SHORT-TERM IMPACTS OF AGRICULTURAL LAND USE CHANGE ON SOIL HEALTH AND NITROGEN CYCLING MICROBIAL COMMUNITIES

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ABSTRACT

Short-term impacts of agricultural land use change on soil health and N-cycling microbial communities

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Glyphosate burndown and tillage, followed by the cultivation of cash crops, are frequently used techniques in LUC from perennial cropping systems (PS) to annual cropping systems (AS). Agricultural LUC can result in the loss of soil nitrogen (N) via emission of nitrous oxide (N_2O), a potent greenhouse gas (GHG). The purpose of this thesis is to investigate the short-term impacts of agricultural LUC from PS to AS on soil health parameters and the nitrogen (N)-cycling bacterial communities responsible for nitrification and denitrification processes that result in the emission of N_2O. The study field site was in Stone Mills, Ontario and comprised of four fields: two annual cropping systems were regularly cultivated for cash crops (AS), and two perennial cropping systems had not been cultivated for cash crops for over 50 years (PS). One PS was left intact while the other PS was subjected to LUC (converted system [CS]) from PS to AS within the study period. The results of this study indicate that PS promotes soil health, as illustrated through higher soil organic matter % (2.3 ± 0.2 %), beta-glucosidase activity (0.41 ± 0.04 mmol g^{-1} dry soil h^{-1}), and N-acetylglucosaminidase activity (0.18 ± 0.03 mmol g^{-1} dry soil h^{-1}). The PS soils exhibited higher nitrifier (6.0 ± 0.3 $\log_{10}$ copies per g dry soil) and denitrifier (*nirS, nirK* and *nosZI*: 7.8 ± 0.05, 8.1 ± 0.1 and 5.0 ± 0.1 $\log_{10}$ copies per g dry soil, respectively) gene abundances compared to AS (*amoA*, *nirS*, *nirK* and *nosZI*: 5.7 ± 0.1,

7.7 ± 0.04, 7.9 ± 0.1 and 4.8 ± 0.1 $\log_{10}$ copies per g dry soil, respectively). Moreover, LUC from PS to AS deteriorated soil health parameters and significantly decreased the *nosZI*/*16S* rRNA gene ratio, leading to potential N loss through N_2O emissions. A laboratory incubation study revealed that the use of N-containing fertilizer in conjunction with easily metabolized C cumulatively resulted in 64.2% increase in N_2O and 42.1% increase in CO_2 fluxes in AS soils compared to PS soils. The AS soils also produced 69.8% more N_2O and 13.4% more CO_2 when compared to CS soils. The results suggest that the availability of C and N promote R-strategists, leading to increased production of CO_2 and N_2O. Additionally, results also suggest that LUC mediates fluxes depending on resource availability. The findings of this research demonstrate the significance of LUC in shaping N-cycling microbial communities and GHG emissions, emphasizing the importance of transitioning towards less intensive management practices to ensure the long-term sustainability of the agri-food system.

Keywords: perennial, annual, denitrification, nitrification, greenhouse gas, laboratory incubation

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Abbreviations

AC	Active carbon
AOA	Ammonium oxidizing archaea
AOB	Ammonium oxidizing bacteria
AS	Annual cropping system
ATP	Adenosine triphosphate
BD	Bulk density
BG	β-glucosidase enzyme
C	Carbon
CFC	Chlorofluorocarbons
CH_4	Methane
C:N	C to N ratio
CO_2	Carbon dioxide
CS	Converted agricultural system
DNA	Deoxyribonucleic acid
EC	Electrical conductivity
EEA	Microbial Extracellular enzyme activity
FAO	United Nations Food and Agricultural Organization
G	Treatment with glucose
GHG	Greenhouse gas
G+U	Treatment with glucose and urea to obtain C: N ratio of 24:1
IPCC	Intergovernmental Panel on Climate Change
KCl	Potassium chloride
$KMnO_4$	Potassium permanganate
KNO_3	Potassium nitrate

LUC	Land use change
N	Nitrogen
N_2	Dinitrogen
NAG_{ase}	N-acetyl-β-D-glycosaminidase enzyme
NaOH	Sodium hydroxide
NFG	Nitrogen-cycling functional genes
NH_4^+	Ammonium
NH_2OH	Hydroxylamine
NO	Nitric oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
N_2O	Nitrous oxide
O_2	Oxygen
PS	Perennial cropping system
qPCR	Quantitative Polymerase chain reactions
RNA	Ribonucleic acid
SIC	Soil inorganic carbon
SMC	Soil microbial community
SOM	Soil organic matter
U	Treatment with urea
WSA	Water stable aggregates

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Chapter 1: General Introduction

1.1 Context

Since the 1700s, there has been a substantial shift in land use from natural land cover (e.g., forest) to agricultural (e.g., cropland and pasture) (Ramankutty & Foley, 1999; Klein Goldewijk & Ramankutty, 2004). The trend of land use change (LUC) is projected to continue, and this will lead to significant environmental and ecological consequences (Lawler et al., 2014). Some of these include biodiversity loss (Reidsma et al., 2006), climate change (Dale, 1997) and soil health depletion (Tilahun et al., 2022) (e.g., nutrient depletion, erosion, and compaction) (Bekele, 2019; Murty et al., 2002; Turley et al., 2020).

Agricultural LUC can result in the loss of organic matter in the soil, which has serious implications for carbon (C) sequestration and nutrient cycling (Wasis, 2012; Benalcazar et al., 2022). During LUC activities, such as conversion to intensive agriculture, the disturbance of soil can result in the release of greenhouse gases (GHGs), including carbon dioxide (CO_2) and nitrous oxide (N_2O) (United States Environmental Protection Agency [USEPA], 2022). Consequently, GHGs contribute to global warming (Chen et al., 2019; Government of Canada, 2022; USEPA, 2022). The loss of natural ecosystems due to LUC can lead to a reduction in biodiversity, which in turn affects ecosystem services (Reidsma et al., 2006). Soil degradation due to agricultural LUC can also lead to a decline in the abundance of soil microbial communities (SMCs) (Merloti et al., 2019; Liu et al., 2022). Soil microbial communities plays a crucial role in driving the biogeochemical cycling of nitrogen (N) in agroecosystems (Cui et al., 2021; Wang et al., 2021), which holds notable importance because N is a key nutrient required for plant biomass production and physiological functioning and its availability in the soil is a determining factor for crop yield (Anas et al., 2020; Hanrahan & Chan,

2005; Wang et al., 2014; Howarth, 2022). In addition, agricultural LUC may result in increased emissions of nitrous oxide (N_2O), a potent GHG (Bouwman, 1996; Hénault et al., 2012; Ruser & Schulz, 2015). Nitrogen cycling SMCs are the primary drivers of the N-cycle in agricultural systems (Gattinger et al., 2008; Schmitz et al., 2015) and are responsible for N transformation processes including nitrification, and denitrification (Galloway et al., 2008; Stein & Klotz, 2016; Takai, 2019), which play a crucial role in converting N into available forms for plants [nitrate (NO_3^-)] and releasing N_2O into the atmosphere (Isobe & Ohte, 2014; Zhang et al., 2022).

Agricultural LUC, particularly the conversion of perennial cropping systems to annual cropping systems that involve chemical herbicide and fertilizer application, and soil disturbance through tillage can significantly impact the soil N-cycle and result in increased emissions of N_2O (Plaza-Bonilla et al., 2014; Bayer et al., 2015; Žurovec et al., 2017; Wang et al., 2019). Transitioning from a perennial to an annual cropping system through chemical burndown (Kanissery et al., 2019; Peillex & Pelletier, 2020), soil tillage, and fertilization (Ziadi et al., 2014; st. Luce et al., 2022) has the potential to reduce SMC abundance (Shang et al., 2020; Singh et al., 2020) and contribute to soil health depletion (Matson et al., 1997; Pinares-Patiño et al., 2009; Zhou & Butterbach-Bahl, 2014).

The C:N ratio of organic matter inputs can indicate whether N is mineralized or immobilized (Bengtsson et al., 2003; Brust, 2019). This ratio refers to the proportion of C to N in organic matter present (Flavel & Murphy, 2006). Previous literature has shown that the availability and rate of application of an external C and N sources can impact N_2O emissions (Yang et al., 2020). Carbon acts as an energy source for SMCs and N acts as electron acceptor for denitrification (Rivett et al., 2008; Zhu et al., 2015).

When soil is amended with an external C and N source, the amended soil can influence the emission of both CO_2 and N_2O (Liao et al., 2016).

While numerous studies have examined the differences in soil health and N-cycling microbial community size between perennial and annual cropping systems (Devkota et al., 2013; Kraut-Cohen et al., 2020; Sokolowski et al., 2020; Ye et al., 2020), only a limited number of studies have specifically focused on examining the transition from a perennial to an annual cropping system (Panettieri et al., 2013). Assessing soil health and N-cycling SMCs are crucial for evaluating the potential impact of management practices on the overall condition of soil and agricultural LUC related N_2O emissions (Yang et al., 2020; Glaze-Corcoran et al., 2020; Sadras et al., 2020; Muhilan & Chattopadhyay, 2022). Additionally, there is a scarcity of research focusing on the impacts of exogenous addition of C and N sources on GHG emissions associated with LUC. This study will contribute to agricultural land use decision making and will address the gaps in knowledge, which are imperative to advance our understanding of these complex interactions and their implications for agricultural management.

1.2 Thesis format and research objectives

This thesis is structured into five main chapters, following the manuscript style. Each chapter outlines specific objectives, reflecting the complementary yet independent nature of the individual studies. The overall aim of the thesis is to investigate the short-term (<3 months) impact of LUC from perennial to annual cropping systems on soil health parameters and N-cycling microbial community abundance.

Chapter 2 presents a comprehensive literature review of the impacts of agricultural LUC on soil health parameters and the abundance of the N-cycling SMCs, including the conversion from perennial to annual cropping systems, and its relationship to soil health and N-dynamics. **Chapter 3** was a field study focused on the

impact of agricultural LUC, specifically conversion from a perennial to an annual cropping system, on soil health parameters and the gene abundances of the total bacterial community (*16S* rRNA), nitrifying bacterial community (a*moA*), and denitrifying bacterial communities (*nirS*, *nirK*, and *nosZI*). Quantitative polymerase chain reaction (qPCR) assays were used to enumerate these genes. In **Chapter 4,** a laboratory incubation study was conducted to explore the interaction between LUC and N_2O and CO_2 fluxes in the presence of C and N. Furthermore, changes in soil health parameters were characterized over the incubation period.

In summary, this thesis achieves the following objectives:

1. Compare soil health parameters and N-cycling SMC abundances between a perennial vs. an annual cropping system and characterize the short-term (<3 months) impacts of agricultural LUC from perennial to annual cropping systems (**Chapter 3**).
2. Investigate the interaction between LUC and N_2O and CO_2 fluxes in the presence of exogenous C and N (**Chapter 4**).

Chapter 2: Literature Review

Land use refers to how land is utilized, both physically and biologically (Lambin et al., 2000; Liu et al., 2014), and encompasses a wide range of human activities such as urbanization and agriculture (Zhang et al., 2012). According to the United Nations Food and Agricultural Organization ([FAO], 2011), land use is a major determinant of the quality of life for both human populations and other macro and microorganisms. Changes in land use can lead to significant impacts on biodiversity (Reidsma et al., 2006) and the climate (Dale, 1997) through the production of GHGs, which trap and emit heat in the atmosphere and contribute to global warming (Intergovernmental Panel on Climate Change [IPCC], 2007).

2.1 Agricultural land use change

Agricultural land use refers to the practice of utilizing the land for agricultural purposes, including the production of crops (Verburg et al., 2011). Agricultural LUC is a continuous process that has occurred for a significant period through the transformation and modification of land for agricultural purposes. Alterations in the way land is used for agricultural purposes over time are referred to as agricultural LUC (Bekele, 2019). This can include conversions from one type of agricultural land use to another, such as cropland to pastureland, or changes in the intensity of land use, such as the expansion of agricultural production areas into areas previously not used for agricultural purposes (Lambin et al., 2000; Lambin & Meyfroidt, 2011; Liu et al., 2014). The consequences of agricultural LUC can be significant, affecting the quality of life for both human populations and other living organisms. Changes in land use can lead to loss of biodiversity (Reidsma et al., 2006), degradation of soil and water resources (Zhang et al., 2012), and increased GHG emissions (IPCC, 2007).

Agricultural LUC has a direct impact on GHG emissions, making a significant contribution to global warming and the overall changes in the climate (Hale et al., 2006; Winkler, 2021). Greenhouse gases, including CO_2, methane (CH_4) and N_2O, play a crucial role in maintaining the Earth's temperature through the greenhouse effect (Dale, 1997; Ito et al., 2020). However, increased GHG emissions have disturbed the heat balance of the Earth's surface, leading to a rise in global temperatures (IPCC, 2007; IPCC, 2021). This has resulted in changes in precipitation patterns, declining snow cover, rising sea levels, ocean acidification, and global climate change (IPCC, 2013; IPCC, 2021). Among GHGs, N_2O is a particularly significant contributor to global warming and ozone layer depletion (Ravishankara et al., 2009; Ito et al., 2020), with a considerably higher global warming potential (298 – 300 times) compared to CO_2 (baseline at 1) and CH_4 (21-25) (Kudeyarov, 2020). In the last five decades, N_2O levels in the atmosphere have increased by 20% (Tian et al., 2020) with approximately 40% of these emissions being the result of human activities, including agriculture and associated LUC (USEPA, 2021). Moreover, some studies report that N_2O emissions are gradually increasing over time (Galloway et al., 2004; del-Grosso et al., 2009; Syakila & Kroeze, 2011; Tian et al., 2020). The emission of GHGs such as N_2O and CO_2 from agricultural soils is influenced by a variety of environmental factors including temperature, moisture, organic C content, N availability, mechanical disturbance, pH, and soil texture (Oertel et al., 2016).

2.2 Perennial and annual agricultural systems

Cropping systems can be broadly classified into two main categories: perennial and annual. Non-disturbed perennial cropping systems involve growing perennial crops in the soil over multiple years (FAO, 2018; Gerke, 2022). This type of system provides a

stable and continuous source of food and income over the long term, while also contributing to soil conservation and biodiversity. These perennial systems are primarily utilized for cultivating feedstocks for livestock (Gerke, 2022). In contrast, annual cropping systems involve disturbing the soil regularly to plant cash crops for one growing season (FAO, 2018; Blickensdörfer et al., 2022). This type of system can present challenges in management compared to the perennial system, but it may offer increased adaptability in response to dynamic market demands (World Food Summit, 1996; Blickensdörfer et al., 2022). Additionally, they can have higher yields compared to perennial systems and can be easier to rotate with other crops to reduce pest and disease pressures. The practice of annual cropping often requires more intensive management of soils such as regular tillage, application of synthetic fertilizers, and pesticides (Matson et al., 1997; Rubio et al., 2022; Xue et al., 2022). Such increased management can potentially have adverse effects on soil conservation and biodiversity (World Food Summit, 1996; Emmerson et al., 2016; Raven & Wagner, 2021).

Soil tillage is a critical aspect of crop management, particularly when transitioning from perennial to annual cropping systems. This process involves the physical turning of the soil, the burial of surface residues, and the control of weeds and pests to prepare the seed bed (Lobb, 2008; Baumhardt & Blanco-Canqui, 2014; Feng & Balkcom, 2017; Lal, 2021). Soil tillage has a profound effect on soil properties, such as soil organic matter (SOM), structure, nutrient availability, and microorganisms (Plaza-Bonilla et al., 2014; Bayer et al., 2015; Wu et al., 2017; Campanha et al., 2019; Chen et al., 2019). On the other hand, glyphosate (*N*-phosphonomethyl glycine [$C_3H_8NO_5P$]) is widely recognized for its highly effective weed control and non-selective herbicidal properties in agriculture (Tarazona et al., 2017). These properties make it useful for targeting and killing a broad range of plant species, facilitating the

conversion of land from perennial to annual cropping systems (Tarazona et al., 2017; Gillezeau et al., 2019). Glyphosate works by inhibiting a key enzyme that is essential for plant growth and reproduction, leading to the withering and eventual death of the plant. Furthermore, glyphosate is valued for its role as a chemical burndown agent, which assists in the removal of existing crops or forage to prepare fields for planting and facilitates agricultural LUC from perennial to annual cropping systems (Belfry et al., 2015; Gillezeau et al., 2019). Chemical burndown and tillage aid in controlling weeds and pests that may otherwise harm crop yield and productivity.

The conversion from perennial to annual cropping through chemical burndown and tillage is widely recognized as having a significant impact on soil health (Zhang et al., 2022). Glyphosate and tillage use have the potential to modify soil structure, fertility, and abundance of microorganisms, which are all critical components of soil health (Ozlu et al., 2019; Thomas et al., 2019). Studying the impact of agricultural LUC through glyphosate burndown and tillage can provide valuable insights into the changes that occur in soil health and their impact on soil microorganisms. This information can be leveraged to implement measures that mitigate negative impacts, ensuring the preservation of soil resources for future generations and the continued sustainability of agriculture.

2.3 Soil Health

Soil is a complex mixture of biotic and abiotic components, including living organisms, non-living organic material, minerals, water, and air (Cannone et al., 2008; Spath et al., 2014). It serves as a vital medium for plant growth and is a natural resource (Barea, 2015). The interactions between the biotic and abiotic factors result in the development of a dynamic ecosystem that provides essential services such as nutrient cycling and

food production (Meulen et al., 2016; Saccá et al., 2017; Prasad et al., 2021). Soil also serves as a habitat for a diverse range of organisms, from microscopic bacteria and fungi to macroscopic insects and earthworms (Lee & Pankhurst, 1992; Pavao-Zuckerman, 2008; Liu et al., 2019; Altieri, 1999; Tiemann et al., 2015; Bender et al., 2016).

Soil health refers to the capacity of the soil to perform vital ecosystem functions that support the growth and survival of plants, animals, and humans (Glaze-Corcoran et al., 2020; Sadras et al., 2020; Muhilan & Chattopadhyay, 2022). As a plant growth substrate, soil provides crucial nutrients and water for the physiological needs of plants (Kearney & Porter, 2009), making soil health a critical factor for the overall well-being of both plants and animals (Das & Varma, 2010). Agricultural LUC from perennial to annual cropping systems by chemical burn down combined with turning the soil and returning remaining residues via shallow tillage has the potential to negatively impact soil health and contribute to increased GHG emissions and NO_3^- leaching (Matson et al., 1997; Doran, 2002; Pinares-Patiño et al., 2009; de Corato, 2020). Characterizing soil health is essential for assessing the impact of LUC on soil function and developing effective strategies to preserve soil health and to ensure the sustained productivity and viability of agricultural lands over the long term.

2.3.1 Soil health parameters in an agroecosystem

Soil health assessment has gained prominence in the field of sustainable agriculture in recent years (Bünemann et al., 2018). This practice involves evaluating a combination of soil physical, chemical, and biological attributes to determine soil health (Lehmann et al., 2020; Simfukwe et al., 2021), which reflects the soil's ability to provide ecosystem services (Williams et al., 2020). The selection of suitable soil health parameters is important as they should be correlated with the targeted function and

responsive to changes in soil management practices (Table 2.1) (Arshad & Coen, 1992; Wu & McGechan, 1998; Pulleman et al., 2000; Karlen et al., 2003; Andrews et al., 2004; Kunhikrishnan et al., 2012; Kavamura et al., 2019).

Table 2.1: Soil health parameters and their functions.

Category	Soil health parameter	Function	References
Physical parameters	Soil texture	Influences water holding capacity, nutrient retention, soil structure, aeration, and microbial activity, all of which are crucial for plant growth and ecosystem functioning.	Huluka & Miller, 2014
	Soil porosity	Allows for the movement of air and water, promoting soil aeration, drainage, and nutrient uptake by plants. It is recommended to maintain a minimum air-filled porosity of 10% in soil.	Lipiec et al., 2006
	Bulk density	Influences soil compaction, root growth, and water movement, which can affect plant growth, nutrient uptake, and soil erosion. An ideal bulk density for agricultural purposes is typically considered to be equal to or less than 1.3 g cm^{-3}.	Logsdon & Karlen, 2004
	Aggregate stability	Improves soil structure and water infiltration, promotes aeration and root growth, and reduces erosion, which contributes to healthy plant growth and ecosystem functioning. For agricultural purposes, it is	Beare et al., 1994

		desirable for soil to possess a minimum aggregate stability of 50% - 80%.	
	Water holding capacity	Determines the amount of water that can be stored in the soil, which affects plant growth and survival, nutrient availability, and soil erosion. It is recommended to maintain a soil water holding capacity of 60%.	Harding & Ross, 1964
Chemical parameters	Soil organic matter	Improves soil fertility, nutrient cycling, water holding capacity, soil structure, and microbial activity, which are critical for healthy plant growth and ecosystem functioning. Agricultural soils typically exhibit a soil organic matter content ranging from 3% to 6%.	Fließbach et al., 2007
	Soil N content	Influences plant growth, microbial diversity, nutrient cycling, and the production of enzymes and other biochemical compounds that are essential for plant productivity and adaptation. The recommended N content for agricultural purposes is typically 40 ppm.	van Groenigen et al., 2015
	Soil pH and electrical conductivity	Improves soil fertility, nutrient availability, microbial activity, and plant growth and health, making them important indicators of soil health and ecosystem functioning. A pH range of 6-6.5 and an electrical	Miller & Curtin, 1982

		conductivity of ≤ 750 µS m^{-1} are considered favorable indicators of good soil quality for agricultural purposes.	
Biological parameters	Soil active carbon	Labile fraction of carbon in soil serves as an important indicator of soil microbial activity, organic matter decomposition, and the supply of carbon for heterotrophic organisms. A range of 1000 to 3000 mg kg^{-1} of active carbon is often regarded as desirable for agricultural soils.	DuPont et al., 2010
	Soil microbial community abundance, diversity, and composition	Influences nutrient cycling, organic matter decomposition, and disease suppression, making them key factors in regulating soil health, plant productivity, and ecosystem functioning.	Gattinger et al., 2008
	Microbial extracellular enzyme activity	Influences organic matter decomposition and nutrient cycling, making it a key factor in regulating ecosystem functioning and plant growth.	Uwituze et al., 2022

2.4 Nitrogen

Nitrogen is a critical component for all living organisms as it plays a vital role in the synthesis of proteins and nucleic acids (Navarro-González et al., 2001). With its abundant presence as the fourth common element in the environment, N can be found in various forms, including dinitrogen (N_2) which accounts for 78% of the atmospheric gas content (Navarro-González et al., 2001; Robertson & Vitousek, 2009; Stein & Klotz, 2016; Bloch et al., 2020). However, for most organisms, N_2 is largely inaccessible and must be transformed into NH_4^+ to become available for primary producers (Knapp et al., 2012). In addition to N_2 and NH_4^+, N also exists in organic forms, such as amino acids and nucleic acids, and inorganic forms, such as NO_3^- (Fowler et al., 2013; Stein & Klotz, 2016). The transformation of N from one form to another is referred to as the N cycle, and it is essential to ensure the availability of N in the ecosystem (Stein & Klotz, 2016; Takai, 2019).

2.4.1 Nitrogen Cycle

The N-cycle is comprised of five main processes: N-fixation, mineralization, immobilization, nitrification, and denitrification (Stein and Klotz, 2016). Atmospheric N_2 enters the soil during the N-fixation process. While N_2 is inaccessible to plants for direct uptake (Knapp et al., 2012), a group of prokaryotes known as diazotrophs can reduce N_2 to NH_4^+ by using a nitrogenase enzyme complex, which is encoded by the *nifH* gene (Wallenstein & Vilgalys, 2005; Penton et al., 2013; Wang et al., 2017). The process of N-fixation requires a significant amount of energy, in the form of 16 moles of adenosine triphosphate (ATP), which is supplied by plants through a symbiotic relationship (Gupta et al., 2019; Shah et al., 2017). The primary processes that transform N into forms that are usable by plants (NH_4^+ / NO_3^-) and result in the release of N_2O and N_2 are mineralization,

nitrification and denitrification (Pajares & Bohannan, 2016). The transformations of N in these pathways are dependent on the activities of microorganisms, such as bacteria, archaea, and fungi (Galloway et al., 2008; Fowler et al., 2013), and edaphic conditions, such as oxygen availability, moisture content, and C and N availability and access (Masclaux-Daubresse et al., 2010; Huber et al., 2011). Enzymes produced by SMCs catalyze N transformations in the denitrification and nitrification pathways and are encoded by specific N-cycling functional genes (NFGs) (Bothe et al., 2006; Pérez-Álvarez et al., 2013; Pajares & Bohannan, 201).

In agricultural soils, the major sources of N are inorganic synthetic N fertilizers and plant and animal debris (Fuertes-Mendizábal et al., 2018; Castillo-Díaz et al., 2022). Synthetic N fertilizers provide readily available forms of N, such as NO_3^- and NH_4^+, for direct uptake by plants (Pan et al., 2016). On the other hand, the return of organic matter to agricultural soils provides organic N that is subject to degradation through the process of N-mineralization by SMCs (Bengtsson et al., 2003; Butterbach-Bahl & Dannenmann, 2011). This process is essential in breaking down complex organic N into more readily available forms for plant uptake. The energy required for N-mineralization is supplied by SMCs through the degradation of complex organic matter and nutrient absorption (Tarazona & Ramos-Peralonso, 2014). The mineralized N in the form of NH_4^+ can then be absorbed by plants or undergo further transformation reactions (Butterbach-Bahl & Dannenmann, 2011; Risch et al., 2019). Nitrogen immobilization is a process where soil microorganisms consume N for their growth and metabolic processes, leading to a temporary reduction in soil N availability to plants (Bengtsson et al., 2003; Freppaz et al.,

2007). This process ties up N in microbial biomass, making it unavailable for plant uptake until release from cellular death (Dalias & Christou, 2022).

2.4.2 Nitrification

Nitrification (Figure 2.1) is a two-step oxidation process in which NH_4^+ is converted into NO_3^-, with nitrite (NO_2^-) serving as an intermediate (Barnard et al., 2005; Stein & Klotz, 2016). This process is conducted by distinct types of SMCs. The first step, which is the rate-limiting reaction step of the pathway is carried out by ammonia-oxidizers and involves the oxidation of NH_4^+ into NO_2^- via the intermediate hydroxylamine (NH_2OH) (Stams et al., 1991; Stein & Klotz, 2016; Pajares & Ramos, 2019). This reaction requires two enzymes: (i) ammonia monooxygenase, encoded by *amoA* and *crenamoA* in ammonia-oxidizing bacterial (AOB) and ammonia-oxidizing archaea (AOA), respectively (Bothe et al., 2006; Li et al., 2015; Nunes-Alves, 2016; Wang et al., 2021), and (ii) hydroxylamine oxidoreductase, encoded by the *hao* gene (Richardson & Watmough, 1999; Whittaker et al., 2000; Bothe et al., 2006; Pérez-Álvarez et al., 2013).

In anaerobic conditions, nitrite-oxidizing bacteria, such as AOB, can perform nitrifier-denitrification by utilizing NO_2^- as an electron acceptor instead of oxygen (O_2), leading to the production of N_2 or N_2O. During this process, nitric oxide (NO) and N_2O are produced as obligate intermediates and escape from bacterial cells into the atmosphere. The oxidation step in this process occurs due to the action of the hydroxylamine oxidoreductase enzyme (*hao*), which oxidizes NH_2OH (Richardson & Watmough, 1999; Caranto & Lancaster, 2017). Previously, nitrifier-denitrification was thought to be carried out exclusively by AOB species. However, recent research has indicated AOA are also involved in nitrifier-denitrification (Könneke et al., 2005).

The second stage of nitrification involves the oxidation of NO_2^- into NO_3^- by nitrite-oxidizing bacteria (Stams et al., 1991; Pajares & Ramos, 2019;) which is catalyzed by the enzyme nitrite oxidoreductase encoded by *nxr* genes (Norton & Ouyang, 2019; Richardson & Watmough, 1999; Stein & Klotz, 2016). Although this step yields only a minimal amount of energy due to the electrons being partially extracted during the first stage of nitrification, it is essential for completion of the nitrification process (Stams et al.,1990; Richardson and Watmough, 1999; Stein and Klotz, 2016). Nitrite oxidation is energetically unfavorable and requires high activation energy, but without it, toxic NO_2^- can accumulate, inhibiting the growth of other microorganisms involved in N-cycling. Additionally, NO_3^- is a crucial nutrient for many plant species, and completing the nitrification process is necessary for maintaining healthy and productive ecosystems (van Kessel et al., 2015; Norton et al., 2018; Norton & Ouyang, 2019; Pajares & Ramos, 2019).

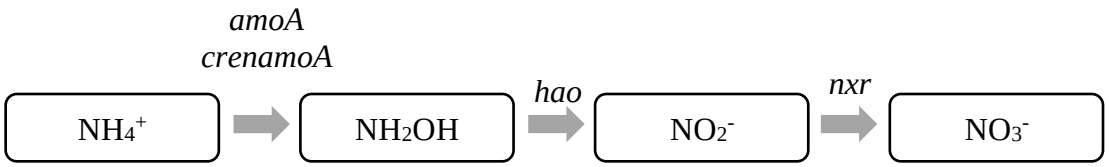


Figure 2.1: Nitrification. Oxidation process of ammonium (NH_4^+) to nitrate (NO_3^-). Genes encoding for nitrification enzymes are shown on the top of each arrow. Ammonium ($NH_4^+ \rightarrow NH_3$) is oxidized via ammonia monooxygenase (encoded by *amoA*) to hydroxylamine (NH_2OH), which is further oxidized via hydroxylamine oxidoreductase (encoded by *hao*) to nitrite (NO_2^-) and to nitrate (NO_3^-) via nitrite oxidoreductase (encoded by *nxr*).

2.4.3 Denitrification

Denitrification (Figure 2.2) is a reduction reaction series that transforms NO_3^- into N_2. This process results in the release of N_2 into the atmosphere and reduces leaching losses of N from the soil (Delwiche & Bryan, 1976; Stein & Klotz, 2016). The end product of denitrification is N_2, however, intermediate gaseous N forms such as NO and N_2O are also produced during this process (Skiba, 2008). When O_2 levels are limiting, facultative and obligate anaerobic SMCs can use denitrification as an alternative respiratory pathway (Bollmann & Conrad, 1998; Richardson & Watmough, 1999; Boyer et al., 2006; Wallenstein et al., 2006; Liou & Madsen, 2008). The denitrification process relies on the presence of C and NO_3^- as essential components. Furthermore, denitrification is particularly important in agriculture, as it helps to reduce the loss of NO_3^- (e.g., applied in fertilizers) from the soil system, which is both harmful and costly (Delwiche and Bryan, 1976; Stein and Klotz, 2016).

The reactions in denitrification are catalyzed by specific enzymes, namely nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase (Richardson and Watmough, 1999; Bothe et al., 2006; Perez-Alvarez et al., 2013). These enzymes are encoded by *nar*, *nir*, *nor,* and *nos* genes respectively in bacterial communities (Bothe et al., 2007; Perez-Alvarez et al., 2013; Wang et al., 2017). The NO_2^- reduction gene, referred to as *nir*, occurs as two distinct types, namely *nirS* and *nirK*, which are functionally equivalent but possess different structural features. The active site of *nirK* is comprised of copper (Cu type), whereas *nirS* contains cadmium as a cofactor (cytochrome-cd1 type) (Lin et al., 2022). Additionally, *nir* genes are present as a single copy per genome, and the simultaneous presence of both the *nirS* and *nirK* genes within a single bacterial species is

a rare occurrence (Sun and Jiang, 2022). The *nosZ* gene encodes the N_2O reductase enzyme, which plays a critical role in the degradation of N_2O in the N-cycle. This gene has been classified into two phylogenetic clades, *nosZI*, and *nosZII*, based on their sequence similarities and evolutionary relationships (Orellana et al., 2014). The inhibition of *nosZII* gene expression by high NO_3^- concentrations could lead to a potential increase in N_2O emissions (Semedo et al., 2021).

The detection of *nar* genes and *nor* genes is also important in understanding the complexities of N-cycling. The *nar* gene encodes the nitrate reductase enzyme, which is fundamental in transforming NO_3^- to NO_2^-, and it consists of various types of enzyme subunits (Richardson and Watmough, 1999; Bothe et al., 2006; Perez-Alvarez et al., 2013). The *nor* gene encodes the nitric oxide reductase enzyme, which plays a crucial role in denitrification by catalyzing the reduction of NO to N_2O (Bothe et al., 2006; Perez-Alvarez et al., 2013). However, high concentrations of NO can have cytotoxic effects (Bothe et al., 2006). Identifying specific *nor* genes responsible solely for NO reduction through denitrification is a challenge, as non-denitrifying organisms may possess *nor* genes with additional functions, such as involvement in pathways beyond denitrification. These difficulties with regards to *nar* and *nor* genes are further compounded by the genetic diversity and low abundance of these genes, making polymerase chain reaction (PCR)-based detection methods less sensitive and less specific (Dandie et al., 2007).

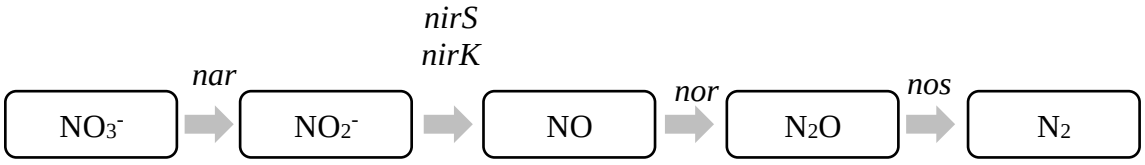


Figure 2.2: Denitrification. Reduction of nitrate (NO_3^-) to dinitrogen (N_2) and the genes that encode specific enzymes. Nitrate (NO_3^-) is reduced to nitrite (NO_2^-) by a nitrate

reductase (encoded by *nar* genes), which is further reduced to nitric oxide (NO) via a nitrite reductase (encoded by *nirS* or *nirK*). NO is then reduced to nitrous oxide (N_2O) by a nitric oxide reductase (encoded by *nor* genes) and in some species, N_2O is further reduced to dinitrogen (N_2) via a nitrous oxide reductase (encoded by *nosZ*).

2.5 Impacts of agricultural land conversion on N-cycling SMCs

The conversion of perennial to annual cropping systems through chemical burndown and tillage modifies the habitats of SMCs (Young and Ritz, 2000; Kraut-Cohen et al., 2020). The composition of these communities is shown to be sensitive to changes in land use (Frøslev et al., 2022). In studies carried out by Smith et al. (2016) and Singh et al. (2021) the effects of soil tillage and crop type on SMC abundances were investigated. Smith et al. (2016) discovered that bacterial communities involved in N-cycling were more abundant in perennial cropping systems when compared to annual cropping systems. Singh et al. (2021) found that soils planted with perennial species have a greater abundance of genes related to N-cycling, specifically *amoA*, and *nirK*, compared to soils with annual cropping. Furthermore, they suggest perennial agricultural systems lead to enhancements in soil structure, organic matter content, and C content, ultimately providing an environment conducive to the growth and activity of SMCs.

Research conducted by Wang et al. (2019) and Grave et al. (2018) investigated the impact of agricultural practices on the abundance of nitrifier and denitrifier genes as well as N_2O emissions. Wang et al. (2019) found no significant difference in bacterial *16S* rRNA gene abundance (representing the size of the total bacterial community) between a perennial system with zero tillage and an annual system with conventional tillage that was planted to winter wheat. However, archaean ammonia oxidizer (*crenamoA*) gene

abundances were highest in the soil from the annual cropping system. In contrast, they observed the lowest bacterial *amoA* gene abundances in the annual system compared to the perennial system, concluding that bacteria and archaeal nitrifying SMCs are differently regulated by the cropping system. Denitrifier *nirK* and *nirS* gene abundances were higher in disturbed soils, whereas *nosZ* gene abundances were highest in non-disturbed soils. On the other hand, Grave et al. (2018) conducted a study to investigate the impact of perennial and annual cropping systems on denitrifying communities in a corn-wheat double-crop rotation field in Brazil. They examined fields that were either tilled or untilled. They quantified bacterial *16S* rRNA, *amoA*, *nirS*, and *nosZ* genes from both annual and perennial agricultural soils and observed no significant differences in the abundance of *16S* rRNA, *amoA*, or *nosZ* genes between cropping systems. However, they did observe a greater abundance of the *nirS* gene in the perennial cropping system than the annual system. Overall, both studies highlight the complexity of the impacts of agricultural practices on SMCs and the potential for these practices to affect soil functioning and GHG emissions.

In Italy, a long-term (23 year) field experiment was conducted to assess the potential impact of non-tilled and tilled fields planted with wheat and faba bean respectively on N_2O emissions from agricultural soils. Researchers found that perennial soils had higher abundances of *16S* rRNA, *amoA*, and *nosZ* genes compared to annual soils. The findings suggest that perennial cropping systems may enhance the abundance of total bacteria and NFGs in soil, which could in turn influence N_2O emissions (Badagliacca et al., 2018). Previous research has also shown that soil disturbance by tillage can affect the size and activity of N-cycling microbial communities. The disturbance caused by tillage may promote the movement of SMCs in the soil or favor their tolerance of such

disturbance, potentially impacting the abundance and diversity of microbial communities (Tirol et al., 2018). These results indicate that differences between perennial and annual cropping systems may impact both the abundance and diversity of soil microbial communities, which could subsequently affect N_2O emissions from agricultural soil (Smith et al., 2016).

A study conducted by Thompson et al. (2016) investigated changes in the population size and community structure of nitrifiers and denitrifiers in annual (corn) and perennial grass (timothy, *Phleum pratense* L.) and legume (alfalfa) mixture cropping systems, as well as after tillage in a perennial cropping system. Authors found significantly higher *nos*Z and *16*S rRNA gene abundances in perennial fields compared to annual fields, but no significant differences in the abundance of *amoA*, *crenamoA*, *nirS*, or *nirK* genes between the two systems were observed. Additionally, *nosZ* gene abundances were significantly decreased in the perennial cropping system after tillage, which was associated with a 10-fold increase in N_2O emissions, while there were no significant changes in the abundance of *16S* rRNA, *amoA*, *crenamoA*, *nirS*, or *nirK* genes. The study results revealed alterations in the compositions of *amoA*, *creanamoA*, *nirK*, and *nosZ*-bearing nitrifier and denitrifier communities after tillage practices. These observations show that different cropping systems might support specific N-cycling SMCs that exhibit resilience to disturbances.

A study by Haney et al. (2002) examined the effect of glyphosate addition on soils from annual cash crop and perennial pasture grass cropping systems in a microcosm study. They found that SMC biomass C and N, as well as C and N mineralization rates, were significantly higher in annual cash crop systems compared to pasture grass systems. This

suggests that the microbial communities in annual cash crop systems may be more efficient in organic matter decomposition. In another microcosm study by Lancaster et al. (2010), the effect of repeated addition of glyphosate on SMC composition was observed. They reported that repeated application of glyphosate resulted in an increase in the incorporation of glyphosate into microbial biomass. In China, a 3-year experiment was conducted on an established 12-year field study to investigate the impact of tillage on *in situ* N_2O emissions following the addition of N-containing fertilizer. The study comprised both perennial cropping systems with no tillage and annual cropping systems with tillage twice a year, which were fertilized with urea and planted to corn. The researchers used the static chamber method to measure *in situ* N_2O emissions. Results indicated that the annual cropping systems had substantially higher N_2O fluxes compared to the perennial cropping systems throughout the study period. Additionally, they noted that application of urea significantly increased the observed N_2O fluxes (Wang et al., 2021).

In the North of China, a two-year experimental study was conducted to investigate the emissions of N_2O from a wheat-maize annual cropping system (Hu et al., 2013). The study encompassed six distinct treatments, including a control treatment with no amendments, a urea-only amendment (following recommended dosage), urea combined with straw, manure combined with urea and straw, urea combined with a nitrification inhibitor, and polymer-coated slow-releasing urea. Emissions of N_2O were measured in the field using the static chamber method. The study revealed that direct N_2O emissions resulting from the recommended application of urea accounted for 0.39% of the annual urea N input in the North China region. Notably, the combination of slow release urea with straw resulted in relatively higher N_2O emissions compared to the other treatments. Hence,

understanding the ways in which agriculture contributes to N_2O emissions, particularly intensified agricultural practices, is important for develop effective strategies to mitigate the impact on the environment and address climate change concerns.

In China, a study was carried out to assess the impact of C and N amendment to the soil via plant residues and fertilizer on CO_2 and N_2O emissions (Huang et al., 2004). The researchers collected soil samples from a wheat-cultivated field and performed a microcosm study by amending plant residues and urea to obtain varying C:N ratios. The study was conducted for a period of 21 days, during which gas fluxes were measured regularly using a modified gas chromatography method. Their findings indicated that the application of soil amendments containing C and N with a C:N ratio of 8:1 resulted in the highest CO_2 and N_2O fluxes, whereas a ratio of 118:1 yielded the lowest fluxes. Furthermore, it was observed that the cumulative gas fluxes increased progressively over time. Therefore, it is important to understand the relationship between soil C and N availability to develop effective strategies for mitigating GHG emissions.

Based on the studies presented, it appears that the abundances of N-cycling SMCs in agricultural soil are influenced by a variety of factors, including crop types, tillage practices, use of synthetic chemicals, and soil physicochemical properties. Different management practices can also affect the abundance of specific microbial gene targets such as *16S* rRNA, *amoA*, *nirS*, *nirK*, and *nosZ*. Previous studies also suggest that perennial cropping systems may have the potential to increase total bacterial and NFG abundances in soil, while chemical burndown and soil disturbance used to transition from perennial to annual agricultural land use can significantly impact SMCs. These findings have implications for soil health, as changes in microbial community composition can affect

nutrient cycling and other ecosystem services provided by soil. However, there is a lack of field studies that have examined the combined impact of chemical burndown and tillage on soil health parameters and the abundance of NFGs (Panettieri et al., 2013). Therefore, further research is needed to better understand the interactions between management practices, microbial communities, and soil health.

Chapter 3: Impacts of agricultural land use change on soil health parameters and N-cycling soil microbial community size.

ABSTRACT

Nitrogen-cycling SMCs play a crucial role in nitrification and denitrification in soils, leading to the emission of N_2O, a GHG that contributes to global warming. These SMCs have the potential to respond to changes in agricultural land use, including the conversion from perennial to annual cropping systems, where chemical burndown, tillage, and fertilization are often applied. In this study, soil health parameters and the abundance of N-cycling SMCs were compared between a perennial and an annual cropping system, and the short-term impacts of agricultural LUC from a perennial to an annual cropping system on these communities were characterized. The study field site was in Stone Mills, Ontario, Canada, and was comprised of four fields. Two annual systems (AS) were regularly cultivated for ten years and planted to corn (*Zea mays* L.) in 2020. The remaining two perennial systems (PS) had not been cultivated for cash crop production in over 50 years; these fields were both orchard grass (*Dactylis glomerata*). One PS was left intact, while the remaining field was converted to an annual cropping (soybean, *Glycine max* L.) within the study period and classified as a 'converted' system (CS). Baseline soil sampling was done on May 14th, 2021, before planting (AS) or conversion of the perennial to an annual system (CS). The AS and CS fields were sprayed with glyphosate, shallowly tilled (5-8 cm in depth), and planted to soybean on May 25th, while one field remained as a PS and was not disturbed. Post-conversion sampling occurred on June 1st, 2021. Late-conversion

sampling occurred mid-growing season on July 21st, 2021. Results indicate PS soils supported higher nitrification (*amoA*, 6.0 ± 0.3 log_{10} gene copies per g dry soil) and denitrification (*nirS*, *nirK* and *nosZI*, 7.8 ± 0.05, 8.1 ± 0.1 and 5.0 ± 0.1 log_{10} gene copies per g dry soil, respectively) gene abundances and better soil health, as evidenced by significantly higher SOM % (13.0%), beta-glucosidase (BG, 26.8%), and N-acetylglucosaminidase (NAGase, 33.3%) activities than AS soils. Furthermore, the conversion of a perennial to an annual system resulted in a significant decrease in SOM % (-45.5%), active carbon (-46.2%), BG activity (-21.9%), and NO_3^- (-42.1%) levels, which paralleled a significant decrease in *amoA*, *nirS*, and *nosZI* gene copies, indicating a potential effect of agricultural LUC on the N_2O/N_2 balance. Overall, results suggest that agricultural LUC from perennial to annual cropping systems can have a significant impact on soil health parameters and N-cycling SMCs in the short-term, highlighting the importance of considering biotic factors when assessing the sustainability of agricultural LUC.

Keywords: perennial, annual, denitrifier, nitrifier, intensification, greenhouse gas

3.1 Introduction

Since the 1700s, there has been a substantial transition from natural land cover, such as forests, to agricultural land use, including croplands and pastures (Ramankutty and Foley, 1999; Goldewijk and Ramankutty, 2004). This historical shift is expected to continue, resulting in notable environmental and ecological impacts including biodiversity decline (Reidsma et al., 2006), contributions to climate change (Dale, 1997) and the depletion of soil health (Tilahun et al., 2022).

A common type of agricultural LUC is the shift from perennial to annual cropping systems. Perennial cropping systems grow crops for several years, with a mixture of grasses and forages intended to feed livestock (Siller et al., 2016; Stewart, 2018; Galindo et al., 2022), while annual cropping systems grow crops for one season and are then replanted each year. While annual cropping systems may involve intensive soil management practices and the application of synthetic fertilizers and herbicides, they can also yield high-value cash crops to meet market demand and ensure profitability (Malézieux, 2012). Several challenges arise concerning the sustainability of continued agricultural LUC efforts aimed at increasing food production achieved through chemical burndown (Kanissery et al., 2019; Peillex and Pelletier, 2020), and tillage (Ziadi et al., 2014; St.Luce et al., 2022). These challenges primarily arise from the potential effects on ecosystem attributes such as soil health parameters (Padbhushan et al., 2022) and SMCs (Schlautman et al., 2021), and on ecosystem processes like biogeochemical cycling. Therefore, it is important to assess soil health and SMC abundances to understand the impacts of agricultural LUC (Busari et al., 2015; Castellini et al., 2019).

The assessment of soil health is a crucial component in understanding and reducing environmental impacts and maintaining the long-term sustainability of agricultural systems (Kinyangi, 2007; Lorenz and Lal, 2016; Hatten and Liles, 2019; Yang et al., 2020). Nitrous oxide, a GHG, is 298 times more potent than CO_2 (baseline at 1) (Kudeyarov, 2020) and is produced during both nitrification (Wrage et al., 2001) and denitrification (Butterbach-Bahl and Dannenmann, 2011) processes that are mediated by SMCs (Isobe and Ohte, 2014; Zhang et al., 2022). Nitrification involves the oxidation of NH_4^+ to NH_2OH by the ammonia monooxygenase enzyme (Sahrawat, 2008; van Groenigen et al., 2015; Wendeborn, 2020) which is encoded by the *amoA* gene in AOB (Offre et al., 2009; Morimoto et al., 2011; Li et al., 2015; Nunes-Alves, 2016). Denitrification involves the reduction of NO_3^- to N_2. In denitrification, NO_2^- is reduced into NO by nitrite reductase enzymes encoded by *nirS* and *nirK* genes (Cabello et al., 2009). Moreover, N_2O is reduced to N_2 in soil by nitrous oxide reductase enzymes encoded by *nosZ* genes (Henry et al., 2006; Orellana et al., 2014). Chemical burndown, fertilization, and tillage practices may affect the soil N-cycle, N-cycling SMCs, and N_2O emissions (Plaza-Bonilla et al., 2014; Bayer et al., 2015; Žurovec et al., 2017; Wang et al., 2019). According to Canada's 2022 National Inventory Report, the agricultural sector in Canada accounts for 75% of annual N_2O emissions. Therefore, investigating potential sources of N_2O emissions is crucial in the development of mitigation strategies.

While numerous studies have examined the differences in soil health and N-cycling SMCs between perennial and annual cropping systems (Devkota et al., 2013; Kraut-Cohen et al., 2020; Sokolowski et al., 2020; Ye et al., 2020), only a limited number of studies have

specifically focused on examining the transition from perennial to annual cropping systems.

In this chapter, the short-term impacts of agricultural LUC from perennial to annual cropping systems were investigated. This study was designed (i) to compare soil health parameters and N-cycling SMC abundances between perennial and annual cropping systems, and (ii) to characterize the short-term (<3 months) impacts of agricultural LUC on soil health parameters and N-cycling SMC abundance. To assess the impact of perennial to annual LUC, the size of the total bacterial community (*16S* rRNA), the bacterial nitrifying community (*amoA*), and select bacterial denitrifying communities (*nirS*, *nirK*, and *nosZI*) were quantified using quantitative polymerase chain reactions (qPCR). I predicted that (i) the prolonged and regular application of chemical herbicides and fertilizers, and the use of tillage in annual cropping systems decrease soil C content and soil health, which impacts the gene abundances of nitrifiers (*amoA*) and denitrifiers (*nirS*, *nirK* and *nosZI*) which contribute to N_2O production and consumption, and (ii) agricultural LUC from a perennial to an annual cropping systems, through chemical burndown and tillage, decrease nitrifier (*amoA*) gene abundance, decrease soil C, and increase the denitrifier *nirS+nirK*/*nosZI* gene ratio that is associated with N_2O emissions. This study's findings can provide valuable insights into the impact of agricultural LUC on soil health parameters and the abundance of NFGs addressing a knowledge gap in understanding the effects of LUC on soil health and GHG emissions.

3.2 Materials and Methods

3.2 1 Site description and soil sampling

The study field site was in Stone Mills, Ontario (44° 44’ 65.17’’ N and 76° 83’58.65’’ W). This location falls within the Great Lakes Central Lowland physiographical region and resides within the mixedwood plains ecoregion. The prevailing climatic conditions within this region were characterized by a mean annual temperature of 7.8°C and a mean annual precipitation of 951.4 mm (Environment and Climate Change, Canada: Kingston Pumping Station, 1981-2010). The prevailing soil composition at the study site was taxonomically classified as the Otonabee series, falling within the Brunisolic Order. This soil order was further categorized as a constituent of the brown forest great soil group, and the degraded brown forest soil group and the family is Otonabee, as per the Canadian system of soil classification. The field site was comprised with four fields (Supplementary figure 3.1). Two annual cropping systems (AS-1 and AS-2) were regularly cultivated for ten years and planted to corn (*Zea mays* L.) in 2020. In the year 2020, urea [$CO(NH_2)_2$] was applied to these annual cropping systems during the corn plantation. These annual cropping systems had a history of being planted with corn and received mono-ammonium phosphate fertilizer (MAP: 12-52-0 NPK ratio) from 2015-2019. The remaining two perennial cropping systems (PS) had not been cultivated for cash crop production in over 50 years and were both orchardgrass (*Dactylis glomerata*) systems. They were managed as non-grazed and non-pasture fields, undergoing an annual harvest. One perennial system was left intact, while the remaining perennial field was planted as an annual (soybean, *Glycine max* L.) system within the study period (in 2021) and classified as a “converted” agricultural system (CS).

Soil sampling was conducted on May 14th (pre-conversion), 2021, before planting (AS) or converting from perennial to annual systems (CS). After pre-conversion sampling, AS-1, AS-2, and CS fields were treated with glyphosate, followed by superphosphate fertilizer (6-32-23 NPK ratio) at 225, 280, and 280 kg per ha, respectively. The two AS fields and the CS field were shallowly tilled (5 to 8 cm in depth) on May 25th, 2021, and planted to soybean, while the PS field remained as a perennial system without any disturbance. Soil sampling was conducted again on June 1st, 2021, in all fields after the conversion process ("post-conversion" sampling). Another round of soil sampling was conducted during the mid-growing season on July 21st, 2021 to monitor any changes in soil properties after the conversion process ("late conversion" sampling). At each sampling date, ten soil cores (0 – 20 cm depth) per transect (pseudo replicates, n=4) were collected within each field. These transects were each 100 meters in length and spaced 100 meters apart. Soil cores were evenly spaced within each transect, and soil samples were composited along each of these transects (Thompson et al., 2016). Efforts were made to maintain consistent factors, including slope aspect. The treeline was not extensive and was located at the edge of the fields. Soils were stored at -20°C until analyses for soil health and extraction of soil DNA.

3.2.2 Soil bulk density

Three soil cores (0-5 cm depth, 2.5 cm diameter) per transect were collected and stored at 4°C for bulk density (BD) analysis. Stones and gravel were removed from cores and the cores were weighed. The soil cores were dried in the oven at 105°C for 24 h and the dry weight was measured to calculate the BD (Equation 3.1) (Erbach, 1987). Volume corrections for stones and gravel were conducted using the displacement method, in which

the volume of water displaced by these materials was measured to determine their actual volume occupied in the soil. These corrected volumes were then utilized to calculate the BD of the soil.

Equation 3.1:

$$\text{Bulk density } (gcm^{-3}) = \frac{\text{Weight of the dry soil (g)}}{\text{Total volume of the soil } (cm^3)}$$

3.2.3 Soil water stable aggregates

To determine the percentage of water stable aggregates (WSA), 4.0 g air-dried soil (<4 mm) was placed on a wet sieving apparatus (Eijkeikamp, Forestry Suppliers Inc., USA) and moistened with deionized water. The tin under each sieve (0.25 mm) was filled with water and the soils were repeatedly mechanically submerged in the water for 3 mins as recommended by the manufacturer. New weighed tins were filled with a dispersion solution, (containing 2 g sodium hexametaphosphate/L for soils with pH > 7 or 2 g sodium hydroxide/L for soils with pH < 7) and were placed under the sieve and the remaining soils were repeatedly submerged until all the soil particles passed through the sieve. The tins which contained the dispersed soil were oven dried at 105°C for 8 h and weighed to calculate the percentage of WSA present in the soil according to Equation 3.2.

Equation 3.2:

$$\text{Water stable fraction (\%)} \frac{\text{Stable soil weight (g)}}{\text{Total soil weight (g)}} \text{ X } 100\%$$

3.2.4 Soil pH and electrical conductivity

For soil pH and electrical conductivity (EC), 10.0 g of air-dried (<2 mm) soil was suspended in 20.0 mL of deionized water. Before the instrument reading, the soil solution was stirred for 30 mins and left to stand for 1 h for pH measurements (Accumet AB150, Fisher Scientific, Ontario, Canada) or 4 h for EC measurements (Laqua, Horiba Instruments Inc., NJ, USA) before taking readings (Miller & Curtin, 1982; Hendershot et al., 2008).

3.2.5 Soil organic matter content

Soil organic matter was estimated following the loss on ignition method (Jensen et al., 2018). For each transect, 5.00 g of oven-dried soil (<2 mm) was placed on a ceramic crucible and placed in the muffle furnace at 550°C for 4 h and re-weighed to estimate SOM.

3.2.6 Soil extractable NO_3^--N

Soil exchangeable NO_3^--N was determined for each of the soil samples by potassium chloride (KCl) extraction (Nelson, 1983). Field moist soil samples (10.0 g) were placed into a 125 mL conical flask and 100.0 mL of 2.0 M KCl was added into each flask. Flasks were stoppered and shaken for 1 h at 160 oscillations per min. The solutions were allowed to settle for 30 mins and filtered through Whatman No.42 filter paper (Whatman Plc, ME, USA). Extractable NO_3^--N was determined colorimetrically with a flow injection analyzer (Lachat Quickchem Flow Injection Analysis System, Hach Sales and Service LP, London, Ontario, Canada). In parallel, a triplicate standard curve was constructed by performing a serial dilution of 200 mg N/L potassium nitrate (KNO_3) to a final concentration of 0.025, 0,05, 0.10, 0.2, 0.50, 1.00, 2 and 10 mg N/L, as recommended by manufacturer.

Furthermore, a KCl reagent blank was run for every four samples. To assess extraction efficiency, a triplicated spike test was performed using a 10 mg N/L KNO_3 solution, yielding a recovery percentage of 99.9%.

3.2.7 Soil extractable NH_4^+-N

The determination of soil exchangeable NH_4^+-N was carried out through the KCl extraction methodology, as outlined in section 3.2.6 (Nelson, 1983). Extractable NH_4^+-N was determined colorimetrically with a Perkin Elmer Lambda 2 UV/VIS Spectrophotometer (Perkin Elmer, Woodbridge, Ontario, Canada) at a wavelength of 667 nm. In parallel, a triplicate standard curve was constructed by performing a serial dilution of 5 mM ammonium sulfate to a final concentration of 50, 100, 150, 200, and 300 μM (Diatloff & Rengel, 2001).

3.2.8 Active Carbon

To measure the labile fraction of C, or "active carbon" (AC) of the soil, the permanganate oxidizable C method was conducted as described by Culman et al. (2012). For each transect, 2.5 g of soil was measured into 50.0 mL falcon tubes containing 18.0 mL of deionized water and 2.0 mL of 0.2 M potassium permanganate ($KMnO_4$). The tube was placed on a horizontal shaker for 2 mins at a rate of 240 oscillations per min, then allowed to settle for 10 mins, and 0.5 mL of the supernatant was transferred to a 50.0 mL falcon tube with 49.5 mL deionized water. A volume of 200 μL was transferred into the wells of the 96-well microplate. In parallel, a standard curve was constructed and triplicated by performing a serial dilution of 0.2 M $KMnO_4$ to final concentrations of 0.005, 0.01, 0.015, and 0.02 M. The absorbances of the samples and standards were measured with an Epoch

spectrophotometer (Biotek Instruments Inc., Winooski, VT) at 550 nm wavelength to determine soil AC.

3.2.9 Soil microbial extracellular enzyme assays

Microbial extracellular enzyme assays (EEAs) for BG and NAGase were performed by creating a soil slurry using 5.0 g of air-dried (<2 mm) soil and 7.5 mL of 50 mM acetate buffer solution. A volume of 150 μL of this soil slurry was pipetted out to a centrifuge tube containing 150 μL of 50 mM acetate buffer. Subsequently, 150 μL of the substrate solutions (*p*NP-β-D-glucopyranoside for BG and *p*NP-β-N-acetylglucosaminide for NAGase) were added to the soil solutions, which were incubated at 37°C for 1 h for BG and 3 h for NAGase. At the end of the incubation period, 100 μL of the supernatant from each soil sample for each enzyme was added to a 96-well microplate containing 10 μL of 1 M NaOH and 190 μL of deionized water. In parallel, a standard curve was constructed by performing a serial dilution of 4-nitrophenol into 50 mM acetate buffer to final concentrations of 0.025, 0.05, 0.1, 0.25, 0.5, and 1 mM (Jackson et al., 2013; Acosta-Martinez et al., 2018). The absorbances of the samples and standards were measured with an Epoch spectrophotometer (Biotek Instruments Inc., Winooski, VT, USA) at 410 nm wavelength.

3.2.10 Soil DNA extraction

Field moist soil (approximately 0.25 g) was used to extract DNA from soils. The DNA was extracted as described in the manufacturer's protocol using the PowerSoil Pro kit (Qiagen, Toronto, Ontario, Canada) as a single replicate per transect to a final volume of 100 μL. Soil DNA extracts were stored at -80°C until use in downstream applications.

3.2.11 Quantification of total and N-cycling functional genes

Quantitative PCR assays were used to enumerate the size of the total bacterial community by targeting the *16S* rRNA gene. The nitrifier community was quantified by targeting ammonium monooxygenase encoded by the *amoA* gene in bacterial communities, and communities of bacterial denitrifiers were quantified by targeting nitrite reductase (*nirS* and *nirK*) and nitrous oxide reductase (*nosZI*) genes, using primer pairs listed in Table 3.1. The gene targets were enumerated on a QuantStudio3 Real-Time PCR system (ThermoFisher Scientific, Markham, Ontario, Canada). The qPCR reaction mixture contained 10 µL of either SsoAdvancedTM Universal SYBR Green Supermix (Bio-Rad Laboratories, Inc.) for bacterial *16S* rRNA, *nirS*, and *nirK*, or PowerUpTM SYBR Green Master mix (ThermoFisher Scientific (Mississauga) Inc.) for *amoA* and *nosZI*, 1 µL of 10 µM of each forward and reverse primer (Table 3.1), 2 µL of DNA template, and nuclease-free water to a final volume of 20 µL. No-template samples were used as a negative control with each qPCR assay. In parallel with triplicated unknown samples, triplicate standard curves were included on each qPCR plate, which were constructed by serially diluting gBlocks™ gene fragments (Integrated DNA Technologies, Inc.) containing the target genes (10^1-10^8 copies). The targeted bacterial *16S* rRNA, *amoA*, *nirS*, *nirK*, and *nosZI* gene blocks were based on published gene sequences that originated from genomic DNA of *Clostridium thermocellum* spp., *Nitrosomonas europaea* spp., *Alcaligenes faecalis* spp., *Pseudomonas aeruginosa* spp., and *Pseudomonas fluorescens* spp. respectively (Thompson et al., 2016). The qPCR assays had efficiencies ranging from 90.0 - 102.1%, with an R^2 ranging from 0.990 - 1.000, and slopes ranging from -3.344 to -3.594. SMC abundances were expressed in gene copy numbers per gram of dry soil.

Table 3.1: Primer pairs used in qPCR assays to enumerate target gene sequences.

Gene	**Forward primer**	**Reverse primer**	**Primer Reference**	**qPCR protocol**	**qPCR protocol Reference**
Bacterial 16S rRNA	338f	518r	Fierer et al., 2005	1 cycle: 98°C 2 min; 40 cycles: 98°C 15 s, 55°C 15 s; Melt curve: 60-95°C	Thompson et al. (2016)
amoA	1F	2R	Rotthauwe et al., 1997	1 cycle: 50°C 2 min; 95°C 10 min; 40 cycles: 95°C 15 s, 57°C 15 s; 72°C 1 min; Melt curve: 60-95°C	Thompson et al. (2016)
nirS	Cd3af	R3Cd	Throback et al., 2004	1 cycle: 98°C 2 min; 40 cycles: 98°C 10 s, 57°C 10 s; Melt curve: 60-95°C	Thompson et al. (2016)

nirK	F1aCu	R3Cu	Hallin et al., 1999	1 cycle: 98°C 2 min; 40 cycles: 98°C 10s, 56°C 10 s; Melt curve: 60-95°C	Thompson et al. (2016)
nosZI	1F	1R	Henry et al., 2006	1 cycle: 50°C 2 min; 95°C 10 min; 40 cycles: 95°C 15 s, 61.5°C 30 s; 72°C 30 s; Melt curve: 60-95°C	Ligi et al. (2014)

3.2.12 Statistical analysis

To determine whether the soil health parameters and N-cycling SMCs differed between perennial and annual cropping systems, analysis of variance (ANOVA) was performed using R Version 4.2.1 (R Core Team 2023) (Integrated Development Environment for R. R Studio, PBC, Boston, MA). The Shapiro-Wilks test was used to test for the normality of data and confirmed the absence of outliers. Soil BD, SOM %, extractable NO_3^--N, NH_4^+-N, and AC, WSA %, pH, EC, BG, and NAGase data were normally distributed, whereas the probability distributions of gene abundance data sets were log-normal or highly skewed, and values were normalized to $\log_{10}$ gene copy number per gram of dry soil. Fixed effects in each dataset were the cropping system (AS, PS), while the transects were considered random effects. Statistical significance was assessed using p values, where the rejection of the null hypothesis (H_0) was determined at $p < 0.05$. To assess the short-term impact of agricultural land conversion from PS to AS systems, ANOVA was conducted as above using a mixed model, where sampling time was considered a repeated measure. Tukey's honestly significant difference was used to compare individual treatment means in all pairwise comparisons. P-values were used to determine significant differences among and between means, with the rejection of the null hypothesis (H_0) determined at $p < 0.05$. Pearson's correlation analysis was conducted to assess the statistical associations between gene abundances and soil health parameters tested. Finally, to visually demonstrate the multiple relationships between soil health parameters, total bacterial gene abundance, and NFG abundances, a multivariate ANOVA (MANOVA) followed by a principal components analysis (PCA) was carried out in R Version 4.2.1 (R Core Team 2023). In the PCA, a scree plot was examined for breaks, and PC components with eigenvalues ≥ 1 (PC1 and PC2) were retained in the 2-dimensional plot for visual analysis.

3.3 Results

3.3.1 Soil health parameters and SMC abundances in perennial and annual cropping systems

In this study, a comparative analysis was conducted between the PS and the AS soils. In the PS soil, pH (6.5 ± 0.02), SOM % (2.3 ± 0.2 %), BG activity (0.41 ± 0.04 mmol g^{-1} dry soil h^{-1}), NAGase activity (0.18 ± 0.03 mmol g^{-1} dry soil h^{-1}), and NO_3^- levels (5.0 ± 2 μg N g^{-1} dry soil) were significantly higher compared to the pH (6.2 ± 0.09), SOM % (2.0 ± 0.1 %), BG activity (0.28 ± 0.03 mmol g^{-1} dry soil h^{-1}), NAGase activity (0.13 ± 0.003 mmol g^{-1} dry soil h^{-1}), and NO_3^- levels (1.3 ± 1 μg g^{-1} dry soil) in the AS soil (Table 3.2). However, in the PS soils, NH_4^+ levels (5.4 ± 2 μg N g^{-1} dry soil) were significantly lower compared to NH_4^+ levels (8.5 ± 1 μg N g^{-1} dry soil) in the AS. There were no significant differences in EC, AC, BD, and WSA % levels between PS and AS soils ($p < 0.05$) (Table 3.2).

Additionally, in the PS soil, gene abundances of the total bacterial community (*16S* rRNA, 9.6 ± 0.1 log_{10} gene copies g^{-1} dry soil), nitrifying community (*amoA*, 6.0 ± 0.3 log_{10} gene copies g^{-1} dry soil), and denitrifying communities (*nirS*,7.8 ± 0.05 log_{10} gene copies g^{-1} dry soil, *nirK*, 8.1 ± 0.1 log_{10} gene copies g^{-1} dry soil, and *nosZI*, 5.0 ± 0.1 log_{10} gene copies g^{-1} dry soil) were significantly higher than the total bacterial community (*16S* rRNA 9.3 ± 0.1 log_{10} gene copies g^{-1} dry soil), nitrifying community (*amoA*, 5.7 ± 0.1 log_{10} gene copies g^{-1} dry soil), and denitrifying communities (*nirS*, 7.7 ± 0.04 log_{10} gene copies g^{-1} dry soil, *nirK*, 7.9 ± 0.1log_{10} gene copies g^{-1} dry soil, and *nosZI*, 4.8 ± 0.1 log_{10} gene copies g^{-1} dry soil) in the AS soil (Table 3.3).

Table 3.2: Comparisons of soil parameters among PS (perennial cropping systems) and AS (Annual cropping systems) at the pre-conversion stage. Parameters are bulk density (BD, gcm^{-3}), pH, electrical conductivity (EC, $\mu S\ cm^{-1}$), active carbon (AC, $mg\ kg^{-1}$), water stable aggregates % (WSA %), soil organic matter content % (SOM %), nitrates (NO_3^-, $\mu g\ NO_3^-N\ g^{-1}$ dry soil), and ammonium ions (NH_4^+, $\mu g\ NH_4^+$-$N\ g^{-1}$ dry soil), and extracellular enzyme activities (EEA) of β-glucosidase (BG, $mmol\ g^{-1}$ dry soil h^{-1}), and N-acetylglucosaminidase (NAGase, $mmol\ g^{-1}$ dry soil h^{-1}). The comparisons were done using one-way ANOVA and the data are means (n=4) ± SE (standard error); parameters indicated as NS are not significantly different ($p < 0.05$).

Parameter	**BD ($g\ cm^{-3}$)** (NS)	**pH**	**EC ($\mu S\ cm^{-1}$)** (NS)	**AC ($mg\ kg^{-1}$)** (NS)	**WSA %** (NS)	**SOM %**	**BG ($mmol\ g^{-1}$ dry soil h^{-1})**	**NAGase ($mmol\ g^{-1}$ dry soil h^{-1})**	**NO_3^- ($\mu g\ N\ g^{-1}$ dry soil)**	**NH_4^+ ($\mu g\ N\ g^{-1}$ dry soil)**
PS	0.92 ± 0.04	6.5^a ± 0.02	104 ± 8	825 ± 25	59 ± 4	2.3^a ± 0 .2	0.41^a ± 0.03	0.15^a ± 0.002	5.0^a ± 2	5.4^b ± 2
AS	1.1 ± 0.1	6.2^b ± 0.09	93 ± 14	818 ± 30	59 ± 7	2.0^b ± 0.1	0.30^b ± 0.02	0.10^b ± 0.003	1.3^b ± 1	8.5^a ± 1

Table 3.3: Mean abundances (log_{10} copies per g dry soil) of total bacterial (*16S* rRNA), nitrifying (*amoA*), and denitrifying (*nirS, nirK,* and *nosZI*) genes and the ratio between *nirS+nirK/nosZI, nir/16S* rRNA *and nosZI/16S* rRNA genes in PS (perennial cropping systems) and AS (annual cropping systems) at the pre-conversion stage. The comparisons were done using one-way ANOVA and the data are means (n=4) ± SE; gene abundances and ratio indicated as NS are not significantly different ($p < 0.05$).

Gene	***16S* rRNA**	***amoA***	***nirS***	***nirK***	***nosZI***	***nirS+nirK/nosZI***	***nirS+nirK/16S* (NS)**	***nosZI/16S***
PS	$9.6^{a} \pm 0.1$	$6.0^{a} \pm 0.3$	$7.8^{a} \pm 0.05$	$8.1^{a} \pm 0.1$	$5.0^{a} \pm 0.1$	$3.3^{a} \pm 0.1$	1.7 ± 0.01	$0.52^{a} \pm 0.01$
AS	$9.3^{b} \pm 0.1$	$5.7^{b} \pm 0.1$	$7.7^{b} \pm 0.04$	$7.9^{b} \pm 0.1$	$4.8^{b} \pm 0.1$	$3.0^{b} \pm 0.1$	1.7 ± 0.02	$0.51^{b} \pm 0.01$

Correlation and multivariate analyses were conducted to assess potential interactions between soil health parameters and SMC gene abundances and showed that total bacterial community size and NFG abundances were positively correlated with SOM (correlation coefficient of 0.50, $p < 0.05$). In addition, there were positive correlations between NO_3^- levels and both *nirS* gene abundance (+0.78) and *nirK* gene abundance (+0.60). Pearson's correlation analysis demonstrated correlations among parameters tested and abundances of total bacterial (*16S* rRNA), nitrifying (*amoA*), and denitrifying (*nirS*, *nirK*, and *nosZI*) genes (Figure 3.1) which prompted the creation of a PCA loading plot (Figure 3.2) to visualize the multivariate relationships among these parameters. The plot revealed that the first two principal components, PC1 and PC2, accounted for 42.2% and 15.7% of the dataset variance, respectively (Figure 3.2). Notably, pH, EC, AC, SOM %, NAGase, NH_4^+, NO_3^-, BD, and gene abundances for the total bacterial community (*16S* rRNA), *amoA*, *nirS*, and *nirK* were identified as significant variables associated with PC1, while WSA %, BG and *nosZI* gene abundances loaded on PC2 (Figure 3.2). The MANOVA analysis (Supplementary table 3.2) yielded significant effects (Pillai index = 0.83) for various soil parameters, including pH, SOM %, EEA (BG and NAGase), NO_3^-, and NH_4^+ levels ($p < 0.05$). Additionally, the analysis revealed significant separation among the groups for total bacterial community abundance and abundances of NFGs including *amoA*, *nirS*, *nirK*, and *nosZI*.

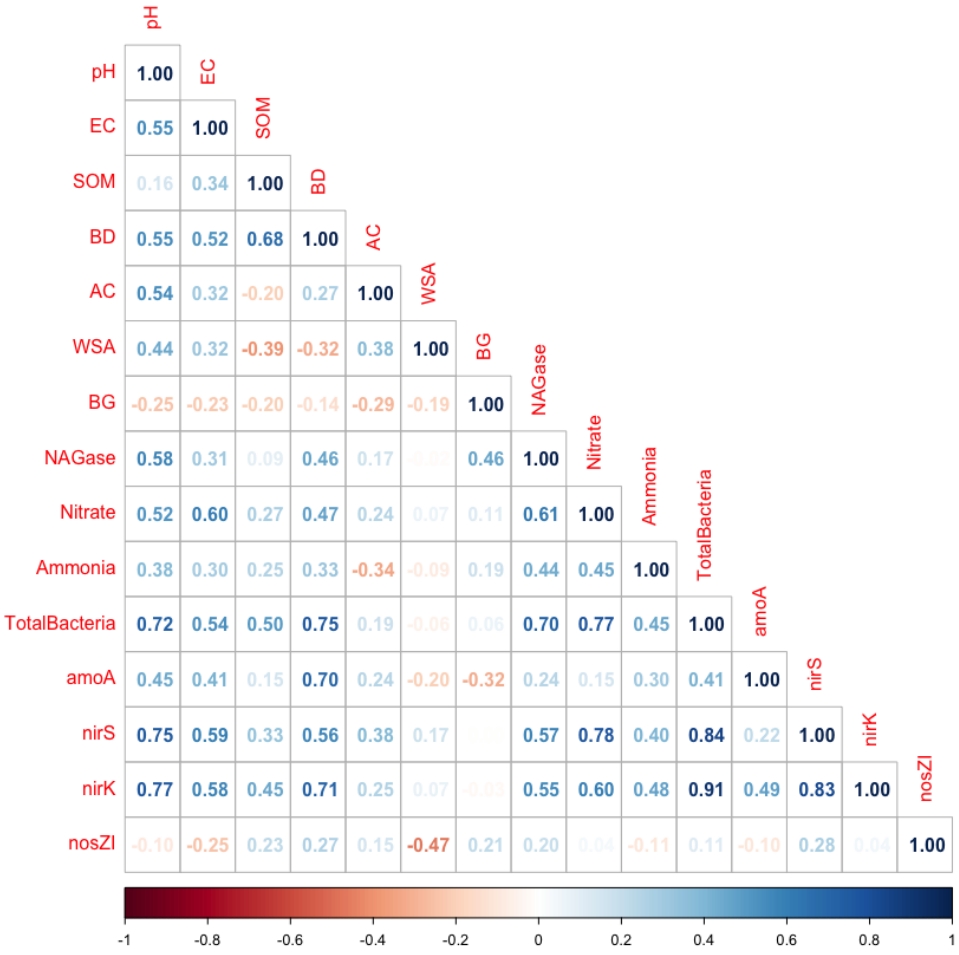


Figure 3.1: Pearson correlation results between soil health parameters- soil pH, electrical conductivity (EC), soil organic matter (SOM), bulk density (BD), active carbon (AC), water stable aggregates (WSA), enzyme activities of β-glucosidase (BG), N-acetylglucosaminidase (NAGase), nitrate ions (Nitrate), ammonium ions (Ammonia), *16S* rRNA abundance ("TotalBacteria"), nitrifying bacterial (*amoA*), denitrifying bacteria (*nirS*, *nirK* and *nosZI*) gene abundances in soils samples in the PS (perennial cropping system) and AS (annual cropping system) before land use change.

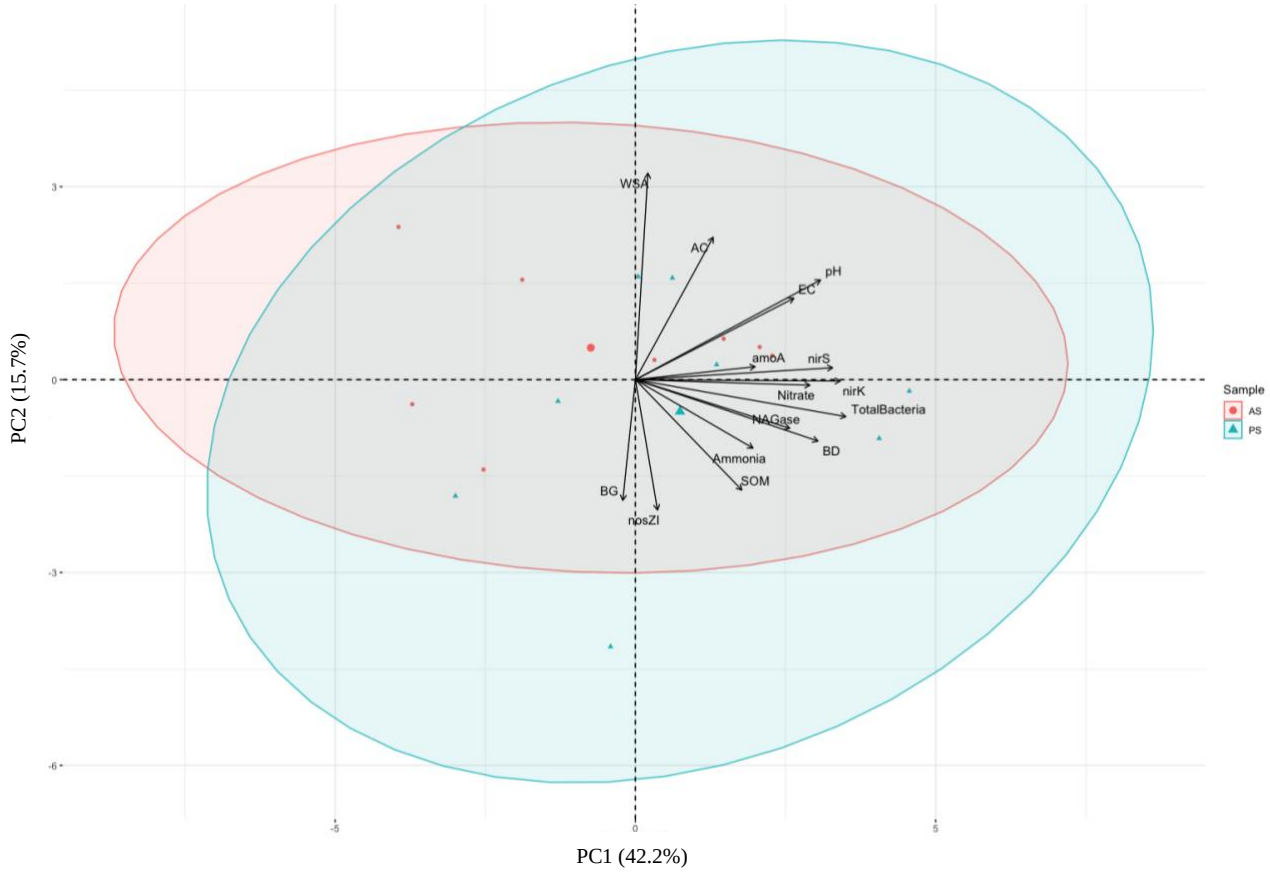


Figure 3.2: A principal component analysis (PCA) of soil parameters in the PS (perennial cropping system) and AS (annual cropping system) before land use change. Variables included were soil pH, electrical conductivity (EC), active carbon (AC), bulk density (BD), water stable aggregates (WSA), soil organic matter (SOM), nitrate ions ("Nitrate"), ammonium ions ("Ammonia"), and enzyme activities of β-glucosidase (BG), N-acetylglucosaminidase (NAGase), total bacterial (16S rRNA, "TotalBacteria"), nitrifying bacterial (*amo*A), denitrifying (*nir*S, *nirK* and *nosZI*) gene abundances. The first two principal components (PC1 and PC2) are shown. Blue triangles represent PS and red circles represent AS. Soil pH, EC, BD, SOM, ammonia, nitrate, NAGase and gene abundances for the total bacterial community (*16S* rRNA), *amoA*, *nirS*, and *nirK* were loaded onto PC1 while, BG and *nosZI* gene abundances loaded onto PC2. The two ellipses represent the two clusters: the blue ellipse illustrate the cluster of PS samples, and the red ellipse illustrates the cluster of AS samples.

3.3.2 Short-term impacts of agricultural land use change on soil health parameters and soil N-cycling SMC abundances

This study focused on the agricultural LUC within the CS field, specifically the transition from a perennial to an annual cropping system. The conversion from a perennial to an annual cropping system did not significantly impact WSA levels (%, $p < 0.05$) (Table 3.4). However, pH and NH_4^+ levels showed a significant increase after conversion from perennial to annual cropping (CS field) (Table 3.4). In contrast, EC, AC, SOM %, and NO_3^- levels decreased significantly following the LUC to an annual cropping system (Table 3.4). The activity of BG significantly decreased immediately after the conversion (post-conversion sampling), which was later restored by the late-conversion sampling date in CS (Table 3.4). On the other hand, NAGase activity showed a significant decrease from pre-conversion to post-conversion sampling dates, followed by a significant increase at the late-conversion sampling date, with notably higher levels than that of the pre-conversion sampling. Bulk density significantly increased after the conversion, which was later restored during the late conversion (mid-growing season) (Table 3.4).

In terms of bacterial community abundance, the total bacterial community (*16S* rRNA) size did not exhibit any significant changes after the conversion from a perennial to an annual cropping system ($p < 0.05$) (Table 3.5). However, nitrifier *amoA* gene abundances and denitrifier *nirS* gene abundances significantly decreased following agricultural LUC (Table 3.5). The *nirK* denitrifier community increased significantly in abundance after LUC but returned to pre-conversion levels later in the growing season, whereas *nosZI* gene abundances significantly decreased and then returned to higher levels later in the growing season (Table 3.5).

Table 3.4: Comparisons of soil parameters among the stages of conversion from perennial to annual cropping system in CS (converted agricultural system) soils at pre-conversion, post-conversion, and late-conversion stages. Parameters are bulk density (BD, g cm^{-3}), pH, electrical conductivity (EC, μS cm^{-1}), active carbon (AC, mg kg^{-1}), water stable aggregates % (WSA %), soil organic matter % (SOM %), nitrates (NO_3^-, μg NO_3^-N g^{-1} dry soil), and ammonium ions (NH_4^+, μg NH_4^+-N g^{-1} dry soil), and extracellular enzyme activities (EEA) of β-glucosidase (BG, mmol g^{-1} dry soil h^{-1}) and N-acetylglucosaminidase (NAGase, mmol g^{-1} dry soil h^{-1}). The comparisons were done using repeated measures ANOVA and the data are means (n=4) ± SE; parameters indicated as NS are not significantly different ($p < 0.05$).

Parameter	BD (g cm^{-3})	pH	EC (μS cm^{-1})	AC (mg kg^{-1})	WSA % (%)	SOM % (%)	BG (mmol g^{-1} dry soil h^{-1})	NAGase (mmol g^{-1} dry soil h^{-1})	NO_3^- (μg N g^{-1} dry soil)	NH_4^+ (μg N g^{-1} dry soil)
Pre-conversion	$0.98^{b} \pm 0.1$	$6.5^{b} \pm 0.02$	$104^{a} \pm 7.7$	$847^{a} \pm 51$	$67^{a} \pm 6.4$	$2.2^{a} \pm 0.2$	$0.32^{a} \pm 0.03$	$0.11^{b} \pm 0.01$	$8.8^{a} \pm 2$	$4.9^{b} \pm 0.6$
Post-conversion	$1.1^{a} \pm 0.0$	$6.5^{b} \pm 0.03$	$94^{ab} \pm 36$	$782^{a} \pm 43$	$65^{a} \pm 4.5$	$2.0^{a} \pm 0.3$	$0.25^{b} \pm 0.02$	$0.07^{c} \pm 0.01$	$7.0^{a} \pm 1$	$8.3^{a} \pm 0.1$
Late conversion	$0.98^{b} \pm 0.0$	$6.7^{a} \pm 0.04$	$53^{b} \pm 3.8$	$456^{b} \pm 74$	$59^{a} \pm 2.3$	$1.2^{b} \pm 0.1$	$0.39^{a} \pm 0.05$	$0.15^{a} \pm 0.02$	$5.1^{b} \pm 1$	$6.2^{b} \pm 0.2$
Sampling stage	*	*	*	*	NS	*	*	*	*	*

*statistical significance of F value at $p<0.05$

Table 3.5: Mean abundances (log_{10} copies per g dry soil) of total bacterial (*16S* rRNA), nitrifying (*amoA*), and denitrifying (*nirS, nirK,* and *nosZI*) genes and the ratio between *nirS+nirK*/*nosZI, nirS+nirK*/*16S* rRNA and *nosZI*/*16S* rRNA genes in CS (converted agricultural system) at the pre-conversion, post-conversion and late-conversion stages. The comparisons were done using repeated measures ANOVA and the data are means (n=4) ± SE; gene abundances and ratio indicated as NS are not significantly different ($p < 0.05$).

Gene	***16S* rRNA**	***amoA***	***nirS***	***nirK***	***nosZI***	***nirS+nirK/nosZI***	***nirS+nirK/16S***	***nosZI/16S***
Pre-conversion	$9.2^{a} \pm 0.08$	$5.8^{a} \pm 0.3$	$7.6^{a} \pm 0.03$	$7.8^{b} \pm 0.09$	$4.7^{a} \pm 0.2$	$3.3^{a} \pm 0.1$	$1.7^{a} \pm 0.00$	$0.51^{a} \pm 0.02$
Post-conversion	$9.3^{a} \pm 0.03$	$5.4^{ab} \pm 0.2$	$5.7^{c} \pm 0.1$	$8.3^{a} \pm 0.04$	$4.1^{b} \pm 0.1$	$2.8^{b} \pm 0.1$	$1.5^{b} \pm 0.02$	$0.44^{b} \pm 0.01$
Late-conversion	$9.4^{a} \pm 0.2$	$5.0^{b} \pm 0.3$	$5.9^{b} \pm 0.1$	$7.8^{b} \pm 0.2$	$4.5^{a} \pm 0.2$	$3.0^{b} \pm 0.1$	$1.5^{b} \pm 0.04$	$0.48^{a} \pm 0.02$
Sampling stage	NS	*	*	*	*	*	*	*

*statistical significance of F value at $p < 0.05$

Correlation and multivariate analyses were conducted to assess potential interactions among soil health parameters and SMC gene abundances. According to Pearson's correlation analysis (Figure 3.3), NH_4^+ and *amoA* gene abundances were positively correlated (+0.59). Additionally, a weak positive correlation was observed between NO_3^- levels, and *nirK* copy numbers (coefficient of +0.13), while a negative correlation was observed between NO_3^- levels and *nirS* gene copies (coefficient of -0.59). Pearson's correlation analysis demonstrated correlations among soil parameters tested and abundances of total bacterial (*16S* rRNA), nitrifying (*amoA*), and denitrifying (*nirS*, *nirK*, and *nosZI*) genes (Figure 3.3), which prompted the creation of a PCA loading plot (Figure 3.4) to visualize the multivariate relationships among these parameters. The plot revealed that the first two principal components, PC1 and PC2, accounted for 40.9% and 19.3% of the dataset variance, respectively (Figure 3.4). Notably, pH, BG, NAGase, NH_4^+, NO_3^-, AC, SOM %, WSA %, EC, and gene abundances for the total bacterial community (total bacteria), and *amoA* abundances were identified as significant variables loading on PC1, while BD, and *nirS*, *nirK*, and *nosZI* gene abundances loaded on PC2 (Figure 3.4). The MANOVA analysis (Supplementary table 3.2) revealed a significant effect (Pillai index = 1.9) across a range of soil parameters including pH, EC, SOM %, BD, AC, BG, NAGase, NH_4^+, NO_3^-, and NFG abundances including *amoA*, *nirK*, *nirS*, and *nosZI* ($p < 0.05$). These results indicate a significant separation among the groups, suggesting distinct differences in the overall pattern of variation across these soil parameters.

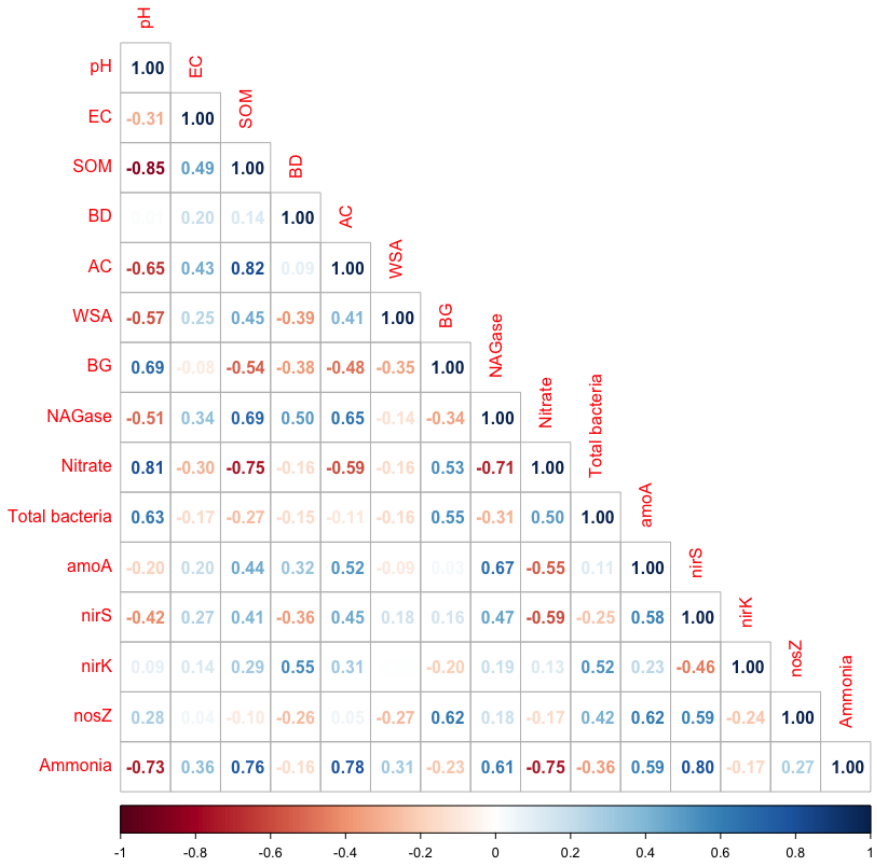


Figure 3.3: Pearson correlation results in soil health parameters after agricultural land conversion from perennial to annual cropping system with the aid of glyphosate burndown and tillage. Soil health parameters- soil pH, electrical conductivity (EC), soil organic matter (SOM), bulk density (BD), active carbon (AC), water stable aggregates (WSA), enzyme activities of β-glucosidase (BG), N-acetylglucosaminidase (NAGase), nitrate ions ("Nitrate"), ammonium ions ("Ammonia"), *16S* rRNA abundance ("Total bacteria"), nitrifying (*amoA*), denitrifying (*nirS*, *nirK* and *nosZI*) gene abundances in soils samples after the in the land use change.

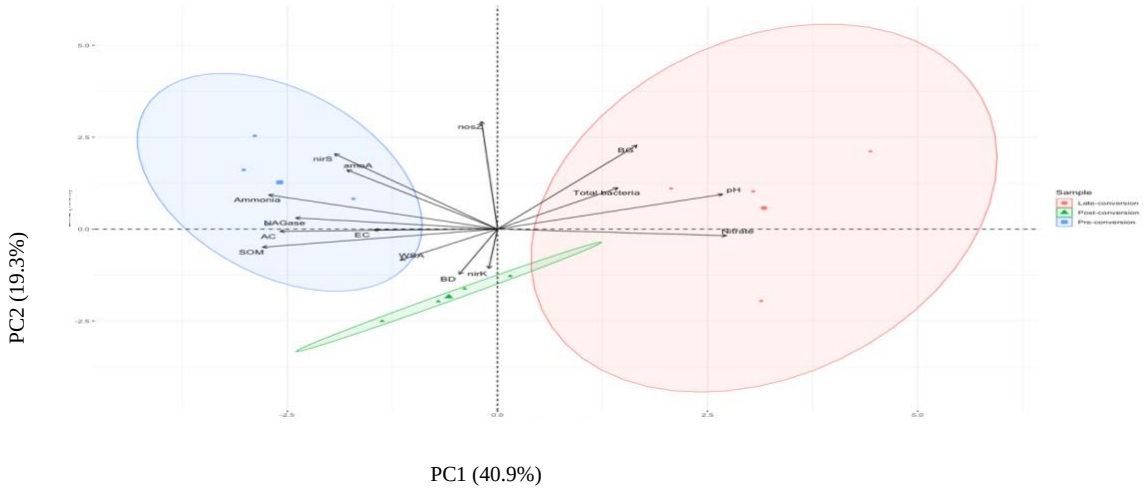


Figure 3.4: A principal component analysis (PCA) of soil parameters after agricultural land conversion from perennial to annual cropping system with the aid of glyphosate burndown and tillage. Variables included were soil pH, electrical conductivity (EC), active carbon (AC), bulk density (BD), water stable aggregates (WSA), soil organic matter (SOM), nitrate ions ("Nitrate"), ammonium ions ("Ammonia"), and enzyme activities of β-glucosidase (BG), N-acetylglucosaminidase (NAGase), total bacterial (*16S* rRNA, "Total bacteria"), nitrifying (*amo*A), denitrifying (*nir*S, *nirK* and *nosZI*) gene abundances. The first two principal components (PC1 and PC2) are shown. Blue squares represent pre-conversion, green triangles represent post-conversion and red circles represent late-conversion. Soil pH, BG, NAGase, NH_4^+, NO_3^-, AC, SOM%, WSA%, EC, and gene abundances for the total bacterial community (*16S* rRNA "Total Bacteria"), and *amoA* were loaded onto PC1 while BD, and gene abundances for *nirS*, *nirK*, and *nosZI* loaded onto PC2. The three ellipses represent the three clusters: the blue ellipse illustrate the cluster of pre-conversion samples, the green ellipse illustrates the cluster of post-conversion samples, and the red ellipse illustrates the cluster of late-conversion samples.

3.4 Discussion

Agricultural LUC from perennial to annual cropping systems can have significant impacts on the physical, chemical, and biological attributes of the soil, as well as on the abundance of SMCs. In this study, I aimed to evaluate differences in soil health and gene abundances of N-cycling SMCs between annual and perennial cropping systems and sought to assess the effects of agricultural LUC from a perennial to an annual cropping system by analyzing soil health and N-cycling SMC abundances in a field pre- and post-conversion.

This study yielded results with two notable observations: (i) more favorable soil health parameters were observed in PS vs. AS soils. Higher SOM levels in the PS provide an energy source for heterotrophic denitrifiers that play a crucial role in N_2O emissions and consumption. Secondly, (ii) the conversion from a perennial to an annual cropping system via chemical burndown and soil disruption resulted in a decreased abundance of the *amoA* gene, which potentially led to reduced nitrification, resulting in lower levels of NO_3^-. Additionally, a decrease in soil C content (SOM % (-45.5%) and AC (-46.1%) was also observed following LUC. This reduction in soil C was associated with a decreased ratio of *nirS+nirK*/*nosZI* genes, and a decreased ratio of *nosZI/16S* rRNA. The findings of this study provide valuable insights into the potential benefits of perennial cropping systems in promoting soil health. Furthermore, these findings highlight the importance of incorporating soil management practices into agricultural systems. Such integration helps both soil conservation and N-cycling, contributing to reduced GHG emissions.

3.4.1 Soil health parameters and SMC abundance in perennial and annual cropping systems

In this study, an assessment was conducted to compare soil health parameters and the abundance of N-cycling SMCs between perennial and annual cropping systems. The PS soils exhibited a significantly higher SOM content (2.3%) compared to the AS (2.0%) soils, aligning with findings from prior research (Hamza & Anderson, 2005; van Eerd et al., 2014; Morrow et al., 2016). However, there were no notable differences observed in AC levels, representing microbially available C, between the two cropping systems. This result contrasts with the research by Thomas et al. (2019), who reported higher AC levels in perennial cropping systems compared to 21-year annual cropping systems in coastal British Columbia. The statistical difference in SOM % between these systems has important implications for soil health and microbial activity. The undisturbed PS soils can accumulate more SOM due to the buildup of surface residues. In contrast, the frequently disturbed AS soils exhibit lower SOM levels. Within the PS soils, orchardgrass contributes N-rich proteins that undergo rapid decomposition, while live roots release organic acids (Angers et al., 1995; Ruan et al., 2019), collectively enhancing SOM %. In contrast, the AS soils contain corn stover residues characterized by high cellulose and lignin content, rendering them more resistant to decomposition (Bolinder et al., 1999).

The higher SOM content in the PS soils serves as a valuable nutrient source for both plants and microbes, providing a habitat and nutrient supply for diverse SMCs, including various bacterial and fungal species. The ample SOM in PS soils ensures energy supply for these SMCs, as evidenced by higher abundance of total bacterial communities (bacterial *16S* rRNA) and heterotrophic denitrifier communities (*nirS*, *nirK*, and *nosZI*)

assessed via qPCR analysis. Furthermore, the PS soils exhibit significantly higher levels of BG and NAGase activities, indicating potentially greater microbial activity and enhanced nutrient acquisition in PS soils (Ekenler & Tabatabai, 2003; Chen et al., 2011). This higher microbial activity can lead to increased biomass production, resulting in higher necromass levels. These factors may have collectively contributed to the observed elevation in SOM % compared to the AS soils (Ruan et al., 2019; Liu et al., 2021).

While significant statistical differences weren't observed when comparing EC, WSA %, and BD between PS and AS soils, a notable difference in pH was detected, with the PS soils exhibiting a significantly higher pH. Despite the relatively small pH difference (0.3 units, equivalent to 0.5 mol dm^{-3} of hydrogen ion $[H^+]$ concentration), both values remained within the recommended range by Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). The pH and EC variations can exhibit considerable influence on soil health and the activities of N-cycling SMCs (Piotrowska-Długosz et al., 2022). These factors impact soil nutrient availability and ion concentrations, consequently affecting the composition and metabolic functions of SMCs, with potential impacts on nutrient cycling dynamics and overall soil health.

Microbial extracellular enzymes play a crucial role in soil processes by driving nutrient cycling, including breaking down SOM, releasing minerals, and transforming N (Stege et al., 2010; Yang et al., 2012). These enzyme activities respond quickly to changes in soil management, like input of fertilizers or tilling, making them a valuable indicator of soil health changes. In the current study, BG which catalyzes the breakdown of complex carbohydrates such as cellulose and hemicellulose into simpler sugars such as glucose (Stege et al., 2010; Vazquez et al., 2019), and NAGase activity which catalyzes N-

mineralization (Yang et al., 2012) which can be used by SMCs as an energy source (Gougoulias et al., 2014) showed higher levels (BG 26.8% and NAGase 33.3%) in the PS than AS soils. These observations aligned with Tyler, (2019) and Vazquez et al. (2019) who similarly reported significantly higher levels of EEAs in perennial cropping systems compared to annual cropping systems that underwent tillage. Microbial EEAs indicate the abundance and functioning of SMCs. The differences in EEAs between PS and AS could be related to differences in nutrient availability between the two systems. PS did not receive synthetic fertilizer inputs, which may have resulted in PS SMCs investing more energy in acquiring nutrients from existing organic sources. Conversely, AS which was regularly exposed to synthetic fertilizers, may have resulted in reduced microbial energy expenditures for nutrient acquisition.

In this study, significantly higher levels of NH_4^+ in the AS (36.5%) compared to the PS soils were observed, while the levels of NO_3^- in the PS (74.0%) were significantly higher compared to the AS soils. This may be due to the higher *amoA* gene abundances in the PS (Hussain et al., 2019; Zhang et al., 2020). The significantly higher abundance of the *amoA* gene associated with nitrifying microorganisms in the PS soils indicates a greater potential for efficient conversion of NH_4^+ to NO_3^- compared to the AS soils resulting lower NH_4^+ and higher NO_3^- levels in the PS soils (Sahrawat, 2008). The higher NAGase activity observed in the PS soils which promotes N-mineralization and the release of NH_4^+ (Das & Varma, 2010; Tabatabai et al., 2010), the higher *amoA* abundance may have contributed to the lower NH_4^+ levels observed in the PS soils (Amoo & Babalola, 2017). The addition of NPK fertilizer to the AS soils stimulate plant growth (Mashamaite et al., 2022), and resulted in higher NH_4^+ levels compared to the PS soils (Jin et al., 2022), which did not

receive any fertilizer application. The external addition of N through fertilizer, along with the natural processes of N-cycling may have contributed to the increased NH_4^+ content in the AS soils.

Conversely, the PS soils exhibited significantly higher levels of NO_3^- compared to the AS soils. This difference can be attributed to lower *amoA* gene abundance and the regular tillage practices in the AS, which have a detrimental impact on soil structure (Castellini et al., 2019) and can potentially lead to leaching of NO_3^- (Beaudoin et al., 2005). While NH_4^+ levels exhibited a significantly higher values in the AS soils, the lower abundance of *amoA*, responsible for NH_4^+ oxidation to NO_3^- in the soil, may have contributed to the observed lower levels of NO_3^- in the AS. Furthermore, it is important to consider that the AS field was mono cropped with corn in the previous year prior to sample collection. Corn is known for its efficient uptake and utilization of NO_3^- (Hussain et al., 2019; Zhang et al., 2020). Therefore, the enhanced NO_3^- uptake by corn plants may have depleted the available NO_3^- in the AS soils, contributing to the observed lower NO_3^- levels. In contrast, the PS soils exhibit a significantly higher abundance of *amoA* genes, resulting in an higher capacity for NH_4^+ oxidation to NO_3^- and an associated higher levels of NO_3^-. Additionally, PS soils having experienced no disturbance, maintained a better soil structure (Zhang et al., 2020), reducing the likelihood of NO_3^- leaching and resulting in higher NO_3^- levels.

Denitrification, which involves the reduction of NO_3^- to N_2 (Hanrahan & Chan, 2005; Cabello et al., 2009; Stein & Klotz, 2016) is an essential process for mitigating environmental NO_3^- pollution (Skiba, 2008). In this study, the PS soil exhibited higher levels of NO_3^- compared to the AS soil, which is necessary for initiating the denitrification

process (Braker et al., 2000; Butterbach-Bahl & Dannenmann, 2011). The higher abundance of *nirS* and *nirK* genes in the PS soil suggests a higher potential for denitrification to occur in the PS soils, whereas lower abundances of *nirS* and *nirK* in the AS soils suggested a lower potential for denitrification to occur (Wrage et al., 2001). Furthermore, the PS soil exhibited a significantly higher abundance of *nosZI* genes, which encode the enzyme responsible for reducing N_2O to N_2, suggesting a higher capacity for potential N_2O reduction in the PS soils compared to the AS soils (Skiba, 2008).

The significantly higher ratio of *nirS+nirK*/*nosZI* genes in the PS soil, compared to the AS soil, indicates a greater capacity for the reduction of NO_3^- to N_2O than N_2O to N_2, consequently leading to higher potential N_2O emissions. However, the *nosZI*/*16S* rRNA gene ratio was significantly higher in the PS compared to AS soils, indicating that a larger proportion of the SMC has the capacity to reduce N_2O to N_2. In contrast, the AS soils exhibited a significantly lower *nirS+nirK*/*nosZI* and *nosZI*/*16S* rRNA ratio, suggesting a potentially lower capacity for N_2O emissions and a potentially lower ability for a complete denitrification. In contrast, the PS soils may feature a potentially active internal N-cycle, ultimately resulting in reduced N_2O.

3.4.2 Short-term impacts of agricultural land use change on soil health parameters and soil N-cycling SMC abundance.

This study results demonstrate the agricultural LUC from a perennial to an annual cropping system by way of shallow tillage of perennial biomass combined with glyphosate application, fertilization and replanting to soybean has an impact on soil health parameters and the abundance of N-cycling SMCs. Although there is a wealth of literature comparing perennial and annual cropping systems, research specifically examining the impacts of

transitioning from a perennial to an annual cropping system on SMCs is scarce (DuPont et al., 2010; Partoazar, 2011).

Results indicate a significant decline in SOM content following the LUC in the CS soils, particularly during the late-conversion phase. Agricultural LUC that includes the application of glyphosate has been demonstrated to have a direct adverse effect on the abundance and composition of SMCs, as reported by Lancaster et al. (2010). However, when synthetic fertilizer is added, providing essential nutrients such as N, P, and K, the microbial population known as R-strategists benefits from these nutrient inputs (Ho et al., 2017). These nutrients act as a stimulus, promoting microbial growth and activity, as microorganisms rely on nutrients for their metabolic processes. The increase in BG and NAGase enzyme levels following the LUC may further enhance microbial activity (Tabatabai et al., 2010; Uwituze et al., 2022). The likely increased activity of R-strategists and BG and NAGase activities in the CS soils may lead to accelerated rates of decomposition. This acceleration can result in a rapid breakdown of organic matter in the soil (Tabatabai et al., 2010). The greater availability of nutrients and energy sources further amplifies microbial activity, potentially leading to increased decomposition and mineralization of organic matter (Flavel & Murphy, 2006). Consequently, this process could contribute to the observed significant reduction in SOM % in the CS soils following the LUC. Additionally, AC may have been utilized by microbes due to higher microbial activity on the topsoil, leading to a decrease (-46.2%) in AC following the conversion. Decreased levels of AC can result in increased competition between the microbes, ultimately favoring the proliferation of heterotrophic nitrifier (*amoA*) and denitrifier (*nirS*, *nirK* and *nosZI*) communities which are best adapted to the new habitat conditions

(Hibbing et al., 2010). Therefore, the observed decrease in gene abundances after LUC may be explained by the competitive exclusion of less adapted microbes.

The current study showed that the transition from a perennial to an annual soybean cropping system had a significant impact on soil NO_3^-, NH_4^+, pH, and EC. The decrease in NO_3^- and increase in NH_4^+ levels observed after the field conversion could be attributed to several factors. The application of NPK fertilizer to the CS field during LUC may have resulted in a significant increase (+41.0%) in NH_4^+ levels compared to the pre-conversion phase (Jin et al., 2022; Mashamaite et al., 2022). However, the abundance of nitrifier genes (*amoA*) was significantly decreased following LUC. This reduction in nitrifier gene abundances suggests a potential decrease in nitrification capacity (Wrage et al., 2001). The combination of increased NH_4^+ levels and decreased *amoA* gene abundance in the CS soils (Wrage et al., 2001; Hart et al., 2018; Feng et al., 2020), likely contributed to the substantial reduction (-42.1%) in NO_3^- levels, indicating a reduced potential for nitrification to occur. The first sampling day after the LUC (post-conversion) revealed a noteworthy increase in BD (+12.2%). Increased soil BD following agricultural LUC can lead to compaction, which reduces the size of soil pores. Compacted soils typically have fewer and smaller pores, limiting the diffusion of O_2 into the soil, which limits available O_2 for the SMCs. Under O_2-limiting conditions, anaerobic microbes, which drive denitrification processes, can become more active. This can increase the potential denitrification, a process that reduces NO_3^- to N_2 (Stein & Klotz, 2016). The abundance of denitrifiers bearing the *nirS* and *nosZI* genes decreased, while the *nirK*-bearing gene abundance increased in compacted soils. This suggests more *nirK*-bearing denitrifiers may contribute to N_2O production after agricultural LUC. A decrease in soil BD following at the late-conversion phase indicates

an increase in soil pore spaces and O_2 availability. This potentially facilitates heterotrophic nitrification processes which increases N_2O emissions (Stein & Klotz, 2016). The findings of this study reveal that there were no significant differences in the WSA % following the LUC from a perennial to an annual cropping system. These results are consistent with previous microcosm studies that also reported no substantial changes in WSA % when glyphosate was applied to intensively managed soils (Panettieri et al., 2013). The presence of pre-incubation artifacts could have influenced this study, contributing to the absence of a difference in the WSA.

The findings of this research indicate that the agricultural LUC resulted in a decrease in the activity of NAGase (-36.4%) and BG (-21.9%) enzymes. To my knowledge, this phenomenon has received limited attention in previous research (Panettieri et al., 2013). The addition of glyphosate and the reduction in SOM % (Stege et al., 2010) may have collectively contributed to the decline in the microbial community responsible for BG and NAGase production, resulting in decreased enzyme activities. Furthermore, the availability of N from added fertilizers may have provided microbes with a readily available N source, potentially reducing their need to produce NAGase (Kraft et al., 2011). There is a relative scarcity of research focused on examining the impacts of LUC on bacterial community abundance (Partoazar, 2011). The absence of significant difference in the abundance of the total bacterial community after the LUC suggests the presence of a diverse range of bacterial species with adaptability to varying conditions, including pH, EC and nutrient availability (Ho et al., 2017). These bacteria can access and utilize available resources, such as nutrients added through fertilizer, for their growth and reproduction. However, due to its universal nature, the quantification of bacterial community via qPCR

of *16S* rRNA targets not only live microbes but also includes dead cells. Furthermore, the presence of multiple copies of the bacterial *16S* rRNA gene per genome (1 to 13 copies) (Větrovský & Baldrian, 2013; Hassler et al., 2022) further complicates the estimation. Therefore, there is a potential risk of overestimating total bacterial community abundance, emphasizing the importance of interpreting the results cautiously.

Following LUC, a notable decline (-13.8%) was observed in the abundance of the nitrifier *amoA* gene. The decrease in *amoA* gene abundance may be due to the application of glyphosate, which has demonstrated direct effects on SMCs (Lancaster et al., 2010). Furthermore, significant changes in the abundance of *nir* genes following the LUC were observed. Specifically, there was a significant decrease in the *nirS* denitrifier abundance while the *nirK* denitrifier abundance significantly increased after LUC. The functions of *nir* genes are similar, however, they vary in sensitivity to management practices and differ in structure (Sun & Jiang, 2022), with *nirS* being more sensitive, whereas *nirK* being less sensitive to environmental changes (Azziz et al., 2017). The rhizosphere effect in agricultural soil (Hou et al., 2018) may have a greater impact on the abundance of *nirS* denitrifiers, suggesting that the transition from orchardgrass to soybean could contribute to a reduction in the *nirS* gene abundance. Conversely, the increase in *nirK* denitrifiers following LUC could be due to the availability of N sources in the soil. It is possible that *nirK* denitrifiers represent K-strategists, characterized by their higher survivability in the environment (Bohn et al., 2014). However, the increased *nirK* community may increase the capacity for potential N_2O production. Additionally, a significant decrease in *nosZI* gene abundance was observed. A significant decrease in *nosZI* abundance may affect the reduction of N_2O to N_2 (Domeignoz-Horta et al., 2015). Furthermore, the significant

decrease in the *nosZI*/*16S* rRNA ratio suggests a potentially reduced capacity for N_2O reduction to N_2, thereby increasing the potential for N_2O emissions following agricultural LUC.

Finally, the abundance of *nirK* and *nosZI* genes in the late-conversion phase returned to levels similar to the pre-conversion phase following LUC. This phenomenon may be related to the ability of SMCs to adapt to the new environment over time (Chase et al., 2021), and the ubiquitous nature of *nir* genes in SMCs (Jacoby et al., 2017). Although glyphosate can directly impact SMCs, its half-life is approximately 30 days, and thus its toxicity diminishes over time (Duke, 2020), allowing microbes to adapt to the new environmental conditions.

3.5 Conclusion

Firstly, in comparing PS and AS soils, the prediction was that the frequent use of chemical herbicides, fertilizers, and tillage in AS would reduce soil C content and overall soil health, consequently lowering the gene abundances of N-cycling SMCs responsible for N_2O emissions. The PS exhibited significantly higher SOM content, serving as an energy source for heterotrophic microbial communities, as evidenced by markedly higher BG and NAGase activities. Moreover, lower NH_4^+ levels and higher NO_3^- levels in the PS suggested higher nitrification potential. Although the *nirS+nirK/nosZI* ratio was notably lower in the AS soils compared to the PS soils, the potential denitrifier community activity in the AS soils may be significantly higher, potentially resulting in higher N_2O emissions. Conversely, the PS soils harbor greater SMC abundances, indicating a more robust internal N-cycle, which may lead to lower N_2O production compared to the AS soils.

Secondly, the impact of agricultural LUC from perennial to annual cropping systems was investigated, with the prediction that this transition, involving chemical burndown and tillage, would decrease the abundance of nitrifier (*amoA*) genes while increasing the *nirS+nirK/nosZI* gene ratio associated with N_2O emissions. The results indicated a reduction in the soil C pool, including SOM and AC, following LUC, with distinct responses among other soil health parameters. Furthermore, the results indicated an increase in NH_4^+ levels and a decrease in NO_3^- levels after LUC which is likely related to the decreased abundance of *amoA*, which plays a pivotal role in nitrification and results in the oxidation of NH_4^+ to NO_3^-. CS exhibited unique trends in BG and NAGase activities, which decreased and then increased after LUC, in contrast to the PS and AS soils collected on the same sampling dates. This difference suggested that the changes were not solely attributable to environmental factors. While the total bacterial community (*16S* rRNA) abundance remained stable, N-cycling SMCs responded differentially to LUC, with an increase in *nirK* and a decrease in *nosZI* abundances, indicating the potential for increased N_2O emissions following agricultural LUC. To establish a direct link between the effects of LUC on the N-cycling SMCs and N_2O to N_2 reduction, future studies measuring N_2O emissions, denitrifier activity (mRNA), and microbial community structure would provide insights into the microbial taxa present, their functional potential, and their interactions within the community.

Appendix:

Supplementary table 3.1: Particle size distributions of sand (50 µm – 2 mm), silt (2 µm – 50 µm), and clay (<2 µm) in PS (perennial cropping system), CS (converted system), and AS (annual cropping system) soils (n=4).

Field	Soil particle size distribution
PS	Sandy Clay (46% Sand, 11% Silt, 43% Clay)
CS	Sandy Clay (54% Sand, 5% Silt, 41% Clay)
AS	Clay (44% Sand, 9% Silt, 47% Clay)

Supplementary table 3.2: Results of a multivariate analysis of variance (MANOVA) comparing the perennial cropping system (PS) and annual cropping system (AS) in the prior to land use change (LUC) and the converted agricultural system (CS) subjected to land use change (post LUC).

	Df	Pillai index	F value	Pr (>F)	
Prior to LUC	1	0.8	2.4	0.18	NS
Post LUC	2	1.9	33.1	3.10E-14	***

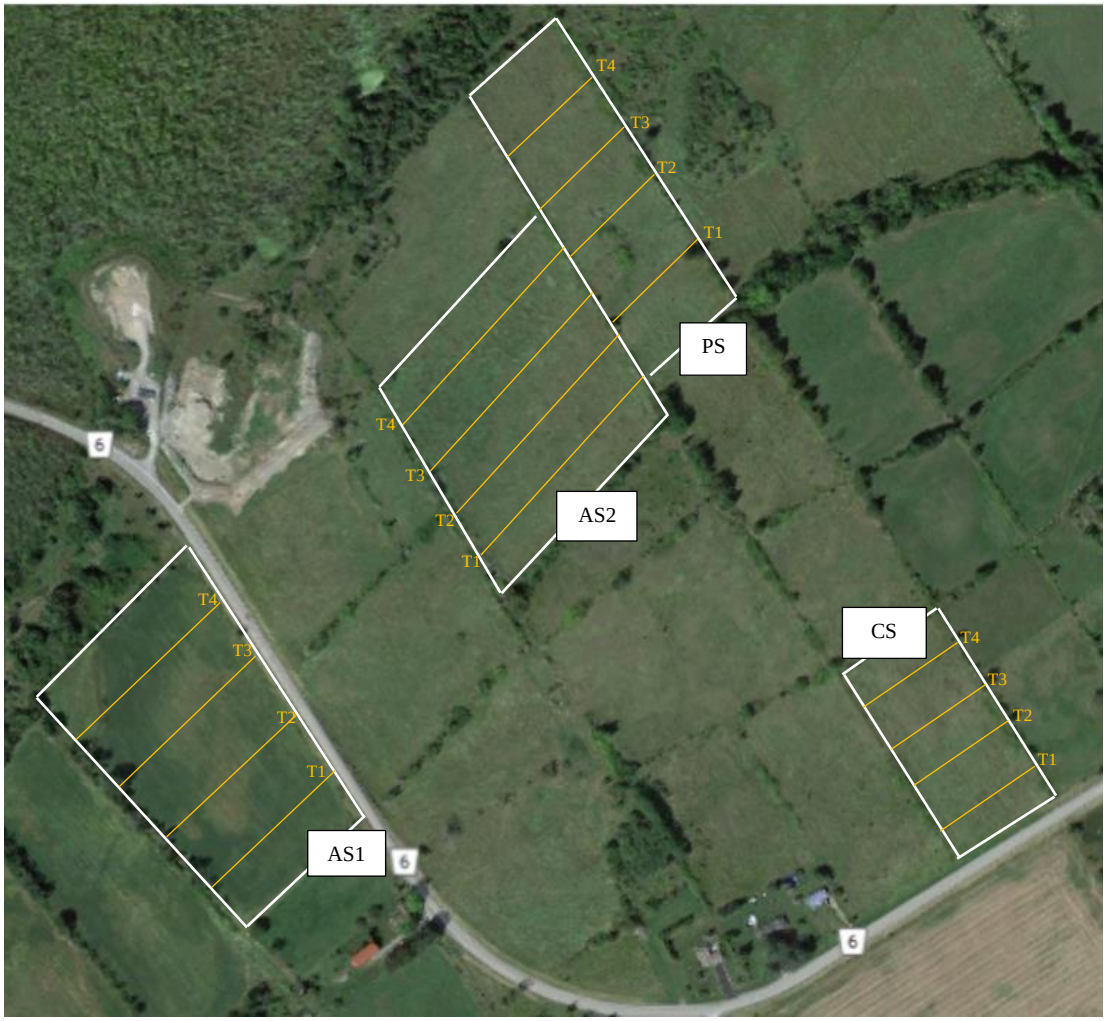
NS indicates there is no significant difference ($p < 0.05$); ***statistical significance of F value at $p < 0.001$.

Supplementary table 3.3: Comparisons of soil parameters among the stages of LUC in PS (perennial cropping system) and AS (annual cropping system) at pre-conversion, post-conversion, and late-conversion stages. Parameters are bulk density (BD, gcm^{-3}), pH, electrical conductivity (EC, $\mu S\ cm^{-1}$), active carbon (AC, $mg\ kg^{-1}$), water stable aggregates % (WSA %), soil organic matter % (SOM %), nitrates (NO_3^-, $\mu g\ NO_3^-N\ g^{-1}$ dry soil), and ammonium ions (NH_4^+, $\mu g\ NH_4^+$-$N\ g^{-1}$ dry soil), and extracellular enzyme activities (EEA) of β-glucosidase (BG, $mmol\ g^{-1}$ dry soil h^{-1}) and N-acetylglucosaminidase (NAGase, $mmol\ g^{-1}$ dry soil h^{-1}). The comparisons were done using repeated measures ANOVA and the data are means (n=4) ± SE; parameters share the same letter are not significantly different ($p < 0.05$).

Field	Sampling stage	BD ($g\ cm^{-3}$)	pH	EC ($\mu S\ cm^{-1}$)	AC ($mg\ kg^{-1}$)	WSA % (%)	SOM % (%)	BG ($mmol\ g^{-1}$ dry soil h^{-1})	NAGase ($mmol\ g^{-1}$ dry soil h^{-1})	NO_3^- ($\mu g\ N\ g^{-1}$ dry soil)	NH_4^+ ($\mu g\ N\ g^{-1}$ dry soil)
PS	Pre-conversion	$1.1^a \pm 0.04$	$6.3^b \pm 0.08$	$64^a \pm 10$	$791^a \pm 36$	$62^b \pm 4$	$2.4^a \pm 0.3$	$0.26^b \pm 0.02$	$0.07^c \pm 0.02$	$1.3^a \pm 0.4$	$5.5^a \pm 0.3$
	Post-conversion	$0.9^b \pm 0.03$	$6.4^a \pm 0.06$	$47^c \pm 8$	$746^b \pm 20$	$86^a \pm 2$	$2.7^b \pm 0.4$	$0.28^{ab} \pm 0.03$	$0.13^b \pm 0.00$	$1.0^a \pm 0.4$	$4.9^a \pm 0.1$
	Late-conversion	$0.9^b \pm 0.3$	$6.4^a \pm 0.07$	$53^b \pm 9$	$750^{ab} \pm 41$	$62^b \pm 7$	$2.0^a \pm 0.3$	$0.37^a \pm 0.03$	$0.16^a \pm 0.01$	$1.0^a \pm 0.2$	$5.3^a \pm 0.2$
AS	Pre-conversion	$1.3^a \pm 0.04$	$6.5^a \pm 0.03$	$93^a \pm 14$	$863^a \pm 33$	$52^b \pm 11$	$1.9^{ab} \pm 0.1$	$0.39^a \pm 0.04$	$0.13^a \pm 0.02$	$2.1^a \pm 0.8$	$9.6^a \pm 0.4$
	Post-conversion	$1.0^b \pm 0.02$	$6.3^b \pm 0.02$	$39^b \pm 6$	$787^a \pm 48$	$76^a \pm 10$	$2.2^a \pm 0.2$	$0.40^a \pm 0.03$	$0.13^a \pm 0.01$	$3.0^a \pm 0.8$	$6.5^a \pm 0.4$
	Late-conversion	$1.1^b \pm 0.05$	$6.5^a \pm 0.04$	$73^a \pm 7$	$681^b \pm 36$	$63^b \pm 4$	$1.2^b \pm 0.1$	$0.41^a \pm 0.05$	$0.10^b \pm 0.01$	$4.0^a \pm 0.6$	$6.6^a \pm 0.5$

Supplementary table 3.4: Mean abundances (log_{10} copies per g dry soil) of total bacterial (*16S* rRNA), nitrifying (*amoA*), and denitrifying (*nirS, nirK,* and *nosZI*) genes in PS (perennial cropping system) and AS (annual cropping system) at the pre-conversion, post-conversion and late-conversion stages. The comparisons were done using repeated measures ANOVA and the data are means (n=4) ± SE; gene abundances share the same letter are not significantly different (p <0. 05).

		Gene abundances (log_{10} copies g^{-1} dry soil)				
Field	**Sampling stage**	***16S* rRNA**	***amoA***	***nirS***	***nirK***	***nosZI***
PS	**Pre-conversion**	$9.7^{a} \pm 0.1$	$4.9^{b} \pm 0.1$	$7.8^{a} \pm 0.1$	$8.2^{b} \pm 0.1$	$5.0^{a} \pm 0.2$
	Post-conversion	$9.9^{a} \pm 0.1$	$4.9^{b} \pm 0.2$	$6.1^{b} \pm 0.2$	$8.2^{b} \pm 0.1$	$4.8^{a} \pm 0.1$
	Late-conversion	$9.7^{a} \pm 0.1$	$5.7^{a} \pm 0.2$	$5.7^{c} \pm 0.1$	$9.0^{a} \pm 0.1$	$4.8^{a} \pm 0.1$
AS	**Pre-conversion**	$9.3^{a} \pm 0.1$	$5.4^{a} \pm 0.6$	$7.9^{a} \pm 0.1$	$7.9^{b} \pm 0.2$	$5.2^{a} \pm 0.2$
	Post-conversion	$9.3^{a} \pm 0.1$	$4.9^{a} \pm 0.4$	$6.1^{b} \pm 0.2$	$7.9^{ab} \pm 0.3$	$5.2^{a} \pm 0.4$
	Late-conversion	$9.6^{a} \pm 0.4$	$5.1^{a} \pm 0.1$	$5.8^{b} \pm 0.1$	$8.7^{a} \pm 0.4$	$5.1^{a} \pm 0.2$



Supplementary figure 3.1: Google Maps. (2023). [County Road 6, Enterprise, Ontario, Canada] [Satellite Map]. Retrieved July, 2023, from https://www.google.com/maps/place/4965+County+Rd+6,+Enterprise,+ON+K0K+1Z0

The sampling site was situated in Stone Mills, Ontario, Canada, and compassed of four distinct fields: PS (perennial cropping systems), CS (converted system, previously a PS and underwent land-use change within the study period), AS1, and AS2 (annual cropping systems). The sampling was conducted along four transects, each spaced approximately 100 m apart from one another.

Chapter 4: Linking land use change with N_2O and CO_2 fluxes in the presence of C and N: An incubation study.

ABSTRACT

Agricultural LUC involving conventional agricultural practices, such as synthetic fertilizer application and tillage can have significant impacts on GHG emissions. In the current laboratory incubation study, I aimed to investigate the interaction between exogenous C and N availability and N_2O and CO_2 fluxes in soils collected from agricultural fields impacted by LUC. The soils used in the incubation study were obtained from a field site located in Stone Mills, Ontario, Canada, consisting of three fields. One annual cropping system (AS) was regularly cultivated and planted to cash crops. One of two perennial cropping systems (PS) was left intact, as a PS system, while the other was converted into an annual cropping system and planted to soybean (*Glycine max* L.) (CS) on May 25th, 2021, after chemical burndown and shallow tillage of perennial biomass. Soil sampling was conducted on October 30th, 2021, during plant maturation, and bulk soil was collected from each field. Soils were placed in mason jars and had either zero exogenous C or N added (control), or had glucose (G), urea (U), or both glucose and urea (G+U) applied (C:N = 24:1) to soils (n=3). The soils were destructively sampled over time to characterize short-term changes in biological soil health indicators (e.g., SOM, AC, extracellular enzyme activities and plant available N levels); a parallel set of jars were used to measure net N_2O and CO_2 fluxes over time. The PS soils emitted the lowest fluxes of net N_2O and CO_2, whereas AS soils produced the highest net fluxes under the given experimental conditions. The net rates of N-mineralization and nitrification were significantly higher in the AS soils. The addition of C and N had a significant impact on the fluxes of N_2O and CO_2 in the PS, CS, and AS soils. Measured soil N_2O fluxes ranged

from 2.1 ± 0.6 to 4.4 ± 1.3 µg N kg^{-1} dry soil m^{-2} s^{-1} across all treatments evaluated, with the G+U treatment having the highest levels of N_2O fluxes of all the treatments. Measured soil CO_2 fluxes ranged from 587 ± 176 to 1900 ± 571 µg C kg^{-1} dry soil m^{-2} s^{-1} across all treatments evaluated, with the G+U treatment having the highest levels of CO_2 fluxes despite soils being exposed to varying degrees of agricultural intensification. The AS soils exhibited the highest levels of N_2O fluxes (4.4 ± 1.3 µg N kg^{-1} dry soil m^{-2} s^{-1}) and CO_2 fluxes (1900 ± 571 µg C kg^{-1} dry soil m^{-2} s^{-1}), followed by CS (max N_2O fluxes of 2.8 ± 0.8 µg N kg^{-1} dry soil m^{-2} s^{-1} and max CO_2 fluxes of 1754 ± 524 µg C kg^{-1} dry soil m^{-2} s^{-1}) and PS (max N_2O fluxes of 2.4 ± 0.7 µg N kg^{-1} dry soil m^{-2} s^{-1} and max CO_2 fluxes 1221 ± 362 µg C kg^{-1} dry soil m^{-2} s^{-1}) soils. These results support the prediction that agricultural land use can have a significant impact on GHG fluxes, and application of exogenous C and N would influence the fluxes of N_2O and CO_2.

Keywords: glucose, urea, annual cropping system, perennial cropping system, nitrification, nitrogen mineralization, soil organic matter

Introduction

Land use encompasses the various ways in which humans utilize or manage land, including agriculture, urban development, and other activities (Ramankutty & Foley, 1999;Ritchie & Roser, 2013; Winkler et al., 2021). Agricultural land use involves cultivating crops and performing other activities related to food production (Dolly et al., 2018), and it is a significant component of land use worldwide, covering roughly 40% of the global land area (USEPA, 2022). The conversion of perennial to annual cropping systems constitutes significant agricultural LUC that can be classified as an intensification strategy (FAO, 2018), and in Ontario often involves the use of chemical burndown and tillage techniques to prepare the soil for planting crops (Lambin et al., 2000; Malézieux, 2012; Bessou et al., 2013; Padbhushan et al., 2022).

The use of these techniques can lead to soil degradation and loss of SOM, which can adversely affect soil health and SMCs (Murty et al., 2002; Bekele, 2019; Kanissery et al., 2019; Turley et al., 2020). In addition, the transition from perennial to annual cropping systems can also contribute to increased GHG emissions into the atmosphere (Burney et al., 2010; Okada et al., 2019) which is a pressing environmental concern (Chen et al., 2019; Government of Canada, 2022; USEPA, 2022) due to global warming, and other manifestations of global climate change (IPCC, 2013; IPCC, 2021). The primary GHGs include CO_2, and N_2O (Hale et al., 2008; Dale et al., 2011; Ito & Inatomi, 2018). Nitrous oxide has a notably higher global warming potential (298-310 times) compared to CO_2 (baseline at 1) (Kudeyarov, 2020), and is the most important GHG contributing to ozone layer depletion in the stratosphere (Ravishankara et al., 2009; Ito & Inatomi, 2018). Furthermore, N_2O emissions predominantly occur through the processes of nitrification (Sahrawat, 2008; Nunes-Alves, 2016) and denitrification (Skiba, 2008), both of which are mediated by SMCs. Soil management practices, including tillage (Bayer et al., 2015), the use of herbicides (van

Bruggen et al., 2021), and the addition of synthetic fertilizers such as urea (Wang et al., 2019; Rubio et al., 2022), have been identified as notable sources of GHG emissions. These practices can influence the activity of SMCs, increasing N_2O emission potential.

Soil C availability plays a significant role in the emission of GHGs (O'Neill et al., 2020). The decomposition of SOM can release C and other nutrients that promote microbial activity, ultimately leading to increased N_2O and CO_2 production (Dijkstra et al., 2010). Additionally, the denitrification and heterotrophic nitrification processes require C as an energy source for microbial growth and activity. Agricultural practices such as tillage and crop residue management can have a significant impact on soil C and N availability and thereby, N_2O and CO_2 emissions (Balesdent et al., 2000). Furthermore, the use of synthetic fertilizers (Kudeyarov, 2020) and soil management practices such as tillage (Bayer et al., 2015), can alter the availability of C:N ratio within the soil. Such modifications can have significant implications for GHG emissions (Brust, 2019).

This chapter outlines the results of a laboratory incubation study that examined the interaction between agricultural LUC and N_2O and CO_2 fluxes in the presence of exogenous C and N. I predicted that agricultural intensification involving the addition of C and N through external amendments, can increase microbial activity, ultimately resulting in elevated fluxes of N_2O and CO_2. To test this, soils (i.e., perennial cropping system [PS], annual cropping system [AS] and converted system [CS]) were amended with glucose as a C source and urea as a N source and a Gasmet Terra5000 Fourier transmission infra-red (FTIR) portable gas analyzer was used to measure N_2O and CO_2 emissions at different time intervals. Additionally, SOM %, microbial extracellular enzyme assays, particularly BG, and NAGase activities and soil AC were measured at the end of the incubation period. The results demonstrated that agricultural soils with higher inputs of exogenous C and N exhibited higher N_2O and CO_2 fluxes compared to soils with no

inputs. Moreover, the amendment of both C and N resulted in elevated GHG fluxes, with the highest fluxes observed in the combined C and N treatment, while the lowest fluxes were recorded in the soil with no amendments. Furthermore, the study results demonstrated that N_2O fluxes were notably higher in the AS soils compared to both CS and PS, whereas CS and PS showed more similar trends. CO_2 fluxes were notably higher in the AS soils, followed by CS and PS. While previous studies have investigated different amendments at the field level (Huang et al., 2002: Huang et al., 2004; Jha et al., 2020), there is a limited number of studies specifically examining the combined effects of simple sugars and N-fertilizer on GHG fluxes during LUC.

4.2 Materials and Methods

4.2.1 Site description and soil sampling

The soils for the incubation study were collected from an operational farm located in Stone Mills, Ontario (44° 44’ 65.17’’ N and 76° 83’58.65’’). This location falls within the Great Lakes Central Lowland physiographical region and resides within the mixedwood plains ecoregion. The prevailing climatic conditions within this region were characterized by a mean annual temperature of 7.8°C and a mean annual precipitation of 951.4 mm (Environment and Climate Change, Canada: Kingston Pumping Station, 1981-2010). The prevailing soil composition at the study site was taxonomically classified as the Otonabee series, falling within the Brunisolic Order. This soil order was further categorized as a constituent of the brown forest great soil group, and the degraded brown forest soil group and the family is Otonabee, as per the Canadian system of soil classification. The field site was comprised of three fields. One annual cropping system (AS) was regularly cultivated for cash crops for ten years and planted to corn (*Zea mays* L.) in 2020 and was fertilized with urea [$CO(NH_2)_2$]. The AS had a history of being planted with corn and received

mono ammonium phosphate fertilizer (MAP: 12-52-0 NPK ratio) from 2015-2019. The remaining two perennial cropping systems had not been cultivated for cash crop production in over 50 years. These fields were both orchardgrass (*Dactylis glomerata*) perennial systems. One perennial system was left intact (PS), while the remaining field was converted to annual cropping (soybean, *Glycine max* L.) and classified as a "converted system" (CS). On May 25th, 2021, glyphosate was applied to the AS and CS fields, while the PS field was left intact. The AS and CS fields were shallowly tilled to a depth of 5-8 cm, superphosphate fertilizer (6-32-23 NPK ratio) was then applied at a rate of 280 kg per ha and planted to soybeans (*Glycine max* L.). Soil sampling was conducted on October 30th, 2021, during the plant maturation stage in the AS and CS fields. Bulk soil samples were collected from a depth of 0–20 cm within each field and transported to Trent University for storage at -20°C until further use.

4.2.2 Experimental design

In this laboratory incubation study, soils (<2 mm) were pre-incubated prior to the start of the experiment. Specifically, 200 g of soil was placed in 1 L mason jars and deionized water was added to adjust the water holding capacity (WHC) to 60% (Harding & Ross, 1964). These mason jars were then incubated at 22°C (Li et al., 2018) for three days before any treatment was introduced. The incubation experiment involved four treatments, each of which was conducted in triplicate using four mason jars: (i) addition of glucose (G) (2.5 g C kg^{-1} dry soil), (ii) addition of urea (U) (0.11 g N kg^{-1} dry soil), (iii) addition of both glucose and urea (G+U), (iv) served as the control and involved no amendments. In the G+U treatment, a C:N ratio of 24:1 was maintained, and the added C and N sources were mixed homogenously with the soil. Soil WHC was also regularly monitored and maintained at 60%, and the soil was packed to mimic the standardized field bulk density (1.1 g cm^{-3}). During the incubation period, the mason jars containing the soils were closed

with their caps and incubated at 22°C, and 24 h intervals, the jar lids were removed to facilitate ventilation. In addition, a set of mason jars with similar treatments were maintained in parallel to monitor net N_2O and CO_2 fluxes regularly. At specific time intervals (0, 6, 12, 24, 48, 72, 96 and 168 h), soils were destructively sampled for laboratory analysis, and GHG emissions were measured using a GT5000 Terra Portable FTIR gas analyzer (Gasmet Technologies Oy, Mestarinitie, Finland). A closed-loop system was used to measure GHG emissions, with a sampling time of 1 min, and continuous sampling was conducted for 5 mins and the net N_2O and CO_2 fluxes were calculated as recommended by the manufacturer (Equation 4.1).

Equation 4.1:

$$\text{Soil } N_2O / CO_2 \text{ fluxes } (\mu\text{mol m}^{-2}\text{ s}^{-1}) = \left\{\frac{C2 - C1}{t2 - t1}\right\} \times \frac{v}{v_a . \frac{T2}{T1}} \times \frac{1}{A}$$

where, C1 is starting concentration of N_2O / CO_2 in ppm, C2 is final concentration of N_2O / CO_2 in ppm, t1 is starting time of the analysis in s, t2 is finishing time of the analysis in s, v is volume of the vessel including cell, sample lines and headspace in m^3, v_a is molar volume for the ideal gas at 273 K, T1 is air temperature in K, T2 is standard temperature in K and A is footprint area of the chamber in m^2.

4.2.3 Soil organic matter content

Soil organic matter was estimated following the loss on ignition method (Jensen et al., 2018). For each transect, 5.00 g of oven-dried soil (<2 mm) was placed on a ceramic crucible and placed in the muffle furnace at 550°C for 4 h and re-weighed to estimate SOM.

4.2.4 Soil extractable NO_3^--N

Soil exchangeable NO_3^--N were determined for each of the soil samples by KCl extraction (Nelson, 1983). Field moist soil samples (10.0 g) were placed into a 125 mL conical flask and 100.0 mL of

2.0 M KCl was added into each flask. Flasks were stoppered and shaken for 1 h at 160 oscillations per min. The solutions were allowed to settle for 30 mins and were filtered through Whatman No.42 filter paper (Whatman Plc, ME, USA). Extractable NO_3^--N was determined colorimetrically with a flow injection analyzer (Lachat Quickchem Flow Injection Analysis System, Hach Sales and Service LP, London, Ontario, Canada). In parallel, a triplicate standard curve was constructed by performing a serial dilution of 200 mg N/L KNO_3 to a final concentration of 0.025, 0.05, 0.10, 0.20, 0.50, 1.00, 2 and 10 mg N/L, as recommended by manufacturer. Furthermore, a KCl reagent blank was run for every four samples. To assess extraction efficiency, a triplicated spike test was performed using a 10 mg N/L KNO_3 solution, yielding a recovery percentage of 99.7%. The net nitrification (NN) was calculated (Equation 4.2) (Hart et al., 2018; Vazquez et al., 2019).

Equation 4.2:

$$\text{Net nitrification (NN)} \left(\mu g\ NO_3^-\text{-N}\ g^{-1}\ d^{-1}\right) = \frac{[NO_3^-\text{-N}_{\text{final}}] - [NO_3^-\text{-N}_{\text{initial}}]}{\text{Days of incubation}}$$

4.2.5 Soil extractable NH_4^+-N

Following the methodology described in section 4.2.4, I employed KCl extractions to determine the levels of exchangeable NH_4^+-N in the soil (Nelson, 1983). In parallel, a standard curve was constructed and triplicated by performing a serial dilution of 5 mM ammonium sulfate to a final concentration of 50, 100, 150, 200, and 300 μM (Diatloff & Rengel, 2001). Extractable NH_4^+-N was determined colorimetrically with a Perkin Elmer Lambda 2 UV/VIS Spectrophotometer (Perkin Elmer, Woodbridge, Ontario, Canada) at a wavelength of 667 nm. The net nitrogen mineralization (NNM) was calculated (Equation 4.3) (Hart et al., 2018; Vazquez et al., 2019).

Equation 4.3:

$$\text{Net N-mineralization (NNM) } (\mu g\ N\ g^{-1}\ d^{-1}) = \frac{[NH_4^+\text{-}N_{final} + NO_3^-\text{-}N_{final}] - [NH_4^+\text{-}N_{initial} + NO_3^-\text{-}N_{initial}]}{\text{Days of incubation}}$$

4.2.6 Active Carbon

To measure the AC the permanganate oxidizable C method was conducted as described by Culman et al. (2012). For each sample, 2.5 g of soil was measured into 50 mL falcon tubes containing 18.0 mL of deionized water and 2.0 mL of 0.2 M $KMnO_4$. The tube was placed on a horizontal shaker for 2 mins at a rate of 240 oscillations per min, then allowed to settle for 10 mins and 0.5 mL of the supernatant was transferred to a separate 50 mL falcon tube with 49.5 mL deionized water. A volume of 200 µL was transferred into the wells of the 96-well microplate. In parallel, a standard curve was constructed and triplicated by performing a serial dilution of 0.2 M $KMnO_4$ to final concentrations of 0.005, 0.01, 0.015, and 0.02 M. The absorbances of the samples were measured with an Epoch spectrophotometer (Biotek Instruments Inc., Winooski, VT) at 550 nm wavelength to determine the AC.

4.2.7 Soil microbial extracellular enzyme assays for β-glucosidase enzyme and N-acetyl-β-D-glucosaminidase enzyme

Microbial EEAs for BG and NAGase were performed by creating a soil slurry using 5.0 g of air-dried (<2 mm) soil and 7.5 mL of 50 mM acetate buffer solution. A volume of 150 µL of this soil slurry was pipetted out to a centrifuge tube containing 150 µL of 50 mM acetate buffer. Subsequently, 150 µL of the substrate solutions (*p*NP-β-D-glucopyranoside for BG and *p*NP-β-N-acetylglucosaminide for NAGase) were added and the soil solutions were incubated at 37°C for 1

h for BG and 3 h for NAGase. By the end of the incubation period, 100 μL of the supernatant from each soil sample was added to a 96-well microplate containing 10 μL of 1 M NaOH and 190 μL of deionized water. In parallel, a standard curve was constructed by performing a serial dilution of 4-nitrophenol into 50 mM acetate buffer to final concentrations of 0.025, 0.05, 0.1, 0.25, 0.5, and 1 mM (Jackson et al., 2013; Acosta-Martinez et al., 2018). The absorbances of the samples were measured with an Epoch spectrophotometer (Biotek Instruments Inc., Winooski, VT) at 410 nm wavelength to determine the microbial extracellular enzyme activity.

4.2.8 Statistical analysis

The statistical analysis was conducted using R Studio 1.4 (R Studio: Integrated Development Environment for R, R Studio, PBC, Boston, MA). To compare soil N_2O fluxes and CO_2 fluxes, among different treatments, repeated measures ANOVA with a linear mixed model was used. To compare SOM %, NNM, NN, AC, BG, and NAGase among different treatments, one-way ANOVA with a generalized linear model was used. Tukey's Honestly Significant Difference test (HSD) was employed to determine the mean separation between control and treated soils, with significance declared at $p < 0.05$. The Shapiro-Wilks test was used to test for the normality of data, confirming the absence of outliers. Significant differences among and between least-square means were identified using p-values, with Ho rejected at $p < 0.05$ unless otherwise stated.

Pearson's correlation analysis was conducted to assess the statistical associations between N_2O fluxes, CO_2 fluxes and soil health parameters tested. Finally, to visually demonstrate the multiple relationships of soil health parameters between treatments a MANOVA followed by a PCA analysis was carried out. In the PCA, a scree plot was examined for breaks, and PC components

with eigenvalues ≥ 1 (PC1 and PC2) were retained for the build-up of the 2-dimensional plot for visual analysis.

4.3 Results:

Measured soil N_2O fluxes ranged from 2.1 ± 0.6 to 4.4 ± 1.3 µg N kg^{-1} dry soil m^{-2} s^{-1} across all treatments evaluated, with the G+U treatment having the highest levels of N_2O fluxes of all the treatments. The AS soils exhibited the highest levels of N_2O flux (4.4 ± 1.3 µg N kg^{-1} dry soil m^{-2} s^{-1}), followed by CS (2.8 ± 0.8 µg N kg^{-1} dry soil m^{-2} s^{-1}) and PS (2.4 ± 0.7 µg N kg^{-1} dry soil m^{-2} s^{-1}) soils, under the specific incubation conditions (Figure 4.1).

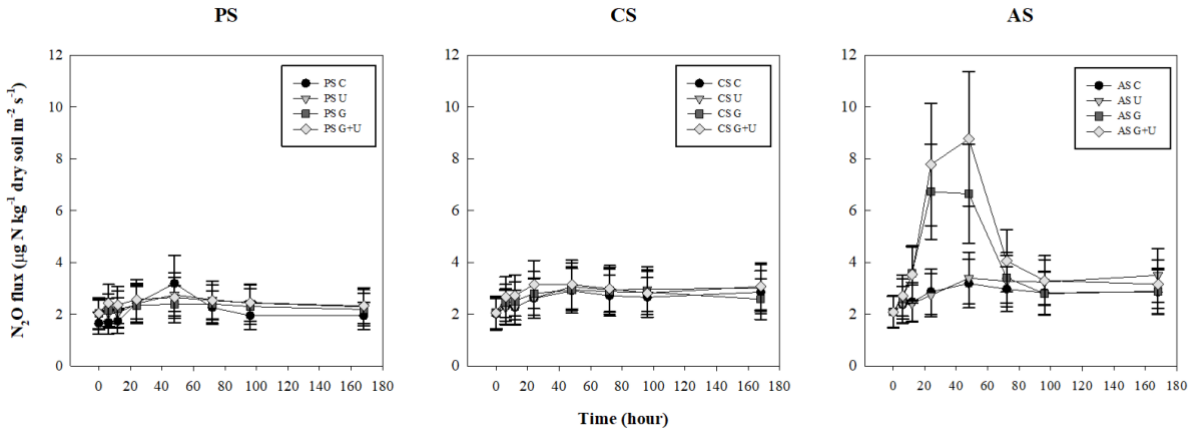


Figure 4.1: Nitrous oxide fluxes (µg N kg^{-1} dry soil m^{-2} s^{-1}) in differently intensified soils over the incubation period (hour). Soils used included soils from PS (perennial cropping system), CS (converted system), and AS (annual cropping system). Treatments included Control (unamended control), G (amendments only with glucose), U (amendments only with urea), and G+U (amendments of glucose and urea to obtain a C: N ratio of 24:1).

Measured soil CO_2 fluxes ranged from 587 ± 176 to 1900 ± 571 µg C kg^{-1} dry soil m^{-2} s^{-1} across all treatments evaluated, with the G+U treatment having the highest levels of CO_2 fluxes

regardless of the varying degrees of agricultural intensification. Furthermore, the AS soils exhibited the highest levels of CO_2 fluxes (1900 ± 571 µg C kg^{-1} dry soil m^{-2} s^{-1}), followed by CS (1754 ± 524 µg C kg^{-1} dry soil m^{-2} s^{-1}) and PS (1221 ± 362 µg C kg^{-1} dry soil m^{-2} s^{-1}) soils, under the specific incubation conditions (Figure 4.2).

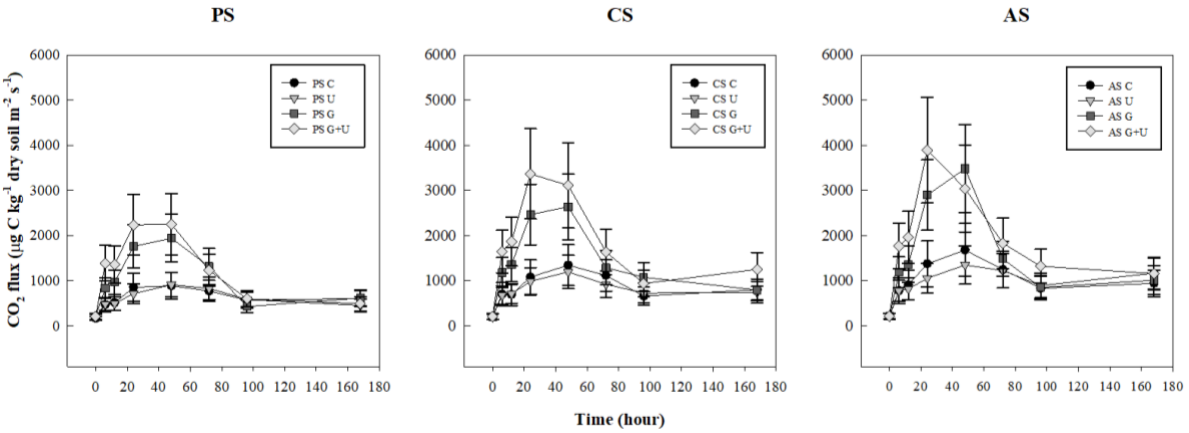


Figure 4.2: Carbon dioxide fluxes (µg C kg^{-1} dry soil m^{-2} s^{-1}) in differently intensified soils over the incubation period (hour). Soils used included soils from PS (perennial cropping system), CS (converted system), and AS (annual cropping system). Treatments included Control (unamended control), G (amendments only with glucose), U (amendments only with urea), and G+U (amendments of glucose and urea to obtain a C: N ratio of 24:1).

There were no significant differences in SOM % between the different treatments in the PS and CS soils (Table 4.1). However, in the AS soil, the G+U treatment soils had significantly higher SOM % compared to the other treatments (Table 4.1). Furthermore, there was no significant difference in SOM % between the PS, CS, and AS soils. The PS soil had significantly higher AC levels compared to both the CS (+9.0%) and AS (+6.1%) treatments, and there were no significant differences observed among the different C/N treatments (Table 4.1). Additionally, there were no

significant differences in AC levels between AS and CS soils or within different C/N treatments in the AS and CS soils (Table 4.1).

There were no significant differences found in BG levels between different C/N treatments in PS and CS soils (Table 4.1). However, in the AS soils, the G+U treatment exhibited significantly higher BG levels compared to the control (+32.7%), U (+26.5%) or G (+24.5%) treatments in the AS soils (Table 4.1). In terms of NAGase activity, no significant differences were found between the PS and AS soils, while the CS soils exhibited the lowest NAGase levels (Table 4.1). Additionally, in the PS soils, there were no significant differences observed in NNM within treatments, whereas, in the CS soils, the G+U treatment showed the highest rates of NNM compared to the other treatments (Table 4.1). In the AS soils, both the U and G+U treatments exhibited the highest rates of NNM compared to the other treatments (Table 4.1). Notably, the AS soils had significantly higher rates of NNM compared to the PS soils in the G+U treatment, which exhibited the lowest rates of NNM (Table 4.1). Furthermore, the G+U treatment exhibited the highest NN rates in the PS and CS soils (Table 4.1)

Table 4.1: Comparisons of soil parameters among PS (perennial cropping systems), CS (converted system), and AS (Annual cropping systems) at the pre-conversion phase. Parameters are soil organic matter % (SOM %), active carbon (AC), extracellular enzyme activities of β-glucosidase (BG, mmol g^{-1} dry soil h^{-1}), N-acetylglucosaminidase (NAGase, mmol g^{-1} dry soil h^{-1}), net N-mineralization (NNM, μg N g^{-1} dry soil d^{-1}) and net nitrification (NN, μg N g^{-1} dry soil d^{-1}). The reported values correspond to the measurements taken at the end of the study. The data are means (n=3) ± SE; parameters share the same letters are not significantly different ($p < 0.05$).

Sample	Treatment	SOM %	AC (mg kg^{-1})	BG (mmol g^{-1} h^{-1})	NAGase (mmol g^{-1} h^{-1})	NNM (μg N g^{-1} d^{-1})	NN (μg N g^{-1} d^{-1})
PS	Control	$3.9^{ab} \pm 0.3$	$1292^{a} \pm 7$	$0.45^{abcd} \pm 0.06$	$0.19^{ab} \pm 0.04$	$2.5^{d} \pm 0.7$	$0.19^{d} \pm 0.1$
	U	$4.0^{ab} \pm 0.3$	$1279^{a} \pm 9$	$0.53^{a} \pm 0.05$	$0.20^{a} \pm 0.05$	$10^{bcd} \pm 2.3$	$0.29^{abcd} \pm 0.1$
	G	$4.0^{ab} \pm 0.3$	$1279^{a} \pm 9$	$0.53^{a} \pm 0.06$	$0.21^{a} \pm 0.05$	$5.6^{d} \pm 1.1$	$0.25^{bcd} \pm 0.1$
	G+U	$4.3^{a} \pm 0.4$	$1302^{a} \pm 10$	$0.54^{a} \pm 0.06$	$0.21^{a} \pm 0.06$	$11^{bcd} \pm 3.5$	$0.82^{a} \pm 0.2$
CS	Control	$3.4^{bcd} \pm 0.2$	$1118^{d} \pm 16$	$0.38^{bcd} \pm 0.02$	$0.10^{c} \pm 0.01$	$6.6^{cd} \pm 3.0$	$0.38^{cd} \pm 0.1$

	U	$3.8^{abc} \pm 0.3$	$1157^{cd} \pm 21$	$0.43^{abcd} \pm 0.02$	$0.11^{bc} \pm 0.01$	$15^{abc} \pm 3.4$	$0.99^{ab} \pm 0.4$
	G	$3.4^{bcd} \pm 0.3$	$1175^{bcd} \pm 22$	$0.50^{abc} \pm 0.04$	$0.11^{bc} \pm 0.01$	$8.9^{bcd} \pm 2.2$	$0.85^{bc} \pm 0.3$
	G+U	$3.9^{ab} \pm 0.3$	$1185^{bcd} \pm 17$	$0.50^{ab} \pm 0.06$	$0.12^{bc} \pm 0.01$	$17^{ab} \pm 4.6$	$1.4^{a} \pm 0.4$
AS	Control	$3.1^{d} \pm 0.1$	$1203^{bcd} \pm 16$	$0.33^{d} \pm 0.02$	$0.15^{abc} \pm 0.02$	$5.3^{d} \pm 1.6$	$0.45^{abc} \pm 0.2$
	U	$3.4^{bcd} \pm 0.2$	$1211^{bcd} \pm 20$	$0.36^{cd} \pm 0.03$	$0.15^{abc} \pm 0.01$	$17^{ab} \pm 4.1$	$1.1^{ab} \pm 0.6$
	G	$3.2^{cd} \pm 0.2$	$1205^{bcd} \pm 32$	$0.37^{bcd} \pm 0.05$	$0.16^{abc} \pm 0.02$	$10^{bcd} \pm 2.3$	$0.86^{abcd} \pm 0.6$
	G+U	$3.9^{ab} \pm 0.3$	$1223^{b} \pm 9$	$0.49^{ab} \pm 0.06$	$0.17^{abc} \pm 0.02$	$22^{a} \pm 6.1$	$1.7^{a} \pm 0.7$

Pearson's correlation analysis demonstrated a number of correlations among parameters tested and CO_2 and N_2O fluxes (Figure 4.3). There was a positive correlation between N_2O fluxes and both NNM and NN with correlation coefficients of +0.66 and +0.75. Moreover, a positive correlation was found between NNM and NN with a correlation coefficient of +0.93. Additionally, there were positive correlations between CO_2 and N_2O fluxes with a correlation coefficient of +0.73. A PCA (Figure 4.4) was conducted to visualize the correlations among soil health parameters (SOM %, AC, BG, NAGase, NNM, and NN) and CO_2 and N_2O fluxes in different treatments/soil types. The first two principal components, PC1 and PC2 accounted for 50.7% cumulative variance. Fluxes of N_2O, CO_2 and NNM, NN, SOM and BG loaded on to PC1, while AC and NAGase loaded on to PC2. The MANOVA analysis (Pillai index = 0.64, $F = 19.1$, $p < 0.001$) yielded significant multivariate effects for various soil parameters, including EEA (BG and NAGase), NN, and NNM ($p < 0.05$). Additionally, the analysis revealed significant separation among N_2O and CO_2 fluxes.

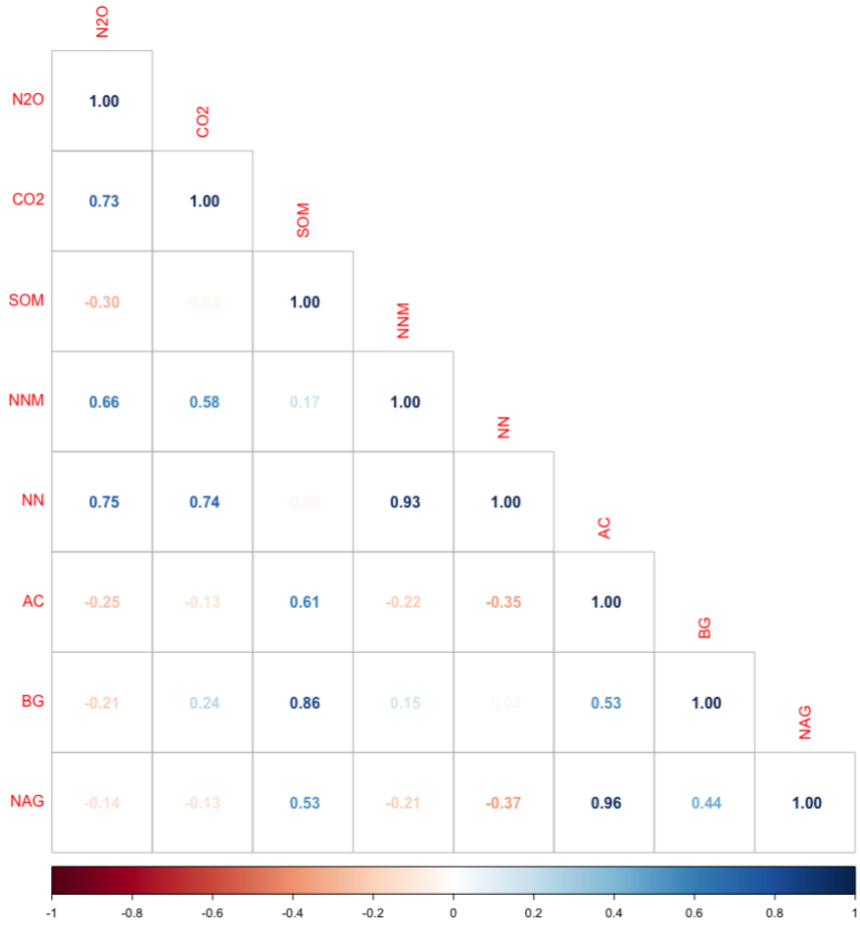


Figure 4.3: Pearson arson correlation of N_2O and CO_2 fluxes and soil health parameters, namely active carbon (AC), soil organic matter (SOM), net N-mineralization (NNM), net nitrification (NN), and enzyme activities of β-glucosidase (BG), and N-acetylglucosaminidase (NAGase).

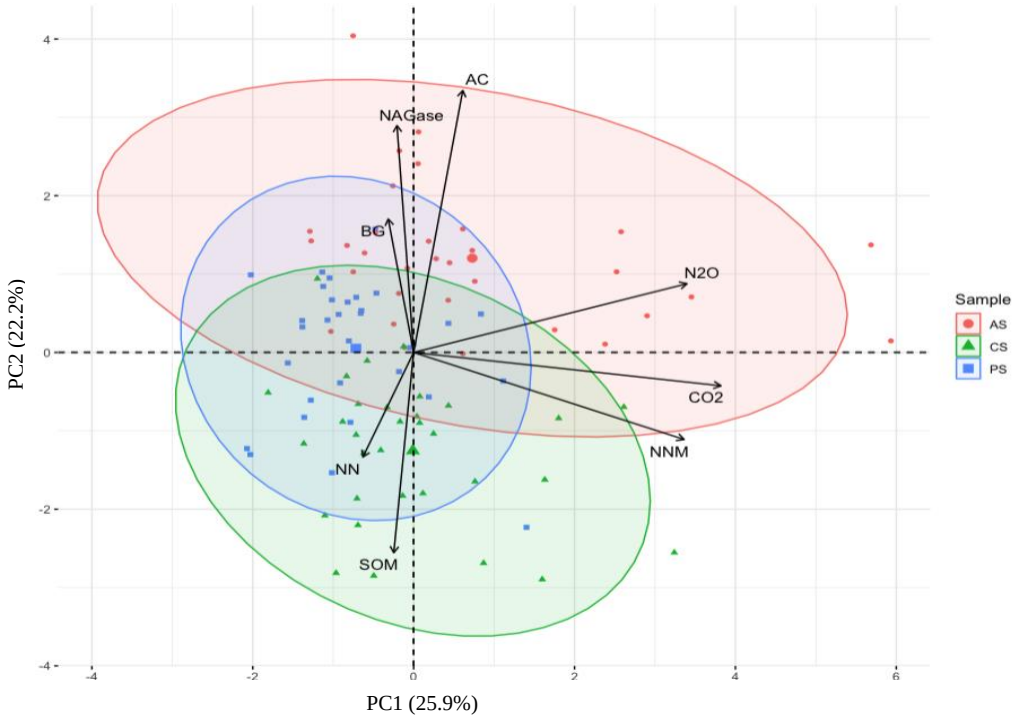


Figure 4.4: A principal component analysis (PCA) of N_2O and CO_2 fluxes and the soil health parameters tested in PS (perennial cropping system), CS (converted system), and AS (annual cropping system) soils that were treated with glucose (G), urea (U), Glucose, and N to obtain a C: N ratio of 24:1 and control treatment with no amendments. Variables included were soil N_2O and CO_2 fluxes, active carbon (AC), soil organic matter (SOM), net N-mineralization (NNM), net nitrification (NN), and enzyme activities of β-glucosidase (BG) and N-acetylglucosaminidase (NAGase). Fluxes of N_2O and CO_2 and NNM were loaded onto PC1 while SOM, AC, BG, NAGase and NN loaded onto PC2. The three ellipses represent the three clusters: the blue ellipse illustrate the cluster of PS, the green ellipse illustrates the cluster of CS, and the red ellipse illustrates the cluster of AS.

4.4 Discussion:

Agricultural LUC from perennial to annual cropping systems can significantly impact the physical, chemical, and biological attributes of soil (Reidsma et al., 2006; Ruf et al., 2018; Ito & Hajima, 2020; Padbhushan et al., 2022). In this laboratory incubation study, I investigated the interaction between LUC and N_2O and CO_2 fluxes in the presence of exogenous C and N. Results supported the initial prediction, showing that soils under intensive management practices leads to faster N-mineralization in the presence of readily available C and N, resulting in increased net N_2O and CO_2 fluxes. The scientific community has conducted numerous studies investigating the impact of tillage and glyphosate addition on soil N-loss (Mkhabela et al., 2008; Panettieri et al., 2013; Bayer et al., 2015; Jha et al., 2020). However, research examining the impact of LUC on GHG emissions is limited (Panettieri et al., 2013; Okada et al., 2019). Therefore, the findings of this study contribute to filling this research gap and provide valuable insights to the existing literature focusing on the effects of LUC on N_2O and CO_2 fluxes.

The net N_2O (Figure 4.1) and CO_2 (Figure 4.2) fluxes gradually increased before reaching a peak and subsequently decreased to reach a plateau within the study period. Soils that were under various degrees of intensification i.e., soils from PS, CS, and AS fields, were investigated in this study. The results of the study revealed significant differences in maximum N_2O fluxes among the tested soil types, specifically, the AS soils exhibited notably higher maximum N_2O fluxes compared to both PS (69.8% higher) and CS (64.2% higher) soils. Among the various C/N treatments, the G+U treatment within the AS soils displayed the highest N_2O fluxes, surpassing all other treatments. In the AS soils, the second highest N_2O fluxes were observed in the G treatment, followed by the U treatment, while the control treatment exhibited the lowest N_2O fluxes. These outcomes suggest a clear influence of exogenous C and N inputs on N_2O emissions

from AS soils. Availability of readily accessible C sources in the G treatment in soil provides the necessary fuel for denitrifiers, thereby promoting potential denitrification, and subsequently, this increased potential denitrification activity has the ability to contribute to N_2O fluxes in the soil. As mineralization and urea degradation increase the available NH_4^+ levels (Bengtsson et al., 2003; Hart et al., 2018), they can support potential nitrification (Nunes-Alves, 2016; Sahrawat, 2008). The fluxes of N_2O also showed a positive correlation with NN, indicating that increased nitrification activities may contribute to elevated N_2O fluxes. The end product of nitrification serves as a substrate for denitrification, which produces N_2O as a by-product, contributing to the observed increase in N_2O fluxes (Skiba, 2008). Additionally, N_2O fluxes can be directly related to nitrification, where it is produced as a by-product, as well as co-denitrification, which occurs when O_2 levels are limited (Nunes-Alves, 2016). Additionally, the N_2O fluxes observed in different soils suggest differences in the microbial community composition or differences in the microbial activity responsible for N_2O fluxes in the AS soils compared to the PS and CS soils. Interestingly, PS and CS exhibited similar trends in N_2O fluxes (Figure 4.1), suggesting a potential legacy effect of the PS soils on the N_2O fluxes observed in the CS soils.

Additionally, similar trends were observed in CO_2 fluxes, with the G+U treatment in the AS soils exhibited the highest fluxes. These fluxes were higher than those observed in the CS and PS soils by 13.4% and 42.1%, respectively. Furthermore, across all soil types, the CO_2 fluxes were notably higher in the G+U treatment, followed by the G and U treatments, while the control treatment displayed the lowest CO_2 fluxes. This pattern suggests that the addition of exogenous C and N sources significantly affected CO_2 fluxes in all three soil types. The present study's findings are consistent with previous research at controlled environmental conditions (Flessa & Beese, 1995; Huang et al., 2004) that utilized soil amendments of C and N to investigate their potential

impact on GHG emissions, particularly CO_2 and N_2O. The significantly higher GHG fluxes in the G+U treatment could be attributed to the presence of an optimal diet for microbes with a C:N ratio of 24:1, providing sufficient energy sources for microbial utilization (Bengtsson et al., 2003; Brust, 2019; Melis et al., 2023). The hydrolysis of urea in the U treatment, facilitated by the urease enzyme, likely contributed to CO_2 fluxes (Qin & Cabral, 2002). The C:N ratio is a useful predictor of GHG fluxes, as noted in a previous study by Huang et al., (2002), which found that seasonal N_2O and CO_2 fluxes from wheat-cultivated soil negatively correlated with soil C:N ratio in an outdoor pot experiment with 18 paddy soils.

The AS showed the highest CO_2 fluxes, which were 7.6% higher than CS and 35.7% higher than PS soils. In the AS, annual inputs of root exudates, plant residues, fertilizers and the exposure and oxidation of SOM resulting from tillage practices have the potential to enhance the availability of resources for soil microbes. This increased availability of C sources may have created favorable conditions for copiotrophic bacteria. Copiotrophic bacteria, characterized by their capacity for rapid growth in nutrient-rich environments, can experience enhanced proliferation and increase microbial biomass, leading to higher respiratory rates in response to the availability of abundant C sources (Koch, 2001; Wolińska et al., 2015; Wang et al., 2021). The energetic demands associated with their rapid growth and maintenance processes may have contributed to a higher proportion of C being respired rather than being converted into biomass in the AS (Sinsabaugh et al., 2013). Therefore, a greater amount of C may have released as CO_2 during respiration, potentially reducing the overall C use efficiency (CUE). However, it is noteworthy that the CO_2 fluxes observed in the CS soils were higher than those in the PS soils, which may be due to the effects of LUC and fertilizer application in the CS soils, which promote the growth of R-strategists and stimulate their respiration (Ho et al., 2017).

The results of the incubation study indicated no significant difference in SOM % between the PS and CS soils. However, significant differences in SOM % were observed between cropping systems in Chapter 3. Jha et al. (2020) conducted a study comparing the effects of residue management and synthetic fertilizer addition on soil C and N dynamics in perennial and annual cropping systems. They found that perennial cropping systems exhibited an 18-30% increase in SOM content compared to annual cropping systems. This suggests that effective residue management can contribute to significant improvements in SOM levels in perennial cropping systems (Jha et al., 2020). Additionally, there were no significant differences in the levels of AC within the same soil across the various C/N treatments. Under incubation conditions, the PS soils exhibited the highest AC levels compared to the AS and CS soils, showing 6.1% and 9.0% higher, respectively. This finding is consistent to Plaza-Bonilla et al. (2014), who observed variations in AC levels in perennial and annual cropping systems, reporting that perennial cropping systems had significantly higher levels of AC compared to that of annual cropping systems. This difference in statistical significance between the field study and the incubation study may have arisen due to variations in microbial activity in the pre-incubation period and at the time of sampling. The field study involved sampling in May, while the incubation study collected samples in October. These differences in timing introduced seasonal variations, potential incubation artifacts and microbial respiration within pre-incubation period may have contributed to the observed variations in results. In the PS soils, the control, U, and G treatments displayed significantly higher SOM % compared to the corresponding treatments in the AS soils, with 20.5%, 15.0%, and 20.0% higher SOM respectively. This difference could be attributed to stimulated microbial activity, likely resulting from the greater root mass of orchardgrass, which exceeds that of soybean in the AS soils (Aoyama

& Kuroyanagi, 1996). This higher microbial activity likely played a pivotal role in enhancing mineralization and the decomposition of organic matter.

The impact of agricultural LUC on microbial extracellular enzyme activities, specifically BG and NAGase, is a research area that has received limited attention (Deng & Tabatabai, 1996; Uwituze et al., 2022). Beta-glucosidase is an enzyme that plays a vital role in the breakdown of complex carbohydrates in soil, facilitating the conversion of cellulose and hemicellulose into glucose (Stege et al., 2010), whereas NAGase is an enzyme involved in the breakdown of chitin, a complex polysaccharide (Peters et al., 1999) which can be used as energy sources by microorganisms. A significant increase in BG activity in the AS soils that were amended with G+U compared to the control soil was observed, whereas, NAGase activity were found to be similar between the AS and the PS soils, while CS soils exhibited significantly lower NAGase activity compared to AS and PS. Observed higher BG activity in AS G+U treatment could be due to the amendment of an optimal diet with a C:N ratio of 24:1 that provides a better substrate of simple sugars for the SMCs (Bengtsson et al., 2003; Brust, 2019). In the AS G+U treatment, despite the availability of easily accessible C and N sources, certain soil microbes may exhibit preferences for specific C and N substrates (Zhalnina et al., 2018). This preference for specific substrates may have contributed to the observed increase in BG enzyme activity within the microbial communities of the system. Another possible explanation is the adaptation of microbes to the specific environmental conditions of the AS soils, particularly related to the change in vegetation. In the past, the AS soils were planted with corn, which contained higher levels of lignin and cellulose, components that are more resistant to decomposition. However, the shift in vegetation to soybean, which has more easily decomposable plant residues with a lower C:N ratio compared to corn, may have influenced microbial activity. Microbes in the AS soils may have efficiently utilized the

readily available C and N inputs provided through glucose and urea in the beginning, resulting in a decreased requirement for breaking down complex organic matter. Consequently, the increased availability of easily decomposable organic matter in soybean residues could have stimulated the activity of BG enzymes (Ekenler & Tabatabai, 2003). This higher enzyme activity suggests enhanced decomposition and utilization of C compounds by the microbial community. The lower NAGase activity observed in the CS may be attributed to the adverse effects of glyphosate on microbial abundance and activity (Wardle & Parkinson, 1990; Lancaster et al., 2010), leading to a reduction in the activity of chitin-degrading microbes. During the process of LUC, the application of NPK fertilizer in the CS soils, which had not previously been exposed to synthetic fertilizers, introduces a nutrient-rich N source for the microbial community (Koch, 2001; Kremer & Means, 2009). The availability of abundant N resources through fertilizer application may satisfy the N requirements of the microbes in the CS soils, thereby reducing the need to break down complex organic matter to obtain N sources.

Nitrogen mineralization is a vital process in soil ecosystems, involving the conversion of organic N into inorganic forms (Bengtsson et al., 2003; Hart et al., 2018). In this study, the application of a G+U treatment to the AS soils resulted in the highest NNM. Net N mineralization serves as a quantitative measure, reflecting the difference between potential N mineralization and immobilization rates occurring in the soil (Hart et al., 2018; Vazquez et al., 2019). Through the application of exogenous N as to the AS soils, there is a notable increase in the concentration of N (Ayoola & Makinde, 2009). Subsequently, this enriched N undergoes mineralization, leading to the conversion of organic N compounds into NH_4^+ ions within the soil. Moreover, in the presence of exogenous C/N amendments, the degradation of urea can occur, resulting in the release of NH_4^+ into the soil (Qin & Cabral, 2002). Higher plant-available N in the soil can increase the risk of N-

leaching from the soil in the form of NO_3^- (Huang et al., 2004). Li et al. (2018) conducted a study to investigate the effect of N availability and its interaction with soil organic C mineralization. The study comprised an incubation experiment wherein soils were collected from two distinct cropping systems: an annual cropping system characterized by a wheat-tomato rotation, and a perennial grass cropping system. Various soil amendments were applied during the experiment, including mineral N, ryegrass residue, ryegrass residue combined with mineral N, water-extracted ryegrass residues, extracted residues combined with mineral N, and a control soil with no amendments. Results revealed that NNM was significantly higher in the control treatment, as well as in treatments involving C amendments and combinations of C and N, as compared to the N-only amendment treatment. These results were similar across both cropping systems. However, Li et al. (2018) used different C:N ratios (10:1) and incubation conditions than in current study, which showed higher NNM in the G+U amendment treatment compared to other treatments.

Although potential NN in AS soils were not significantly different among the various treatments, a significantly higher NN was observed in CS and PS soils treated with G+U compared to controlled treatment with no amendments. Nitrifiers are commonly classified as autotrophs; however, it is important to note the existence of heterotrophic nitrifiers capable of conducting nitrification using external energy sources, such as organic C (Martikainen, 2022). The presence of heterotrophic nitrifiers in the system may have played a role in the observed increase in NN in the G+U treatment. Additionally, the availability of plant residues and root exudates further influences the availability of C for heterotrophic nitrifiers (Lian et al., 2019) which may have contributed to the higher NN in the PS soils. Furthermore, the study revealed a significant correlation between NN and NNM, highlighting the strong influence of NH_4^+ derived from N-

mineralization or from urea degradation on the process of nitrification. The higher NH_4^+ levels observed in the PS soils likely promoted nitrification.

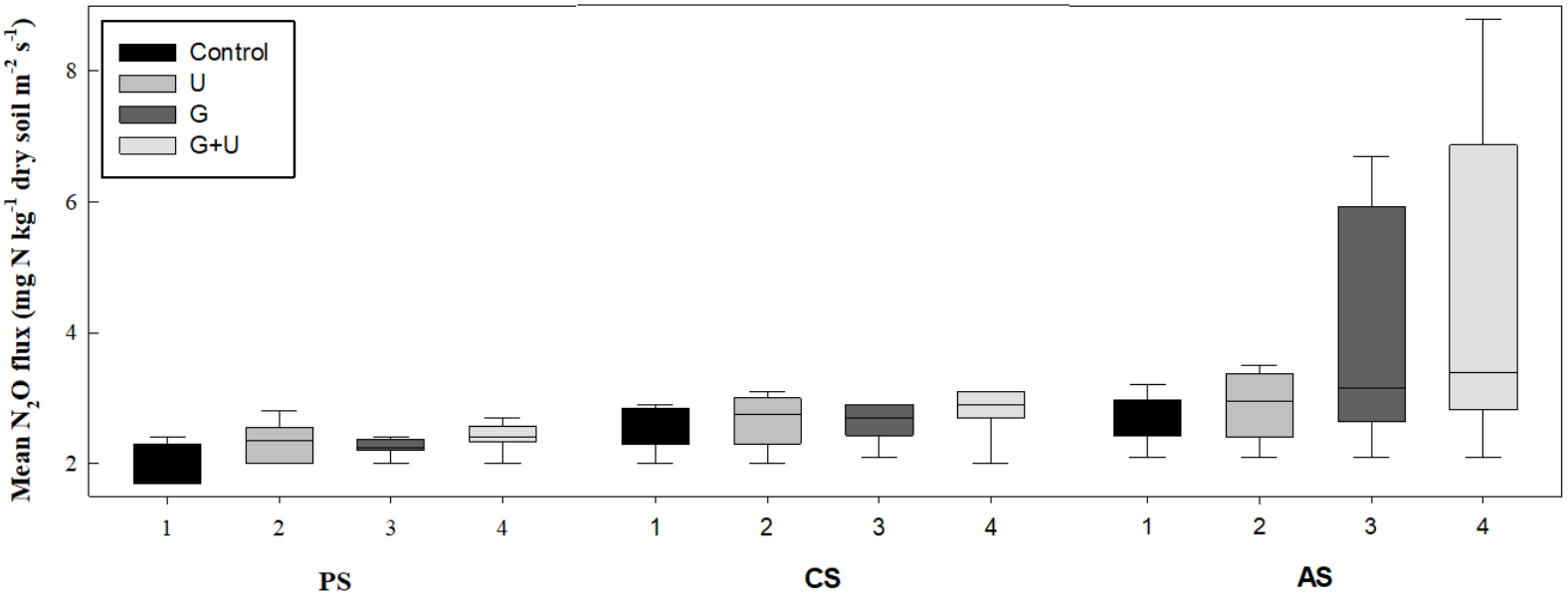
The results of the PCA analysis and Pearson's correlation analysis indicate a strong relationship between N-transformation processes and N_2O fluxes, particularly between N_2O fluxes and NNM and NN. Nitrogen mineralization and nitrification are influenced by various environmental factors, including moisture and O_2 levels (Skiba, 2008; Nunes-Alves, 2016; Hart et al., 2018). In this study, the soil samples were found to be moderately moist, and the adjusted WHC, which refers to the ability of soil to retain water against gravity, was set at 60% (Brischke & Wegener, 2019). Previous researchers (Ghezzehei et al., 2019; Stres et al., 2008) have demonstrated that microbial activity in soil is typically highest when the WHC is close to or at field capacity, which generally occurs at around 60% in most soils. When soil moisture is too low, microbial activity can be hindered by a lack of water, which can lead to dehydration stress and decreased metabolic rates (Manzoni et al., 2012). Conversely, when soil moisture is too high, microbial activity can be constrained by insufficient O_2, resulting in anaerobic conditions that encourage the growth of anaerobic microorganisms, thereby facilitating denitrification and the production of GHGs like N_2O and CH_4 (Stres et al., 2008; Brischke & Wegener, 2019; Ghezzehei et al., 2019). The moderate moisture and aerobic headspace in the jars used in this study are favorable conditions for nitrification (Nunes-Alves, 2016; Sahrawat, 2008). The availability of resources with a suitable C:N ratio of 24:1, along with favorable WHC and moderate temperatures (22°C) (Li et al., 2018; Nottingham et al., 2019), may have contributed to the higher NNM and NN levels observed in the G+U treatment compared to the control. Considering the combined results, it can be inferred that both denitrification and nitrification processes likely contributed to the observed N_2O fluxes.

4.5 Conclusion

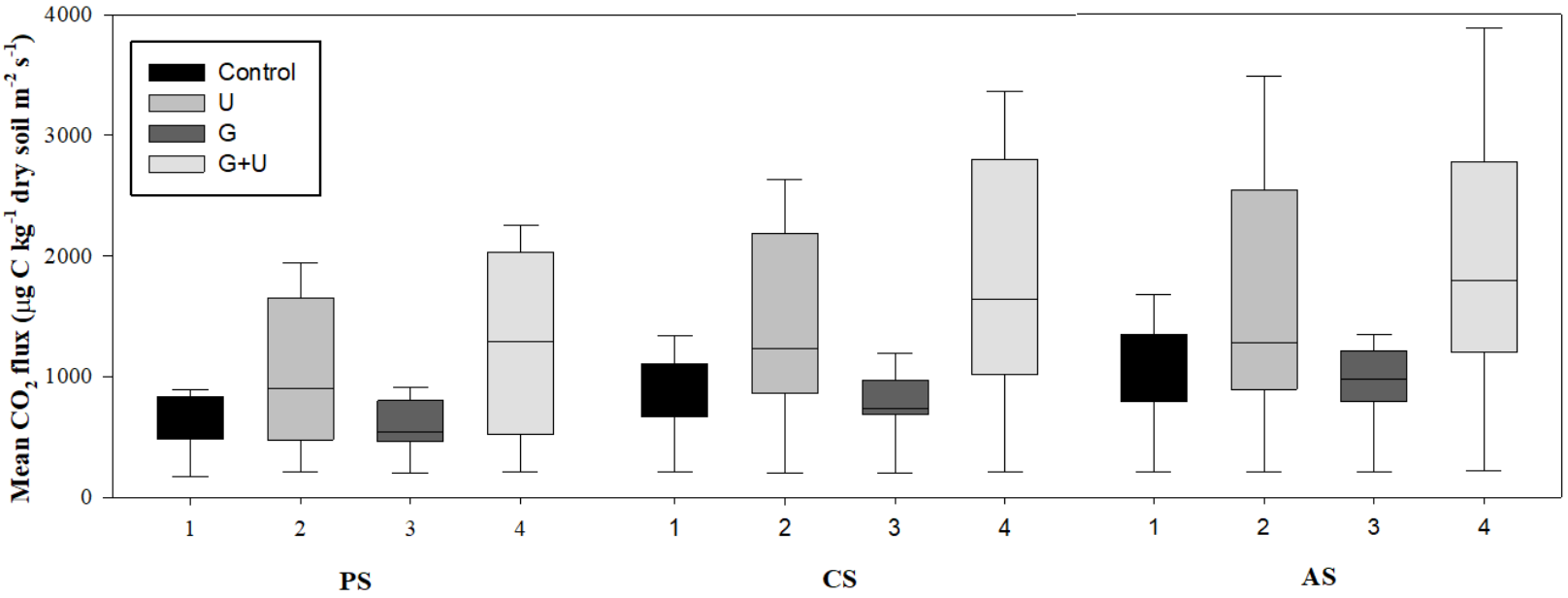
The objective of this study was to investigate the interaction between LUC and soil N_2O and CO_2 fluxes when exposed to readily available sources of C and N. The study's findings indicated that AS soils exhibited the highest N_2O fluxes in comparison to both PS and CS soils. These outcomes suggest that AS soils may harbor a distinct microbial community with a different functional profile or microbial composition contributing to N_2O fluxes. The similarity in N_2O flux trends between PS and CS soils implies that CS soils might retain a historical legacy from their PS counterparts regarding N_2O emissions. CO_2 fluxes displayed largely similar patterns across all three soil types, with the highest CO_2 fluxes observed when both C and N were added. This shows the role of exogenous C and N amendments in supporting microbial communities, thereby driving CO_2 and N_2O fluxes. This study underscores the potential influence of LUC and the application of available C and N on N and C losses from soil, manifesting as N_2O and CO_2 fluxes, which are significant environmental pollutants.

To gain a more comprehensive understanding of these phenomena, future investigations could incorporate molecular biological assays such as qPCR for microbial quantification, mRNA analysis to assess the activity of microbes involved in CO_2 and N_2O fluxes, and sequencing techniques to elucidate the composition of microbial communities participating in these processes. Such approaches would enable a more detailed exploration of the underlying mechanisms driving GHG fluxes from soils under agricultural LUC.

Appendix:



Supplementary figure 4.1: Cumulative mean fluxes of N_2O (µg N kg^{-1} dry soil m^{-2} s^{-1}) over the incubation period (hour) in differently intensified soils. Soils used included soils from PS (perennial cropping system), CS (converted system), and AS (annual cropping system). Treatments included Control (unamended control), G (amendments only with glucose), U (amendments only with urea), and G+U (amendments of glucose and urea to obtain a C: N ratio of 24:1).



Supplementary figure 4.2: Cumulative mean fluxes of CO_2 (µg C kg^{-1} dry soil m^{-2} s^{-1}) over the incubation period (hour) in differently intensified soils. Soils used included soils from PS (perennial cropping system), CS (converted system), and AS (annual cropping system). Treatments included Control (unamended control), G (amendments only with glucose), U (amendments only with urea), and G+U (amendments of glucose and urea to obtain a C: N ratio of 24:1).

Chapter 5: General Discussion and Conclusions

The trend of agricultural LUC is projected to continue, and this will lead to significant environmental and ecological consequences (Lawler et al., 2014). One example of agricultural LUC is the shift from perennial to annual cropping systems (Lambin et al., 2000). This LUC typically involves intensified agricultural practices, including chemical burndown of the previous vegetation and soil disturbance (Blickensdörfer et al., 2022; Gerke, 2022). In addition, agricultural practices aid in LUC, including fertilizer application, tillage, and other management practices can contribute to N-losses as N_2O emissions, which can have significant environmental impacts (Ellis et al., 1996; Rivett et al., 2008; Wang et al., 2019). In addition, these practices can also result in soil health depletion (Benalcazar et al., 2022; Morrow et al., 2016) and impact the abundance of SMCs (Tyler, 2019; Singh et al., 2021; Wang et al., 2021).

Several studies have investigated the effects of either tillage or glyphosate application on soil health parameters and SMC abundances (Mkhabela et al., 2008; Kremer & Means, 2009; Mathew et al., 2012; Martínez et al., 2013; Singh et al., 2020). However, studies examined the impact of LUC on soil health and N-cycling SMCs is rather limited (DuPont et al., 2010). Therefore, the primary objective of this thesis was to investigate the short-term effects of agricultural LUC on soil health parameters and the abundance of N-cycling SMCs during the conversion from a perennial to an annual cropping system. The study aimed to provide insights into the short-term impacts of LUC on soil health and the dynamics of nitrifying and denitrifying microbial communities, which play a crucial role in potential N losses, particularly in the form of N_2O. Additionally, the research explored the relationship between C and N availability and their potential influence on N_2O and CO_2 fluxes on agricultural LUC.

In Chapter 3, the impacts of agricultural LUC on soil health parameters and N-cycling SMC abundance were assessed using soil samples collected from a field site located in Ontario. The soil health parameters and N-cycling SMC abundance of PS soils, which had not been intensified or cultivated with cash crops for over 50 years, were compared with those of AS soils, which had been regularly used for cash crop production for around 10 years. The results showed significantly higher SOM % and BG activity in the PS compared to the AS. The higher BG enzyme activity in the PS, known for catalyzing C degradation (Stege et al., 2010), suggests that SOM serves as a C source for microbial communities, in addition to the contributions of orchardgrass residue inputs. The PS soils exhibited significantly higher NAGase activity, indicating a higher potential for N mineralization due to low available N for SMCs and thereby leading to increased availability of NH_4^+ (Chen et al., 2011). The NH_4^+ concentrations were found to be significantly lower in the PS soils. The PS soils showed significantly higher abundance of the *amoA* gene, suggesting a potentially more active nitrifier community capable of effectively oxidizing NH_4^+ to NO_3^- (Nunes-Alves, 2016). This indicates that the nitrifier SMCs in the PS soils are potentially using NH_4^+ and depleting its levels. This observation is supported by the higher levels of NO_3^- detected in the PS soils compared to the AS soils. The presence of a well-structured and undisturbed soil in the PS likely contributed to reduced leaching of NO_3^- (Hussain et al., 2019). Furthermore, the PS soils demonstrated a significantly higher denitrification capacity, as evidenced by the significantly higher *nirS*+*nirK*/*nosZI* gene ratio, which suggest a greater potential for complete denitrification to occur (Skiba, 2008). However, the higher levels of NO_3^- indicate potentially lower activity of *nir* denitrifiers, resulting in potentially slower NO_3^- reduction. The gene ratio of *nosZI*/*16S* rRNA (Skiba, 2008), was significantly higher in the PS soils. The higher *nosZI/16S* rRNA ratio in the PS soils suggests a greater potential for N_2O reduction to N_2, implying

a potentially lower N_2O emissions. Overall, these results indicate that the PS soils possess more robust internal N-cycling than AS soils.

In the AS soils, NAGase activity was found to be significantly lower compared to the PS soils. This indicates that the addition of NH_4^+ through synthetic fertilizer application in the AS soils may provide a readily available N source (Liu et al., 2018), resulting in a reduced need for microbes to degrade external chitin-like compounds (Chen et al., 2011) to fulfill their N requirements. The AS soils exhibited significantly higher levels of NH_4^+, while the abundance of the *amoA* gene was significantly lower compared to the PS soils. The NO_3^- levels in the AS soils were significantly lower than those in the PS soils, likely due to NO_3^- uptake by annual crops and regular tillage which disrupts soil particles (Perez-Brandán et al., 2012), indicating that N-loss from AS in the form of NO_3^- leaching is also a possibility (Hussain et al., 2019; Wang et al., 2019). Lower *amoA* gene abundance and NO_3^- levels suggest that although there is an ample amount of NH_4^+ present in the AS soils, the potential activity of the nitrifier bacterial community may be lower, leading to a less effective nitrification process. Furthermore, the AS soils displayed significantly lower *nirS+nirK/nosZI* gene ratios, along with lower NO_3^- levels, indicating a potentially lower capacity for denitrification. However, the significantly lower gene ratio of the *nosZI/16S* rRNA in the AS soils suggests a lower potential for the reduction of N_2O to N_2 compared to the PS soils. Collectively, the significantly lower *nirS+nirK /nosZI* ratio and the lower relative abundance of *nosZI* gene in the AS soils supports these observations, indicating a lower potential for complete denitrification and a higher likelihood of N_2O emissions from the AS soils.

Further in Chapter 3, the short-term impacts of agricultural LUC from a perennial to an annual cropping system was studied. The changes in soil health parameters and N-cycling SMC abundances in an agricultural field that underwent LUC were studied. The results of the study

demonstrated that the LUC had a significant impact on SOM and AC levels. The change in vegetation from orchardgrass to soybean could be a potential factor contributing to the observed effects on SOM contents. The application of synthetic fertilizers may have stimulated the growth and activity of R-strategists, which are known for their rapid decomposition of organic matter. Additionally, the activity of BG and NAGase enzymes showed an increase following LUC. These enzymes are associated with the breakdown of organic compounds, suggesting enhanced organic matter degradation in the CS soils. This increased microbial activity may have contributed to a significant reduction in the SOM and AC levels observed in the CS soils after LUC. The addition of fertilizer following LUC had a significant impact on the NH_4^+ levels in the CS soils, increasing their concentrations. In contrast, the levels of NO_3^- showed a significant reduction after LUC. This reduction can be due to soil disruption, which can lead to NO_3^- leaching (Hussain et al., 2019) and increased access of NO_3^- to SMCs. The decreased levels of NO_3^- observed, along with the reduced abundance of the *amoA* gene, suggest a potential decline in nitrifier activity following LUC. Additionally, there was a noteworthy decrease in *nirS* gene abundance, which is known to be more responsive to changes in soil environments compared to the *nirK* community (Sun & Jiang, 2022). Interestingly, *nirK* gene abundance showed a significant increase after the conversion, suggesting the presence of K-strategist that were able to survive and persist with the remaining SOM. The reduced levels of NO_3^-, coupled with the increased abundance of the *nirK* gene, indicate a potentially higher activity of *nirK* denitrifiers, resulting in NO_3^- reduction to N_2O. Additionally, the *nosZI*/*16S* rRNA gene ratio showed a significant decrease, following the LUC. These findings collectively suggest that the potential for reducing NO_3^- to N_2O may be increased, while the potential for reducing N_2O to N_2 is decreased. Therefore, the potential capacity for N_2O emissions following LUC may be increased.

The laboratory incubation study conducted in Chapter 4 was aimed to investigate the relationship between LUC and N_2O and CO_2 fluxes in the presence of readily available C and N. The study involved amending soil samples from differently intensified agricultural soils with glucose as a C source and urea as an N source and measuring N_2O and CO_2 fluxes. The AS soils demonstrated significantly higher N_2O fluxes in comparison to the PS and CS soils, with the G+U treatment exhibiting the highest fluxes among all treatments. This observation suggests a direct influence of the presence of both C and N on N_2O fluxes from the AS soils. It is possible that the AS soils bear a distinct N cycling microbial community than PS soils. Furthermore, the similarity in N_2O flux trends between the CS and PS soils suggests a historical influence of PS on the N_2O flux dynamics in CS soils. The CO_2 flux trends were similar across all three soil types, with AS exhibiting the highest fluxes, followed by CS and PS. The addition of exogenous C and N sources resulted in the highest CO_2 fluxes in all three soils. These findings suggest the significant influence of C and N on CO_2 flux dynamics in soil systems.

The study revealed that the addition of readily available C and N sources resulted in increased net N-mineralization (NNM) in both the CS and AS soils. Moreover, the addition of exogenous C and N increased net nitrification (NN) in the PS and CS soils, indicating the influence of LUC on nitrification and available NH_4^+ utilization when supplied with external C and N sources. Increased rates of mineralization and nitrification can lead to higher levels of NH_4^+ and NO_3^- availability in the soil. In the AS soils, although NNM was significantly higher with the addition of exogenous C and N, the lower abundance and potentially lower activity of the *amoA* community (Chapter 3) resulted in no significant increase in NN. In contrast, in the PS soils, while there was no significant increase in NNM, NN was significantly higher, potentially due to the higher abundance of *amoA* genes (Chapter 3) which may have resulted in potentially higher

nitrification activity. Notably, the correlation analysis demonstrated a strong positive correlation between NN and N_2O fluxes. This finding suggests that, in addition to denitrification, there could be a significant contribution of nitrification to the observed N_2O fluxes.

This study highlights the impacts of agricultural LUC from a perennial cropping system to an annual cropping system on soil health parameters and the abundance of N-cycling SMCs. The research also sheds light on the potential impact of C and N-containing fertilizers on this conversion process, and how C and N availability may contribute to GHG fluxes, particularly N_2O and CO_2. The findings presented here have the potential to pave the way for extensive studies that delve deeper into the relationship between agricultural LUC, SMCs, and N-losses.

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