}$	$11.64 \pm 0.672^{B,a}$	$11.01 \pm 0.962^{A,a}$
10–20	Macro-sized fractions	$160.85 \pm 3.215^{A,a}$	$126.50 \pm 20.432^{A,a}$	$123.39 \pm 7.908^{A,a}$	$125.26 \pm 5.369^{A,a}$
	Micro-sized fractions	$43.21 \pm 2.697^{A,a}$	$22.54 \pm 3.371^{B,b}$	$35.89 \pm 2.660^{A,a}$	$39.03 \pm 6.162^{A,a}$
	Silt + Clay-sized fractions	$17.18 \pm 1.100^{A,a}$	$8.34 \pm 1.909^{B,b}$	$27.53 \pm 14.088^{A,a}$	$10.81 \pm 1.221^{A,b}$
20–30	Macro-sized fractions	$154.66 \pm 13.315^{A,a}$	$156.27 \pm 16.011^{A,a}$	$112.74 \pm 11.855^{A,a}$	$105.86 \pm 13.708^{A,a}$
	Micro-sized fractions	$42.20 \pm 3.878^{A,ab}$	$51.14 \pm 3.661^{A,a}$	$32.13 \pm 6.157^{A,b}$	$32.88 \pm 7.787^{A,b}$
	Silt + Clay-sized fractions	$17.71 \pm 2.444^{A,a}$	$16.03 \pm 0.458^{A,ab}$	$12.07 \pm 2.800^{B,b}$	$12.10 \pm 2.081^{A,ab}$
0–30	Macro-sized fractions	472.76 ± 35.209^{a}	413.27 ± 27.548^{a}	389.72 ± 30.575^{a}	369.15 ± 16.111^{a}
	Micro-sized fractions	136.46 ± 2.031^{a}	102.18 ± 11.288^{a}	106.85 ± 6.689^{a}	110.45 ± 18.860^{a}
	Silt + Clay-sized fractions	48.25 ± 3.023^{a}	34.30 ± 3.629^{ab}	51.24 ± 16.031^{ab}	33.92 ± 3.978^{b}

Means followed by the same lowercase letter, comparing differences in the same soil-sized fraction among land use type for a given depth are not significantly different ($p>0.05$) and means followed by the same uppercase letter, comparing differences in the same soil-sized fraction between depths for a given land use type are not significantly different ($p>0.05$) according to Fisher's least significant difference (LSD).

4.3.4 Biomass and soil $\delta^{13}C$ and $\delta^{15}N$ dynamics in soil-sized fractions

The $\delta^{13}C$ for biomass from the bioenergy crops and successional site ranged from –30.40‰ in willow to –12.80‰ in miscanthus, whereas $\delta^{15}N$ ranged from –1.88‰ in miscanthus to –0.53‰ in willow (Table 4.4). Differences among $\delta^{13}C$ in soil-sized fractions for all the land use types were first analysed by depth. Since the $\delta^{13}C$ did not differ with depth, differences between land use type in $\delta^{13}C$ for each soil-sized fraction was analyzed for the entire 0–30 cm depth. The $\delta^{13}C$ signatures of the different soil-sized fractions to 30 cm depth differed significantly ($p<0.05$) between C_3 and C_4 vegetation (Table 4.5). The C_4 crops of miscanthus and switchgrass were 1.17‰ to 3.28‰ enriched in ^{13}C in the soil-sized fractions compared to the successional site and willow with C_3 vegetation. Only miscanthus showed significant difference ($p<0.05$) in $\delta^{13}C$ among soil-sized fractions with significantly enriched ($p<0.05$) ^{13}C in micro-sized fractions compared to the macro-sized and silt + clay fractions (Table 4.5). Unlike the bioenergy crops which did not differ ($p>0.05$) in ^{15}N, the successional site was significantly enriched ($p<0.05$) in ^{15}N in micro-sized fraction compared to the switchgrass and in silt + clay fractions compared to the switchgrass and miscanthus (Table 4.5).

Table 4.4: $\delta^{13}C$ and $\delta^{15}N$ of aboveground biomass from different perennial bioenergy crops and vegetation from a successional site on a low productive agricultural land at the University of Guelph Agroforestry Research Station in southern Canada

Land use type	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Successional site	–28.83 ± 0.024	–0.63 ± 0.807
Miscanthus	–12.80 ± 0.138	–1.88 ± 1.288
Switchgrass	–12.98 ± 0.080	–1.04 ± 0.896
Willow	–30.40 ± 0.027	–0.53 ± 0.122

Table 4.5: $\delta^{13}C$ and $\delta^{15}N$ signatures of soil to 30 cm depth in different perennial bioenergy crops and a successional site on a low productive agricultural land at the University of Guelph Agroforestry Research Station in southern Canada

	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
	Macro-sized fractions	Micro-sized fractions	Silt + Clay-sized fractions	Macro-sized fractions	Micro-sized fractions	Silt + Clay-sized fractions
Successional site	$-24.88 \pm 0.205^{A,b}$	$-24.98 \pm 0.293^{A,b}$	$-24.25 \pm 0.355^{A,b}$	$6.24 \pm 0.261^{B,a}$	$6.39 \pm 0.305^{AB,a}$	$6.76 \pm 0.174^{A,a}$
Miscanthus	$-22.56 \pm 0.595^{B,a}$	$-21.70 \pm 0.353^{A,a}$	$-22.73 \pm 0.252^{B,a}$	$6.25 \pm 0.218^{A,a}$	$6.11 \pm 0.268^{A,ab}$	$6.30 \pm 0.248^{A,b}$
Switchgrass	$-22.45 \pm 0.317^{A,a}$	$-22.29 \pm 0.333^{A,a}$	$-23.08 \pm 0.227^{A,a}$	$6.02 \pm 0.193^{A,a}$	$5.87 \pm 0.226^{A,b}$	$6.23 \pm 0.273^{A,b}$
Willow	$-24.89 \pm 0.157^{A,b}$	$-24.89 \pm 0.145^{A,b}$	$-24.62 \pm 0.130^{A,b}$	$6.26 \pm 0.231^{A,a}$	$6.10 \pm 0.233^{A,ab}$	$6.42 \pm 0.221^{A,ab}$

For $\delta^{13}C$ and $\delta^{15}N$, means followed by the same lowercase letter, comparing differences among land use type for a given soil-sized fraction are not significantly different ($p>0.05$) and means followed by the same uppercase letter, comparing differences among soi-sized fractions for a given land use type are not significantly different ($p>0.05$) according to Fisher's least significant difference (LSD).

4.3.5 Proportion of carbon contribution from bioenergy crops to soil-sized fractions

Even though, higher C contributions from the bioenergy crops by stocks were contained in the macro-sized fractions, the greater C contribution by percentage occurred in micro-sized fractions followed by macro-sized and silt + clay fractions (Table 4.6). While C contribution from miscanthus to the different soil fractions were higher and constituted 20%, 22%, and 11% to macro-, micro-sized, and silt + clay fractions, respectively, the corresponding contributions from switchgrass and willow were 17%, 19%, 9% and 12%, 13%, 6%.

Table 4.6: The proportion and stock of carbon contribution from the different perennial bioenergy crops to the different soil-sized fractions to 30 cm depth on a low productive agricultural land at the University of Guelph Agroforestry Research Station in southern Canada.

		Soil Organic Carbon (g C m^{-2})	% Contribution of Carbon from bioenergy crop	Soil Organic Carbon contribution from bioenergy crop (g C m^{-2})
Miscanthus	Macro-sized fractions	4294.03	19.89	875.95 ± 135.088
	Micro-sized fractions	1185.43	21.88	259.56 ± 32.63
	Silt + Clay-sized fractions	366.40	11.08	41.50 ± 9.61
Switchgrass	Macro-sized fractions	4074.77	17.02	706.47 ± 159.86
	Micro-sized fractions	1179.37	18.68	221.99 ± 49.50
	Silt + Clay-sized fractions	365.44	9.36	33.85 ± 13.33
Willow	Macro-sized fractions	3947.76	12.49	514.44 ± 56.64
	Micro-sized fractions	1196.91	13.23	156.32 ± 18.26
	Silt + Clay-sized fractions	343.70	6.04	20.62 ± 2.16

4.4 Discussion

4.4.1 Bulk density, soil organic carbon and nitrogen contents

The average 1.15 to 1.23 g cm^{-3} soil bulk density values recorded in the bioenergy crops in the 0–30 cm soil depth were lower than the 1.32 g cm^{-3} baseline soil bulk density value from the study site in 2009 prior to the bioenergy crops cultivation (Bazrgar et al., 2020). Soil bulk density which reflects soil compaction is impacted by management practices and soil factors such as texture and organic matter content (Blanco-Canqui & Ruis, 2018). The use of farm machinery can cause soil compaction resulting in higher bulk density values (Wang et al., 2022b). Since the site prior to the bioenergy crops cultivation in 2009 was under annual row crop production (Marsal et al., 2016), the possible use of heavy farm machinery which is a common feature in annual row crop systems in the study region may have caused soil compaction which could have led to the relatively higher bulk density value compared to the bulk density after 12 years of the bioenergy crop cultivation. Additionally, soil bulk density decreases with increased organic matter input and increased root concentration (Lal, 2020; Topa et al., 2021). Gauder et al. (2016) observed soil bulk density reduced in the upper 20 cm soil layers with increased root concentration. Hence, potential higher root network and organic matter input to the soil in the bioenergy crops may have caused the reduction in the soil bulk density over the years that the bioenergy crops occupied the site compared to 2009 when the site was under annual row production.

Also, the minimal soil disturbance in the bioenergy crops may explain the lack of significant differences in soil bulk densities in the bioenergy crops compared to the successional site. Aside the roto tillage that was performed prior to growing the bioenergy crops more than a decade before this present study was conducted (Marsal et al., 2016) and the occasional biomass harvest, the successional site and bioenergy crop sites had been under the same management

system and with the same soil texture. It appears the occasional biomass harvest was not enough to have caused any significant disturbance in the soil resulting in a significant change in bulk density compared to the successional site. Wang et al. (2022b) studied that the effect of farm machinery use on soil bulk density is dependent on the weight and the number of passes of the farm machinery, with increased number of passes resulting in higher bulk density. Hence, while potential frequent use of heavy farm machinery in the annual row crops prior to the bioenergy crop cultivation may have caused an increased in the soil bulk density, the once a year and once every three years biomass harvest in the herbaceous crops and willow, respectively, may not have generated any significant compaction to have increased soil bulk density in the bioenergy crops similar to the baseline bulk density value or higher than the successional site.

The SOC concentration to 30 cm depth in the current study site in 2009 before the bioenergy crop cultivation was 1.62% (Bazrgar et al., 2020). This SOC concentration is lower than the 1.81%, 1.66%, and 1.66% measured in the successional site, miscanthus, and willow, respectively, but higher than the 1.56% in the switchgrass. The higher SOC concentrations in the miscanthus and willow, although marginal, support the hypothesis that cultivation of the bioenergy crops would increase SOC contents despite frequent biomass harvest and a lack of fertilization. However, the 3.7% decrease in SOC concentrations in the switchgrass compared to the 2009 baseline SOC concentration rejects this hypothesis. The marginal gains and loss of SOC in the bioenergy crops relative to the 2009 SOC concentration can be attributed to lower biomass yield due to the absence of fertilization, resulting in lower biomass input to the soil (Lemus & Lal, 2005). Soil organic C storage is a function of biomass inputs to the soil from net primary productivity and organic C losses (Lemus & Lal, 2005). At the time of this current study, the bioenergy crops had not received any form of fertilizer for almost half a decade. A previous study from the research site

(Marsal et al., 2016) revealed biomass yields of 23.81, 14.79, and 21.80 odt ha^{-1} yr^{-1} in fertilized miscanthus, switchgrass, and willow, respectively, compared to 13.2, 5.94, and 9.74 odt ha^{-1} yr^{-1} biomass yields in the unfertilized crops. Kering et al. (2012) also observed 12.82 Mg ha^{-1} biomass yield in N-fertilized switchgrass compared to 7.99 Mg ha^{-1} in the unfertilized crop. Moreover, Gauder et al. (2016) studied that after 11 years of bioenergy crop cultivation, SOC stocks in miscanthus, switchgrass, and willow fertilized with 40 kg N ha^{-1} yr^{-1} were 94.8, 91.6, and 93.5 Mg ha^{-1}, respectively compared to 85.4, 89.4, and 90.7 Mg ha^{-1} in their respective unfertilized crops.

Findings from these cited studies suggest the positive impact fertilization of perennial bioenergy crops could have on biomass yield and SOC storage. Hence, with the absence of fertilization and consequent lower biomass yield in the bioenergy crops in this current study, the loss or marginal SOC gained over the 2009 SOC concentration would be due to reduced biomass inputs from lower biomass yield coupled with frequent aboveground biomass harvests. This implies that maintaining adequate levels of soil fertility in perennial bioenergy crops on low productive agricultural lands can be very important in enhancing long-term SOC stocks.

Despite the higher SOC concentrations in the successional site than the miscanthus, SOC concentrations were not significantly different ($p>0.05$) between the successional site and the bioenergy crops and further supports the hypothesis that perennial bioenergy crops could have similar effects on SOC accumulation to successional sites on low productive agricultural lands. In spite of the absence of significant difference between SOC concentrations, the marginally higher bulk density value in the successional site resulted in SOC stocks that were higher ($p<0.05$) in the successional site compared to the switchgrass and willow. Soil organic C concentrations and stocks, however, did not differ ($p>0.05$) between the successional site and miscanthus. The ability

of miscanthus unlike the switchgrass and willow to maintain SOC stocks comparable to the successional site may be due to the ability of miscanthus to produce higher biomass than switchgrass and willow (Borzecka-Walker et al., 2008; Heaton et al., 2008; Marsal et al., 2016; Sanford et al., 2017). For instance, the study by Marsal et al. (2016) at the same site that evaluated biomass productivity found that miscanthus had the greatest biomass (17.03 Mg ha^{-1} yr^{-1}) compared to switchgrass (5.99 Mg ha^{-1} yr^{-1}) and willow (12.15 Mg ha^{-1} yr^{-1}). In a global meta-analysis, Xu et al. (2024) observed 16% SOC increase in miscanthus compared to 7% in switchgrass and attributed the higher SOC gains in miscanthus to greater biomass in miscanthus compared with switchgrass.

Moreover, miscanthus has higher concentration of root biomass in the upper soil layer compared to switchgrass (McGowan et al., 2019). Monti & Zatta (2009) found that ~90% of miscanthus roots were concentrated in the top 35cm soil depth compared to switchgrass which had only 35% of their roots in the same soil depth. Richter et al. (2015) also found 78% of miscanthus roots in the top 30cm soil depth, with Garten et al. (2010) observing 69% of switchgrass live roots in the top 30cm depth. McGowan et al. (2019) studied SOC under miscanthus increased by 1.3 Mg C ha^{-1} yr^{-1} to a 15-cm depth compared to 0.8 Mg C ha^{-1} yr^{-1} under a switchgrass and attributed this finding to higher root biomass concentrations in upper soil layers in miscanthus than switchgrass. Considering the soil carbon analyses in this current study was limited to the top 30 cm depth due to limitations posed by the bedrock in the study site, it is possible that majority of the switchgrass roots may have been below the 30 cm studied depth. This may have obscured any increase in soil organic carbon stocks from the switchgrass relative to the successional site. Hence, future studies should consider soil C in deeper than 30 cm depth to fully understand how SOC concentrations and stocks may differ between switchgrass and a successional site.

Analyses of SOC by depth revealed higher SOC concentrations in 0–10 cm compared to 20–30 cm. Higher SOC in the upper soil layer may reflect higher organic matter addition in surface soil resulting in SOC enrichment in the 0–10 cm than the 20–30 cm depth. A number of studies (*e.g.*, Cardinael et al., 2015; Christensen et al., 2016; Mayer et al., 2022) observed higher SOC in surface than subsoil in temperate regions due to increased organic matter input in the surface soils. Analyses by Topa et al. (2021) also revealed that SOC stocks in undisturbed soils increased in 0–10 cm soil depth but remained relatively stable in 10–20 cm and 20–30 cm depths due to surface placement of crop residues and a lack of soil disturbance that kept residues isolated from the lower depths. A study by Chimento et al. (2016) equally observed that continuous C inputs to the soil in perennial bioenergy crops and minimal soil disturbance increased SOC stocks in the top 10 cm of soil, but not in deeper soil layers. Hence, in the less disturbed soils under the perennial bioenergy crops such as those in this current study, the possible accumulation of organic residue in the upper soil layers and the lack of soil disturbance to incorporate organic residue in lower soil depths may have contributed to the higher SOC stocks in 0–10 cm than 20–30 cm depth.

Additionally, fresh C inputs from root exudates can stimulate SOC loss in subsoils (Fontaine et al., 2007; Henneron et al., 2022; Shahzad et al., 2018). After 6 years of growing miscanthus on a former grassland, Zatta et al. (2014) observed decrease in initial SOC in the presence of higher belowground biomass and attributed this to rhizosphere priming effect triggered by easily available C sources. It is possible that this mechanism may have also contributed to the lower SOC stocks in the lower soil depths in the bioenergy crops in this study. As soil organic matter is the storehouse of N in the soil (Singh et al., 2018), it is expected that the total soil N content would follow similar trend as the SOC stocks, especially, in these systems where the bioenergy crops were not receiving any form of N fertilization. Getino-Álvarez et al. (2023)

observed the same trend in total soil N to SOC stocks in temperate soils in Europe, with higher total N in topsoil layer than in deeper layers.

While lower soil C/N ratios can represent microorganisms and microbially processed and/or derived organic matter, higher C/N ratios can indicate greater presence of non-decomposed plant residues or particulate organic matter (Fernández-Ugalde et al., 2013; Hontoria et al., 2016). As explained earlier, in undisturbed systems such as the perennial bioenergy crops in this study, the lack of organic residue mixing and incorporation of surface litter in lower soil depths (Topa et al., 2021) can increase the presence of particulate organic matter in surface soils compared to lower depths. This increased presence of non-decomposed organic residue may have resulted in higher C/N ratios in the 0–10 cm soil depths under the bioenergy crops and successional site compared to the significantly lower C/N ratios in the lower soil depths. Getino-Álvarez et al. (2023) observed higher C/N ratio in topsoil layer (0–10 cm) which slightly decreased with depth. Sun et al. (2020) equally found soil C/N ratios decreased with depth and attributed this observation to the potential gradual decrease of plant-derived C and enrichment of microbial-derived C from surface to subsoils.

Additionally, lower soil C/N ratios in the 0–10 cm depths in the two herbaceous bioenergy crops (miscanthus and switchgrass) than the willow may be due to difference in organic litter chemistry (Cools et al., 2014; Lovett et al., 2002). Booth et al. (2005) found higher soil C/N ratio in woodlands than grasslands from a wide range of ecological systems and attributed this observation to potential dominance of bacteria in the grasslands compared to the woodlands which dominated in fungi. Mafa-Attoye et al. (2020) found higher fungi to bacteria ratio in willow than switchgrass and miscanthus from this study site. Due to higher C/N ratios in fungi than bacteria (Fierer et al., 2009; Li et al., 2016; Wan et al., 2015), it can, therefore, be inferred that the higher

association of the herbaceous bioenergy crop species with bacteria compared to the willow may have caused the lower soil C/N ratio in the upper soil layers of the herbaceous species compared to the willow.

4.4.2 Distribution of soil-sized fractions

As postulated, the percentage mass distribution of the various soil-sized fractions under the bioenergy crops and successional site showed greater abundance of the macro-sized fractions followed by the micro-sized fractions with silt + clay being the least. The extent to which soil aggregation is promoted depends on the soil fauna, microorganisms, roots, organic and inorganic binding agents, and environmental and physical forces (Six et al., 2004). Higher proportion of the macro-sized soil fractions from the study site reflects the lack of soil disturbance and organic residue inputs which enhanced the formation of the macro-sized fractions (Six et al., 2000). The positive impact of no soil disturbance on macroaggregate formation has been observed in temperate soils (*e.g.,* McGowan et al., 2019; Mikha & Rice, 2004; Plaza-Bonilla et al., 2013; Six et al., 2000). This is because in undisturbed soils, the increased presence of hyphae, roots and fresh organic residues serve as binding agents for the formation of macro-sized soil fractions (Six et al., 2000). Tisdall & Oades (1982) referred to these as transient and temporary binding agents which can be impacted by soil disturbance. Hence, in this present study with undisturbed soils under the perennial bioenergy crops and successional sites, the potential increased availability of these transient and temporary binding agents may have increased the formation and proportion of macro-sized fractions relative to micro-sized and silt + clay fractions. Mikha & Rice (2004) observed that the addition of manure in the form of fresh organic residue increased the proportion of macro-sized soil aggregates relative to micro-sized aggregates. Conversely, soil disturbance through tillage

operations reduced the proportion of the macro-sized aggregates with a concomitant increase in microaggregates.

Whereas the micro-sized and silt + clay fractions were significantly different between miscanthus and the other land use types, no significant difference in macro-sized fractions among land use types was observed. This observation supported the hypothesis of this study and consistent with findings from Schrama et al. (2016) who did not observe any significant difference in macroaggregates between switchgrass, miscanthus, and willow in a fine sandy loam textured soil but observed significantly higher microaggregates in switchgrass and willow compared to miscanthus. McGowan et al. (2019) did not also observe any significant difference in macroaggregates between switchgrass and miscanthus, although, both perennial grasses had significantly higher macroaggregates compared to soil under annual row crops. The lack of significant difference in the macroaggregates in the study by Schrama et al. (2016) may have been due to higher abundance of coarser soil fractions (with same size range as macroaggregates) from their study site which may have obscured any potential differences in macroaggregates. This same reason may explain the lack of any observable difference in the macro-sized fractions between the different land use types in this current study as the soil in the present study site had the same soil texture (fine sandy loam) as that of Schrama et al. (2016).

Whereas the higher micro-sized and silt + clay fractions in the successional site in the 10–20 cm depth compared to the miscanthus may be linked to potential increased microbial activities in the successional site, it is not clear what may have caused the higher micro-sized fractions in switchgrass and willow in the 10–20 cm soil depth than the miscanthus. It can only be speculated that a possible shift in microbial biomass and activities (Schrama et al., 2016) may have resulted in the lower micro-sized aggregates in the miscanthus compared to the switchgrass and willow.

This is because the micro-sized soil fractions are bonded by polysaccharides and humic materials from microbial activities which increase in the soil with increase in microbial activities (Nair et al., 2010; Six et al 2004; Tisdall & Oades, 1982). It is, therefore, possible that difference in microbial biomass and activities as influenced by chemistry of root exudates contributed to the formation of microbial by-products which induced the formation of micro-sized fractions differently in the miscanthus soil. This may also explain why the miscanthus was the only land use with enhanced proportion of micro-sized and silt + clay fractions in deeper soil layer (20–30 cm) than in the upper soil layers (0–10 cm and 10–20 cm), hence, requires further studies.

4.4.3 Distribution of soil organic carbon and total nitrogen in soil-sized fractions

Consistent with the hypothesis of this study, the highest SOC and total N in the macro-sized fractions followed by micro-sized and lowest in the silt + clay fractions in the bioenergy crops and successional site throughout each depth class and the entire 0–30 cm depth agrees with previous studies on SOC distribution in soil aggregates (Fernández-Ugalde et al., 2013; Hontoria et al., 2016; Mikha & Rice, 2004; Zeng et al., 2021). Results of SOC distribution in the soil fractions in this study also agrees with the aggregate hierarchy model, which suggests that in temperate soils such as these, where the major binding agents for aggregate formation are organic, SOC is expected to be higher in macro-sized than micro- and silt + clay fractions (Tisdall & Oades, 1982). Greater SOC and total N in the macro-sized fractions can be attributed to the fact that this soil-sized fractions contain the new C addition from biomass inputs to the soil (Zeng et al., 2021). Additionally, in these undisturbed systems, increased litter addition in the macro-sized fractions and abundance of roots and hyphae possibly contributed to the higher SOC and total N pools.

As the root network plays vital role in soil macroaggregation (Six et al., 2000), potential higher concentration of root biomass in miscanthus in the top 30 cm soil depth compared to

switchgrass and willow (McGowan et al., 2019; Monti & Zatta, 2009) as already explained, could have enhanced SOC stocks in the macro-sized fractions with depth compared to the switchgrass and willow whose macro-sized-associated SOC reduced with depth. It is equally possible that higher biomass production (Heaton et al., 2008; Marsal et al., 2016; Sanford et al., 2017) and potential increased biological activities in the miscanthus as mentioned earlier enhanced SOC and total N in the macro-sized fractions in deeper soil layers relative to the switchgrass and willow. This potential increased biological activities in the deeper soil layers of the miscanthus may have also resulted in the higher micro- and silt + clay-associated SOC stocks in the lower soil depths compared to the switchgrass and willow, as microbial cell residues and products which can constitute a greater part of organic C in soil microaggregates is influenced by vegetation (Jing et al., 2023).

Although, the macro-sized fractions contained the largest amount of SOC and total N contents in this study, since macro-sized fractions offer weaker physical protection to SOC and N compared to the micro-sized and silt + clay fractions (Tisdall & Oades, 1982), the significantly higher micro-sized and silt + clay-associated SOC in the 20–30 cm depth in miscanthus compared to the switchgrass and willow indicates the potential vital contribution miscanthus could play in long-term C stabilization in deeper soil layers.

4.4.4 Biomass and soil $\delta^{13}C$ and $\delta^{15}N$ dynamics in soil-sized fractions

The –30.40‰ and –28.83‰ $\delta^{13}C$ values in the biomasses in the successional site and willow, respectively are typical of C_3 vegetations, whereas the –12.98‰ and –12.80‰ in switchgrass and miscanthus are representative of C_4 vegetations (Wang et al., 2012; Chalk et al., 2021). The –30.40‰ to –12.80‰ $\delta^{13}C$ values for the C_3 and C_4 biomasses in this study fall within the range reported by Oelbermann et al. (2006) and O'Leary (1988). For instance, Poeplau & Don

(2014) measured $\delta^{13}C$ values of miscanthus plants which ranged from –11.61‰ to –12.93‰ which agrees with the –12.80‰ measured for the miscanthus biomass in this study, whereas the –12.98‰ measured for switchgrass in this study was within the range of –12.60‰ and –13.41‰ $\delta^{13}C$ values reported for switchgrass biomass (Garten & Wullschleger, 2000). The measured $\delta^{13}C$ value of biomass from the successional site was –28.83‰, which is within the range of $\delta^{13}C$ reported for C_3 plants of –26.8‰ to –29.05‰ in Oelbermann et al. (2006).

Contrary to the assumption that SOC in the soil-sized fractions from the miscanthus and switchgrass will show C_4-dominant $\delta^{13}C$ signatures, the –23.08‰ to –21.70‰ $\delta^{13}C$ values in the soil fractions under miscanthus and switchgrass showed C_3-dominant $\delta^{13}C$ signatures. Although, the miscanthus and switchgrass were 1.17‰ to 3.28‰ ^{13}C enriched in soil fractions compared to the successional site, these enrichments in ^{13}C were not enough to shift $\delta^{13}C$ values to C_4-dominant signatures. A possible reason for this might be due to reduced biomass input from the miscanthus and switchgrass which underwent frequent biomass harvests. This frequent biomass harvest may have been a major contributing factor in reducing the amount of C_4 biomass return to the soil to contribute to enrichment of the soil with C_4 signatures. Moreover, considering that the site had been under C_3 vegetation over several millennia before conversion to agricultural land for 25 years prior to the bioenergy crops during which wheat and soybeans (C_3 crops) were also grown on the site (Hoffman et al., 1963; Marsal et al., 2016), it would likely take much longer than the 12 years the miscanthus and switchgrass occupied the site to cause any significant shift from C_3 to C_4 dominant $\delta^{13}C$ signatures. Hansen et al. (2004) observed in a study that soil beneath a 9-year miscanthus to 20 cm depth led to a shift of $\delta^{13}C$ signature from –27.6‰ in the reference to –25.6‰ compared to –22.8‰ in the soil under the 16-year-old miscanthus. Oelbermann et al. (2004) also studied that under temperate conditions, a period much greater than 10 years may be

needed to observe any changes in SOC pools following the implementation of conservation practices such as agroforestry systems. This indicates that it would most likely require much longer period for organic residue inputs from C_4 crops such as miscanthus and switchgrass to result in C_4-dominant $\delta^{13}C$ signatures in the SOC pools when grown on low productive agricultural lands previously occupied by C_3 vegetation.

The enrichment in ^{13}C in the micro-sized fraction in the miscanthus compared to the macro-sized and silt + clay fractions could mean that miscanthus contributed more C to the micro-sized fractions than the macro-sized and silt + clay fractions. This potential increased C contribution from the miscanthus to the micro-sized fraction may have possibly enriched this fraction with ^{13}C by 0.86‰ and 1.03‰ over the macro-sized and silt + clay fractions, respectively. Additionally, ^{13}C enrichments have been linked to older organic matter and increased soil organic matter humification (Cheng et al., 2013; Wang et al., 2015). As micro-sized fractions relatively constitute much of the older and more humified organic C in the soil compared to macro-sized fractions (Tisdall & Oades, 1982), it is possible that the ^{13}C enrichment as observed in the micro-sized fraction in the miscanthus compared to the macro-sized fraction was due to the aforementioned suspected increased microbial activities in the miscanthus which may have resulted in more humified organic matter in the micro-sized soil fraction. Liu et al. (2018) observed ^{13}C enrichment in micro- than macro-aggregates and attributed this finding to increased microbial decomposition of organic matter.

The $\delta^{15}N$ in foliage and soil were within range reported by Sheng et al. (2014) in natural temperate forest ecosystems. While lower $\delta^{15}N$ values showed depletion of ^{15}N in the bioenergy crop biomasses, the higher $\delta^{15}N$ values in their soils showed ^{15}N enrichment. In the absence of N fertilization, much of the crop N supply comes from organic matter and atmospheric N deposition

(Bobbink et al., 2010; Walker et al., 2023; Zhang et al., 2021c). Nitrogen mineralization and nitrification discriminates against ^{15}N (Craine et al., 2015; Wang et al., 2021b), which can cause an increased uptake of ^{15}N-depleted N by plants (Chen et al., 2024). This increased uptake of ^{15}N-depleted N results in higher ^{15}N-depleted N (lower $\delta^{15}N$ values) in plant biomass and a consequent enrichment of ^{15}N in the soil (higher $\delta^{15}N$ values) (Chen et al., 2024). Additionally, N from atmospheric deposition through rain contains ^{15}N-depleted N (Xiao & Liu, 2002). Therefore, in unfertilized systems such as the bioenergy crops in this study where wet deposition of N may constitute a major N source for the crops, biomass would be expected to be depleted in ^{15}N. This may explain the lower $\delta^{15}N$ values in the biomasses of the bioenergy crops in this study relative to the soil.

While biomass from the bioenergy crops and successional sites all showed depletion in ^{15}N, the miscanthus and switchgrass biomass showed higher depletion in biomass ^{15}N compared to the willow and successional site. Herbaceous crops such as miscanthus and switchgrass have greater capacity to form association with arbuscular mycorrhizae fungi (AMF) compared to woody crops such as willow (Bahulikar et al., 2014; Gushgari-Doyle et al., 2021; Jesus et al., 2016; Mafa-Attoye et al., 2020). Mycorrhizae fungi have been observed to supply ^{15}N-depleted N to plants causing ^{15}N depletion in plant biomass (Craine et al., 2015). For instance, Craine et al. (2009) found that plants with mycorrhizal fungi were depleted in foliar ^{15}N by 2‰ to 5.9‰ compared to nonmycorrhizal plants. Hence, the higher ^{15}N depletion in the miscanthus and switchgrass biomass as indicated by their lower $\delta^{15}N$ values can be attributed to the potential effect from their association with mycorrhizae fungi compared to the willow and successional site.

The increased acquisition and supply of the ^{15}N-depleted N by the AMF (Craine et al., 2009, 2015) to miscanthus and switchgrass might have equally caused the lower $\delta^{15}N$ values in

the micro-sized and silt + clay fractions in the miscanthus and switchgrass compared to the successional site. It is also possible that the more frequent aboveground biomass harvest in the miscanthus and switchgrass which results in translocation of C and N to belowground parts (Lemus & Lal, 2005; Pugliese et al., 2019) contributed to the lower $\delta^{15}N$ values in the micro-sized and silt + clay fractions in miscanthus and switchgrass compared to the successional site. Considering the biomass N consisted of ^{15}N-depleted N, it is only logical that much of the translocated N from aboveground to belowground would constitute this ^{15}N-depleted N. The possibility of this translocated N in belowground biomass to contribute to the micro-sized and silt + clay fractions-associated organic N pool is enhanced by its proximity to the soil matrix which is a precursor for micro-aggregation and formation of organo-mineral interaction in the soil (Anderson-Teixeira et al., 2013). This potential mechanism may have resulted in the lower $\delta^{15}N$ values in the micro-sized and silt + clay fractions in the miscanthus and switchgrass compared to the successional site.

4.4.5 Proportion of carbon contribution from bioenergy crops to soil-sized fractions

The lack of available data on C contributions from miscanthus, switchgrass, and willow to different soil-sized fractions on low productive agricultural lands using the ^{13}C natural abundance technique made it challenging to compare results from this study to others conducted in similar environments. Studies conducted using this approach in perennial bioenergy crops have been in whole soils and herbaceous species, with none in willow. The 9 to 22% C_4-derived C contribution from the switchgrass and miscanthus to the different soil-sized fractions to 30 cm depth are comparable to results of C_4-derived C contributions from these crops in whole soils reported in other studies. Garten & Willschleger (2000) observed that 19–31% of SOC was C_4-carbon contribution from a 5-year-old switchgrass in southeastern USA to 40 cm depth whereas Leifeld et al. (2021) measured 14–37% C_4-derived C contribution from miscanthus to 30 cm soil depth

from five different sites in Switzerland after 19 to 24 years of establishment. Meanwhile, after 9 and 16 years of miscanthus cultivation on a coarse loamy sand soil in Denmark, Hansen et al. (2004) studied that 13% and 31% of the SOC present in 0–20 cm soil was derived from miscanthus. While variation in soil sampling depths contributed to the wide range of C_4-derived C from miscanthus and switchgrass reported in the different studies, differences in management systems, stand age, climatic and edaphic factors may have also contributed to these variations in results.

Among the bioenergy crops in this study, miscanthus contributed the most C to the SOC pool with willow contributing the least. The higher C contribution from miscanthus to the SOC pool compared to switchgrass and willow in this study is consistent with observation from Xu et al. (2022) who found 13% of SOC to 60 cm depth was C_4-derived C from miscanthus compared to 7% from switchgrass and attributed this to higher concentration of belowground biomass in miscanthus in upper soil depth than switchgrass. Borzecka-Walker et al. (2008) also compared the contribution of C_4 miscanthus and C_3 coppice willow to net soil C sequestration and observed that the miscanthus (0.64 Mg C ha^{-1} yr^{-1}) accumulated two times more C than the willow (0.30 Mg C ha^{-1} yr^{-1}) after 3 years of cultivation. The higher C_4-derived C from the miscanthus than the switchgrass and willow in this study may be attributed to the potential of miscanthus to produce higher biomass than switchgrass than willow (Borzecka-Walker et al., 2008; Heaton et al., 2008; Marsal et al., 2016; Sanford et al., 2017; Xu et al., 2024). Additionally, due to frequent aboveground biomass harvest in perennial energy crops, belowground biomass production and root exudates contribute majorly to SOC in perennial energy crops (McGowan et al., 2019; Xu et al., 2022). As already mentioned, since majority of miscanthus roots are contained in the top 30cm soil depth compared to switchgrass (McGowan et al., 2019; Monti & Zatta, 2009), it is logical to

expect that the miscanthus would contribute higher C to the SOC pool compared to switchgrass and willow.

Although, C contributions in terms of stocks were highest in the macro-sized fractions, the C contribution by proportion-wise in all three energy crops were higher in the micro-sized than macro-sized fractions. This implies that the bioenergy crops in this study on the fine sandy loam-textured soil contributed more C to the more stable micro-sized fractions than the less stable macro-sized fractions. This could be crucial in long-term carbon storage in these energy crops on low productive agricultural lands, contributing to climate change mitigation.

4.5 Conclusions

Soil organic C concentration to 30 cm depth were 2.5% higher in miscanthus and willow and 3.7% lower in switchgrass compared to the baseline SOC concentrations in 2009 prior to the bioenergy crops cultivation. The marginal gain and loss in SOC in the bioenergy crops were due to lower biomass yield and input due to absence of fertilization. Soil organic C stocks ranged from 5686 to 7002 g C m^{-2} and were significantly higher in successional site than switchgrass and willow but not in miscanthus. This ability of miscanthus unlike the switchgrass and willow to maintain SOC stocks comparable to the successional site was attributed to the ability of miscanthus to produce higher biomass than switchgrass and willow. Surface placement of crop residues and the lack of soil disturbance that may have kept residues isolated from the lower depths contributed to higher SOC concentrations and stocks in the upper 10 cm soil layers than 20–30 cm depth. Soil-sized fractions as well as SOC and total N distribution in soil-sized fractions followed an increasing order in the silt + clay > micro- > and macro-sized fractions, supporting the proposed aggregate hierarchy model for temperate soils proposed by Tisdall & Oades (1982). Significantly higher SOC in miscanthus in micro-sized and silt + clay-sized fractions were contained in 20–30 cm depth

compared to surface soil layers. These higher micro-sized and silt + clay-associated SOC in miscanthus in deeper soil depths compared to the switchgrass and willow indicates the vital contribution miscanthus could play in long-term C stabilization in deeper soil layers. The $\delta^{13}C$ signatures of the different soil-sized fractions to 30 cm depth differed between C_3 and C_4 vegetation. Although the C_4 crops of miscanthus and switchgrass had 1.17‰ to 3.28‰ higher $\delta^{13}C$ values in soil-sized fractions than the successional site and willow, these enrichments in ^{13}C were not enough to substantially shift the $\delta^{13}C$ values to C_4-dominant signatures under the miscanthus and switchgrass due to potential reduction in biomass input into the SOC pool. The proportion of C contribution from the bioenergy crops ranged from 6% in silt + clay fractions in willow to 22% in the micro-sized fractions in miscanthus. Among the three bioenergy crops, miscanthus contributed the most to the SOC pool with willow contributing the least. The higher proportion of C contribution by the three bioenergy crops to the micro-sized fractions compared to macro-sized fractions indicates the contribution of C in these bioenergy crops to more stabilized pools with positive impact on long-term C storage and climate change mitigation.

CHAPTER 5

5.0 Soil carbon dynamics in perennial biomass crops on low productive agricultural land in southern Canada

This chapter is an adaptation of the following manuscript under review in Geoderma Regional: Osei, A. K., Thevathasan, N.V., and Oelbermann, M. Soil carbon dynamics in perennial biomass crops on low productive agricultural land in southern Canada.

5.1 Introduction

Low productive agricultural lands have been recommended globally for growing perennial bioenergy crops including switchgrass, miscanthus, and willow (Lewandowski, 2015). Perennial bioenergy crops are temperate agroforestry systems which offer rehabilitation benefits when grown on low productive agricultural lands and can be produced as single crops or intercropped in tree-based systems (Newman & Gordon, 2018; Thevathasan et al., 2012, 2018). Converting low productive agricultural lands to perennial bioenergy crops, which eliminate competition with food crops, can also provide climate change mitigation benefits through soil C sequestration (Chimento et al., 2016). However, the capacity of perennial bioenergy crops to sequester C depends on several factors including the type of bioenergy crop produced, the inputs (*e.g.*, fertilizer) required for its successful production, and the quality of the land (*e.g.*, productive versus marginal) (Norgrove, 2010). For example, Searchinger & Heimlich (2015) suggest that growing bioenergy crops on low productive agricultural land may not always result in soil C sequestration. This is because low productive agricultural lands, once agricultural activities are discontinued, can regrow vegetation (i.e., secondary regrowth) thereby providing an opportunity to rehabilitate these lands through soil C sequestration (Searchinger & Heimlich, 2015). Thus, offsetting C emissions and rejuvenating low productive agricultural land with bioenergy crops can only occur if more C is assimilated by the bioenergy plantation compared to the regrowth vegetation (Searchinger & Heimlich, 2015).

Since the benefits of soil C sequestration are realized only with time (Ontl & Schulte, 2012), it is important to understand how changes in land management practices and (or) different land-use systems impact long-term SOC stocks. For example, perennial bioenergy crops typically have productive lifespan of ~30 years (Amichev et al., 2014). However, the majority of studies evaluating the SOC sequestration potential in perennial bioenergy crops on low productive agricultural lands have been short-term (≤ 3 years) in young systems (*e.g.*, established less than 10 years ago) (Coleman et al., 2018) or in older systems (Agostini et al., 2015). Moreover, there is lack of comprehensive long-term studies on SOC sequestration in perennial bioenergy crops, especially those on low productive agricultural lands. This is because there is a lack of well-designed and replicated field studies comparing different bioenergy crops to help understand long-term SOC dynamics (Sartori et al., 2006).

Furthermore, most studies investigated SOC sequestration in the whole soil with minimal emphasis on the dynamics of the various soil C fractions. Since different SOC fractions differ in their mean residence time in the soil (Parton et al., 1988), it is imperative to understand if and how different types of perennial bioenergy crops can increase these C fractions. And if so, does this differ among bioenergy crop species. This also provides further insight into the design and management of bioenergy plantations to help maximize long-term C sequestration.

Process-based models such as the Century soil organic matter model have been successfully used to predict long-term changes in SOC stocks and associated pools of active, slow, and passive C fractions in various land management systems (Hashimoto et al., 2012; Oelbermann et al., 2017; Zhang et al., 2021a). Even though, Century was originally developed to simulate SOC in grasslands (Parton et al., 1988), the model has been calibrated to accurately predict and verify SOC stocks in a wide range of land management systems, including agroecosystems (Brandani et

al., 2014; Lugato & Jones, 2015; Oelbermann et al., 2017; Zhang et al., 2021a), agroforestry systems (Oelbermann & Voroney, 2011) and forest ecosystems (Hashimoto et al., 2012). Furthermore, the Century model has been adapted to build other process-based models such as the SCSOC (Kwon et al., 2013), EPIC (Izaurralde et al., 2006), and Agro-IBIS (Kucharik et al., 2000) to simulate SOC dynamics in perennial biomass crops (Nair et al., 2012). Despite this, there is a limited application of the Century model itself to predict SOC dynamics in different perennial bioenergy crops, especially, those grown on low productive agricultural lands in temperate regions. Hence, the objective of this study was to use Century to predict and compare long-term SOC dynamics in different perennial bioenergy crops to a secondary growth successional site and a row crop agroecosystem.

The study hypothesized that despite continuous biomass harvest, the perennial bioenergy crops would accumulate similar or more SOC than the successional site. Also, due to reduced biomass return to the soil and increased organic matter degradation from soil tillage in the row crop system, SOC would be higher in the perennial bioenergy crops than in row crop system. It was also hypothesized that reduced soil disturbance in the perennial bioenergy crops would enhance slow and passive SOC fractions, representing the more stabilized and long-lived soil C pools relative to the much-disturbed soil in the row crop system.

5.2 Materials and Methods

The study took place at the University of Guelph Agroforestry Research Station in Guelph, Ontario, Canada (cf. Chapter 1, Section 1.9).

5.2.1 Century model parameterization and simulation

The Century model (version 4.0) was used to simulate long-term changes in SOC (0–20 cm) in perennial bioenergy crops. Since the Century model is site-specific (Oelbermann et al., 2017), soil biophysical characteristics, climate and management practices were adjusted to reflect the specific conditions and land management practices encountered at the study site. The initial site-specific soil parameters including texture, pH, bulk density, and SOC were used to calibrate the model (Table 5.1). Climate data, including average, maximum and minimum air temperature, and monthly total precipitation (Table 5.2) were obtained from a nearby weather station in Guelph (Latitude 43^{o} 31’ 00” N, longitude 80^{o} 14’ 00” W), and were based on a 120-year average (Environment and Climate Change Canada, 2022).

Table 5.1: Soil parameters (0–20 cm) prior to the establishment of perennial bioenergy crops at the University of Guelph Agroforestry Research Station in southern Canada (adapted from Mann, 2012).

Sand (%)	56
Silt (%)	34
Clay (%)	10
Bulk density (g cm^{-3})	1.3
pH	7.2
SOC (g kg^{-1})	16.9

Table 5.2: Climate data for the University of Guelph Agroforestry Research Station in southern Canada used for the Century simulation based on a 120-year average (Source: Environment and Climate Change Canada, 2022).

	Temperature (ºC)		Precipitation (cm)
	Minimum	**Maximum**	
January	–12.2	–4.1	5.8
February	–11.7	–2.9	4.9
March	–6.9	1.5	7.4
April	0.0	10.2	7.0
May	5.3	17.4	7.8
June	11.0	23.2	8.7
July	12.7	25.5	7.3
August	11.6	24.6	7.2
September	8.2	20.2	7.1
October	3.1	13.8	6.6
November	–1.8	5.5	6.6
December	–8.7	–1.6	7.2

Prior to simulating the land management systems, the Century forest sub-model was used to initialize the SOC equilibrium for 1900 years (Table 5.3). A temperate deciduous-coniferous mixed forest, which is typical for southern Canada (Dil & Oelbermann, 2014) was used to estimate equilibrium SOC levels and plant productivity. The initialization of SOC equilibrium was used to bring the model to its first year of simulation of the historical land use management practices (Table 5.3).

Beginning in 2009, perennial bioenergy crops including willow, miscanthus and switchgrass were simulated for 162 years until 2170 (Table 5.3). Since none of these perennial crops were available as a default crop option in Century 4.0, a new crop file in CROP.100 was

created for each bioenergy crop. The warm season perennial grass CROP.100 file was modified for switchgrass and miscanthus, and the cotton crop file was modified for willow, using the Century defaults for perennials. For all bioenergy crops, the potential aboveground monthly production (g m^{-2}) was based on biomass yields obtained at this site from a previous study by Bazrgar et al. (2020) and other studies with similar management systems, geographic region, and edaphic characteristics (Nissim et al., 2013). Biomass yields were averaged over a five-month period, representing the number of active growing months of these crops in a climatic region corresponding to southern Canada (Nunn et al., 2017). Based on these values, the aboveground monthly production for miscanthus was 454 g m^{-2} and 184 g m^{-2} for switchgrass. These values were based on their respective potential biomass production capacities of 23 and 9 Mg ha^{-1} yr^{-1} as measured from the study site (Bazrgar et al., 2020). Meanwhile, the potential monthly production for willow was calculated as 336 g m^{-2} based on its biomass production capacity of 17 Mg ha^{-1} yr^{-1} in unfertilized systems in southern Quebec, Canada (Nissim et al., 2013). These biomass yields were within reported yields in other studies (*e.g.*, Kwon et al., 2013; Volk et al., 2011).

Additionally, an organic matter addition (OMAD.100) file was created for each of the crops, where it was assumed that, before, during, and after harvest, crop litter and residue from senescent leaves and roots will be added to the soil as organic matter inputs. About 26% of harvested aboveground biomass in miscanthus and between 21% to 30% of switchgrass can be lost through leaves and shoot-tip senescent before and after harvest as organic matter input (Kahle et al., 2001; Anderson et al., 2013). Organic matter inputs were calculated as 25% of aboveground biomass produced in the OMAD.100 file.

Woody crops like willow can be productive for more than two decades (Volk et al., 2004) and more than a decade for switchgrass and miscanthus (Guzman & Lal, 2014). Therefore, a 21-

year stand rotation, representing 7 harvesting cycles at 3-year intervals were adopted for willow with 10-year stand rotations for the miscanthus and switchgrass (harvested annually). This provided an opportunity to evaluate root biomass turnover after each rotation cycle (Jarecki et al., 2020). Root biomass for the willow (2.34 Mg ha^{-1} yr^{-1}), switchgrass (1.98 Mg ha^{-1} yr^{-1}), and miscanthus (1.72 Mg ha^{-1} yr^{-1}) were based on data from the study site (Bazrgar et al., 2020).

Two reference scenarios were created and simulated. The first scenario included a corn–soybean–winter wheat rotation that ceased in 2008 and the site underwent secondary succession to temperate mixed forest, herein referred to as successional site. The second reference scenario included a corn–soybean–winter wheat rotation, herein referred to as row crop system.

Table 5.3: Scheduling of historical land-use changes and management practices at the University of Guelph Agroforestry Research Station in southern Canada used in the Century Model simulation [(Source: Hoffman et al. (1963); Mann, (2012); Jarecki et al. (2020)].

Date	Management practices
0–1910	Temperate mixed forest with 50/50 deciduous and coniferous species
1911	Forest clearance by clear cutting method
1912–1958	Corn–Barley rotation.
1959–1983	Continuous corn
1984–2008	Corn–Soybean–Winter wheat rotation.
	Corn: Moldboard plow in October; soil disking in April; corn planting in May with 11 kg ha^{-1} N at planting as starter and 146 kg ha^{-1} as side-dress in June. Grain and 50% straw harvest in October.
	Soybean: Moldboard plow in October; soil disking in April; soybean planting in May with no fertilizer application; grain only harvest in October
	Winter wheat: Moldboard plow in October to incorporate soybean residue into soil; wheat planting in November and 100 kg ha^{-1} N applied in April the following year. Grain and 50% straw harvest in August.
	Row crop system
2009–2170	Corn–Soybean–Winter wheat (same management as from 1984 to 2008)
	Successional site

2009–2170	Temperate mixed forest with 50/50 deciduous and coniferous species
	Perennial biomass crops
2009–2170	**Switchgrass:** Planted in April; no soil tillage; no fertilizer application; hay harvest in October; organic matter addition from switchgrass litter after harvest in October. This cycle continues till 9 years. In the 10th year, representing one growing stand cycle, after hay harvest in October, soil is plowed to incorporate switchgrass root into the soil and new stand is grown in April the following year for another 10 years.
	Miscanthus: Planted in April; no soil tillage; no fertilizer application; hay harvest in October; organic matter addition from miscanthus litter after harvest in October. This cycle continues till 9 years. In the 10th year, representing one growing stand cycle, after hay harvest in October, soil is plowed to incorporate miscanthus root into the soil and new stand is grown in April the following year for another 10 years.
	Willow: Planted in April; no soil tillage; no fertilizer application; willow harvested in October of every 3rd year; organic matter addition from willow leaves in October every year. This cycle continues till 20 years. In the 21st year, representing one growing stand cycle, after willow harvest in October, soil is plowed to incorporate willow root into the soil and new stand is grown in April the following year for another 21 years.

5.2.2 Soil organic carbon measurements

In May 2021 and April 2022, three soil samples were extracted randomly from each treatment replicate to a 20 cm depth. Soil samples from each replicate were combined, air dried, sieved (2 mm) and analyzed for its SOC content using an elemental analyzer (Costech 4010, Cernusco, CA, USA). Prior to elemental analysis, soil carbonates were removed using 0.5 M HCl according to Dyer et al. (2012). Soil bulk density was also determined at the time of soil sampling using bulk density ring with a 5 cm inner diameter and 5 cm height. Three soil bulk density samples were collected for each treatment replicate and for each replicate, the three bulk density values were averaged to get a representative value. The averaged soil bulk density values for each replicate were then used to convert SOC concentrations (%) for each replicate to SOC stock (g C m^{-2}) according to Osei et al. (2018).

5.2.3 Model validation and statistical evaluation

Although the Century model partitions SOC stocks into active, slow, and passive fractions, all the field-measured SOC stocks from our sites were total stocks, not categorized into these fractions. Hence, measured total SOC stocks in 2021 and 2022, along with values from previous studies at the same site (Abohassan, 2004; Mann, 2012; Coleman et al., 2018; Bazrgar et al., 2020; Mafa-Attoye et al., 2020) which measured total SOC stocks from 2002 to 2018, were used to validate and evaluate the Century model. The model was validated by comparing the measured field total SOC stocks to total SOC stocks simulated by Century using the regression function in SPSS (IBM SPSS Inc.). Model validation and performance was evaluated using the following statistical tests: correlation coefficient (r); coefficient of determination (r^2); adjusted r^2; root mean square deviation (RMSD), representing estimate of the mean deviation of simulated values with respect to the measured values; coefficient of residual mass (CRM), which measured the tendency of the model to over- or underestimate the measurements; and modeling efficiency (EF) which compared measured values to simulated data (Steel et al., 1997; Piñeiro et al., 2008) (Table 5.4).

$$RMSD = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(S_i - M_i)^2} \quad (5.1)$$

where S_i is the simulated (predicted) value, M_i is the measured (observed) value and n is the number of observations.

$$CRM = [(\sum_{measured} - \sum_{simulated})/\sum_{measured}] \quad (5.2)$$

$$EF = [1 - (\sum_{simulated} - \sum_{measured})^2/(\sum_{measured} - \bar{X}_{measured})^2] \quad (5.3)$$

5.3 Results

5.3.1 Century model performance

Statistical evaluation revealed that model performance, based on SOC stocks, was good with a significant relationship (p = 0.003 to 0.024) between measured versus simulated data (Table 5.4, Figure 5.1). For instance, SOC stocks prior to the establishment of the bioenergy crops, in 2009, was 4394 g C m^{-2} (Mann, 2012), and was similar to the SOC simulated by Century (4252 g C m^{-2}) (Figure 5.2). The coefficient of residual mass (CRM) was near zero, ranging from –0.071 to 0.046 (Table 5.4). CRM values close to zero indicated that the distribution of simulated data, in relation to measured data, was not biased (Oelbermann et al., 2017). Furthermore, negative CRM values represented model overestimation, and positive CRM values indicated underestimation. Based on the CRM values from this study, Century overestimated SOC stock by 7.1% for miscanthus and by 2.4% for switchgrass (Table 5.4). However, Century underestimated SOC stocks for willow (–3.0%), the successional site (–4.6%), and the row-crop system (–3.7%) (Table 5.4). It was determined that EF values were close to 1.0, with values ranging from 0.993 to 0.999. This indicated a near perfect modelling outcome (Table 5.4). Model performance is considered good when EF values are close to 1.0 but of poor fit when less than 0 (Steel et al., 1997).

Table 5.4: Statistical tests used to assess Century model performance and agreement between measured and simulated values of soil organic carbon to 20 cm depth in perennial bioenergy crops, successional site and the row crop system at the University of Guelph Agroforestry Research Station in southern Canada.

	Miscanthus	**Switchgrass**	**Willow**	**Successional site**	**Row crop system**
n	7	5	5	5	3
r	0.891	0.926	0.980	0.930	1.000
r^2	0.794	0.858	0.960	0.864	0.999
Adjusted r^2	0.752	0.810	0.946	0.819	0.998
p	0.007	0.024	0.003	0.022	0.018
RMSD (g C m^{-2})	605	296	171	259	226
CRM	–0.071	–0.024	0.030	0.046	0.037
EF	0.993	0.999	0.999	0.997	0.997

n, number of samples (number of studies); *r*, correlation coefficient; r^2, coefficient of determination; RMSD, root mean square deviation; CRM, coefficient of residual mass and EF, model efficiency.

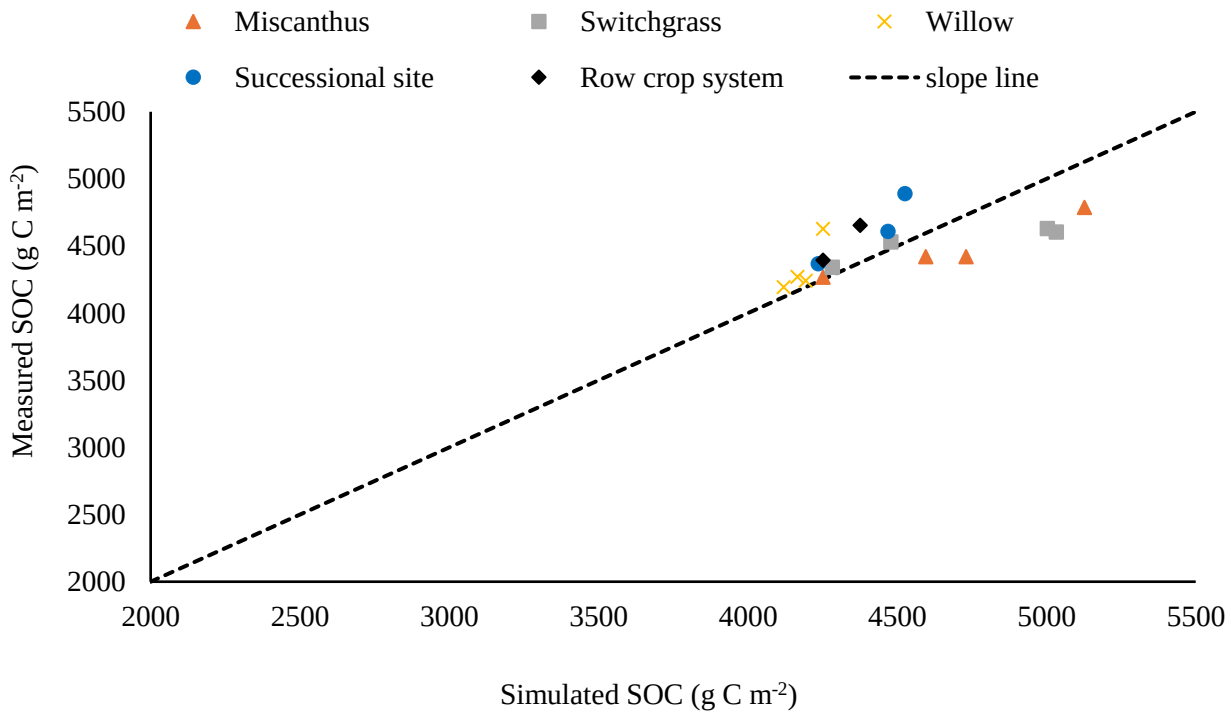


Figure 5.1: Relationship between observed (field measurements) and predicted (simulated) values of soil organic carbon in perennial biomass crops, successional site and the row crop system at the University of Guelph Agroforestry Research Station in southern Canada. Observed data are adapted from Abohassan (2004), Mann (2012), Coleman et al. (2018), Bazrgar et al. (2020), Mafa-Attoye et al. (2020) and current unpublished data.

5.3.2 Soil organic carbon stocks

Prior to forest clearing in 1911, the SOC stock was 8288 g C m^{-2}. Following deforestation and land conversion to agriculture, the SOC stock spiked briefly to 8589 g C m^{-2} in 1914, but rapidly declined thereafter (Figure 5.2). This decline in SOC continued until 2009, after which bioenergy crops were established (Figure 5.2). For example, Century simulated a decline in the SOC stock by 48% between 1911 and 2009. However, converting the site to the bioenergy crops and (or) allowing the site to undergo a secondary regrowth (successional site), increased SOC stocks by 100% for miscanthus, 62% for the successional site, 52% for switchgrass and 28% for willow, whereas the row crop system had a 6% loss of SOC after 162 years. Among the bioenergy crops, miscanthus averaged the highest (8521 g C m^{-2}) SOC stock between the years 2009 and 2170, followed by switchgrass (6480 g C m^{-2}) and willow (5448 g C m^{-2}) (Table 5.5, Figure 5.2).

Whereas miscanthus averaged 24% more SOC than the successional site, switchgrass and willow averaged 6% and 21% less SOC, than the successional site within the same period (Table 5.5, Figure 5.2). Results from this study revealed that growing miscanthus, switchgrass, and willow for the 162-year period will contribute 114, 63, and 37% more SOC than if the site were to remain under row crop production for the same period (Table 5.5, Figure 5.2). Meanwhile, 73% more SOC will accumulate if the land were left to undergo secondary regrowth (successional site) with temperate mixed forest (Table 5.5, Figure 5.2). Furthermore, the SOC stock for miscanthus reached pre-cultivation levels (8288 g C m^{-2}) only 55 years after its establishment (Figure 5.2). The other bioenergy crops and the successional site or row crop system were not able to reach the pre-cultivation SOC stocks throughout the 162-year simulation (Figure 5.2).

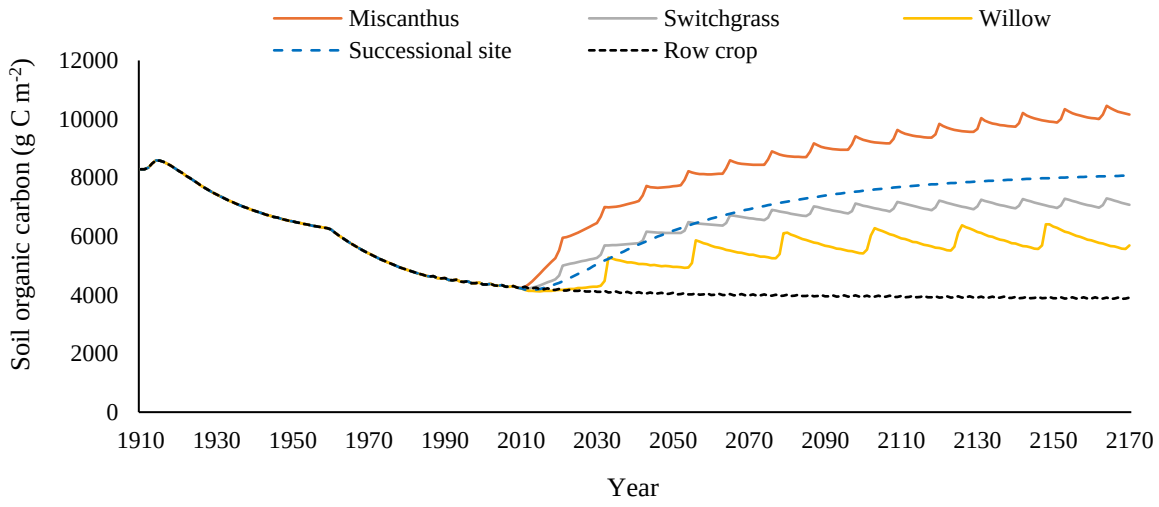


Figure 5.2: Soil organic carbon to 20 cm depth simulated by Century in different perennial biomass crops, successional site and the row crop system at the University of Guelph Agroforestry Research Station in southern Canada.

Table 5.5: Mean (g C m^{-2}) and percentage (%) soil organic carbon fractions to 20 cm depth simulated by Century for different perennial biomass crops, successional site and the row crop system at the University of Guelph Agroforestry Research Station in southern Canada over the entire simulation period (2009–2170).

Land Management	Active Fraction	Slow Fraction	Passive Fraction	Total (SOC)
Miscanthus	541.81 ± 10.79^{a†} (6.36%)	5811.34 ± 112.31^{a} (68.20%)	2168.02 ± 3.73^{a} (25.44%)	8521.17 ± 122.62^{a} (100%)
Switchgrass	364.02 ± 7.08^{b} (5.62%)	4009.31 ± 61.29^{c} (61.87%)	2106.84 ± 0.49^{b} (32.51%)	6480.17 ± 65.35^{c} (100%)
Willow	249.34 ± 13.49^{cd} (4.58%)	3131.05 ± 45.30^{d} (57.47%)	2067.56 ± 1.63^{d} (37.95%)	5447.95 ± 49.11^{d} (100%)
Successional site	268.96 ± 3.70^{c} (3.91%)	4524.79 ± 92.86^{b} (65.79%)	2083.70 ± 0.75^{c} (30.30%)	6877.45 ± 95.86^{b} (100%)
Row crop system	219.37 ± 1.32^{d} (5.49%)	1742.38 ± 4.08^{e} (43.62%)	2033.16 ± 3.70^{e} (50.89%)	3994.91 ± 7.65^{e} (100%)

†Within columns and soil carbon fractions, means followed by the same lower-case letters are not significantly different ($p>0.05$) (n = 162). Percentages in parentheses express the amount of soil organic carbon fraction as a percentage of the overall soil organic carbon (total) simulated for that land management practice.

A bi-decadel analysis of the simulated SOC stocks showed a rapid increase in SOC until the year 2110 when SOC accretion in switchgrass and willow was in equilibrium (Figure 5.3). However, SOC stocks in miscanthus and the successional site increased beyond 2110 whereas the row crop system decreased in SOC from 2011 onward (Figure 5.3). The greatest rate of SOC sequestration occurred within the first 40 years after the bioenergy crops were established (Figure 5.3). For example, miscanthus had a SOC accretion rate of 153 g C m^{-2} yr^{-1} followed by switchgrass (81 g C m^{-2} yr^{-1}), and willow (38 g C m^{-2} yr^{-1}). The successional site had a SOC accretion rate of 71 g C m^{-2} yr^{-1}, whereas that of the row crop system was reduced (–9 g C m^{-2} yr^{-1}) (Figure 5.3). After 2090, SOC stocks were in equilibrium for all sites except for miscanthus, which continued to increase in SOC (Figure 5.3).

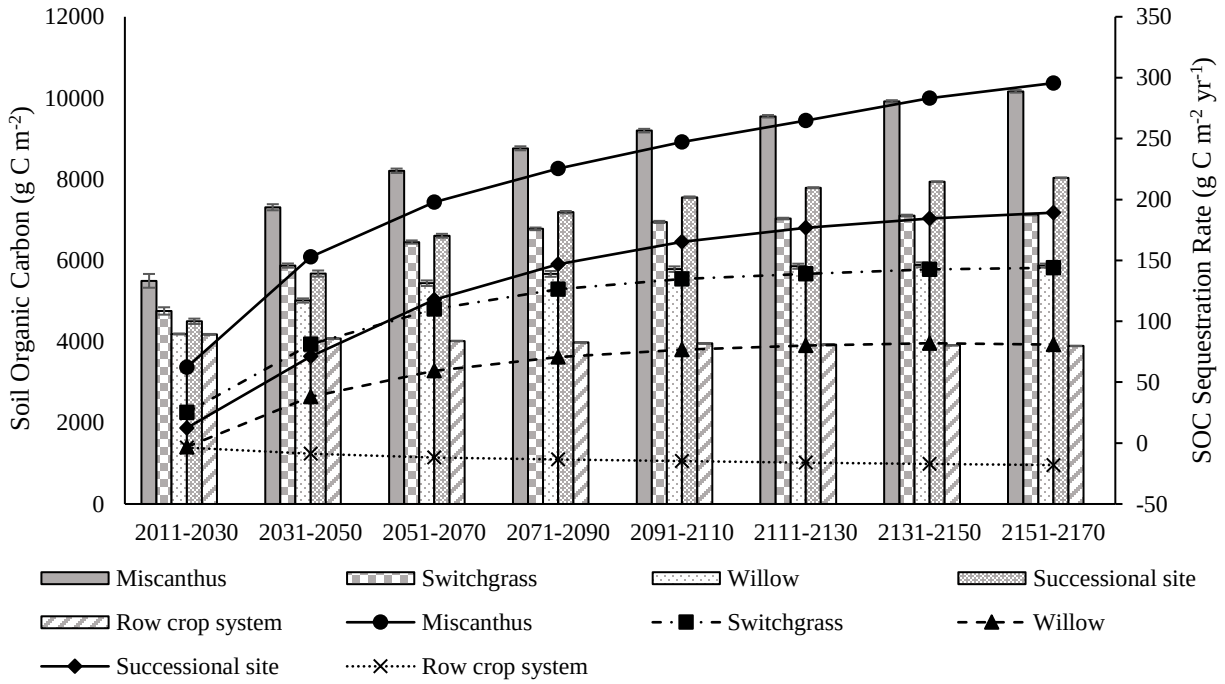


Figure 5.3: Average bi-decadal soil organic carbon stocks and sequestration rates to 20 cm depth for different perennial biomass crops, successional site and the row crop system at the University of Guelph Agroforestry Research Station in southern Canada. Error bars represent standard error of the mean (n = 20).

5.3.3 Soil organic carbon fractions

Evaluating SOC fractions provides further insight into SOC mean residence time and how this impacts long-term SOC sequestration (Blanco-Canqui & Lal, 2004). Carbon in the active SOC fraction, which constitutes soil microbes and microbial products, have short turnover times of about 1–3 months (Parton et al., 1988). The slow fraction, made up of resistant plant matter derived from structural plant material and stabilized soil microbial products has turnover times ranging from 10 to 50 years (Parton et al., 1988). The passive fraction on the other hand, comprises physically and chemically stabilized soil organic matter that is very resistant to decomposition. The passive fraction has turnover times ranging from about 400 to 4000 years (Parton et al., 1988).

Except for the row crop system, which had more than 50% of its SOC in the passive fraction (Figure 5.4E), all SOC contributions from the bioenergy crops and the successional site had most of their SOC stored in the slow fraction (Table 5.5). The study also determined a significant difference among bioenergy crops, the successional site, and the row crop system in the slow and passive SOC fractions (Table 5.5). Like the mean SOC stocks over the entire simulation period (2009–2170), the slow SOC fraction was greatest in miscanthus (5811 g C m^{-2}), followed by the successional site (4525 g C m^{-2}), switchgrass (4009 g C m^{-2}), willow (3131 g C m^{-2}) and row crop system (1742 g C m^{-2}) (Table 5.5). The passive SOC fraction was greatest in miscanthus (2168 g C m^{-2}) followed by switchgrass (2107 g C m^{-2}), successional site (2084 g C m^{-2}), willow (2068 g C m^{-2}) and row crop system (2033 g C m^{-2}) (Table 5.5). Except for the willow, the active SOC fractions were significantly higher ($p<0.05$) in the bioenergy crops and the successional site than the row crop system (Table 5.5).

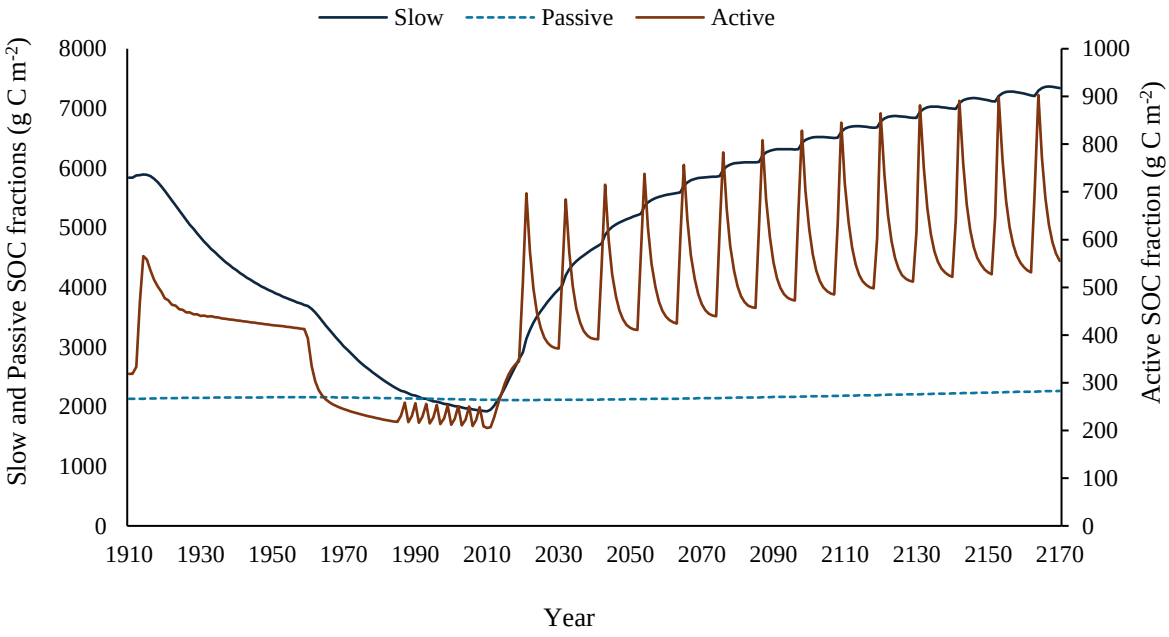


Figure 5.4A: Soil organic carbon fractions to 20 cm depth simulated by Century in miscanthus at the University of Guelph Agroforestry Research Station in southern Canada.

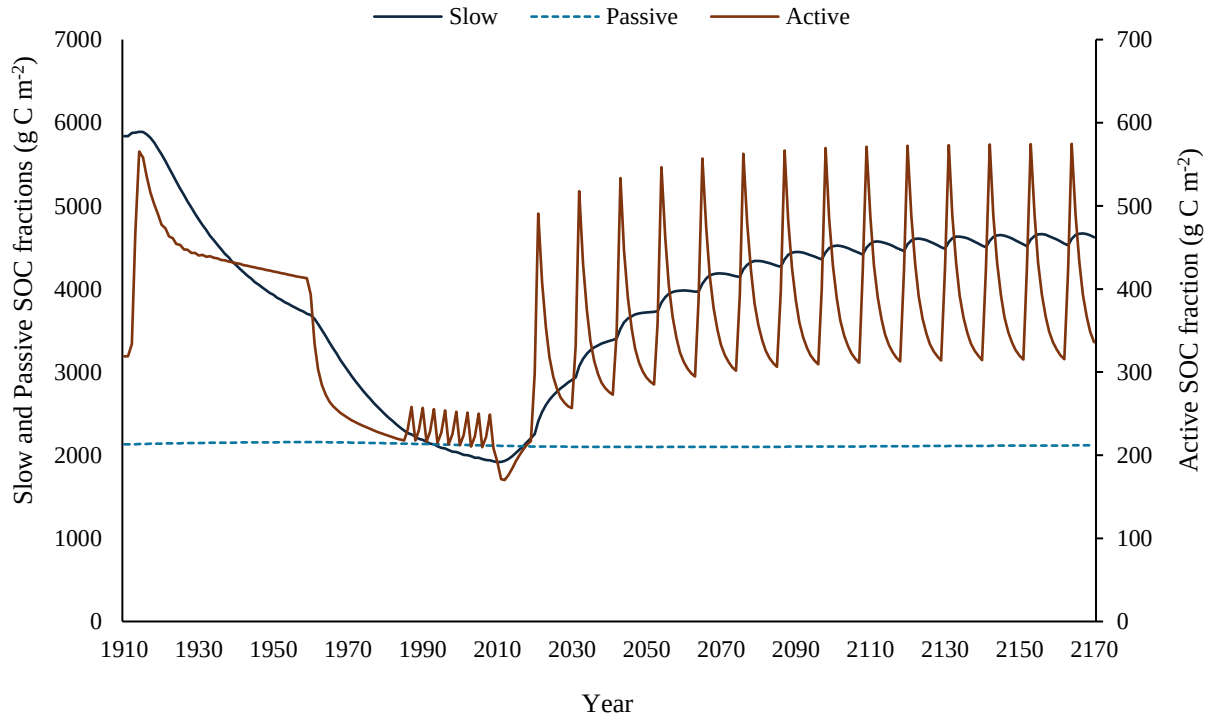


Figure 5.4B: Soil organic carbon fractions to 20 cm depth simulated by Century in switchgrass at the University of Guelph Agroforestry Research Station in southern Canada.

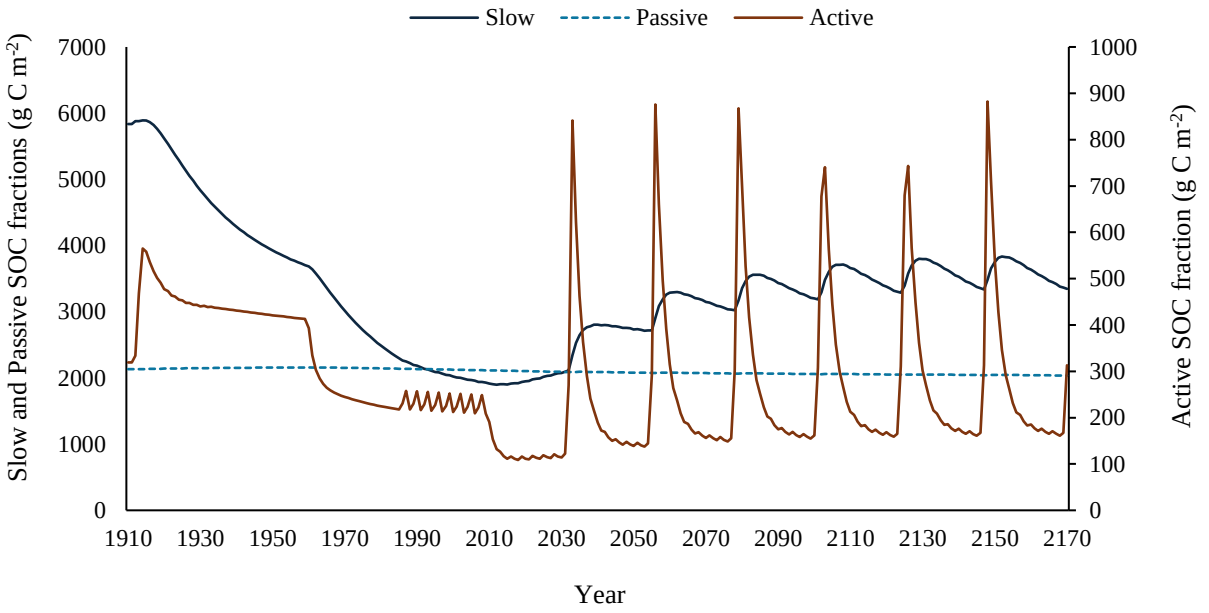


Figure 5.4C: Soil organic carbon fractions to 20 cm depth simulated by Century in willow at the University of Guelph Agroforestry Research Station in southern Canada.

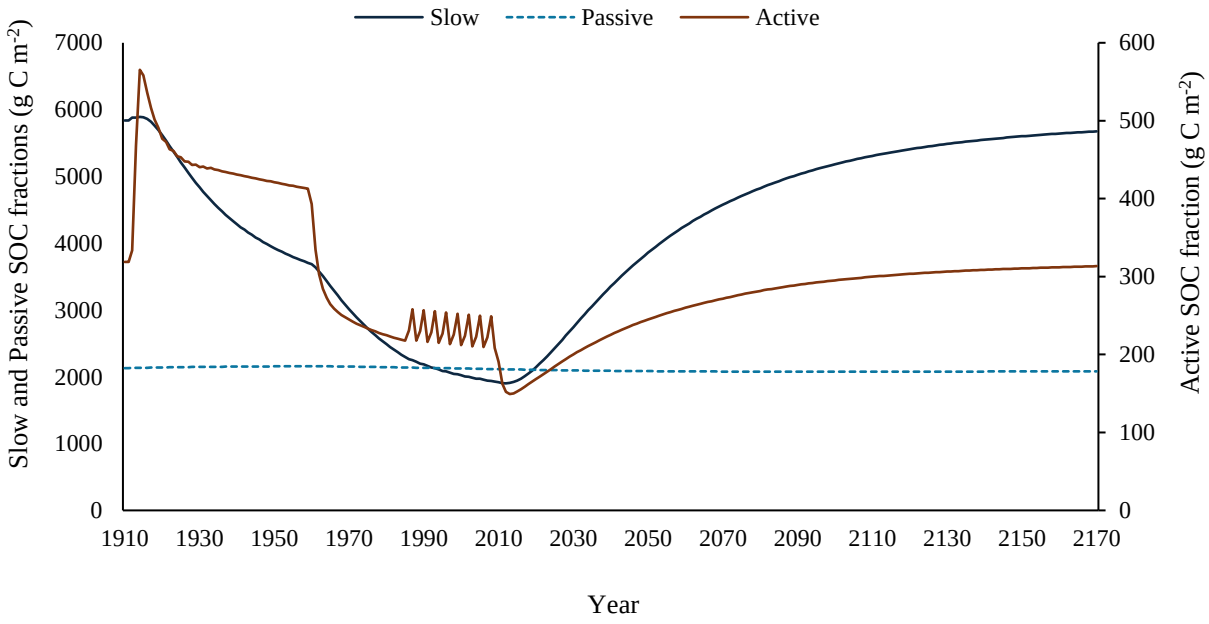


Figure 5.4D: Soil organic carbon fractions to 20 cm depth simulated by Century at the successional site at the University of Guelph Agroforestry Research Station in southern Canada.

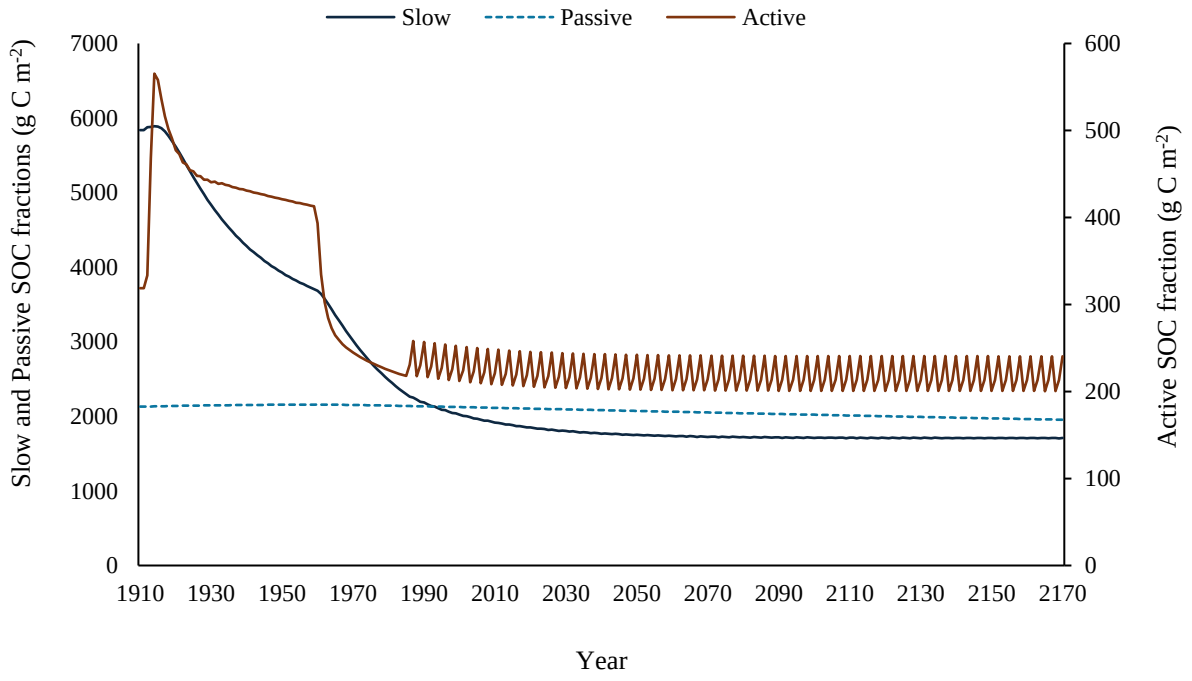


Figure 5.4E: Soil organic carbon fractions to 20 cm depth simulated by Century at the row crop system at the University of Guelph Agroforestry Research Station in southern Canada.

5.4 Discussion

5.4.1 Soil organic carbon stocks

The study determined that the average yearly SOC accumulation over the 162-year period for the bioenergy crops ranged from 34 to 53 g C m^{-2} yr^{-1}. These values were within lower range of the 22 to 155 g C m^{-2} yr^{-1} SOC rates reported in other studies (Don et al., 2012; Poeplau & Don, 2014; Gauder et al., 2016). The lower SOC accumulation rates in this simulation, compared to other studies, may be due to differences in agronomic management practices and site characteristics. For example, Lemus & Lal (2005) determined that fertilization improves biomass production which can result in increased SOC accumulation. Kering et al. (2012) reported that over 60% more biomass accumulated in switchgrass when N fertilizer was added. Marsal et al.

(2016) also reported an increased yield response in willow, miscanthus, and switchgrass when fertilizer was applied. Therefore, it is expected that the average annual SOC accumulation rate observed in this study was lower than that observed in other studies where crops were either established on more fertile soils or where established crops continuously received additional sources of nutrients (Lemus & Lal, 2005; Don et al., 2012; Poeplau & Don, 2014; Gauder et al., 2016).

A higher SOC stock in miscanthus than the successional site in this study supported the hypothesis that despite frequent biomass harvest, perennial bioenergy crops could accumulate similar or more SOC than a successional site. However, lower SOC in the switchgrass and willow, than the successional site, rejected this hypothesis. The higher SOC in the miscanthus than the successional site may be due to high biomass production and input in the miscanthus than the successional site. This is because an increase in SOC depends on the quantity of biomass returned to the soil (Lemus & Lal, 2005). This suggests that higher biomass production in miscanthus could lead to a greater build-up of SOC. This may also explain the higher SOC in miscanthus than switchgrass and willow in this study, as a previous study by Marsal et al. (2016) at the same site evaluated biomass productivity and determined that miscanthus had the greatest biomass (17.03 Mg ha^{-1} yr^{-1}) compared to switchgrass (5.99 Mg ha^{-1} yr^{-1}) and willow (12.15 Mg ha^{-1} yr^{-1}). Jarecki et al. (2020) also observed higher soil C sequestration rate in miscanthus (94 g C m^{-2} yr^{-1}) than switchgrass (26 g C m^{-2} yr^{-1}) and attributed this to higher biomass production in the former than the latter. In a side-by-side study in Illinois, USA, Heaton et al. (2008) observed higher biomass production in miscanthus (29.6–38.2 Mg ha^{-1}) than switchgrass (10.4–12.5 Mg ha^{-1}). Sanford et al. (2017) also determined aboveground biomass productivity of 23.0 Mg ha^{-1} in miscanthus compared to 11.3 Mg ha^{-1} in switchgrass. Hence, higher SOC in miscanthus in this study could be

attributed to higher biomass production which resulted in increased biomass return to the soil as organic matter.

Also, a higher SOC accumulation rate in the successional site (0.42 Mg C ha^{-1} yr^{-1}) than switchgrass and willow was likely due to the frequent disturbance caused by harvesting of the switchgrass and willow, resulting in less biomass return to the soil relative to the undisturbed successional site (Lemus & Lal, 2005; Graham et al., 2019). For example, Graham et al. (2019) observed that frequent biomass harvest in switchgrass resulted in reduced aboveground biomass inputs in the soil, reducing SOC stocks. Further studies may be required to understand how harvest management of different perennial bioenergy crops could impact long-term SOC stocks and dynamics.

It was hypothesized that reduced biomass input to the soil and increased organic matter mineralization due to soil disturbance in the row crop system would result in a lower SOC stock than the perennial bioenergy crops. Lower SOC accumulation rates in the row crop system (0.25 Mg C ha^{-1} yr^{-1}) compared to the bioenergy crops, despite the application of 157 and 100 kg N ha^{-1} yr^{-1} in corn and winter wheat, supported this hypothesis. Soil tillage likely exacerbated soil C loss from the upper 20 cm soil layer in the row crop system than in the perennial systems with no soil tillage. For instance, soil tillage can reduce SOC levels in upper soil layers by incorporating organic matter in deeper soil layers or increase organic matter degradation through soil aggregate disruptions and increased mineralization rate (Congreves et al., 2014). The lack of soil tillage in perennial bioenergy crops allowed for the accumulation of organic matter and contributed to an increase in SOC with time (Lemus & Lal, 2005; Blanco-Canqui, 2010). This may explain the higher SOC in the bioenergy crops than the row crop system.

Although the idea of SOC saturation was not a major focus of this study, it was, however, interesting to observe that highest SOC sequestration rate in the bioenergy crops occurred within the first two decades of their establishment, after which SOC began to stabilize. It was not clear at this stage if the stabilization of SOC accretion after the year 2110 was indicative that after 100 years of continuous C accrual, the soil may have been approaching it C saturation level or because C additions were at equilibrium with C losses. Further studies will be required to understand this observation.

5.4.2 Soil organic carbon fractions

Results from this study showed that whereas the perennial bioenergy crops enhanced SOC in the slow fraction, row crop depleted C in the slow fraction. This observation supported the hypothesis that reduced soil disturbance in the perennial bioenergy crops would enhance SOC in the slow fraction compared to the much-disturbed soil in the row crop system. As perennial bioenergy crops undergo continuous harvesting of aboveground biomass parts, a greater part of C is allocated to the root system, including rhizomes. This allows for a greater incorporation of the root or rhizome-assimilated C in the slow and passive fractions (Lemus & Lal, 2005; Don et al., 2012). Anderson-Teixeira et al. (2013) determined that C input from roots and rhizomes are more chemically recalcitrant, more stabilized through organo-mineral interactions, and are more physically protected in stable soil aggregates. These are the conditions required to build SOC in slow and passive fractions (Parton et al., 1988). This may explain why perennial bioenergy crops enhanced SOC in the slow fraction compared to row crop system.

Moreover, land management systems with greater return of biomass than its rate of decomposition leads to the preservation of recalcitrant C in the slow and passive fractions (Helfrich et al., 2006). In this study, the biomass crops and the successional site had minimal soil disturbance

(e.g., no tillage), which allowed the accumulation of organic matter in the root system and the accumulation of C in the slow and passive fractions (Lemus & Lal, 2005; Blanco-Canqui, 2010). Contrary to the bioenergy crops and the successional site, the row crop system had lower return of residues to the soil and experienced frequent disturbance through tillage, which caused a slower accumulation of C in the slow and passive fractions.

5.4.3 Limitations and future directions

The Century model simulates SOC in the upper 20 cm (Parton et al., 1988). However perennial plants such as the biomass crops evaluated in our study are characterized by a well-developed root system that grows beyond a 20 cm depth (Anderson-Teixeira et al., 2013). For example, Neukirchen et al. (1999) determined that only 28% of the miscanthus root system was located in the top 30 cm of soil and more than 50% of the root system was located at a depth greater than 90 cm. Lemus and Lal (2005) also determined that 39% of switchgrass roots were located at a depth greater than 35 cm. Follett et al. (2012) equally measured changes in SOC from 0 – 150 cm and demonstrated that over 50% of the increase in SOC in switchgrass was below the 30 cm depth. Furthermore, a substantial quantity of SOC was found at depths greater than 40 cm in woody biomass crops including willow (Rytter 2001; Dondini et al. 2009; Felten and Emmerling, 2012; Amichev et al., 2014; Gauder et al., 2016). Taking this into consideration, the Century model likely underestimated the SOC stocks in the biomass crops in our study since the model only quantified carbon in the top 20 cm of soil.

Century assumes soil bulk density and weather data as constant parameters and does not account for changes in bulk density due to changes in land management practices or account for a changing climate (Oelbermann and Voroney, 2011; Badewa et al., 2023). For instance, greater organic matter input to soil may decrease soil bulk density affecting soil carbon stocks over the

long-term (Oelbermann and Voroney, 2011). This likely also influenced the results of our study because bulk density would change with time as organic matter accumulated in the biomass crops and the successional site.

Another limitation of the Century model in our study is that the model assumes organic matter decomposition based on first-order kinetics which may tend to oversimplify soil organic matter decomposition and stabilization by not accurately representing the various biological and complex interactions involved in SOC decomposition (Kwon et al., 2017). However, despite these potential shortcomings, the Century model was still able to accurately predict SOC in our study site as indicated by our statistical evaluators when simulated SOC values were compared to measured data from the site. Since the Century partitions simulated SOC values into active, slow, and passive fractions, we suggest validating Century model results in future studies should consider including measured SOC fractions.

5.5 Conclusions

Results from this study suggest that maintaining long-term soil productivity on low productive agricultural lands is attainable when high yielding perennial bioenergy crops such as miscanthus is grown without the addition of any fertilizer. This is even possible with repeated biomass harvests which results in offsite nutrient exports and reduced litter inputs to the soil. Over the 162-year simulation period, miscanthus averaged the highest (8521 g C m^{-2}) SOC stocks among the perennial bioenergy crops, followed by switchgrass (6480 g C m^{-2}) with willow averaging the least (5448 g C m^{-2}). Whereas miscanthus averaged 24% more SOC than the successional site, switchgrass and willow averaged 6% and 21% less SOC, respectively than the successional site within the same period. Higher SOC stock in the miscanthus than the successional site in this study supports the hypothesis that despite frequent biomass harvest, perennial bioenergy

crops can accumulate similar or more SOC than if the site is left to undergo secondary regrowth. This may, however, depend on the type of crop as among the bioenergy crops, only miscanthus had higher SOC than the successional site. For instance, it took miscanthus 55 years after establishment to reach pre-cultivation SOC levels of 8288 g C m^{-2}. Neither of the bioenergy crops nor successional site reached the pre-cultivation SOC levels, even after 162 years. Hence, the type of crop or species selected is very crucial in achieving climate change mitigation goals. Also, whereas perennial bioenergy crops enhanced SOC in the slow fraction, row crop activities resulted in SOC depletion in the slow fraction. This was attributed to reduced soil disturbance in the perennial bioenergy crops compared to the much-disturbed soil in the row crop system. Enrichment of SOC in the more stabilized fractions in the perennial bioenergy crops indicate the crucial role of these crops in long-term SOC sequestration and positive impact on climate change mitigation. Although, the study recognizes that crop residue management in row crops, such as 100% crop residue return to the soil, can result in SOC gains. The choice of 50% residue removal in corn and winter wheat (Table 5.3) in this study is typical of row crop management in the region of the study site. Therefore, future SOC simulation studies from the site using row crop system as reference should consider different crop residue management strategies to provide further understanding on how residue management can impact slow C fractions. Again, future long-term SOC studies in perennial biomass crops from the site should look beyond the upper soil layers by increasing soil sampling depths to capture C associated with deep roots from these crops. Further studies will also be needed to ascertain the long-term impact of harvest management of perennial biomass crops on SOC stocks.

CHAPTER 6

6.0 Conclusions

6.1 Major Research Findings

This dissertation assessed the potential capabilities of unfertilized miscanthus, switchgrass, and willow on low productive agricultural lands in providing climate change mitigation benefits compared to leaving these lands to undergo their natural succession with natural regrowth vegetation. This was achieved by quantifying GHG emissions and soil C, and N dynamics as influenced by these different bioenergy crops compared to the natural regrowth vegetation site. Additionally, the influence of soil processes and environmental factors such soil temperature fluctuations (i.e., freeze-thaw cycles) that impact GHG emissions of these crops were assessed. A key strength of the studies outlined in this dissertation lies in the comparison of the different bioenergy crops and natural regrowth at one site as described in Chapter 1, since contrasting climates, cropping history and soil textures have strong influence on soil C and N dynamics and related GHG emissions.

Chapter 1 explored the sustainability climate benefits of using bioenergy to decarbonize our energy sector which is a major contributor to global warming. However, the sustainability and climate benefits derived from bioenergy would depend on the type of crop grown, the land used for growing them, and agronomic practices that go into producing these crops. For instance, the food security and environmental consequences of producing energy from first-generation bioenergy crops have been discussed and how using second-generation bioenergy crops such as perennial biomass crops which can thrive and produce biomass on low productive agricultural lands can be a solution to the challenges posed by the first-generation crops.

Chapter 2 quantified and compared GHG emissions in different unfertilized perennial bioenergy crops and a successional site over two growing seasons. Findings of the study from this chapter showed that overall combined emissions in miscanthus and willow did not differ from the successional site. However, switchgrass over the two growing seasons had higher overall combined emissions than the successional site. This implies that unlike miscanthus and willow, converting low productive agricultural land to switchgrass may result in an overall higher emission with negative implications on the global climate.

Chapter 3 evaluated the effects of repeated soil freeze-thaw on soil C and N dynamics, as well as GHG emissions, in different perennial bioenergy crops species in a laboratory incubation study. Freeze-thaw impacted soil and dissolved C and N differently under the different bioenergy crops with higher dissolved and soil N observed in switchgrass and the successional site than miscanthus and willow after the 49-day incubation study. Unlike in the findings in Chapter 2 where GHG emissions over the growing season were highest in switchgrass, under the freeze-thaw conditions, highest emissions were observed in miscanthus and willow, with lower emissions in switchgrass. These contradictory findings in GHG emissions following these contrasting conditions could be assigned to the potential shift in microbial community and microbial biomass which may have responded differently under the two contrasting conditions, as the N_2O and CO_2 emitted during freeze-thaw was assumed to originate mostly from the lysis and death of microbial biomass.

Chapter 4 determined SOC and N pools and dynamics in different soil-sized fractions in different perennial bioenergy crops using stable isotope. Due to the absence of fertilization and potential decreased organic residue input to the soil from lower biomass yield, after 12 years of cultivation, the miscanthus and willow only posted marginal gains in SOC concentrations with the

switchgrass recording a loss. Among the three bioenergy crops, only the miscanthus maintained SOC stocks comparable to the successional site owing to the ability of unfertilized miscanthus to produce higher biomass on low productive agricultural lands compared to switchgrass and willow. The distribution of soil-sized fractions and SOC in the soil-sized fractions under the perennial bioenergy crops were consistent with the aggregate hierarchy model proposed by Tisdall and Oades (1982) and followed an increasing order of silt + clay > micro- > and macro-sized fractions. The δ13C signatures of the different soil-sized fractions under the perennial bioenergy crops to 30cm revealed a higher proportion of C contribution by the crops went to the micro-sized fractions compared to macro-sized fractions, indicating their significant contribution to more stabilized C pools with long-term positive impact on climate change mitigation.

Chapter 5 evaluated SOC dynamics in different perennial biomass crops after more than 100 years of cultivation on a low productive agricultural land. Results from the study over the 162-year simulation period indicated that maintaining long-term soil productivity on low productive agricultural lands can be attainable by cultivating high yielding perennial bioenergy crops, particularly, miscanthus, even with repeated biomass harvests that reduces litter inputs to the soil. The study again revealed the enrichment of SOC in more stabilized fractions in the perennial biomass crops with positive impact on long-term C storage and climate change mitigation benefit.

6.2 Key Research Contributions

Aside from the significant and original contribution to the literature, this research contributes new knowledge to the current discussion on the climate benefits of converting low productive agricultural lands to perennial bioenergy crops of miscanthus, switchgrass, and willow and to also examine the hypothesis that leaving low productive lands for regrowth vegetation can

yield greater climate benefits than converting them to bioenergy crops. This research provides new knowledge on the climate benefits of the different perennial bioenergy crops by quantitatively evaluating N_2O and CO_2 emissions, and SOC pools and dynamics in different soil-sized fractions with different stabilities. Knowledge from this study will be important in providing information in the selection of bioenergy crops and management strategies that can be adopted for greater climate benefits. For instance, results throughout the various chapters in this dissertation revealed that miscanthus has the highest SOC storage capacity with greater potential for lower GHG emissions during growing seasons.

However, the higher N_2O emissions in miscanthus under freeze-thaw conditions revealed that despite the higher SOC storage potential of miscanthus, freeze-thaw can stimulate increased N_2O emissions negating any positive benefit the miscanthus may provide through SOC storage. With the knowledge from this study that reducing soil freezing intensity could reduce the freeze-thaw-related N_2O emissions in miscanthus, adoption of management strategies such as maintaining adequate stubble to enhance snow capture could insulate soils under miscanthus from severe freezing to reduce N_2O emissions. Hence, this research provides not just information on suitable bioenergy crops but also on management strategies to maximize their climate benefits.

6.3 Future Research

The quantitative evaluation of GHG emissions and SOC pools and dynamics in different perennial bioenergy crops on low productive agricultural land provided further insight into understanding the contribution of these crops to climate change mitigation, while also contributing to future management strategies that can be adopted to increase their climate benefits. Future research should include studies on farmers' perspectives on bioenergy crop cultivation on low

productive agricultural lands in the study region to provide a better understanding on what the motivations for farmers are in growing these crops.

Also, with evidence from studies showing that freeze-thaw and bioenergy crop species influence soil microbial biomass and microbial community structure, the current lack of understanding on how soil freeze-thaw under different perennial bioenergy crops would influence the soil microbial community and structure needs further studies. This knowledge will provide a better understanding on how these changes in microbial community and structure would influence C and N cycling leading to N_2O and CO_2 emissions in different bioenergy crops. Moreover, findings from this research showed varied responses of different perennial bioenergy crops to GHG emissions under repeated freeze-thaw events in laboratory conditions. There is the need to extend this study to assess GHG emissions under field conditions as crops may respond differently to freeze-thaw under field conditions than in controlled laboratory settings. Another area that needs further evaluation is the adoption of management strategies such as stubble management during biomass harvest for increased snow retention and its impact on soil freezing and related GHG emissions. Knowledge of this will provide insight into management strategies capable of reducing GHG emissions from soil freeze-thaw.

Furthermore, future studies evaluating SOC pools and dynamics should include soil depths beyond the surface 30 cm if site conditions permit as some of the bioenergy crops such as switchgrass have a greater mass of their roots beyond the surface 30 cm depth. Hence, restricting SOC studies to the upper soil layers may underestimate the SOC storage potential and climate benefits of these crops with much deeper rooting systems.

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Appendices

Appendix A

Table A1: Basic characteristics of miscanthus (*Miscanthus giganteus* L.), switchgrass (*Panicum virgatum* L.) and willow (*Salix miyabeana* L.)

	Miscanthus	**Switchgrass**	**Willow**	**Reference**
Crop type	C_4 perennial warm season grass	C_4 perennial warm season grass	C_3 perennial short rotation woody crop	Samson et al. (2016); Withers et al. (2016); Hancock (2009)
Hardiness	Adapts well to temperate and cold climates but lacks winter hardiness	Tolerant to harsh environmental and soil conditions such as heat, cold, drought, and floods	Adapts well in cold and wet areas	Malinen (2012); Hancock (2009)
Propagation	Rhizome	Seed	Cuttings	Samson et al. (2016); Withers et al. (2016); Mann (2012)
Biomass (dry matter yield)	~ 23 Mg ha^{-1} yr^{-1}	~ 9 Mg ha^{-1} yr^{-1}	~ 17 Mg ha^{-1} yr^{-1}	Bazrgar et al. (2020); Nissim et al. (2013)
Harvesting cycle	Annually	Annually	Every 2 to 4 years	Samson et al. (2016); Withers et al. (2016); Mann (2012); Hancock (2009)
Height	3 to 7 meters	0.5 to 3 meters	5 to 10 meters	Wang et al. (2021); Malinen (2012); Hancock (2009); Ledin (1996)
Rooting depth	~ 2.5 meters	~ 3 meters	~ 2 meters	de Koff and Tyler (2012); Neukirchen et al. (1999)
Soil requirements	Adaptable to a wide range of soils, from sands to high organic matter soils with adequate water and	Adaptable to most soils including well- or moderately well-drained soils, moist, sandy or clay	Well drained soils with high moisture contents but adapted to growing in areas that are too wet for most crops.	Loughrey (2023); Samson et al. (2016); Withers et al. (2016); Malinen (2012); Hancock (2009)

	temperature. Also, tolerant of a wide range of pH. Optimum soil pH is between 5.5 and 7.5	soil with a slightly acidic to neutral pH between 5.5 and 7	Suitable soil pH range is 5.5 to 7	
Water requirements	High	Medium	Very high	Borek et al. (2010); McIsaac et al. (2010)
Productive lifespan	~ 15 years	~ 15 years	~ 21 years	de Koff and Tyler (2012); Malinen (2012); Mann (2012)
Climate and environmental benefits	Reduces the risk of groundwater contamination, increased SOC sequestration, reduces GHG emissions, enhances biodiversity and wildlife	Reduces the risk of groundwater contamination, increased SOC sequestration, reduces GHG emissions, enhances biodiversity and wildlife	Increase SOC sequestration, reduce GHG emissions, filter soil leachates from water and increase different wildlife habitats and biodiversity, for stream protection, remediation of contaminated sites or effluent filtration	Malinen (2012)
Soil benefits	Protects soil against erosion, enriches the soil with organic matter, improves soil structure	Protects soil against erosion, enriches the soil with organic matter, improves soil structure	Reduce erosion, improve soil structure, improve soil health	Malinen (2012)

Table A1.1: P-values of analysis of variance (ANOVA) and interactions for Mean N_2O-N fluxes, mean CO_2-C fluxes, cumulative N_2O-N emissions, cumulative CO_2-C emissions, NH_4^+, NO_3^-, soil moisture, soil temperature, and EC in the different perennial bioenergy crops and successional site at the University of Guelph Agroforestry Research Station in southern Canada. The treatment accounts for differences among land use type (miscanthus, switchgrass, willow, and successional site) and year factor accounts for differences between 2020 and 2021.

Factors	**Mean N_2O**	**Mean CO_2**	**Cumulative N_2O**	**Cumulative CO_2**	**Soil NH_4^+**	**Soil NO_3^-**	**Soil Moisture**	**Soil Temperature**	**EC**
Year	ns	ns	ns	0.046	<0.001	<0.001	0.026	<0.001	ns
Treatment	ns	ns	ns	ns	ns	ns	ns	0.003	0.021
Year x Treatment	ns	0.004	ns	0.015	0.031	ns	0.002	ns	ns

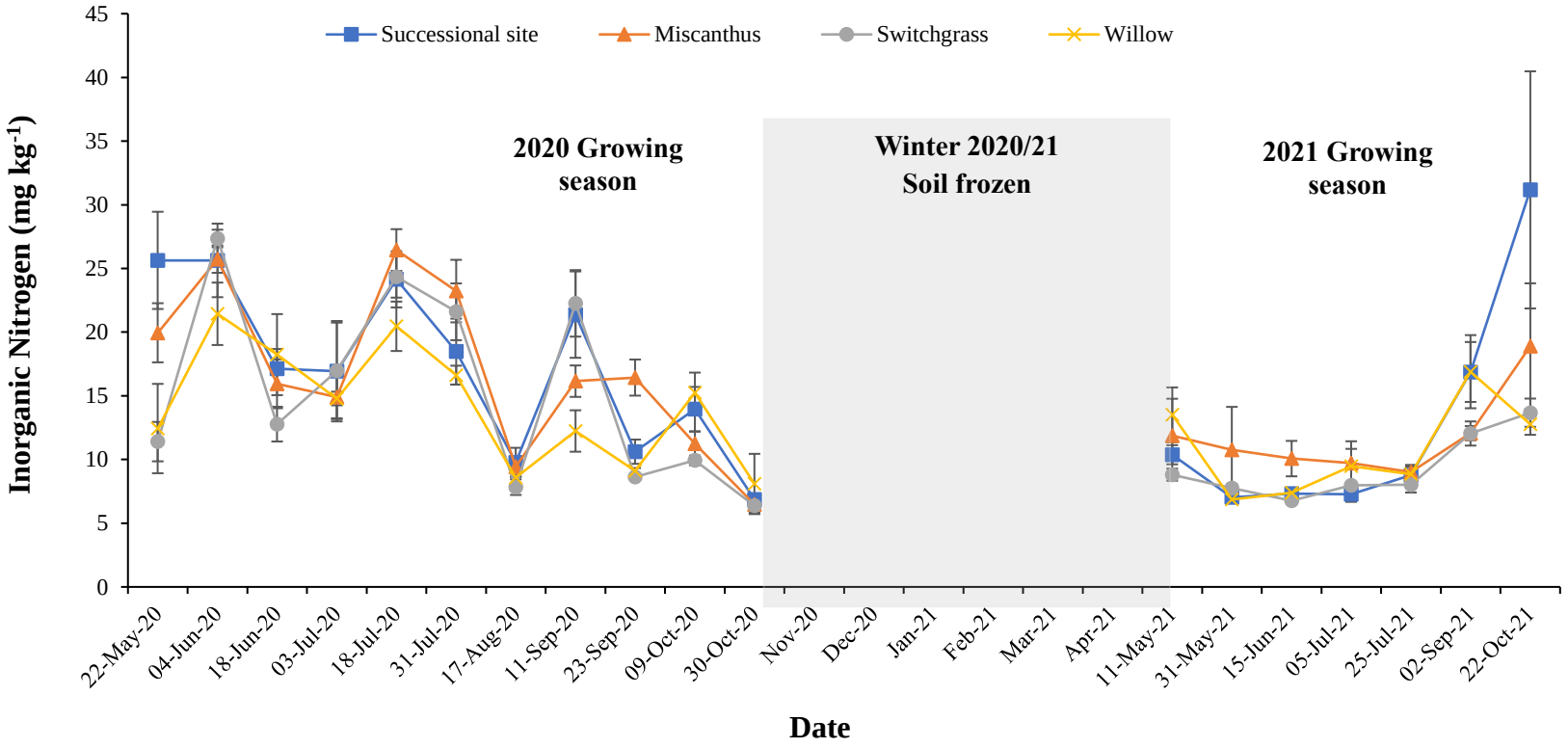


Figure A1.1: Soil inorganic nitrogen measured from soil under the different perennial bioenergy crops and successional site for the 2020 and 2021 growing seasons at the University of Guelph Agroforestry Research Station in southern Canada.

Table A1.2: Pearson correlation coefficient between measured emissions and soil parameters in the successional site during the 2020 and 2021 growing seasons from the University of Guelph Agroforestry Research Station in southern Canada.

Variables	N_2O ($\mu g\ m^{-2}$)	CO_2 ($mg\ m^{-2}$)	Temperature (^oC)	Moisture (%)	EC ($mS\ m^{-1}$)	NH_4^+ ($mg\ kg^{-1}$)	NO_3^- ($mg\ kg^{-1}$)
---------- 2020 ----------							
N_2O	1						
CO_2	-0.265	1					
Temperature	-0.184	0.374	1				
Moisture	0.275	0.255	-0.507	1			
EC	-0.308	0.247	0.950***	-0.686**	1		
NH_4^+	0.560	-0.167	0.008	0.196	-0.121	1	
NO_3^-	-0.134	0.530	0.713*	0.074	0.631*	-0.020	1
---------- 2021 ----------							
N_2O	1						
CO_2	-0.188	1					
Temperature	-0.080	0.346	1				
Moisture	-0.238	0.123	0.203	1			
EC	0.619	-0.306	0.095	-0.721	1		
NH_4^+	-0.113	-0.229	-0.852*	0.195	-0.595	1	
NO_3^-	0.412	-0.661	-0.500	0.373	-0.070	0.478	1

*p<0.05; **p<0.01; ***p<0.001

Table A1.3: Pearson correlation coefficient between measured emissions and soil parameters in miscanthus during the 2020 and 2021 growing seasons from the University of Guelph Agroforestry Research Station in southern Canada.

Variables	N_2O ($\mu g\ m^{-2}$)	CO_2 ($mg\ m^{-2}$)	Temperature (^oC)	Moisture (%)	EC ($mS\ m^{-1}$)	NH_4^+ ($mg\ kg^{-1}$)	NO_3^- ($mg\ kg^{-1}$)
2020							
N_2O	1						
CO_2	-0.069	1					
Temperature	-0.421	0.877***	1				
Moisture	0.587	-0.009	-0.362	1			
EC	-0.403	0.873***	0.971***	-0.355	1		
NH_4^+	-0.346	-0.404	-0.259	-0.099	-0.185	1	
NO_3^-	-0.050	0.674*	0.773**	-0.185	0.836**	-0.331	1
2021							
N_2O	1						
CO_2	0.707	1					
Temperature	-0.647	-0.058	1				
Moisture	-0.341	0.157	0.463	1			
EC	-0.825*	-0.944**	0.137	-0.056	1		
NH_4^+	0.829*	0.398	-0.826*	-0.104	-0.544	1	
NO_3^-	-0.002	-0.098	-0.526	0.166	0.224	0.382	1

*p<0.05; **p<0.01; ***p<0.001

Table A1.4: Pearson correlation coefficient between measured emissions and soil parameters in switchgrass during the 2020 and 2021 growing seasons from the University of Guelph Agroforestry Research Station in southern Canada.

Variables	N_2O ($\mu g\ m^{-2}$)	CO_2 ($mg\ m^{-2}$)	Temperature (^oC)	Moisture (%)	EC ($mS\ m^{-1}$)	NH_4^+ ($mg\ kg^{-1}$)	NO_3^- ($mg\ kg^{-1}$)
---------- 2020 ----------							
N_2O	1						
CO_2	0.217	1					
Temperature	0.016	0.806**	1				
Moisture	0.283	-0.003	-0.385	1			
EC	-0.070	0.731*	0.974***	-0.494	1		
NH_4^+	-0.392	-0.350	-0.150	0.009	-0.104	1	
NO_3^-	-0.498	0.511	0.646*	-0.152	0.734*	-0.123	1
---------- 2021 ----------							
N_2O	1						
CO_2	-0.200	1					
Temperature	0.006	0.198	1				
Moisture	-0.005	-0.480	0.333	1			
EC	0.116	-0.381	0.275	-0.297	1		
NH_4^+	0.056	0.119	-0.833*	-0.046	-0.755	1	
NO_3^-	-0.154	-0.578	-0.185	0.488	0.107	0.147	1

*p<0.05; **p<0.01; ***p<0.001

Table A1.5: Pearson correlation coefficient between measured emissions and soil parameters in willow during the 2020 and 2021 growing seasons from the University of Guelph Agroforestry Research Station in southern Canada.

Variables	N_2O ($\mu g\ m^{-2}$)	CO_2 ($mg\ m^{-2}$)	Temperature (^oC)	Moisture (%)	EC ($mS\ m^{-1}$)	NH_4^+ ($mg\ kg^{-1}$)	NO_3^- ($mg\ kg^{-1}$)
---------- 2020 ----------							
N_2O	1						
CO_2	-0.116	1					
Temperature	0.201	0.622*	1				
Moisture	-0.496	0.355	-0.394	1			
EC	0.475	0.206	0.852**	-0.760**	1		
NH_4^+	-0.037	-0.421	-0.208	-0.103	-0.036	1	
NO_3^-	0.344	0.670*	0.619*	-0.014	0.531	-0.475	1
---------- 2021 ----------							
N_2O	1						
CO_2	-0.215	1					
Temperature	-0.477	0.643	1				
Moisture	-0.105	-0.345	0.465	1			
EC	-0.376	0.417	0.354	-0.180	1		
NH_4^+	0.293	-0.691	-0.866*	-0.175	-0.748	1	
NO_3^-	-0.305	-0.366	-0.205	0.019	-0.140	0.362	1

*p<0.05; **p<0.01; ***p<0.001

Appendix B

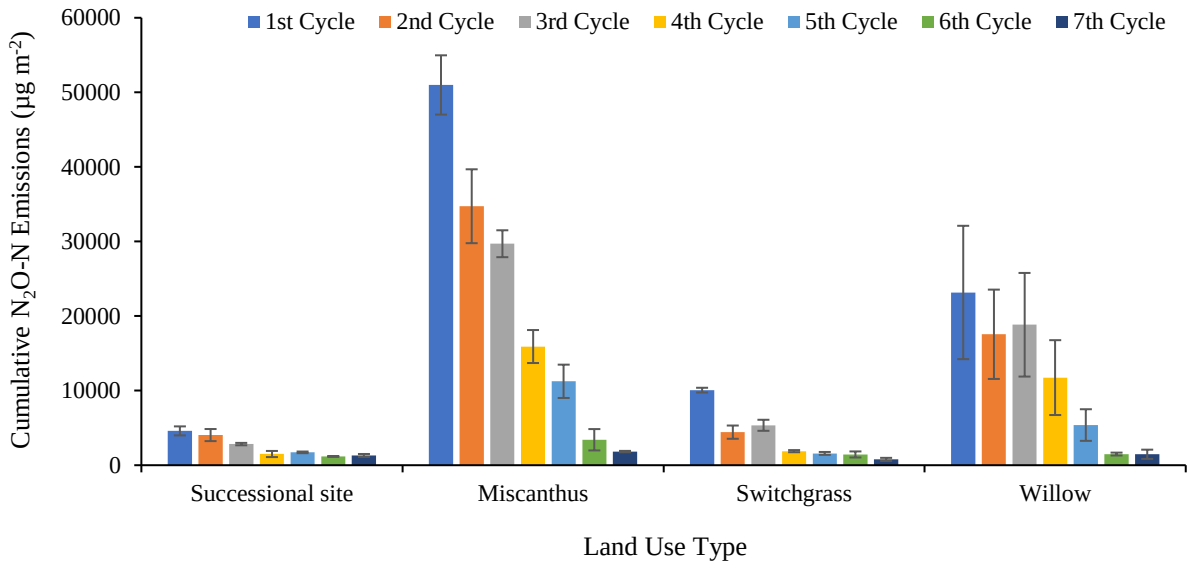


Figure B1.1: Cumulative N_2O-N emissions (µg m^{-2}) over the 7 freeze-thaw cycles of incubated soil from perennial bioenergy crops and successional site. Bars represent standard error of cumulative means (n = 3).

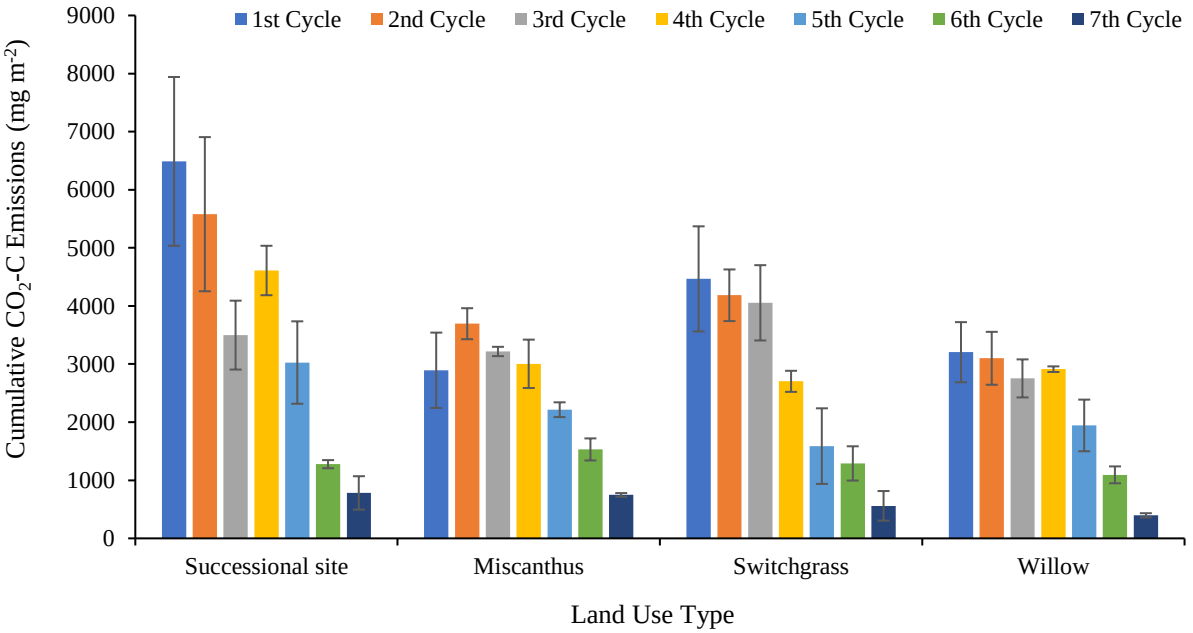


Figure B1.2. Cumulative CO_2-C emissions (mg m^{-2}) over the 7 freeze-thaw cycles of incubated soil from perennial bioenergy crops and successional site. Bars represent standard error of cumulative means (n = 3).

Appendix C

Table C1.1: Analysis of variance of the effects of treatment, depth, soil fractions and their interactions on soil bulk density, soil-sized fractions, carbon and nitrogen pools in different perennial bioenergy crops and successional site at the University of Guelph Agroforestry Research Station in southern Canada.

	Factors	*df*	F	*p*
SOC concentrations (%)	Treatment	3	2.209	0.113
	Depth	2	18.170	**<0.001**
	Treatment x Depth	6	0.138	0.990
Total Nitrogen (%)	Treatment	3	2.045	0.134
	Depth	2	11.814	**<0.001**
	Treatment x Depth	6	0.168	0.983
C/N Ratio	Treatment	3	1.332	0.287
	Depth	2	15.161	**<0.001**
	Treatment x Depth	6	1.149	0.365
Bulk Density	Treatment	3	3.801	**0.023**
	Depth	2	3.788	**0.037**
	Treatment x Depth	6	0.362	0.896
Whole SOC stocks	Treatment	3	5.029	**0.008**
	Depth	2	6.516	**0.005**
	Treatment x Depth	6	0.299	0.931
SOC in soil fractions	Treatment	3	15.047	**<0.001**
	Depth	2	1.975	0.146
	Fractions	2	1211.397	**<0.001**
	Treatment x Depth	6	8.229	**<0.001**
	Treatment x Fractions	6	0.859	0.529
	Depth x Fractions	4	2.407	0.057
	Treatment x Depth x Fractions	12	0.742	0.706
Total N in soil fractions	Treatment	3	7.705	**<0.001**
	Depth	2	0.297	0.744
	Fractions	2	762.291	**<0.001**
	Treatment x Depth	6	5.411	**<0.001**
	Treatment x Fractions	6	1.012	0.424
	Depth x Fractions	4	1.687	0.162
	Treatment x Depth x Fractions	12	1.076	0.393
% Mass Soil-sized Fraction	Treatment	3	1.125	0.345
	Depth	2	1.290	0.281
	Fractions	2	1810.896	**<0.001**

	Treatment x Depth	6	1.395	0.228
	Treatment x Fractions	6	1.018	0.421
	Depth x Fractions	4	1.452	0.226
	Treatment x Depth x Fractions	12	1.059	0.407
$\delta^{13}C$ in Soil Fractions (‰)	Treatment	3	65.772	**<0.001**
	Depth	1	0.219	0.642
	Fractions	2	0.784	0.462
	Treatment x Depth	3	5.615	**0.002**
	Treatment x Fractions	6	2.518	**0.034**
	Depth x Fractions	2	0.630	0.537
	Treatment x Depth x Fractions	6	0.660	0.682
$\delta^{15}N$ in Soil Fractions (‰)	Treatment	3	3.950	**0.013**
	Depth	1	35.014	**<0.001**
	Fractions	2	4.431	**0.017**
	Treatment x Depth	3	21.397	**<0.001**
	Treatment x Fractions	6	0.487	0.815
	Depth x Fractions	2	0.117	0.890
	Treatment x Depth x Fractions	6	0.393	0.880