Effect of Nitrogen Stabilizers and Cropping Sequence on Soil Carbon and Nitrogen Pools, Biochemical Attributes, Active Microbial Populations in Podzolic Soil in Boreal Climate

By

Muhammad Usman

A thesis submitted to the School of Graduate Studies

In partial fulfillment of the requirements for the degree of

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Approved by:

Dean of the Graduate School

Dr. Mumtaz Cheema - Supervisor

Date

Dr. Judith Nyiraneza (Co-supervisor)

Dr. Lakshman Galagedara (Committee member)

Dr. Yeukai Katanda (Committee member)

Abstract

Nitrogen (N) is a fundamental macronutrient required for the growth and yield of crops. N stabilizers mitigate ammonia volatilization, N leaching, and gaseous losses, while enhancing soil organic matter and nutrient cycling. Different crops, with their own unique root systems, have an influence on levels of soil organic carbon (SOC) and microbial activity. A field trial was carried out to examine the impact of N stabilizers and cropping sequence on microbial biomass nitrogen (MBN), SOC, particulate organic matter N (POM-N), microbial biomass carbon (MBC), total N (TN), particulate organic matter C (POM-C), pH, enzyme activities (urease and β glucosidase), and microbial community in podzolic soils in boreal climate. Experimental treatments were five N treatments [control, Urea, urea split, and three N stabilizers (Agrotain, Super-U, and eNtrench)] and three cropping sequences (corn- corn-corn, corn-wheat-corn, and corn-faba bean-corn). Soil samples were collected after harvesting the crop in 2020 and 2021. Results showed N sources significantly impacted active microbial population, SOC, POM-N, TN, pH, and enzyme activities in 2021. In 2020, N sources had significantly influenced TN and β glucosidase enzyme activity only. Cropping sequence significantly affected MBN, MBC, and POM-C in 2021, however, in 2020, MBN, gram positive bacteria (G^+), total bacterial phospholipid fatty acids (Σ B-PLFAs) and total phospholipid fatty acids (Σ PLFAs) were significantly affected. In 2021, cropping sequence and N stabilizers interaction significantly influenced G^+, gram negative bacteria, Σ B-PLFAs and Σ PLFAs. Our results conclude that N stabilizers and cropping sequence improved soil biochemical attributes, microbial activities, and improved soil C pools in podzol soil. However, N stabilizers with different application rates and long-term crop rotation are required to fully understand the effect of these management practices on soil biochemical and microbial processes in podzolic soils under boreal climate.

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Muhammad Usman

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List of Abbreviations

% - Percentage

µl – Microliter

AG – Agrotain

Al^{+3} – Aluminum

AMO - Ammonia monooxygenase

ANOVA – Analysis of variance

BAME – Bacterial acid methyl ester

C – Carbon

C: N – Carbon: Nitrogen ratio

Ca^{+2} – Calcium

CO_2 – Carbon dioxide

DCD - Dicyandiamide

EN - eNtrench

FAME – Fatty acid methyl ester

FAO – Food and Agriculture organization

Fe^{+2} – Iron

g – Gram

G^- - Gram negative bacteria

G^+ - Gram positive bacteria

G^+/G^- - Gram positive bacteria / Gram negative bacteria ratio

GC-FID – Gas chromatography – Flame ionization detection

H^+ - Hydrogen ion

ha - Hectare

L – Liter

m^2 – Square meter

MBC – Microbial biomass carbon

MBN – Microbial biomass nitrogen

mg/L – Milligram per litre

mL – Milliliter

N – Nitrogen

NBPT - N-(n-butyl) thiophosphoric triamide

NH_3 – Ammonia

NH_4^+ - Ammonium

NI – Nitrification inhibitor

NL – Newfoundland and Labrador

NO_2^- - Nitrite

NO_3^- - Nitrate

NUE – Nitrogen use efficiency

ºC – Degree centigrade

p – Probability or level of significance

pH – Power of hydrogen ion

PLFAs – Phospholipids fatty acids

POM-C - Particulate organic matter carbon

POM-N - Particulate organic matter nitrogen

s – Second

SOC – Soil organic carbon

SPE – Solid phase extraction

SU – superU

TMSH – Trimethyl sulfonium hydroxide

TN – Total Nitrogen

UI – Urease inhibitor

USA – United states of America

v/v – Volume over volume

μg/mL – Microgram per milliliter

μl – Microliter

ΣB-PLFAs – Total bacterial phospholipid fatty acids

ΣPLFAs – Total phospholipid fatty acids

Chapter 1

1. General Introduction and literature review

1.1 Introduction

Newfoundland and Labrador (NL) province is confronted with the challenges of local food production to feed people in the Island. The Agriculture Sector Work Plan, a collaborative effort between the Provincial Government of NL, NL Federation of Agriculture (NLFA) and Grenfell Campus Memorial University, put together 43 initiatives with the focus to enhance food production from 10 to 20% and create employment opportunities in NL. The NL soils are mostly podzolic in nature and cover more than 60% of the province's total area. Podzols are darker alluvial acidic soils with high aluminum (Al^{+3}), iron (Fe^{+2}) and organic matter in the B horizon (Sanborn et al., 2011). There is a dire need to improve soil pH, fertility, and physiochemical properties through efficient utilization of natural resources and cost-effective strategies to achieve food self-sufficiency without impacting the environment.

Optimal soil health is crucial for the establishment of economically viable, ecologically sustainable, and environmentally friendly agricultural production systems. A fertile soil facilitates the development of plants by promoting essential processes such as nutrient cycling, vibrant soil microbial populations, and effective regulation of water and air availability (M. Tahat et al., 2020). The functions of soil are affected by the interconnected physio-chemical, and biological attributes of the soil, many of which can be altered by soil beneficial management practises (BMPs) (Raj et al., 2019). These BMPs encompass several techniques, such as cropping sequence, cover crops, conservation tillage methods, and application of organic amendments (such as manure, compost, and bio-char), these practises effectively enhance the physiochemical and biological attributes of soil (Chahal et al., 2021; Farmaha et al., 2022). Nitrogen (N) is important for plants growth, play key role in improving soil fertility. Despite the addition of a significant amount of N fertilisers to the soil, only little portion of applied N is taken by plants (Raun & Johnson, 1999), the remaining N is lost either via leaching of nitrate (NO_3) into groundwater or emission of nitrous oxide (N_2O). For example, 30-40% N is lost through ammonia volatilization, nitrification, and denitrification (Cui et al., 2022; Ladha et al., 2005).

There is a need to enhance nitrogen use efficiency (NUE) in different cropping systems by adopting different BMPs and innovative approaches. For instance, application of 4Rs (right time, right place, right rate, and right source of nutrient) is very useful management practice to reduce NH_3 loss, and increasing N retention in soils (Snyder, 2017). The reduction of greenhouse gas (GHG) emissions can be achieved through the application of modified management practises, which aim to enhance C sequestration (Follett, 2001) and minimize N_2O emissions (Kroeze et al., 1999). Hence, it is imperative to implement novel agricultural management strategies in order to effectively sequester C, enhance soil health, minimize N losses (such as NO_3 leaching and N_2O emissions), while ensuring adequate food production (Pawlak & Kołodziejczak, 2020). Various strategies that have been identified to enhance soil organic carbon (SOC) levels encompass the introduction of cover crops, residue retention, adoption of diverse crop rotations, cultivation of crops with higher root mass, and application of N fertilisers (McDaniel et al., 2014).

The amount of N in soil is determined by fertiliser type, fertilizer rate, soil pH, and organic matter present in soil (Abdelgadir et al., 2010). One of the innovative technologies currently being employed in different cropping systems to reduce N losses and enhance NUE is application of N stabilizers (Snyder, 2017). N stabilisers are chemicals that can be employed alongside N fertilisers to mitigate the likelihood of N loss by retarding the pace of chemical reactions that take place in soil. Urease inhibitors (UIs), nitrification inhibitors (NIs) or double inhibitors have shown success in reducing N losses from soil by delaying the process of urea hydrolysis and suppressing the ammonia oxidizing bacteria activity (Sha, Ma, Loick, et al., 2020). NIs are chemical substances, including 3,4-dimethylpyrazole phosphate (DMPP), Dicyandiamide (DCD), and Nitrapyrin (2-chloro-6-(trichloromethyl) pyridine; NP), that effectively delays the process of bacterial oxidation of ammonium (NH_4^+) in soil. They achieve this by blocking the action of Nitrosomonas bacteria, the inhibitory effect of NIs can last for a duration of 4-10 weeks (Florio et al., 2014; Soares et al., 2012; Weiske et al., 2001). The Nitrobacter and Nitrosolubus, carry out the process of converting NH_4^+ into nitrite (NO_2^-), then further transformed into nitrate (NO_3^-) (Prosser, 2005). The purpose of nitrification inhibitors is to delay nitrification process to reduce the loss of nitrate by leaching or the production of NO_2 through denitrification process by keeping N in the ammonium form for a prolonged period of time, thereby enhancing NUE (Alonso-Ayuso et al., 2016). The application of NIs has the potential to delay soil nitrification, thereby increasing NH_4^+-N and decreasing NO_3^--N content, as well as enhancing crop yield, aboveground biomass, N uptake, and NUE (Cui et al., 2022). UI like N-(n-butyl) thiophosphoric triamide (NBPT) have ability to inhibit conversion of amide-N to ammonium hydroxide and NH_4^+ through the hydrolytic activity of the urease enzyme for a specific duration. Slowing hydrolysis rate of UR can reduce or avoid the volatilization losses of ammonia to atmosphere and the additional leaching losses of nitrate (Klimczyk et al., 2021). By inhibiting the active soil microbial activity or metabolism, N fertilizer stabilizers like Agrotain (AG), eNtrench (EN), and SuperU (SU) are able to delay the urea hydrolysis and nitrification processes (Burton, 2018), hence reducing N losses.

Cropping sequence is vital for increasing amount of soil organic matter (Raphael et al., 2016). Including cover crops into the cropping sequence can enhance soil quality, health, and productivity by enhancing soil C, N, and microbial biomass, this makes cover crops a fundamental component of sustainable agroecosystems. (McDaniel et al., 2014). The cropping sequence improves nutrient cycling, soil fertility, and breaks the pest and disease cycle (Benincasa et al., 2017; Drinkwater & Snapp, 2007). Furthermore, it encourages the growth of a robust soil ecosystem, biodiversity, nitrogen cycling, soil fertility leading to better soil characteristics that enable the sustainable crop production (Karlen et al., 2006). Cropping sequence systems can maximize nutrient uptake and reduce the likelihood of nutrient imbalances by alternating crops with varying nutrient needs. Cropping sequence that are diverse improve soil quality, stabilize crop output, and reduce chemical inputs and environmental concerns (Dias et al., 2015; Gaudin et al., 2015). As reported by Bowles et al. (2020), cropping sequence can successfully increase crop yields and bring about significant economic gains by enhancing soil health and disrupting the cycle of herbivores, weeds, and diseases. Soil compaction, decreased water infiltration, and reduced microbial activity are common results of continuous monocropping (Shah et al., 2017). Cropping sequence breaks up compacted soils, improves plant root penetration, and promotes microbial diversity (Carr et al., 2013).

Carbon (C) sequestration, nitrogen cycling, soil structure, availability of soil nutrients are all improved by cropping sequence (Chahal et al., 2021; Liu et al., 2022). One way to promote microbial activity and organic matter accumulation is to include legume crops in cropping sequence systems (Drinkwater et al., 1998), through a variety of legumes, farmers can replenish soil N levels (Kebede, 2020). Iheshiulo et al. (2023), found that the optimal methods for enhancing the physical condition of soil involves cropping sequence that included a variety of crop species as well as grain legumes. Cereal-legume cropping sequence is widely used in many crop production systems because it increases number of N-fixing bacteria and makes it easier for crops to absorb N from soil (Ghosh et al., 2020; Pandey et al., 2017). According to research by González-Chávez et al. (2010), the authors has observed that implementation of a wheat-soybean rotation strategy results in the attainment of the most significant levels of richness and biodiversity within the microbial community as a whole. Consequently, there has been a subsequent emergence of microbial products that exhibit a significant influence on the sequestration of soil N and C (Cheng et al., 2007; Loranger-Merciris et al., 2006).

Several studies have documented positive impact of various cropping sequences on microbial biomass C (MBC). For example, Borase et al. (2020) found that the maize-wheat-mungbean cropping sequence led to an increase in MBC. Similarly, Benbi et al. (2012) observed that the maize-wheat cropping sequence enhanced MBC. Additionally, Song et al. (2022) reported that the wheat-soybean cropping sequence resulted in higher MBC levels, which can be attributed to the quality and amount of crop residues returned back to the soil and root exudate from plant. Research study conducted by Fu et al. (2019), found corn-winter wheat-winter wheat-millet cropping sequence resulted in an enhancement of microbial biomass N (MBN). Similarly, Borase et al. (2020) reported that the maize-wheat-mungbean cropping sequence also led to an increase in MBN. The various root exudates from different crops in rotation provide a variety of C sources, boosting the proliferation of beneficial soil microorganisms (Larkin, 2008). These microbes enhance soil health and crop productivity by participating in nutrient cycling, decomposing organic debris, and suppressing diseases (Li et al., 2019).

Xiao et al. (2022), reported that ryegrass-cotton-peanut-wheat-maize cropping sequence has the potential to reduce GHG emissions resulting from agricultural activities and have the capacity to enhance C sequestration or the accumulation of SOC. The research study by Drury et al. (2008) demonstrated incorporation of legume crops into cropping sequence resulted in a significant reduction in nitrous oxide (N_2O) emissions, with a five-fold decrease observed in comparison to the continuous cultivation of silage corn Another study conducted by Ortega et al. (2002), stated inclusion of legumes in a rotation, coupled with a high residue crop like silage corn, has the potential to improve SOC levels. Previous studies reported the effect of cropping sequence or N stabilizers on gene abundance, (Fu et al., 2020b; Maul et al., 2019; Munroe et al., 2020) N_2O emission, (Lam et al., 2018; Lan et al., 2013; Ni et al., 2018; Zhao et al., 2017) crop yield and NUE (Cui et al., 2022; F. Xiao et al., 2022). However, effect of N stabilizer and cropping sequence on C and N fractions such as SOC, particulate organic matter C (POM-C), total N, particulate organic matter N (POM-N), MBC and MBN, pH, enzyme activities and active microbial population in podzol soils under boreal climate needs to be investigated. Hence, we hypothesized that N stabilizers and cropping sequence will enhance the soil C and N fractions, enzymatic activities, pH, and microbial community in podzol soils under boreal climate. The specific objectives of study were:

i. To determine effects of N stabilizers and cropping sequence on soil C and N fractions in podzolic soils under boreal climate.
ii. To assess effect of N stabilizers and cropping sequence on soil pH, enzyme activities and active microbial population in podzol soil under boreal climate.

1.2. Review of literature

1.2.1. The role of nitrogen in the growth, development, and biomass yield of crops

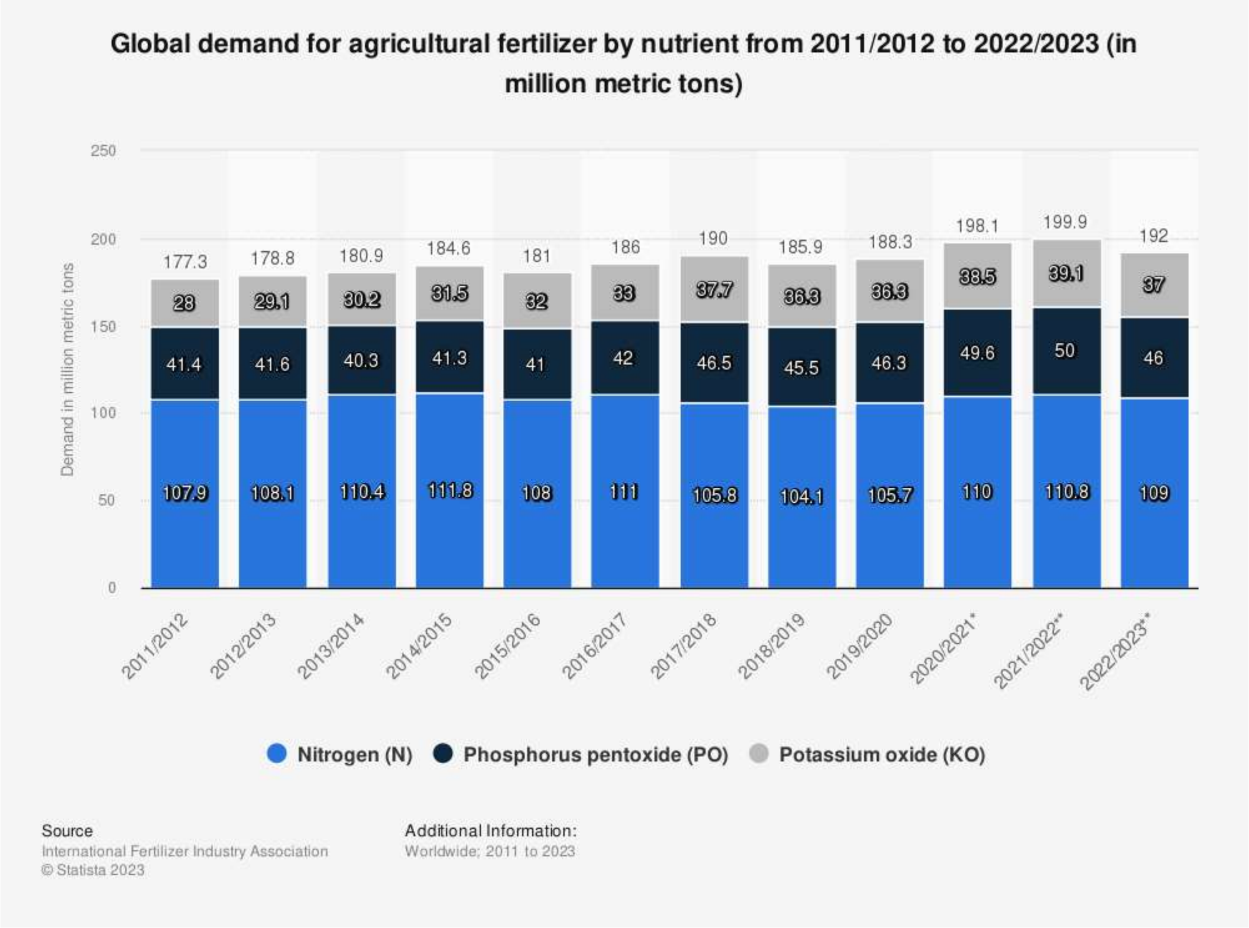
Nitrogen (N) is an essential element and limiting factor in crop growth and yield/biomass (Drinkwater & Snapp, 2007). The availability, adsorption, and transport of nitrogen have a direct impact on fundamental physiological processes related to biomass production and grain yield (Below et al., 1985; Kaizzi et al., 2012). It has been determined that N plays important role on photosynthetic activity (Bange et al., 1997; Dreccer et al., 2000; Muchow, 1988), formation and maintaining sink capacity (seed yield and size) (Miralles et al., 1998; Rajcan & Tollenaar, 1999) and product quality of crops (Cooper & Blakeney, 1990). Due to these effects, N is a crucial nutrient for agriculture and global food security. Effective management of N fertilisers is crucial to ensure sufficient food production to sustain the growing global population of 9 billion by 2050.

The inclusion of N fertilisers considerably enhanced various crop growth metrics, including total grain yield, leaf area index (LAI) and net assimilation rate, (Khan et al., 2021). If there is a significant deficiency of N, LAI and the length of leaf area decrease, resulting in reduced light interception, and photosynthetic rate (Awais et al., 2013; Moosavi, 2012). Hence, it is important to optimise and evaluate N management techniques and cutting-edge technology in order to minimise N losses and enhance N use efficiency (NUE) across various agricultural systems.

1.2.2. Global nitrogen fertilizer scenario

By 2050, 9.7 billion human population is expected, resulting in a roughly 70% surge in the current food consumption. Hence, in order to enhance agricultural output and attain food security, the efficient use of fertilisers becomes crucial, especially considering the constraints of limited arable land. According to FAO (2009), the demand of grains for human consumption is expected to reach 3 billion tonnes in 2050, up from the existing level of approximately 2.1 billion tonnes. The 35% to 56% rise in global food demand from 2010 to 2050 is projected (Van Dijk et al., 2021).

By 2050, there is a possibility of a 5-25% increase in food shortages due to climate change, land degradation, and water scarcity (Ruini et al., 2016). Hence, the utilisation of cutting-edge technologies, and BMPs to improve NUE, increase crop yield, and minimise N losses are regarded as crucial elements in guaranteeing sufficient global food security (Grafton et al., 2015).



Global demand for agricultural fertilizer by nutrient from 2011/2012 to 2022/2023 (in million metric tons)

Source
International Fertilizer Industry Association
© Statista 2023

Additional Information:
Worldwide; 2011 to 2023

Figure 1.1: Global Fertilizer demand in million metric tons (Statista, 2023).

The consumption for urea fertiliser is expected to undergo exponential growth due to its high N content of 46%, cost-effectiveness compared to other N sources, high solubility in water, ease of handling, and safe storage. Additionally, this increased demand is driven by the need to meet global food production requirements (FAO, 2019). A comprehensive assessment of the biogeochemical processes is required to enhance NUE and minimise N losses in various agricultural systems.

1.2.3. Processes involved in the nitrogen cycle

The N cycle refers to the global-scale movement of N between ecosystems. It involves a sequence of redox reactions that transform various N compounds exist in nature, in recent years, it has become evident that the N cycle has undergone significant changes (Ader et al., 2014; Godfrey &

Falkowski, 2009). The processes involved are denitrification, immobilisation, N uptake, mineralization, nitrification, and N fixation. Biological nitrogen fixation (BNF) is a significant natural process that occurs after photosynthesis (Unkovich, 2013), and is closely related to sustainable agriculture (Udvardi & Poole, 2013). Legume crops facilitate the process of N fixation in the soil, which is accomplished by bacteria such as Azotobacter and Bradyrhizobium, this process makes atmospheric N accessible for plant absorption (Aasfar et al., 2021; VanInsberghe et al., 2015).

Plants have the ability to absorb and utilise several forms of N, such as ammonium (NH_4^+) and nitrate (NO_3^-). Among these forms, NH_4^+ and NO_3^- are mostly absorb by higher plants (ZHU et al., 2011). Many crops prefer absorbing NH_4^+ rather than NO_3^- due to the fact that the reduction process of a single NO_3^- nutrient requires a significant amount of energy. The assimilation of NO_3^- elevates the pH level of the solution, resulting in a deficient provision of iron and a reduction in chlorophyll content. Consequently, this has an impact on both the quantity and quality of the yield (Arnold et al., 2015). The energy expenditure associated with the uptake and utilisation of NH_4^+ is less compared to that of NO_3^- (Hachiya & Sakakibara, 2017). Nevertheless, exclusive NH_4^+ nourishment gives rise to numerous complications, including NH_4^+ toxicity, impeded leaf growth, diminished organic acid production, and impaired osmotic regulation (Esteban et al., 2016). The immobilisation of N leads to the transformation of inorganic N form into an organic form, rendering it inaccessible to plant (Bilotto et al., 2021). The N mineralization entails breakdown of organic N into NH_4^+ and NO_3^- ions, rendering it accessible for plant absorption (Pal et al., 2020). Enzymes such as, urease, transaminase, and protease have a direct correlation with MBN, which is a significant source of mineralizable N (Li et al., 2019). Additionally, the rate at which mineral form can be affected by other factors, including precipitation and the overall amount of N in the soil, by altering the biomass of soil microorganisms (Li et al., 2020).

1.2.4. The processes of nitrification, denitrification, and the role of soil microbes

The nitrifying microorganisms encompass bacteria, nitrite-oxidizing bacteria (NOB), ammonia-oxidizing archaea (AOA), and ammonium oxidising bacteria (AOB) (Lehtovirta-Morley, 2018). Nitrification is a process that involves the gradual conversion of ammonium to nitrite by AOB and AOA, followed by the oxidation of nitrite (NO_2^-) to NO_3^- by NOB. The initial stage is facilitated by ammonium monooxygenase (AMO), a copper-containing monooxygenase that is attached to the membrane (Arp et al., 2002), this process is linked to the conversion of oxygen to water, resulting in the production of hydroxylamine. Hydroxylamine undergoes further oxidation through the action of hydroxylamine oxidoreductase (HAO), resulting in the production of NO_2^-. NOB facilitates the second stage of nitrification, which involves the conversion of NO_2^- to NO_3^- through the action of nitrite reductase.

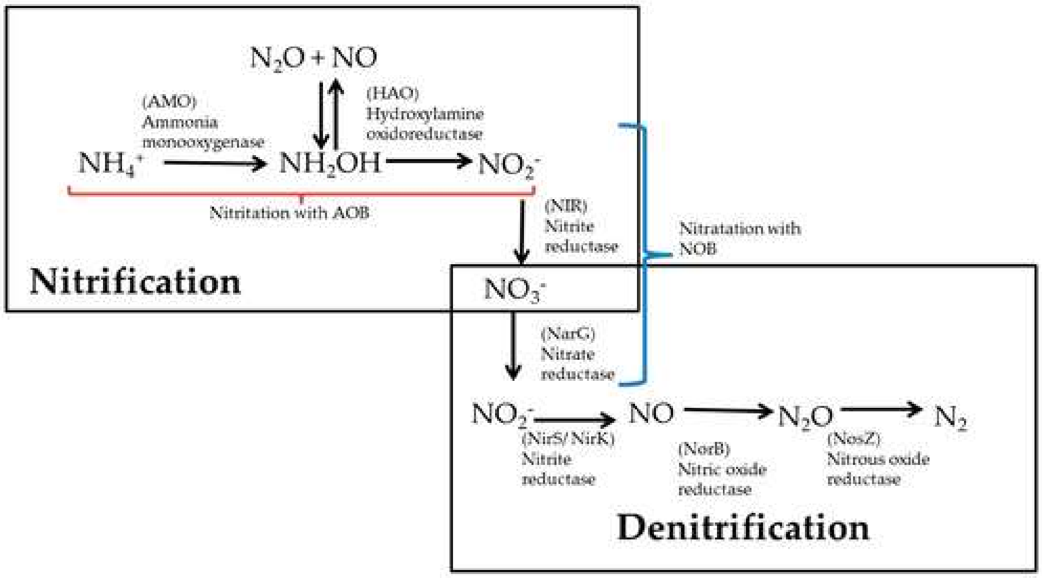


Figure 1.2: Nitrification and denitrification pathways as well as the enzymes involved adopted from Mpongwana et al. (2019).

Denitrification refers to the step-by-step reduction of NO_3^- through microbial respiration in the absence or limited presence of oxygen. The NO_3^- undergoes a series of microbial redox processes, starting with its conversion into NO_2^-, then formation of nitric oxide (NO), and ultimately dinitrogen gas (N_2). Denitrification is a significant process for removing reactive N from soil, especially in agricultural areas that get high amounts of N fertiliser (Lassaletta et al., 2014). Microorganisms have significant impact on process of soil nutrient cycling, which ultimately leads to the accumulation and depletion of N in ecosystems (Van Der Heijden et al., 2008). Microbial activities are frequently utilised as indicators of soil quality and have a substantial impact on mineralization and other ecosystem processes (Gil-Sotres et al., 2005). Soil microbes have a direct impact on soil functionality by playing a crucial part in the cycling of nutrients and the storage of C. Microbial communities exhibit significant spatial and temporal variations, as well as differences between types of soil and under various land management practises (Xue et al., 2018). Knowing the response of microbial enzymes in such conditions is crucial for predicting ecological functioning in future, particularly in context of human-induced environment change (Alster et al., 2013).

1.2.5. Factors influencing the processes of nitrification, denitrification, and immobilization

Nitrification refers to the enzymatic conversion of NH_4^+ to NO_3^- by specific nitrifying microorganisms. As a result, this process is very susceptible to variations in environmental conditions. Various environmental factor, like soil aeration, soil texture, soil moisture, substrate availability to nitrifiers, NH_4^+ abundance and temperature, might influence the populations of nitrifying microorganisms (Allen et al., 2005; Yuan et al., 2005). In agricultural systems, nitrification is the primary N-flow route, with NO_3^- accounting for greater than 95% of the total N absorption (Subbarao et al., 2010). This renders the nitrogen cycle susceptible to reactive N leakage into the environment, making agricultural systems the greatest N polluters (Galloway et al., 2008). Environmental factor like soil moisture, temperature, pH, rainfall, irrigation, and the type of applied N fertilizers not only impact nitrification but also significantly effect denitrification, and N_2O emission(Baggs et al., 2010). Soil pH has a significant impact on nitrification, and rate of nitrification in soils typically achieves its maximum range between soil pH of 8.0 and 9.0 (Shrawat et al., 2008). Due to this, a rise in pH of soil can speed up nitrification rate.

The activity of microorganisms in nitrification and denitrification is heavily influenced by temperature and moisture, making them crucial factors. In addition, soil moisture and temperature have a significant impact on the production of N_2O (Davidson & Swank, 1986). At low temperatures, N conversion rate is minimal, but it gradually increases as the temperature rises (Akiyama et al., 2000; Brentrup et al., 2000; Hao et al., 2001). Nevertheless, when considering a broader spectrum, emission of N_2O demonstrate an exponential growth pattern as soil temperatures rise (Liu et al., 2011). The strong correlation between the seasonal fluctuations of N_2O flux, air temperatures and soil is illustrated by this phenomenon (Wolf & Brumme, 2002; Zhang & Han, 2008). Emphasising the positive correlation between denitrification rate and temperature is crucial. Elevated soil temperatures promote soil respiration, hence creating more anaerobic environments that facilitate denitrification.

Typically, higher levels of soil moisture led to increased N_2O emission (Giacomini et al., 2006) due to the impact of moisture on both nitrification and denitrification processes (Davidson & Swank, 1986). This phenomenon arises due to an increase in microbial activity caused by an increase in soil water content. However, when the moisture levels get excessively high, the microbial activity is hindered. Clayey soils tend to exhibit higher levels of N_2O emissions compared to sandy soils (Brentrup et al., 2000). Additionally, the management of N might further contribute to N_2O release, especially in soil with a finer texture and with no prior mobilisation before sowing (Chen et al., 2008; Tan et al., 2009).

Emission of N_2O is also influenced due to type of fertiliser employed. Ammoniacal fertilisers typically result in more gradual increase in N_2O emissions when compared to nitric fertilisers (Signor & Cerri, 2013). The reason for this is that nitric sources undergo denitrification immediately, whereas ammonia sources require nitrification before denitrification can occur. Carmo et al. (2005) found addition of NO_3-N to soil resulted in higher N_2O emissions compared to the application of NH_4-N. In their study, Zanatta et al. (2010) examined impact of various N fertilisers on emission of N_2O. The findings revealed nitric fertilisers result in higher N_2O emissions compared to urea or NH_4^+ fertilisers. Signor and Cerri (2013) found that N fertilisation in two sugarcane fields in Brazil resulted in more intense and rapid emissions of N_2O when ammonium nitrate was used compared to urea.

1.2.6. Losses of nitrogen in various agricultural production systems

The depletion of N from the soil not just reduces soil fertility and productivity, but also poses possible negative affect on environment. NH_3 released into the atmosphere contributes to the formation of acid rain and serves as an indirect contributor to GHG emissions, eutrophication can be caused by the leaching of NO_3^- into rivers and lakes (Cameron et al., 2013).

Ammonia volatilization is major cause of N loss from agricultural land around the world. The rate of NH_3 volatilization from the soil is affected by soil conditions. Soils with elevated pH levels are prone to significant NH_3 loss, however neutral or acidic soils can also experience NH_3 loss, especially after the use of inorganic fertilisers (Black et al., 1985). Moreover, soil temperature and the environment exert a substantial influence on the process of urea hydrolysis, thereby affecting the pace at which aqueous NH_3 is converted into gaseous NH_3 (McGarry et al., 1987). The emissions of NH_3 and soil N_2O are influenced by factor like supply of N fertiliser, the rate of application, and the temperature of the soil. When fertilisers containing NH_4, like ammonium nitrate, ammonium sulphate or UR are added to soil at various phases of crop growth, they usually undergo quick NH_3 volatilization (Acton, 2007). NH_3 volatilization poses a significant threat to health and the environment because to its ability to react with acidic elements in the environment, such as sulphate or NO_3^-, resulting in the formation of a secondary aerosol (Cameron et al., 2013).

In soil, the concurrent occurrence of nitrification (under aerobic conditions) and denitrification (under anaerobic conditions) results in the production of N_2O as a prevalent byproduct (Caranto & Lancaster, 2017). During the process of nitrification, NH_4^+-N undergoes microbial conversion to hydroxylamine, which is then further transformed to NOH and ultimately to NO_2. Nitrification results in the production of N_2O at both the NH_2OH and NO stages. In contrast, denitrification converts NO_3 or NO into N_2 or N_2O (Mohanty et al., 2020). N_2O is regarded as one of the most powerful GHG since it has a greater capacity to trap heat compared to CO_2. The primary elements contributing to N_2O generation in an agroecosystem are higher rates of fertiliser application (Syakila & Kroeze, 2011). Bouwman et al. (2002) found that NH3 volatilization is greater in warm climates. A field study conducted in climates with temperatures ranging from 25-28°C indicated NH3 volatilization losses of 20-30% (Cantarella et al., 2003), However, in tropical weather conditions, these losses can be 60% high (Sparovek & Jong van Lier, 1997).

Twenty years ago, the global NUE for cereal crops was recorded at 33 %, this means that 67% of the nitrogen given to the crops was not accounted for and could have been lost in the soil system through leaching or in gaseous forms (Omara et al., 2019). The decrease in worldwide NUE in agriculture is mainly attributed to the uneven distribution of nutrients, specifically the excessive use of N fertiliser in certain areas and insufficient use in others (Sutton et al., 2013). Jabloun et al. (2015) observed impacts of temperature and rain differed depending on cropping systems. The rate of NO_3^- depletion from the soil is heavily influenced by the regulating environmental conditions. Excessive usage of fertiliser in, Western Europe, India, China, and North America leads to environmental pollution. In contrast, in Africa and certain regions of Latin America, there is insufficient utilisation of fertiliser, resulting in a phenomenon known as "soil mining," which refers to the gradual loss of nutrients from soil (Austin et al., 2013) adequacy of fertiliser in a country is contingent upon the specific crop and geographical region. For instance, in Argentina, the N balance for wheat cultivation is in equilibrium, but N balance for maize cultivation is in deficit (Alvarez & Grigera, 2005). Switzerland serves as an illustrative case of how regulations have effectively addressed the issue of excessive fertilisation, resulting in a balanced N level. Similarly, the United States has witnessed a positive outcome in terms of enhanced NUE through the implementation of measures in relation to corn production (Spiess, 2011). Hence, it is imperative to employ contemporary technologies in order to minimise N losses, not only for enhancing NUE, but also for enhancing ecological aspects.

1.2.7. Strategies and solutions for mitigating nitrogen losses

A number of management practices, such as no-till, cropping sequence, and cover crops can be utilized to cut down the amount of N loss (Ghosh et al., 2015; Reetz, 2016). The crop must be provided with the necessary amount of nutrients in accordance with the results of soil tests in order to achieve the optimal rate (Ghosh et al., 2015).

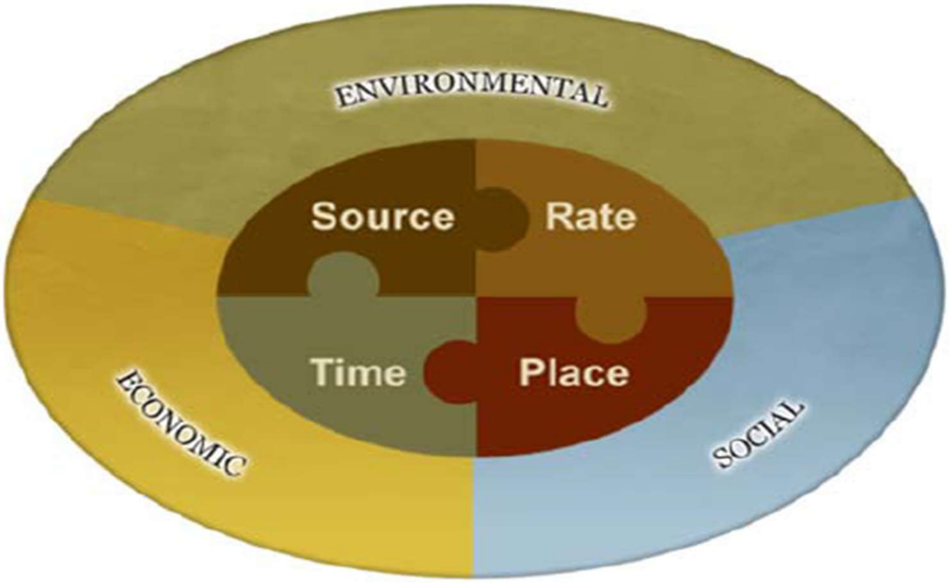


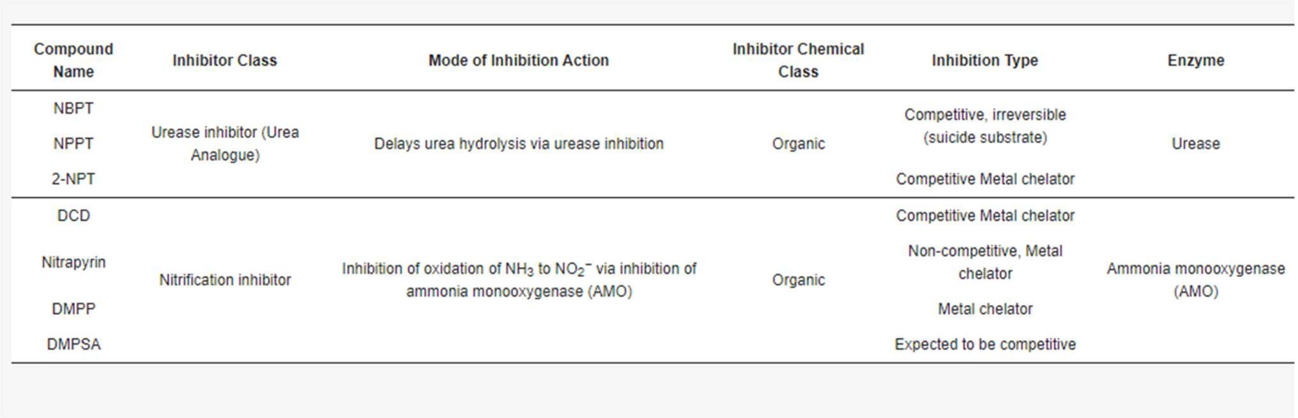
Figure 1.3: The 4R concept for N fertilizer application (Johnston & Bruulsema, 2014).

N stabilisers are effective in reducing losses caused by leaching, denitrification, nitrification, and and volatilization. These tools have been recognised as viable methods to decrease the adverse impacts of N_2O and methane emissions on a worldwide scale (Bedmar et al., 2005). The coating is specifically developed to provide a gradual and synchronised release of the nutrient content in fertilisers, aligning with the nutritional requirements of plants (Naz & Sulaiman, 2016). The primary drawback of N stabiliser is their high cost, which can range from four to eight times that of normal fertilisers (Herrera et al., 2016). In addition, the efficacy of N stabiliser is sometimes hindered by many conditions like as soil pH, moisture, temperature, etc., resulting in limited adoption. Implementing a comprehensive strategy that combines genetic and agronomic methods could enhance NUE in crops (Omara et al., 2019).

N stabilisers consist of layers of impermeable materials that are coated with a NI or UI as an addition. These fertilisers can be employed to inhibit or postpone processes such as nitrification or urea hydrolysis in order to minimise N loss and enhance N absorption by plants by regulating the activities of microrganisms (Mitran et al., 2018; Soares et al., 2012; Verma et al., 2015). Examples of UI include N-(n-butyl) thiophosphoric triamide (NBPT), while dicyandiamide (DCD), phenylphosphorodiamidate, and 3,4-dimethyl pyrazole phosphate (DMPP) are examples of NI. For instance NH_3 emission reduction was seen when sunflower crops were treated with NBPT coated urea, resulting in a drop of 42% compared to the use of uncoated urea (Sanz-Cobena et al., 2008). In New Zealand, the application of NBPT in grazing grasslands resulted in a reduction of NH3 volatilization by 18% to 28% (Rodriguez et al., 2021). The application of NI, specifically DCD and DMPP, in combination with urea, resulted in a reduction of N_2O emissions from agricultural soils by more than 76% and 67% respectively (Meng et al., 2021).

1.2.8. Potential application of urease and nitrification inhibitors

The application of N inhibitors plays significant role in enhancing N availability to plants and is a crucial aspect in optimising N utilisation and enhancing fertiliser quality. For example, the use of urea coated with urease and NIs, as well as the use of controlled release fertilisers, enhance the efficiency of N utilisation in the soil, decrease N leaching and N_2O emissions, and have a significant influence on the biochemical N cycle (Ito et al., 2018; Liu et al., 2019). The application of N inhibitors substantially enhanced the levels of inorganic N in agricultural soils.



Compound Name	Inhibitor Class	Mode of Inhibition Action	Inhibitor Chemical Class	Inhibition Type	Enzyme
NBPT	Urease inhibitor (Urea Analogue)	Delays urea hydrolysis via urease inhibition	Organic	Competitive, irreversible (suicide substrate)	Urease
NPPT					
2-NPT				Competitive Metal chelator	
DCD	Nitrification inhibitor	Inhibition of oxidation of NH_3 to NO_2^- via inhibition of ammonia monooxygenase (AMO)	Organic	Competitive Metal chelator	Ammonia monooxygenase (AMO)
Nitrapyrin				Non-competitive, Metal chelator	
DMPP				Metal chelator	
DMPSA				Expected to be competitive	

Figure 1.4: Classification and properties of N stabilizers used in agricultural production system (Byrne et al., 2020).

The application of NIs has the potential to delay soil nitrification, thereby increasing NH_4^+-N and decreasing NO_3^--N leaching, as well as enhancing crop yield, aboveground biomass, N uptake, and NUE (Cui et al., 2022). SOM can absorb NIs and offer an energy source for microbes, resulting in NI degradation and a decrease in NIs ability to suppress nitrification. In conclusion, combining NIs with N fertilizer is a cost-effective method for reducing the frequency and amount of N fertilizer applied, improving nitrogen availability, and thereby improving NUE (Abalos et al., 2014).

NIs are a group of compounds that impede nitrification process by suppressing activity of nitrifying bacteria responsible for producing the enzymes AMO, nitric oxide reductase, and hydroxylamine oxidoreductase (Ruser & Schulz, 2015). DCD is a widely used NI that efficiently decreases N_2O emissions. DCD has been found to significantly reduce nitrification in alkaline clay soils by 62.4 %. Additionally, it leads to a substantial decrease from silt loam of 93.2% in N_2O emissions. Furthermore, DCD reduces the accumulation of NO_3^- by approximately 20% (Lan et al., 2013). In dry sandy loam soils, up to 40% in reduction of N_2O emission was observed (Skiba

& Smith, 1993). In barley and grassland areas the combination of DCD and urea resulted in a significant decrease of 58-78% in N_2O emissions (McTaggart et al., 1997). In arable soils DCD exhibits greater efficacy in suppressing nitrification and reducing N_2O emissions compared to grassland soils. According to McGeough et al. (2016) in arable soil there was a decrease of 81% in N_2O emissions as compared to 58% in grassland soil. The researchers concluded that this is probably due to the fact that grassland naturally has higher levels of N compared to arable soil.

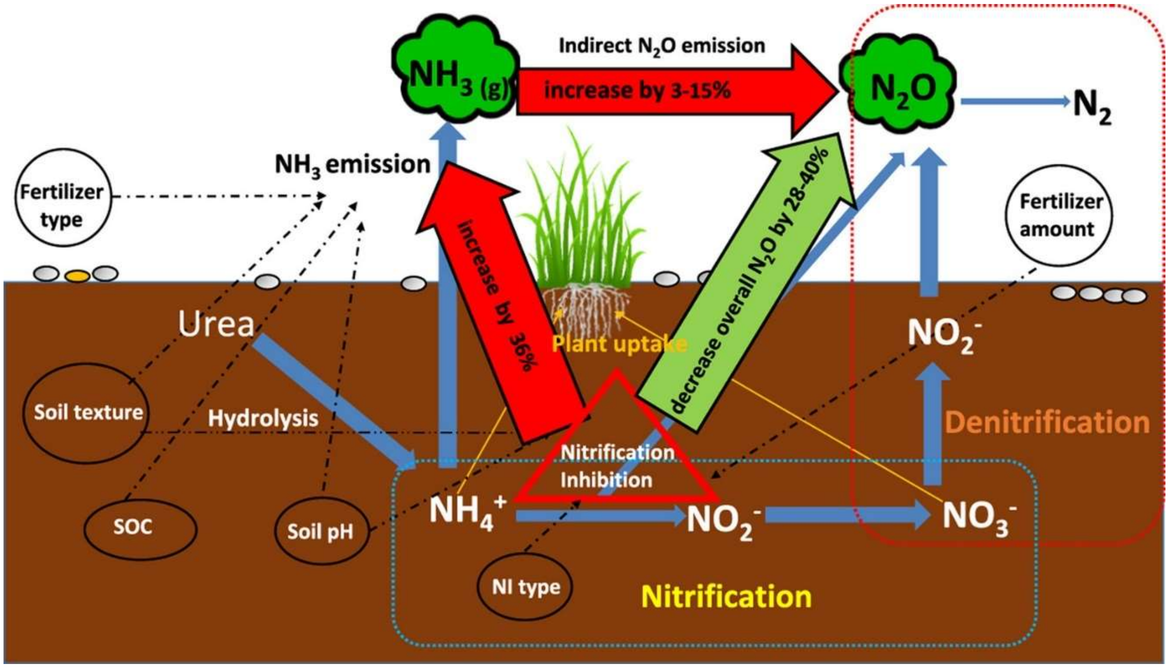


Figure 1.5: A schematic diagram about the role of nitrification inhibitors in reducing N loss during nitrification and denitrification processes in soil (Wu et al., 2021).

The process of breaking down urea into its constituent components is facilitated by the urease enzyme, which is synthesised by bacteria found in soil. This enzymatic reaction leads to the release of NH_3 as a byproduct. The urea molecule acts as a monodentate ligand and occupies the active site of urease, resulting in urea hydrolysis (Manunza et al., 1999). Urease activity is prevalent in eukaryotes and prokaryotes encompassing plants and fungus (Kumari et al., 2016). UIs can hinder urease enzymes and prolong urea hydrolysis through four distinct methods. The types of enzyme inhibition are as follows: (a) irreversible suicide substrate, (b) irreversible binding, (c) reversible non-competitive, and (d) reversible competitive (Hadjipavlou-Litina & Gupta, 2017). Reversible inhibition of urease enzymes occurs through non-covalent interactions, specifically hydrogen bonding. The irreversible urease have functional groups capable of interacting with amino acid at active site of urease enzyme (Shah & Soomro, 2012).

The phosphoramide NBPT, is currently the most successful UI available in the market (Cantarella et al., 2018). NBPT has demonstrated an 84% reduction in NH3 emissions and also contributes to reduction of N_2O release in atmosphere (Forrestal et al., 2016). The increase of 0.8% to 10.2% in crop production achieved by using NBPT in combination with urea ranges (Cantarella et al., 2018). In perennial ryegrass Combination of NBPT and urea resulted in enhanced dry matter production of by optimising the effectiveness of urea. The addition of NBPT to urea resulted in dry matter yields that were similar to those achieved with calcium ammonium nitrate fertilisers, suggesting that the former has the potential to decrease both N_2O and NH_3 emissions (Forrestal et al., 2017; Watson & Miller, 1996). The capacity of UI and NI to decrease GHG emission resulting from widespread use of agricultural fertilisers has been acknowledged for a considerable period of time. The use of UIs and NIs greatly improved the recovery of N by increasing soil retention, enhancing crop absorption, and lowering N losses. Specifically, use of UIs boosted N recovery by 16.4 %, while the use of NIs raised it by 10.2 % (Sha, Ma, Wang, et al., 2020). A comprehensive worldwide study by (Fan et al., 2022) evaluated impact of inhibitors on crop yield and the release of gaseous N in various agricultural practises and environmental contexts. The UI proved very efficient in achieving a 5% increase in crop yields and a 51% reduction in NH_3 volatilization. On the other hand, NI was most effective in reducing N_2O emissions by 49%. The integration of UI and NI can effectively achieve a harmonious balance between crop yield and conservation of environment, without any negative impact on pollutant levels. When adopting inhibitors, it is important to take into account agricultural management practises and environmental conditions (Fan et al., 2022).

1.2.9. Effect of N fertilizer and cropping sequence on soil C and N fractions, pH, enzymatic activities, and active microbial community

N is an essential nutrient for plant growth, but excessive N application can have negative impacts on SOC levels. The impact of N fertilisation on SOC is complicated and contingent upon various aspects, such as the specific N fertiliser type, the application rate, soil type, pH levels, and climatic conditions. Study conducted by Yue et al. (2016) demonstrated applying N fertiliser at the optimal rate is crucial for maximising the storage of SOC, N fertiliser can directly enhance the mineralization of SOM by modifying microbial activity and biomass. N can also indirectly enhance the process of SOM mineralization by promoting net primary productivity (NPP). This impact is especially significant in agroecosystems that rely on maize cultivation, as N inputs can boost NPP by over 200% (Poffenbarger et al., 2017). Enhanced NPP, which encompasses rhizodeposition, can enhance SOM mineralization by boosting microbial biomass or enzyme activity, this phenomenon is commonly known as "positive priming." (R. Chen et al., 2014). Kaur et al. (2008) examined the impacts of fertiliser application in a maize-wheat cropping system, through the comparison of various fertiliser treatments with an unfertilized control, it was revealed that the continual application of fertilisers led to a significant rise in SOC levels in comparison to the original content. This research suggests that fertilisers play a role in the buildup of organic materials in the soil. Secondly, they found that the active fractions of SOC, which are vital for soil health, showed significant improvements when fertilizers were applied, the active components play a crucial role in nutrient cycling and microbial activity. C mineralization, an indicator of organic matter decomposition, increased over time gradually. This suggests, the addition of NPK fertilizers had a substantial impact on the breakdown of organic matter. The findings reflect strong recommendation towards the integrated use of NPK fertilizers to sustain the productivity and health, the nutrient management approach promotes the accumulation of SOC which enhances active SOC fractions and supports the long-term sustainability of agricultural practices in this cropping system. In a study conducted by B. Chen et al. (2014) demonstrated that addition of N resulted in notable decrease in soil pH, from 5.3 to 4.9. Furthermore, the introduction of N led to a reduction in microbial biomass C, as well as a decrease in the overall abundance of microbial, bacterial, and fungal populations. Furthermore, the addition of N did not have a substantial impact on the C and N levels in the overall soil. Notably, the concentration of C in the particulate organic matter fraction was dramatically elevated due to N addition. The research study conducted by Gu et al. (2009) examined MBC and MBN, as well as the structure of microbial community, and their impact on crop yields in long-term fertilisation experiment. Results illustrated that the combined utilisation of N, phosphorus, and potassium enhanced the amount of microorganisms in the soil, enhanced the variety of bacterial communities, and sustained crop yield. The application of N fertilisation influences the composition of soil microbial and nematode communities, as well as the functioning of ecosystems, by altering environmental factors such soil pH and soil organic C levels.

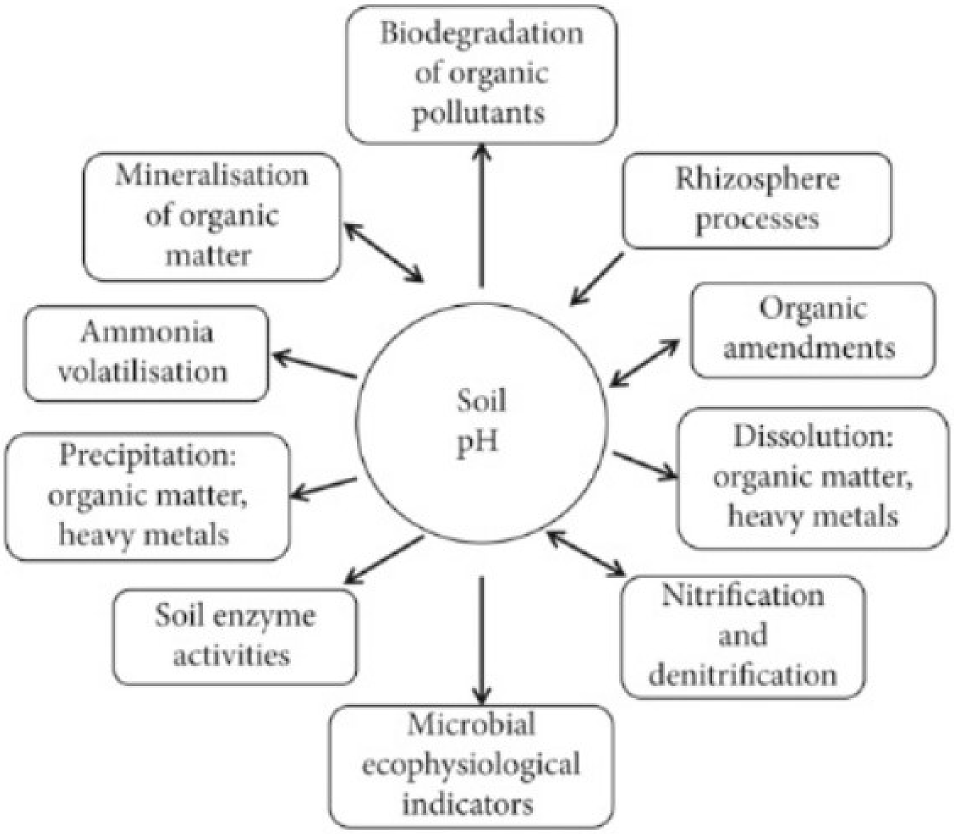


Figure 1.6: Some biogeochemical processes and their relations with soil pH by Neina (2019).

The study conducted by Zhao et al. (2015) using multivariate analysis to examine the profile of PLFA. The results revealed distinct variations in the composition of soil microbial communities across the four treatments. The primary factor influencing these differences was found to be the pH level of the soil. The presence of PLFAs, which are markers for Gram-negative bacteria, showed a correlation with soil pH, while no such correlation was observed for fungus and actinobacteria. The findings indicate that soil pH had a more significant influence on the composition and activity of soil microbial communities in a cropping sequence system that involved legumes, compared to N fertilisation (Zhao et al., 2015).

Agricultural intensification can simplify agroecosystems to single crop plantations, but practices like cropping sequence, intercropping, and companion planting maintain some crop diversity. Reducing diversity can affect ecosystem function. Higher crop diversity in rotation led to greater microbial richness and diversity (Venter et al., 2016), the addition of N fertilisation and organic treatments, including cover crops and composted manure, resulted in an increase in C stored in POM. Among the different treatments, the cover cropping system had the highest proportion of C stored in POM, while reducing C associated with the mineral associated organic matter fraction..

Introducing grain legumes into cereal-based cropping systems improves soil health and fertility through crop diversity. SOC is a fundamental measure of soil health, found in large quantities in aggregate fractions and bulk soils. It is also present in faster cycling SOC pools that are more responsive to management practises, these pools include water extractable organic C, POM-C, possibly mineralizable C, and macroaggregate C (Witcombe et al., 2023). The various root exudates from different crops in rotation provided a variety of C sources, boosting the proliferation of beneficial soil microorganisms (Larkin, 2008). These microbes enhance soil health and increase crop productivity through their contributions to nutrient cycling, breakdown of organic matter, and suppression of diseases (Z. Li et al., 2019). Soil management strategies, along with the prolonged use of N fertilisation, could potentially amplify alterations in soil quality, the most effective indices of soil quality were microbial respiration, microbial biomass, total organic C, urease enzyme activity, metabolic quotient (de Andrade Barbosa et al., 2019).

Soil microbes are essential for the process of mineralization and decomposition of complex organic compounds. The abundance and variety of microorganisms, as well as their functional capabilities, are significantly affected by the amount and type of crop residue. In a research study by Lori et al. (2017) it was discovered organic systems exhibited a significantly higher MBC, MBN, and urease compared to conventional systems. The agricultural techniques had no impact on the metabolic quotient, which serves as an exclusive measure for stressors on microbial populations. Incorporation of legumes in the cropping sequence, and the application of organic inputs are significant farming practises that impact the size and activity of soil microbial communities. The Shannon-Wiener diversity index, which measures species variety in a community, was considerably influenced by the use of cover crops, specifically, the rye treatments showed a higher level of microbial diversity (Nair & Ngouajio, 2012). The findings of Aschi et al. (2017) indicate that altering the cropping sequence by adding faba bean prior to wheat cultivation alters the environment for microbial communities. This is achieved by supplying accessible C and N, as well as maintaining an appropriate soil pH. The introduction of this novel environment may have an influence on the composition and activities of microbial communities. Implementing a diversified cropping system, such as maize-soybean-wheat-oat, has the potential to enhance soil health by increasing activity of β-glucosidase, MBC, activity of urease enzymes, and MBN, (Alhameid et al., 2019). Borase et al. (2020) reported that incorporating pulse crops into cropping sequence result in increased β-glucosidase activity and MBC, as compared to a continuous maize-wheat cycle. Chahal et al. (2021) shown that introducing a diversification of crops enhances the activity of microorganisms in the soil, promotes the absorption of surface SOC, and improves long-term crop productivity. According to a comprehensive study conducted by Jaziri et al. (2022) on a wheat-based system, it has been found that implementing cropping sequence in semi-arid environments significantly improve soil C pools and microbial activity. A study by Muhammad et al. (2021) revealed cover crops are being cultivated more frequently to enhance soil health and agricultural yield, while also reducing environmental harm as compared to the absence of cover crops. When compared to the absence of a cover crop, the presence of a cover crop resulted in an overall increase in PLFA, M C, and MBN.

Most cropping sequence advantages are regulated by soil microorganisms through complex biochemical reactions (Kennedy, 1999; Kennedy & Smith, 1995). There have been many studies explored the effects of cropping sequence on soil microorganisms, which have demonstrated that cropping sequence increases soil microbial biomass (Zuber et al., 2018), changes enzyme activity (Jiao & Yuan, 2019), alters microbial diversity, and mediates community composition. The fact that cropping sequence increases soil microbial biomass C and nitrogen confirmed by several studies (McDaniel et al., 2014; Y. Wang et al., 2017). Cropping sequence can alter soil microbial composition and may be linked to soil enzyme activity by releasing enzymes from living and dead cells, ultimately affecting soil microbial diversity and activity (Jiao & Yuan, 2019). Xuan et al. (2012) observed that the structure, quantity, and variety of microbial communities in cropping sequence exhibited notable distinctions and were greater in comparison to those in monocropping. However, study conducted by Y. Wang et al. (2017) showed that cropping sequence increased soil microbial biomass and activity but did not affect soil bacterial diversity. Meanwhile, more accurate and detailed results have been obtained from the measurements of the soil microbial properties with recent advancements in genetics and bioinformatics (Hamel et al., 2018). Previous studies reported effects of N application on gene abundance, (Fu et al., 2020; Maul et al., 2019; Munroe et al., 2020) N_2O emission, (Lam et al., 2018; Lan et al., 2013; Ni et al., 2018; Zhao et al., 2017) crop yield and nutrient use efficiency (Cui et al., 2022; F. Xiao et al., 2022), soil pH (Aula et al., 2016; Chen et al., 2019; Fu et al., 2019), cropping sequence or N fertilizer on microbial diversity (Ai et al., 2012; Sileshi et al., 2008; P. Wang et al., 2017) β-glucosidase and urease enzyme in different jurisdictions or climate conditions (e.g, tropical and temperate conditions) (Allende-

Montalbán et al., 2021; Ramirez et al., 2012; Tang et al., 2023; F. Xiao et al., 2022; Zhang et al., 2015). However, it was unclear how N stabilizer and cropping sequence can affect these biochemical properties in podzolic soils under boreal climate.

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1.4. Co-authorship statement

Manuscripts based on the chapter 2, entitled “Effects of nitrogen stabilizers and cropping sequence on soil C and N fractions in podzol soils under boreal climate” and chapter 3 “Effect of N stabilizers and cropping sequence on soil biochemical attributes and active microbial population in podzolic soils under boreal climate” will be submitted to “soil biology and biochemistry” and “Science of the total environment” (Usman, M., Abid, H. U., Katanda, Y., Nyiraneza, J., Galagedara, L., Cheema, M. 2023). The primary author will be Muhammad Usman, the author of the thesis, and the corresponding author, the last author will be Dr. Cheema (supervisor). Dr. Judith Nyiraneza (co-supervisor), Dr. Yeukai Katanda (committee member) and Dr. Lakshman Galagedara (committee member) will be third, fourth and fifth authors, respectively. Dr. Cheema wrote the research grants, developed the plan of this field experiment, and assisted in writing the results and discussion for the work in Chapters 2 and 3. The data was collected, analyzed, and the manuscript was written by Muhammad Usman. Hafiz Usama Abid, Dr. Yeukai, helped with experimental setup, field samplings, sample preparation, and lab analysis. Dr. Cheema edited and reviewed the manuscript and supervised overall experimental activities.

Chapter 2

2. Effects of nitrogen stabilizers and cropping sequence on soil C and N fractions in podzol soils under boreal climate

2.1. Abstract

Soil carbon (C) is crucial in the global C cycle as it improve water holding capacity, increases the availability of nutrients, and promotes the growth of microorganisms, resulting in enhanced soil quality and fertility in agricultural soils. Soil C is affected by different factors including land use change, management practices such as nitrogen (N) fertilizer, cover crops, cropping sequence and climate change. Excessive N application result in N losses through leaching, volatilization, and surface run-off, and these N losses can be minimized by using N stabilizers. A field trial was carried out to examine the impact of N stabilizers and cropping sequence on microbial biomass nitrogen (MBN), SOC, particulate organic matter N (POM-N), microbial biomass carbon (MBC), total N (TN), particulate organic matter C (POM-C), in podzolic soils in boreal climate. Experimental treatments were five N sources [control, Urea, urea split, and three N stabilizers (Agrotain, Super-U, and eNtrench)] and three cropping sequences (corn- corn-corn, corn-wheat-corn, and corn-faba bean-corn). Soil samples were collected after harvesting the crop in 2020 and 2021. Results showed that N sources significantly impacted SOC, TN and POM-N, however no significant impact on POM-C, MBC and MBN in 2021. However, N sources had significant effects on TN in 2020. Cropping sequence had significant effects on POM-C, MBC and MBN. The c-fb-c sequence showed 26 % more MBN as compared to c-w-c, while c-w-c sequence exhibited 59 % more MBC as compared to c-fb-c. Additionally, c-c-c showed 9 %, and 11 % higher POM-C as compared to c-w-c and c-fb-c, respectively but no-significant effect on TN, TC, POM-N. In 2020, only MBN was significantly affected by cropping sequence and c-w-c rotation showed 205 % higher soil

MBN compared to c-fb-c cropping sequence. We can conclude that N sources and cropping sequence significantly impacted some soil C pools and played important role in C retention in soil. However, long term studies with different N rates and long-term crop rotation trials are required to expand knowledge on C sequestration in podzol soil under boreal climate.

2.2. Introduction

Soil carbon (C) serves as a prominent indication of soil fertility, and the process of soil C sequestering plays a crucial role in mitigating greenhouse gas emissions (Lal, 2004; Rodrigues et al., 2023). The soil C pool is the most substantial component within terrestrial ecosystems, constituting 81 % of the total C present in the ecosystem (Heimann & Reichstein, 2008). Soil C cycle is highly sensitive to even minor disturbances, as it exhibits a significant response to climate change due to the multifaceted nature of C and its various functions (Smith et al., 1999). Soil C serves as a reservoir for soil nutrients and it enhances soil aggregation (Miner et al., 2018), water-holding capacity and supplements the energy provision for microorganisms (Huang et al., 2021; Soares & Rousk, 2019). Soil C is affected by multiple factors, such as amount and quality of organic matter inputs, activity of soil microbes, and chemical and physical characteristics of the soil (Wei et al., 2020). The relationship between soil C and nitrogen (N) is highly interconnected, with N playing a crucial role in the biogeochemical cycling of C as C serves as an essential energy source for microorganisms and plant growth (Frey et al., 2014). Numerous studies have demonstrated that the excessive use of N fertiliser has adverse effects on the chemical and physical characteristics of soils (Han et al., 2022; Min et al., 2021). This includes the deterioration of soil health, reduction in diversity of microbes, and alteration of microbial community composition (Berlinches de Gea et al., 2023). Hence, the impacts might have far-reaching consequences on global biogeochemical cycles (Duflot et al., 2022). N is lost from soil through different process such as ammonia volatilization, nitrification, and denitrification process (Cameron et al., 2013). Identifying innovative approaches to reduce N losses, enhance crop productivity, preserving soil fertility, are seems to be vital for reaching the goal of sustainable agricultural production. The use of nitrification inhibitors (NIs) and urease inhibitors (UIs) are known to reduce N losses in different cropping systems (Qiao et al., 2015; Zaman et al., 2013). Prior research has demonstrated the efficacy of using UIs and NIs to mitigate N losses (Abalos et al., 2014; Ibarr et al., 2021; Klimczyk et al., 2021). (Yu et al., 2007). NIs are the chemical compounds, including dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP), that have the ability to impede the transformation of NH_4^+ into mobile nitrite and nitrate ions (NO_2^- and NO_3^-) by suppressing the activity of ammonia-oxidizing bacteria (Hill et al., 2015; Zerulla et al., 2001). Subsequently, a tridentate ligand is formed between the UI and the urease enzyme, resulting in the deceleration of urea (UR) hydrolysis (Singh et al., 2013). This process effectively mitigates N losses in the form of ammonia volatilization (Dawar et al., 2011). The application of N fertilizer can have an impact on the levels of soil organic C (SOC) by influencing the influx of newly formed particulate matter organic C (POC) into the soil (Frey et al., 2014) as well as the release of organic C through microbial decomposition (Janssens et al., 2010). Prior research (Romanenkov et al., 2019; Sithole et al., 2019; Zhao et al., 2018) has documented that the sequestration of organic C in response to N addition can exhibit three distinct patterns of change: an increase, a constancy, or a decrease. The availability of N plays a pivotal function in affecting the cycling and storage of C (Feng et al., 2021). For instance, this phenomenon is observed in plant litter, where materials with a low C-to-N ratio (C: N) exhibit a more rapid rate of decomposition compared to those with a high C:N ratio (Zhang et al., 2017). There exists a positive correlation between the rate of soil organic matter (SOM) decomposition and the effectiveness of soil N. Consequently, enhancing N effectiveness leads to an acceleration of SOM decomposition, which in turn has a negative impact on the sequestration of soil organic C (Du & de Vries, 2018). Nevertheless, the presence of N enhances the input of SOC through its positive influence on plant growth and the subsequent formation of plant litter. Simultaneously, the introduction of N also hinders the decomposition of SOC by diminishing soil microbial activity and facilitating the creation of aggregates (Ye et al., 2018). Management techniques aiming at improving the soil C sequestration or increase soil C are necessary for sustainable crop production (Jarecki & Lal, 2003). Cover crops, residual retention, diversified cropping sequences and incorporating crops with more root mass into rotations are examples of agricultural management techniques to raise soil C (McDaniel et al., 2014; Paustian et al., 2016; Poffenbarger et al., 2017; Tiemann et al., 2015). Cropping sequence is a practise that confers numerous benefits to agriculture, enhancing the structure of the soil, availability of water, and the penetration of roots (Bhandari et al., 2020). Consequently, this practise contributes to the overall improvement of soil fertility and the sustenance of high productivity levels. Cropping sequence has been demonstrated to enhance biodiversity on farmland, resulting in a reduction in the occurrence of pests and diseases, while also ensuring the stability of crop yields (Dainese et al., 2019). The presence of plant cover plays a crucial role in mitigating soil erosion, thereby safeguarding the valuable soil nutrients within the cultivated layer of farmland (Mohammed et al., 2021). Additionally, it helps regulate soil temperature and minimize water loss (Stipešević & Kladivko, 2005). Furthermore, plant roots contribute to the breakdown and conversion of SOC through the input of the release of root exudates and plant leftovers (Frasier et al., 2016). Consequently, this process enhances the accumulation of SOC and promotes a more advantageous composition and variety within the soil microbial population (Vukicevich et al., 2016). Cropping sequence affects soil C, soil N, and other agroecosystem processes via influencing below and above-ground biomass (Sindelar et al., 2016). Cropping studies have shown that diversifying cropping sequence increases soil C and total N (Alhameid et al., 2017; Maiga et al., 2019). Legume-based cropping systems have also enhanced soil C (Hobley et al., 2018), though research conducted by (Chen et al., 2018) indicates that adding legumes has a priming effect on the rhizosphere that lessens the impact of residual retention on soil C. Cropping sequence has the potential to improve crop yields and facilitate soil C sequestration, although the extent of their impact varies depending on the specific combination of crops applied (Higashi et al., 2014). The use of a wheat-soybean cropping sequence has been found to yield the highest levels of richness and biodiversity within the overall microbial community (González-Chávez et al., 2010). This, in turn, has led to the production of microbial products that have a notable impact on the retention of soil C and N (Cheng et al., 2007; Loranger-Merciris et al., 2006). (Bowles et al., 2020). Many studies have reported effect of NIs and UIs on N_2O emission ((Lam et al., 2018; Lan et al., 2013; Ni et al., 2018; Zhao et al., 2017), crop yield and nutrient use efficiency (NUE) (Cui et al., 2022; F. Xiao et al., 2022). However, there is little known about effect of N stabilizers and cropping sequence on soil C and N fractions in podzol soils under boreal climate. Therefore, we hypothesized that N stabilizer and cropping sequence will enhance soil C and N fractions in podzolic soils under boreal climatic conditions. The specific objectives of this study were:

i. To investigate effect of N stabilizers on soil C and N fractions in podzol soils under boreal climate.

ii. To evaluate effect of cropping sequence on soil N and C fractions in podzolic soils under boreal climatic conditions.

2.3. Material and methods

2.3.1. Experimental site and treatments

A three-year cropping sequence field research study was commenced in 2019 at the Western Agriculture Centre and Research Station at Pynn's Brook, Newfoundland and Labrador (NL). The experimental treatments consisted of five different sources of N fertiliser: 1) control - C (no fertilizer), 2) Urea - UR, 3) Split Urea – US (30% and 70%), 4) SuperU™-SU (urea coated with DCD and NBPT, 5) eNtrench-EN (urea coated with nitrapyrin), 6) Agrotain-AG (urea coated with NBPT) and three cropping sequences: 1) corn- corn-corn (c-c-c), 2) corn-wheat- corn (c-w-c), 3) corn-faba bean- corn (c-fb-c). The study implemented a randomised complete block design (RCBD) with a split plot configuration and four repetitions. The cropping sequence and N sources were considered as main and subplots, respectively. The dimension of the plots was 3.2 m by 4 m.

TREE LINE

Block 4						
US	CT	EN	SU	UR	AG	**Canola**
EN	SU	AG	US	UR	CT	**Wheat**
SU	AG	UR	EN	US	CT	**Corn**
CT	SU	EN	UR	SU	AG	**Faba beans**
SU	US	EN	AG	CT	UR	**Oat/Peas**

Block 3						
EN	UR	AG	US	CT	SU	**Faba beans**
EN	SU	UR	CT	AG	US	**Oat/Peas**
CT	EN	US	UR	SU	AG	**Canola**
EN	SU	CT	AG	US	UR	**Wheat**
UR	AG	US	EN	CT	SU	**Corn**

Block 2						
SU	EN	UR	US	CT	AG	**Wheat**
AG	US	SU	EN	CT	UR	**Oat/Peas**
AG	EN	UR	SU	CT	US	**Canola**
AG	CT	EN	SU	US	UR	**Faba beans**
US	AG	EN	SU	UR	CT	**Corn**

Block 1						
CT	EN	SU	US	UR	AG	**Oat/Peas**
US	UR	AG	CT	SU	EN	**Wheat**
US	SU	UR	CT	EN	AG	**Corn**
AG	CT	UR	US	SU	EN	**Faba beans**
CT	SU	AG	US	EN	UR	**Canola**

ROAD

Figure 2.1: Experimental site layout at the Western Agriculture Centre and Research Station at Pynn's Brook, NL. CT: Control, UR: Urea, US: Urea split application, SU: Super-U (urease inhibitor + nitrification inhibitor), EN: eNtrench (nitrification inhibitor), and AG: Agrotain (urease inhibitor).

Figure 2.2: Aerial view of experimental site during 2020 growing season at the Western Agriculture Centre and Research Station at Pynn's Brook, NL.

Silage corn was planted as a primary crop during 2019 followed by wheat, faba bean in 2020 and silage corn in 2021. The application of all required fertilisers, including N stabilisers (AG, EN, and SU), phosphorus, and potassium, was done as a basal dose during sowing, except for N which was applied as the full dose of UR along with other fertilisers during sowing. Additionally, UR in split application (US) was applied 30% at seeding and 70% at V6 stage of silage corn. In the 2020 crop season, silage corn, wheat and faba bean crops were fertilised with either UR or N stabilisers at a rate of 115, 100 and 25 kg N ha^{-1}, respectively. In 2021, silage corn was fertilised with either UR or N stabilisers at rate of 115 kg N ha^{-1}.

Table 2.1: Biweekly rainfall data of the experimental site during the 2020 and 2021 growing seasons and historical rainfall data was obtained from the weather station in Deer Lake Airport, NL (YDF 71809).

Growth period	**Rainfall (mm)**		**Last thirty-three year's data (1986-2019)**
	2020	**2021**	**Rainfall (mm)**
May 16-31	35.50	45.40	42.96
June 1-15	32.70	22.80	41.42
June 16-30	37.40	73.70	33.92
July 1-15	29.60	9.80	43.08
July 16-31	30.50	115.80	42.32
August 1-15	19.60	53.60	44.41
August 16-31	115.80	24.80	55.02
September 1-15	27.80	104.80	56.98
September 16-30	26.70	88.70	47.61
October 1-15	79.10	9.70	51.61
October 16-31	58.6	107.2	45.90
Total	493.30	656.30	505.21

SAMCO drill planter (SAMCO 2200 Agricultural Manufacturing, Limerick, Ireland) was used to sow silage corn on June 14, 2019, June 12, 2020, and June 05, 2021, the seeding rate was 9 seeds m^{-2}. On June 12, 2020, wheat and faba bean were sown at seed rate 450 seeds m^{-2} and 45 seeds m^{-2}, respectively. The harvest dates for faba bean, wheat and silage corn were September 17, 2020, October 1, 2020, and November 5, 2020, respectively. On October 25, 2021, silage corn was harvested during the 2021 cropping season.

2.3.2. Soil sampling and analyses

Three soil samples per plot obtained from all plots at depth of 0-20 cm by a soil auger following the harvest of crops in both 2020 and 2021. The three cores combined and homogenized, sieved with 2 mm mesh to make a composite which was subsampled for analyses. First subsample was at room temperature, air-dried for analysis of SOC, particulate organic matter C (POM-C), total N, and particulate organic matter N (POM-N). The second subsample stored at 4 °C to measure microbial biomass C (MBC) and microbial biomass N (MBN). SOC and total N in samples were measured by dry combustion using CHNS elemental analyzer (Al-Kaisi et al., 2005; Jagadamma et al., 2007). The determination of POM-C and POM-N (with diameters ranging from 53 to 2000 μm) was conducted using a modified method based on the procedure described by Moni et al. (2012). In short, 25 g of soil that had been dried in the air and passed through a sieve with a mesh size of less than 2 mm, along with 60 glass beads with a diameter of 5 mm, were vigorously shaken for a duration of 16 hours at a speed of 130 revolutions per minute, in the presence of 100 mL of water. Following agitation, the suspension was carefully transferred onto a sieve with a mesh size of less than 2 mm, which was placed on top of a sieve with a mesh size of 53 μm. This process effectively separated the beads from any remaining POM residues. The remaining material obtained from the <53 μm sieve was subjected to oven drying at temperature of 60 °C, for duration of 48 hours. Subsequently, it was ground finely using ball mill grinder. The POM-C and POM-N were determined through dry combustion utilising a CHNS analyzer (Patra et al., 2020). The fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987) was used to measure soil MBC and MBN. In short, a 10 g soil sample treated for 24 hours with ethanol-free chloroform at a temperature of 25 °C, another sample left unfumigated. The soils, fumigated and non-fumigated, were subjected to extraction using 40 mL of solution containing 0.5 mol L^{-1} of K_2SO_4. The extraction process involved shaking the soils for a duration of 1 hour. The samples passed through 0.45 µm filter paper to remove impurities and then analysed for MBC and MBN using a Shimadzu TOC-LCPH/TN analyzer manufactured by Shimadzu Inc., Japan. The data expressed based on the dry mass, which was obtained by oven-drying at a temperature of 105 °C.

MBC calculated as:

$$\mathrm{MBC} = \frac{\mathrm{E_C}}{\mathrm{k_{EC}}} \text{ .... } (1)$$

where EC = (C from fumigated samples) - (C from non-fumigated samples) and k_{EC} = 0.45 (Wu et al., 1990).

MBN was calculated as:

$$\mathrm{MBN} = \frac{\mathrm{E_N}}{\mathrm{k_{EN}}} \text{............ } (2)$$

where EN = (N from fumigated samples) - (N from non-fumigated samples) and k_{EN} = 0.54 (Brookes et al., 1985).

2.3.3. Statistical analysis

Two-way analysis of variance (ANOVA) was conducted to assess effects of N sources and cropping sequence on SOC, TN, MBC, MBN, POM-C and POM-N using XLSTAT 2021.3.1 software (Lumivero, Denver, USA). Tukey’s post hoc test was employed at the probability level of 0.05 to compare the treatment means. The software Sigma plot 15.0, developed by Systat Software Inc., was utilised for graph creation.

2.4. Results

2.4.1. Effect of nitrogen sources and cropping sequence on soil organic C, total N, microbial biomass C, microbial biomass N, particulate organic matter C and particulate organic matter N

Table 2.2: Two-way analysis of variance (ANOVA) showing the effects of N sources, and cropping sequence, and their interaction (N × C) on soil C and N pools in 2020.

Soil parameters	Nitrogen Sources	Cropping Sequence	N × C
MBC	NS	NS	NS
MBN	NS	**	NS
POM-C	NS	NS	NS
POM-N	NS	NS	NS
SOC	NS	NS	NS
TN	**	NS	NS

**Significant at $p < 0.01$, NS: non-Significant, TN: total nitrogen, POM-N: particulate organic matter nitrogen, MBN: microbial biomass nitrogen, POM-C: particulate organic matter carbon, SOC: soil organic carbon, MBC: microbial biomass carbon.

Table 2.3: Two-way analysis of variance (ANOVA) showing the effects of N sources, cropping sequence, and their interaction (N × C) on soil C and N pools in 2021.

Soil parameters	Nitrogen Sources	Cropping Sequence	N × C
MBC	NS	**	NS
MBN	NS	**	NS
POM-C	NS	**	NS
POM-N	**	NS	NS
SOC	**	NS	NS
TN	**	NS	NS

**Significant at $p < 0.01$, NS: non-Significant, TN: total nitrogen, POM-N: particulate organic matter nitrogen, MBN: microbial biomass nitrogen, POM-C: particulate organic matter carbon, SOC: soil organic carbon, MBC: microbial biomass carbon.

2.4.2. Effect of nitrogen sources on soil organic C, total N, microbial biomass C, microbial biomass N, particulate organic matter C and particulate organic matter N after harvesting the crop in 2020 and 2021

Statistical analysis demonstrated that N sources had significant effects on TN after harvesting the crop in 2020, whereas non-significant effects were found on MBC, MBN, POM-N, POM-C, and SOC (Table 2.1). In 2021, N sources had significant effects on SOC, TN, POM-N, and no significant effects on MBC, MBN and POM-C (Table 2.2).

N sources had significant ($p < 0.042$) effect on SOC after harvesting the crop in 2021 (Table 2.2). N stabilizers (EN and SU) application showed higher SOC compared to UR and control which showed the lowest SOC (Figure 2.1). Application of split urea is statistically at par with N stabilizers and UR. EN and SU stabilizers application enhanced SOC by 10.61 % and 8.82 % compared to UR and 12.58 % and 10.75 % compared to CT, respectively.

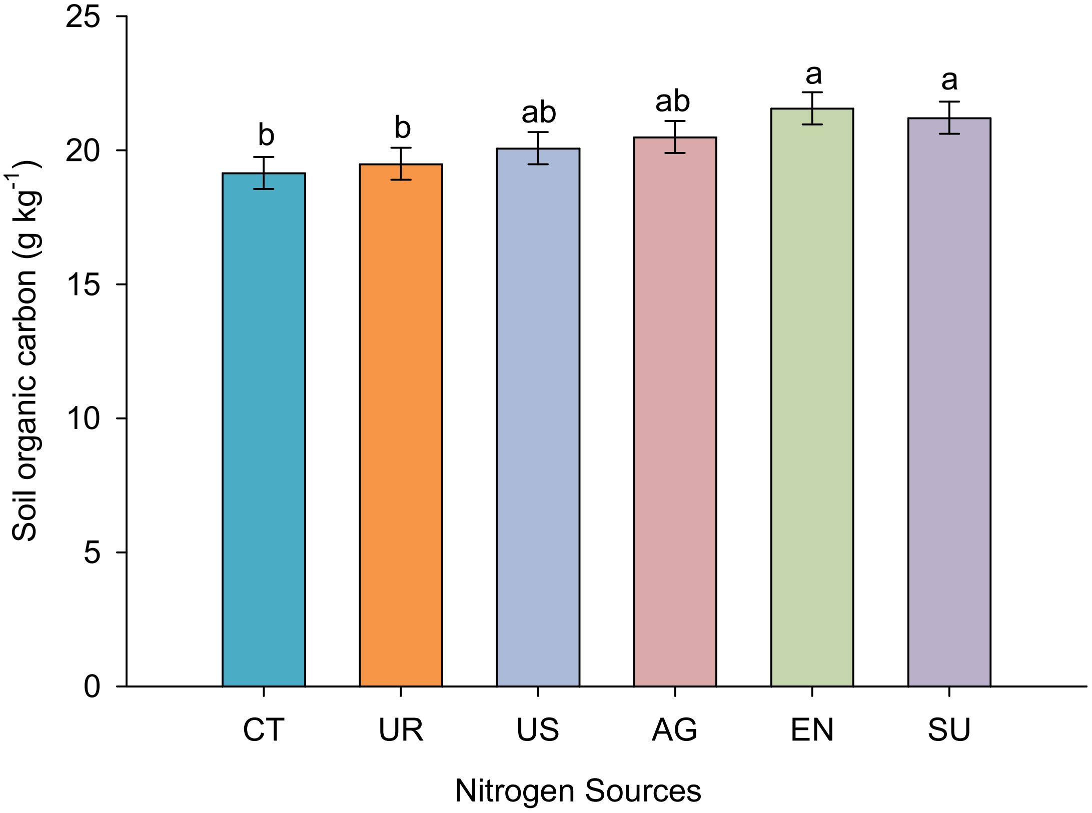

Figure 2.3: Effects of N sources on soil organic C on podzolic soil in boreal climate. The presence of distinct letters on bars indicates statistically significant difference among the treatments and error bars show the standard error. CT: Control, UR: Urea, US: Urea split application, SU: Super-U (urease inhibitor + nitrification inhibitor), EN: eNtrench (nitrification inhibitor), and AG: Agrotain (urease inhibitor).

N sources significantly affected soil TN after harvesting crops in 2020 and 2021 (Tables 2.1 & 2.2). During 2020, SU, a double inhibitor/N stabilizer showed the maximum soil TN and minimum was recorded in CT treatment. There were no significant effects between SU, other N stabilizers and UR application on TN (Figure 2.2a). After crop harvest in 2021, N sources also had significant ($p < 0.03$) effects on TN (Table 2.2). All N stabilizers (AG, EN and SU) application showed higher TN while lower was observed in CT. There were no significant differences between N stabilizers application and UR applications on TN whereas, no significant difference between CT and UR application (Figure 2.2b). SU application enhanced 16 % and 41 % TN compared to UR and control, respectively.

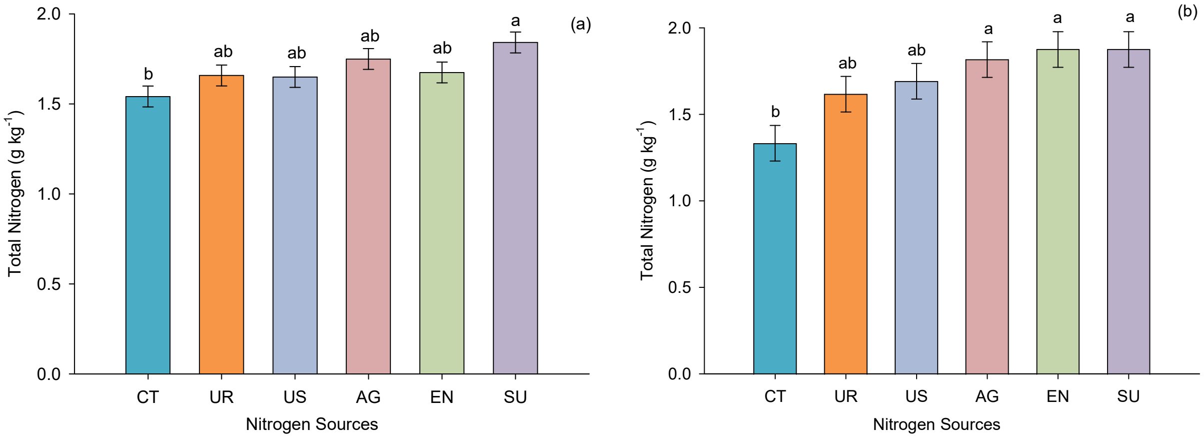

Figure 2.4: Effects of N sources on total soil N on podzolic soil in boreal climate, after harvesting in 2020 (a), and in 2021 (b). The presence of distinct letters on bars indicates statistically significant difference among the treatments and error bars show the standard error. CT: Control, UR: Urea, US: Urea split application, SU: Super-U (urease inhibitor + nitrification inhibitor), EN: eNtrench (nitrification inhibitor), and AG: Agrotain (urease inhibitor).

N sources had significant ($p < 0.043$) effects on soil POM-N in 2021 (Table 2.2). AG application showed higher soil POM-N compared to the lower value observed in control (Figure 2.3). US application had no difference and statistically at par with N stabilizer and UR. However, there was no significant difference among AG, EN and SU application, and UR application on POM-N. AG application enhanced 35 % POM-N compared to control.

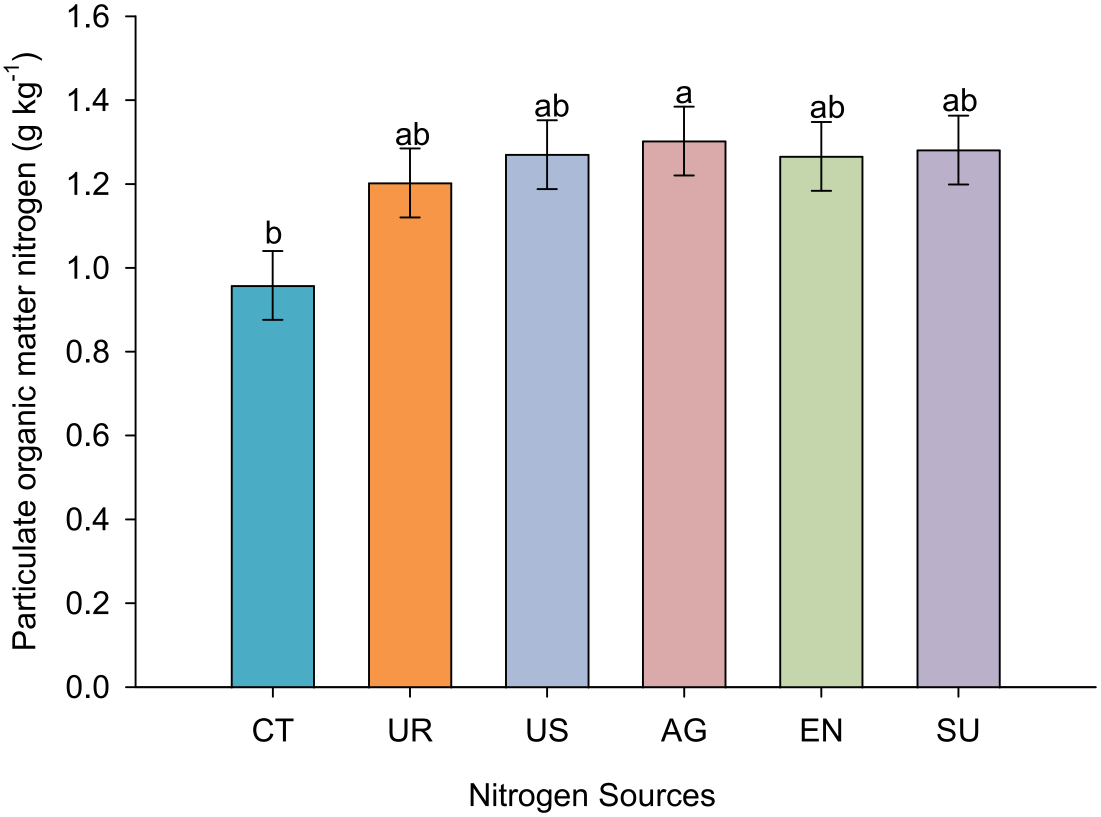

Figure 2.5: Effects N sources on soil particulate organic matter N on podzolic soil in boreal climate in 2021. The presence of distinct letters on bars indicates statistically significant difference among the treatments and error bars show the standard error. CT: Control, UR: Urea, US: Urea split application, SU: Super-U (urease inhibitor + nitrification inhibitor), EN: eNtrench (nitrification inhibitor), and AG: Agrotain (urease inhibitor).

2.4.3. Effect of cropping sequence on soil organic C, total N, microbial biomass C, microbial biomass N, particulate organic matter C and particulate organic matter N after harvesting the crop in 2020 and 2021

The ANOVA showed cropping sequence had significant ($p < 0.000$) effects on MBN whereas no significant effects on MBC, POM-C, POM-N, SOC, TN after harvesting in 2020 (Table 2.1). In 2021, cropping sequence had significant effects on MBC, MBN and POM-C and no significant effects on POM-N, SOC, and TN (Table 2.2).

Cropping sequence had significantly enhanced MBC (Table 2.2), and higher soil MBC (550 mg kg^{-1}) was observed in c-w-c sequence while lowest (345 mg kg^{-1}) was recorded in c-fb-c cropping sequence (Figure 2.4). There was no significant difference between c-fb-c and c-c-c cropping sequence on soil MBC.

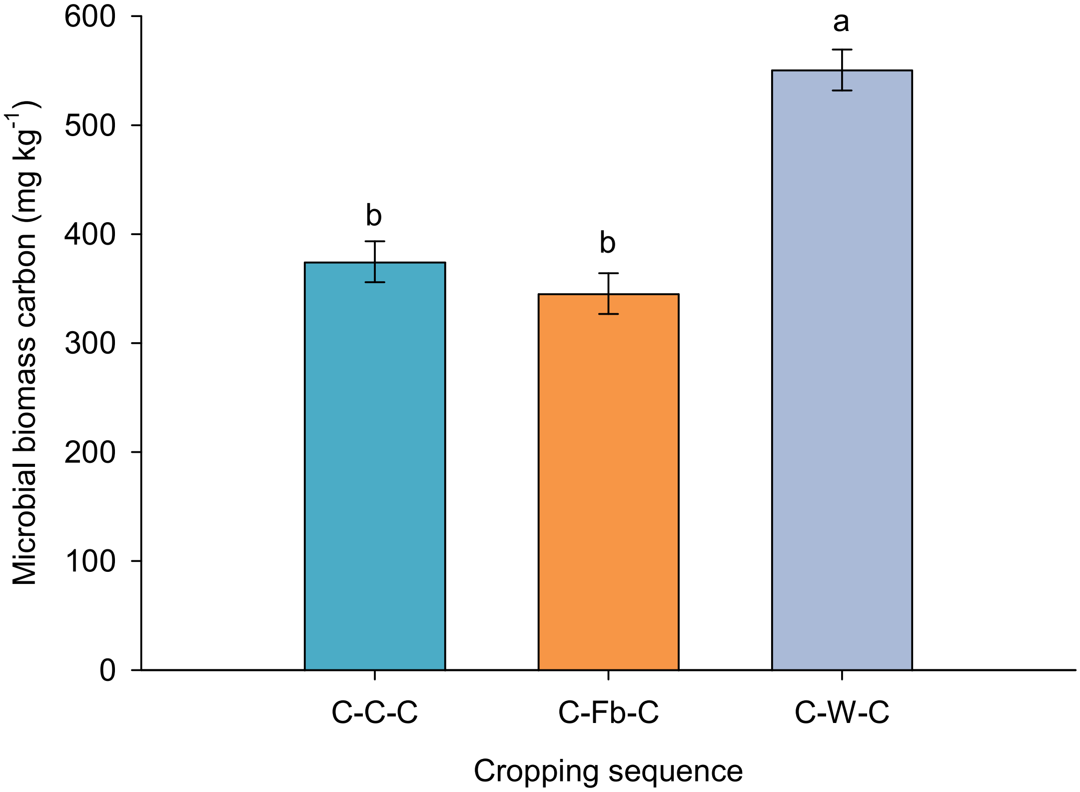

Figure 2.6: Effects of cropping sequence on soil microbial biomass C on podzolic soil in boreal climate. The presence of distinct letters on bars indicates statistically significant difference among the treatments and error bars show the standard error. corn–corn–corn (c–c–c), corn–wheat–corn (c–w–c) and corn–faba bean–corn (c–fb–c).

Cropping sequence had significantly ($p < 0.000$) influenced soil MBN after harvesting the crops in 2020 (Table 2.1). Higher MBN (60 mg kg^{-1}) observed in c-w-c followed by c-c-c (40 mg kg^{-1}) and lowest (19.6 mg kg^{-1}) recorded in c-fb-c. c-w-c sequence showed 205% higher soil MBN compared to C-Fb-C cropping sequence (Figure 2.5a). In 2021, cropping sequence also had significant ($p < 0.014$) effect on soil MBN (Table 2.2). Contrary to 2020, c-fb-c cropping sequence showed 36 % higher MBN compared to c-w-c which produced lowest MBN (Figure 2.5b). There was no significant difference between c-fb-c and c-c-c cropping sequence on MBN.

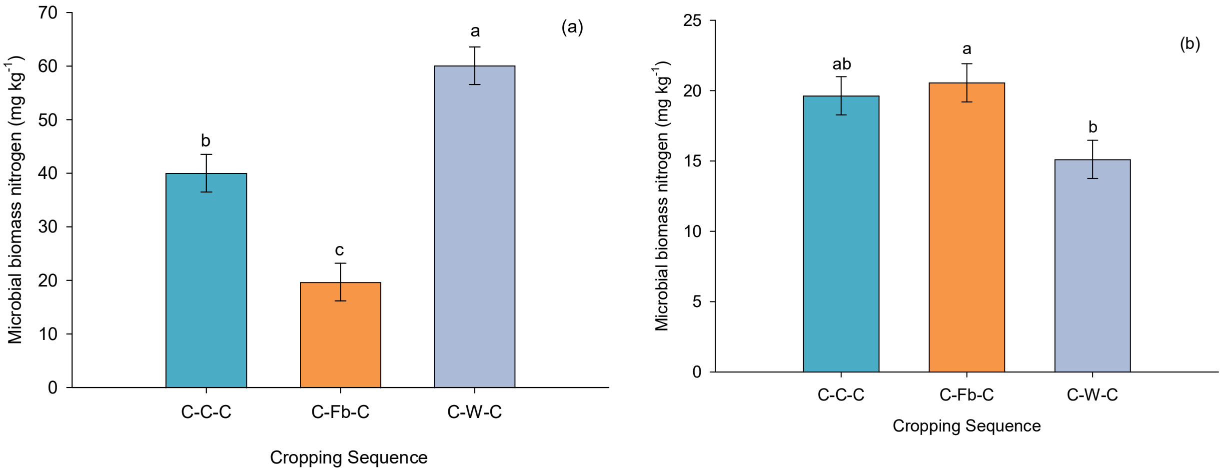

Figure 2.7: Effects of cropping sequence on soil microbial biomass N on podzolic soil in boreal climate (a) after harvesting the crop in 2020, (b) after harvesting the crop in 2021. The presence of distinct letters on bars indicates statistically significant difference among the treatments and error bars show the standard error. corn–corn–corn (c–c–c), corn–wheat–corn (c–w–c) and corn–faba bean–corn (c–fb–c).

Cropping sequence had significant ($p < 0.007$) effects on POM-C (Table 2.2). c-c-c cropping sequence produced significantly higher (16.47 g kg^{-1}) POM-C while lowest (14.72 g kg^{-1}) POM-C recorded in c-fb-c sequence (Figure 2.6). c-c-c cropping sequence exhibited 11.96% higher POM-C as compared to c-fb-c cropping sequence. There was no statistically significant difference between c-fb-c and c-w-c cropping sequence treatments on POM-C.

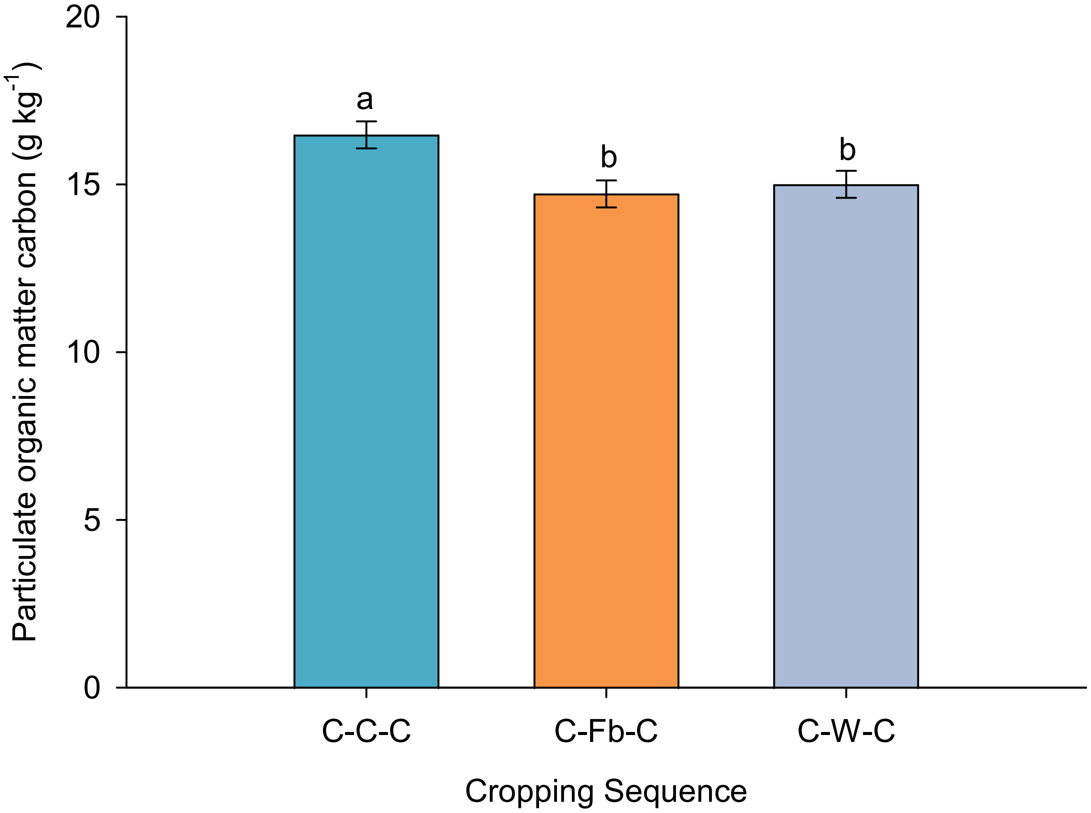

Figure 2.8: Effects of cropping sequence on soil particulate organic matter C on podzolic soil in boreal climate. The presence of distinct letters on bars indicates statistically significant difference among the treatments and error bars show the standard error. corn−corn−corn (c−c−c), corn−wheat−corn (c−w−c) and corn−faba bean−corn (c−fb−c).

2.5. Discussion

2.5.1 Effect of nitrogen sources on soil carbon and nitrogen fractions

Monitoring soil C enhances our understanding about the role of C-cycle in mitigating GHG emissions, ensuring food and energy security and biodiversity protection. SOC accumulation is largely determined by adding organic matter in the soil, such as plant and microbial residue, root exudates, and the output, such as mineralization and the degradation of SOC (Chen et al., 2017;

Lu et al., 2021; Wei et al., 2022). In the present study, N stabilizers, such as EN and SU application produced higher SOC compared to UR and CT. Higher SOC in EN and SU application can be attributed due to the presence of urease and nitrification inhibitors that might have delayed UR hydrolysis (Manunza et al., 1999), and hinder nitrification by decreasing the activity of nitrobacteria or ammonia monooxygenase, resulting in reduced N losses in the environment, enhanced NUE and resulted in an increase in the input of fresh organic C via plant roots to soil . Our findings demonstrated higher SOC in N stabilizers (SU and EN) treatments compared to UR alone due to increased belowground biomass of plants because of N addition owing to UIs limit urease activity in soils. UIs thereby helped in stimulation of root litter intake and enhancement quality of litter with N addition (Aerts et al., 1995; Matsushima & Chang, 2007) might influence process of litter decomposition (Knorr et al., 2005), which in turn can influence soil C storage. When it comes to crop productivity and the C-based processes that are related to soil quality, N is the most important nutrient which enhances C storage belowground. Although C and N are stoichiometrically coupled with SOM to maintain the functional stability of terrestrial ecosystems, it is expected that N fertilization will, over time, have an impact on soil quality and SOC concentration, or vice versa (Hessen et al., 2004). Previous research has reported a range of effects (positive, neutral, or negative) of N fertilization on SOC dynamics under a variety of management practices (Cusack, Silver, Torn, & McDowell, 2011; David et al., 2010; Lu et al., 2011). For instance, it has been observed that the addition of N fertilizer enhances crop yield and root biomass production, which leads to an increase in SOC content. On the other hand, researchers have also reported that the addition of N fertilizer leads to a drop in SOC content by accelerating the mineralization of native SOM (Bowden et al., 2004). However, it typically takes several years for the bulk SOC material to respond to changing management practices to undergo an absolute shift.

Previous studies have reported that N fertilizer boosts litter production and root exudates, which in turn increases the organic matter that help build up the SOC (LeBauer & Treseder, 2008; Lu et al., 2021).

N is important macronutrient required for crop growth, but N deficiency is seen as common in agricultural practises, due to the surplus input and lower utilisation coefficient, a significant quantity of N fertiliser is lost because of NH_3 volatilization, N_2O emissions, and NO_3^- leaching (Allende-Montalbán et al., 2021; Xia et al., 2016; Zhang et al., 2008; Zhang et al., 2015). In our study after harvest 2020, total soil N was significantly higher with SU application compared to control; SU is also known as double inhibitor (contains urease and nitrification inhibitors) and has the potential to maximize NUE, though other N stabilizers (AG, EN), SU and UR treatments were not statistically different from each other. UIs stop UR hydrolysis in soils, which allowed UR to stay in the soil for a few weeks to match crop uptake and nitrification inhibitors inhibit nitrification process, which result in a reduction in the amount of N that could have been lost through leaching. Similar, trend was observed after harvesting crop in 2021, N stabilizers showed higher total soil N but statistically at par with UR application either split application or full dose application at seeding. The presence of UI (NBPT) inhibits three distinct catalytic sites within the urease enzyme, through formation of tridentate bond involving an oxygen atom and two nickel centres, originating from carbamate bridge that connects two metal. This prevents hydrolysis process, which in turn boosts the efficiency of the N supply cycle (Cantarella et al. 2018). The UR hydrolysis process can be delayed up to 14 days due to NBPT application (Zaman et al., 2008). Consistent with the results of this study, Sigurdarson et al. (2018) determined that soil treated with NBPT exhibited a reduced pH. This change likely affected the equilibrium between NH_3 and NH_4^+ in the soil, resulting in a decrease in NH_3 loss and an increase in NH_4^+ concentration. The other reason for higher total soil

N in N stabilized treatments may be due to binding of NIs to active site of ammonia monooxygenase, which is a metallo-enzyme important in catalysing initial step of nitrification (Di & Cameron, 2002; Menneer et al., 2008). This inhibits the activity of ammonia oxidizers, which is necessary for the process of nitrification. This reduction in nitrification could potentially be the result of a limited rate of substrate availability (NH_4^+) as was previously said, NBPT slows down the UR hydrolysis process, which in turn slows down the conversion of UR to NH_4^+, in turn NI reduces the conversion of NH_4^+ to NO_3^- during nitrification process. In our findings, no significant difference among UR application and N stabilizers, due to ineffectiveness of N stabilizers in high moisture levels which were observed in our field experiment. Matczuk and Siczek (2021) observed that UI efficacy was impaired in high moisture content and temperature, while Adhikari et al. (2021) demonstrated NI efficacy was impacted in the same soil conditions. Soil moisture affect N inhibitors efficiency by impacting rate of nitrification and denitrification (Dobbie & Smith, 2001). The activity of urease is subject to the level of moisture present in the soil, where under dry soil conditions, the hydrolysis rate of urease is reduced (Volk, 1966). The aforementioned phenomenon exhibits a gradual increment as the soil's moisture content rises, ultimately get to 20 % (Bremner, 1978). Consequently, the hydrolysis of UR and the resulting production of NH_3 is inclined to be elevated in soils with high moisture content, particularly in conditions of elevated temperature (Terman, 1980). On the other hand, the application of UR on dry soils exhibits a gradual process of hydrolysis, which facilitates the reduction of volatilization losses. After entering soils, UR molecules easily hydrolyzed by urease into the compound $(NH_4)_2CO_3$, which results in the release of NH_3. This is one of primary mechanism that N is lost, and it is also the primary source of NH_3 in airborne aerosols (Ju et al., 2017; Xia et al., 2020). However, excess NH_4^+ in soil solutions and NH_4^+ absorbed on soil colloid are likely to nitrify and can leak along with natural precipitation. Denitrifying bacteria in low-ventilated environments can easily convert NO_3^- to N_2O or N_2, which can subsequently be lost from soils and further reduce N utilisation efficiency (Bouwman et al., 2013). All these N losses can be reduced by N stabilizers such as UIs, NIs or double inhibitors which delayed above mentioned process. The findings of present study are in line with previous studies where authors reported that N application increases soil NH_4^+ concentration due to delayed UR hydrolysis which enhanced soil N (Chen et al., 2019; Shen et al., 2016; Zhou et al., 2015).

The utilisation of particulate organic matter (POM) as a fraction of SOM based on particle size analysis has been established as an effective approach to detect early changes in SOM because SOM in the sand-sized fraction (>53 μm) typically exhibits greater susceptibility to change (Zeller & Dambrine, 2011). The POM is commonly utilised to denote most broken-down plant residues during the initial phases of humification (Besnard et al., 1996). The POM is composed of C and N that are relatively easy to mineralize (Sequeira et al., 2011). In our study, AG, a N stabilizer, exhibited higher POM-N compared to control, though statistically non-significant with other N stabilizers UR, and US. This might be due to the application of N stabiliser leads to an increase in the availability of N in soil, which subsequently impacts growth of plant as well as the biomass of heterotrophic microorganisms and the activity of decomposition in the soil. The fluctuations in N content of POM are influenced by the interplay of plant biomass generation and microbial decomposition. The process of soil acidification caused by N have the effect of inhibiting microbial decomposition which in turn, restrict the conversion of plant litter into organo-mineral fraction and lead to an increase in POM (Ye et al., 2018). The study results indicate that there was no statistically significant distinction observed between the application of N stabilisers, split urea, and UR treatments. This lack of differentiation could potentially be attributed to the inefficacy of N stabilisers in the presence of high moisture levels, as discussed above. Previous studies conducted by (Borges et al., 2019; Valdez et al., 2017) reported that N addition leads to a reduction in the C:N and an increase in crop-derived residues. This, in turn, may have a stimulating effect on the rate of decomposition of SOM mediated by microorganisms and can affect POM which is a vital component in the process of SOM turnover and is more sensitive to changes in soil management practises than other fractions of SOM.

2.5.2 Effect of cropping sequence on soil carbon and nitrogen fractions

Soil microorganisms play a vital role in the processes of SOM breakdown and retention, as well as in the cycling of C and N within the soil (Gessner et al., 2010). The microbial biomass present in soil is of utmost importance in the preservation of soil fertility and is widely acknowledged as a biologically dynamic reservoir within soil systems (Y. Li et al., 2018). The significance of microbial biomass lies in facilitating transformation of soil organic and inorganic reservoirs, thereby exerting a crucial influence on the regulation of plant nutrient assimilation (Liang et al., 2011). In our study, cropping sequence had significantly affected MBC and higher MBC was recorded in c-w-c sequence. This can be attributed to lower priming effect of wheat residues due to reduction in basal mineralization of SOC (Kan et al., 2022). Plant species differ in the amount and quality of substrates secreted in litter and root exudates, which can have profound effects on soil quality and the microbial community. Previous studies reported that intensity of priming effect was shown to be C input rate dependent, with increased inputs leading to increased CO_2 generation (Dimassi et al., 2014; Xu et al., 2016). Priming effect could have been more intense with high maize residue inputs as compared to wheat. Aromatic components like lignin are mostly responsible for the chemical recalcitrance of residue degradation (Schmatz et al., 2017). Lignin can only be broken down by white-rot fungus and requires strong oxidation agents for microbial decomposition. Maize leftovers and roots include a lot of lignin, their addition of C to SOM reduced by maize leftovers (Rasse et al., 2005). The implementation of cereal-legume cropping sequence has been a well-established and efficacious sustainable agricultural technique that offers numerous advantages such as increase crop yields while mitigating the need for additional chemical fertilisers, thereby decreasing dependence on such fertilisers (Cernay et al., 2018). Furthermore, the implementation of a cropping sequence has been shown to enhance soil nutrient levels, including organic C and TN, while also preserving the integrity of the soil structure (X. Li et al., 2019). Many studies reported enhanced MBC by different cropping sequence, for instance a study conducted by Borase et al. (2020) reported that maize-wheat-mungbean cropping sequence enhanced MBC. Another study by Benbi et al. (2012) reported maize-wheat cropping sequence improved MBC, and Song et al. (2022) reported wheat-soybean cropping sequences resulted into higher MBC due to the quality and quantity of different crop residues and root exudates.

The maintenance of soil fertility is heavily reliant on MBN, which is regarded as a biologically active N reservoir within soil (Treseder, 2008). It plays an important role in facilitating transformation of organic and inorganic N pools, thereby regulating the uptake of plant nutrients (Zhou et al., 2017). Alterations in agricultural management practises can impact the magnitude of the MBN reservoir, potentially serving as an early indicator of shifts in soil N stability (Q. Zhang et al., 2017). This is attributable to the MBN pool's greater susceptibility to alterations in the soil environment. In present study, soil MBN was found to be significantly impacted by the cropping sequence after the crop harvesting in the year 2020. The c-w-c sequence exhibited a higher MBN compared to the c-fb-c sequence which produced the lowest soil MBN. This cropping sequence was found to have a noteworthy impact on the soil MBN in 2021. In contrast to the findings of 2020, it was observed that the cropping sequence of c-fb-c exhibited a greater quantity of MBN in comparison to c-w-c. The observed phenomenon could potentially be attributed to enhanced residual root and litter variation within cropping sequences (Peralta et al., 2018). Cropping sequence serve to stimulate the diversity and growth efficacy of soil microbial communities, ultimately promoting soil's stability and enhancing resistance to environmental changes that were affecting the MBN (McDaniel et al., 2014). Cropping sequences has been observed to have an impact on soil-borne microbial communities, resulting in an enhancement of bacterial diversity (Ceja-Navarro et al., 2010) which is deemed advantageous for the maintenance of MBN. The cropping sequence has the potential to improve soil temperature, humidity, and SOM (Castro et al., 2016). This, in turn, can promote the proliferation and metabolic activity of fungi and bacteria, leading to increased diversity and biomass, while simultaneously impeding the growth of harmful microorganisms (Li et al., 2009). A previous study conducted by Fu et al. (2019) reported corn-winter wheat-winter wheat-millet cropping sequence enhanced MBN. Additionally, Borase et al. (2020) reported that maize-wheat-mungbean cropping sequence enhanced MBN because of an increase in the diversity of residual roots and litter, which encourages the diversity of the soil microbial population. The cropping sequences has been observed to yield substantial crop residues that are returned to the soil (Deng et al., 2000). This, in turn, provides organic N to soil microorganisms and promotes the proliferation of Gram-positive bacteria and fungi that facilitate the decomposition of recalcitrant fractions (Zechmeister-Boltenstern et al., 2015).

The SOM is a diverse and complex range of decomposable organic substances that reflect their ongoing decay, which is influenced by microbial activity (Kiani et al., 2017). This continuum encompasses a wide spectrum, from newly added plant residues to more stable humus fractions (Kantola et al., 2017). In order to better understand the dynamics of SOM, it is essential to evaluate the swiftly cycling SOM pools in diverse cropping systems. The implementation of this approach has the potential to facilitate timely identification of the trajectory of alteration and efficacy of pertinent labile SOM fractions resulting from land management strategies (Hernandez-Ramirez et al., 2009). One such labile fraction is known as POM-C, classified as an intermediate decomposable pool (Li et al., 2018) and represents a temporary pool in the ongoing process of SOM decay (Smith et al., 2020). The composition of POM-C comprises of fresh crop residues and microbial residues, making it a highly responsive indicator of recent modifications in cropping practises and their impact on the dynamics of SOM (Kooch & Noghre, 2020). In our experiment, it was found that cropping sequence had significant impact on POM-C, with the highest recorded in c-c-c sequence. The possible explanation of higher POM-C in c-c-c cropping sequence can be greater below-ground and above-ground biomass production that resulted in more C input by greater amount of biomass production (King & Blesh, 2018) that can be associated with elevated root exudation (Daly & Hernandez-Ramirez, 2020), thereby supplying energy as well as nutrients to soil microorganisms (Cates et al., 2019) that facilitates the efficient accumulation of the POM-C by modifying mineralization. The findings of our study align with previous research conducted by (Triberti et al., 2016), which found that diverse cropping sequences led to the accumulation of SOM due to increased supply C from crop residues and root biomass. This is also consistent with the findings of Lorenz and Lal (2005) and Martens (2000), who reported that cereal roots with high C:N ratios, phenol, and lignin contents decompose gradually, resulting in increased POM-C under a c-c-c cropping sequence. Cropping sequence had a substantial impact on the soil POM-C and also brought about alterations in the soil POM components characteristics by means of the buildup of crop residue that is resistant to decomposition and organic matter that has low bioavailability.

2.6. Conclusion

This study demonstrated that nitrogen (N) stabilizers significantly enhanced soil organic carbon (SOC) compared to urea (UR) application. However, split urea (US) application did not differ statistically among N stabilizers and UR application in enhancing SOC. Total N was improved by the application of N stabilizers as compared to control but there was no significant difference noted among UR, US, and N stabilizers. Highest particulate organic matter N (POM-N) was observed in urease inhibitor (Agrotain, though statistically at par with other N stabilizers eNtrench (EN) and superU (SU)). There was no significant difference among UR, split urea and N stabilizer application except AG which showed highest POM-N as compared to control. Cropping sequence also improved Soil C and N fractions. c-w-c cropping sequence exhibited highest microbial biomass C (MBC) and microbial biomass (MBN) compared to c-fb-c cropping sequence. However, c-c-c cropping sequence produced higher particulate organic matter C (POM-C) compared to c-fb-c cropping sequence. Based on results, we may conclude that N stabilizers and cropping sequence have the potential to improve soil C and N fractions in podzolic soils under boreal climate. However, to fully understand changes in soil C and N fractions in short time may not be feasible. Hence a long-term research trial can enhance our understanding of these management practices in improving soil C and N stocks in podzolic soils under boreal climate.

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Chapter 3

3. Effect of N stabilizers and cropping sequence on soil biochemical attributes and active microbial community in podzolic soils under boreal climate

3.1. Abstract

Soil microbes play a crucial role in preservation of soil functions, encompassing the aggregates formation, nutrient cycling, decomposition, organic matter stability, and bioremediation. This valuable contribution is mostly attributed to the existence of various enzymes that serve as catalysts for these diverse reactions. Enzymes play a critical role in the conversion of complex organic matter into plant-accessible nutrients, as well as in the decomposition of organic matter. The measurement of soil enzyme activity provides valuable insights into the rate of soil microbial metabolism and biochemical cycling. A field trial was carried out to examine the impact of N stabilizers and cropping sequence on soil pH, enzyme activities (urease and β glucosidase), active microbial population in podzolic soils in boreal climate. Experimental treatments were five N sources [control, Urea, urea split, and three N stabilizers (Agrotain, Super-U, and eNtrench)] and three cropping sequences (corn- corn-corn, corn-wheat-corn, and corn-faba bean-corn). Soil samples were collected after harvesting the crop in 2020 and 2021. Results demonstrated that N sources significantly impacted soil pH, urease and β glucosidase enzyme activity, active microbial population after harvesting the crop in 2021, however except β glucosidase enzyme activity, N sources had no significant in 2020. Cropping sequence had no significant effects on soil pH and enzyme activities in 2020 and 2021. However, cropping sequence had significant effects on gram positive bacteria (G^+), total bacterial phospholipid fatty acids (Σ B-PLFAs) and total phospholipid fatty acids (Σ PLFAs) in 2020. In 2021, cropping sequence and N stabilizers interaction significantly influenced G^+, G^-, Σ B-PLFAs and Σ PLFAs. Our results demonstrated that N

stabilizers and cropping sequences significantly affected soil pH, enzyme activities, active microbial community structure and abundance in podzolic soils under boreal climate. Long-term crop rotation and N stabilizers studies with different rates are required to fully understand the effects of these management practices on soil biochemical and biological processes in podzolic soils under boreal climate.

3.2. Introduction

Nitrogen (N) is an essential macronutrient, plays a vital role in crop growth and development and hence extensively utilized in agricultural production systems (Galloway et al., 2008). Urea (UR) is widely employed as a N-based fertilizer in crop production due to high N (46%), cost-effectiveness, and user-friendly application (Heffer & Prud'homme, 2016). Nonetheless, the efficacy of UR fertilization is hindered by its rapid hydrolysis rate (Artola et al., 2011), resulting in an excessive production of mineral N that surpasses the crop's capacity for assimilation during the initial phase (Allende-Montalbán et al., 2021). A portion of the N may undergo immobilization within the soil, while any surplus is susceptible to loss through either ammonia (NH_3) volatilization or nitrate (NO^-_3) leaching (Chien et al., 2009) or surface run off (Wang & Huang, 2021). To reduce NH_3 volatilization and NO^-_3 leaching in soil, various approaches have demonstrated their efficacy, such as N stabilizers encompassing urease inhibitors (UI) and nitrification inhibitors (NI) or double inhibitors (DIs). UI delay the hydrolysis of UR by inhibiting the activity of urease enzyme (Drury et al., 2017), upon the application of UR to the soil, a fast hydrolysis process occurs, resulting in the formation of ammonium carbonate (Wang et al., 2020). Ammonium carbonate exhibits inherent instability, leading to its decomposition into NH_3 and carbon dioxide (CO_2). NH_3 can undergo either absorption into the soil or volatilization (Liu et al., 2017). The hydrolysis step is governed by the urease enzyme (Mira et al., 2017), and the function of UI is to impede the activity of urease enzyme, so hindering the transformation of UR into NH_3 (Silva et al., 2017). NIs delays the nitrification process by inhibiting the activity of ammonia monooxygenase (Guo et al., 2014). The enzymatic activity of bacteria that oxidize NH_3 is significantly influenced by the presence of NI (Ruser & Schulz, 2015). The urea coated with NI has been seen in a postponement of the transformation process wherein ammonium ions (NH^+_4) are converted into nitrate ions (NO^-_3) (Di et al., 2014; Liu et al., 2017). The N-(n-butyl) thiophosphoric triamide (NBPT) is commonly used UI to delay UR hydrolysis (F. Xiao et al., 2022), Dicyandiamide (DCD) and 3,4-dimethylpyrazol-phosphate (DMPP) are most common and effective NIs used in delaying the nitrification process by inhibiting the activity of ammonia monooxygenase (Rose et al., 2018; Shi et al., 2017). Previous studies have reported that NI and UI reduced the N losses effectively (Ibarr et al., 2021; Klimczyk et al., 2021), however effectiveness of N stabilizers can be influenced by soil pH (Cui et al., 2021), microbial community (Neufeld & Knowles, 1999), soil texture (Barth et al., 2008), soil moisture (Vitale et al., 2013), and organic matter content (Jacinthe & Pichtel, 1992).

Soil microorganisms are vital in maintaining the soil functions including formation of aggregates, nutrients cycling, decomposition, stability of organic matter, and bioremediation (Dangi et al., 2018). Soil microbial communities play a crucial role in facilitating approximately 80-90% of biochemical processes that occur within the soil environment (Nannipieri et al., 2003). This significant contribution is mostly attributed to the existence of various enzymes that serve as catalysts for these diverse reactions (Bowles et al., 2014). The increased enzyme activities can be attributed to both the stimulation of microbial activity in the rhizosphere caused by deposition (Adetunji et al., 2020), as well as the enzymes released by the root or the lysis of root cells (Rao et al., 2014). Typically, these enzymes facilitate the catalysis of the synthesis of substances that are subsequently taken up by plant roots or microbes (Dotaniya et al., 2019). Enzymes, which are generated by the biological processes occurring in soil, serve as indicators for the nutrient needs of microorganisms (Guan et al., 2020). The activity of soil enzymes serves as an indicator of the rate at which soil microbial metabolism and biochemical cycling activities occur (Zi et al., 2018). The soil enzyme plays a crucial role in the conversion of complicated organic matter into nutrients that may be readily utilized by plants and play key role in decomposition of organic matter (Sinsabaugh et al., 2008). The activities of soil enzymes undergo substantial alterations in response to variations in soil organic matter biochemistry and physical circumstances. Moreover, the mineralization of organic N in soil is primarily governed by the activities of extracellular enzymes present in the soil. The initial stage of soil N mineralization is well acknowledged to involve the complex decomposition of N compounds into readily hydrolysable fractions of N. The soil urease and β-glucosidase enzymes are recognized as influential factors in governing the soil N mineralization (Kumar et al., 2022; Yadav et al., 2023). The measurement of β-glucosidase activities in soil is commonly seen as a reliable indicator of nutrient or energy dynamics and these activities are directly associated with the presence of soil cellobiose hydrolase, which are vital in the breakdown of cellobiose, as well as the availability of energy and carbon (C) in the soil (Tang et al., 2023). Ureases are a class of metalloenzymes that are dependent on nickel and are composed of proteinaceous components and these enzymes exhibit a wide distribution throughout many biological organisms, including bacteria, fungus, algae, invertebrates, and plants (Carlini & Ligabue-Braun, 2016). The urease enzyme activities in soil are significant as they serve as catalysts for the conversion of hydroxyurea, UR, dihydroxyurea, and semicarbazid into NH_3 and CO_2. Previous studies reported positive, neutral, and negative effect of N application on soil enzyme activity (Chen et al., 2017; Gong et al., 2015; Ren et al., 2017; Xiao et al., 2018). Hence needs further detailed investigation.

Soil microbial communities play a pivotal role in the decomposition of organic materials and organic matter mineralization by means of diverse metabolic activities (Stark et al., 2008). The aforementioned processes are contingent upon the size, functionality, and constitution of the soil microbial community (Böhme et al., 2005). This implies that the establishment of various soil microbial communities is of paramount significance to maintain or enhance agricultural productivity through the incorporation of crop residues (Tu et al., 2006). Phospholipid fatty acids (PLFAs), encompassing gram-positive (G^+) or gram-negative bacteria (G^-), fungi (F), and protozoa (P), serve as biomarkers for evaluating the composition of the active microbial population in the soil (He et al., 2007). Soil PLFA profiles and microbial communities exhibit high sensitivity to even slight alterations in soil conditions (Ai et al., 2012). Consequently, they have been widely employed to evaluate and contrast various agricultural management strategies and land utilization systems (Helgason et al., 2010). Additionally, these tools are utilized to evaluate the presence of nutrient-related stress in the soil (Bossio et al., 1998). Hence, the utilization of PLFA profiling presents a highly effective approach for evaluating the dynamic microbial population within the soil, serving as a viable proxy for evaluating both soil health and soil quality (De Vries et al., 2012). Previous studies have reported the effects of N application on soil pH (Aula et al., 2016; Chen et al., 2019; Fu et al., 2019), cropping sequence or N fertilizer on microbial community diversity (Ai et al., 2012; Sileshi et al., 2008; P. Wang et al., 2017), β-glucosidase and urease enzyme in different jurisdictions or climate conditions (e.g, tropical and temperate conditions) (Allende-Montalbán et al., 2021; Ramirez et al., 2012; Tang et al., 2023; Xiao et al., 2022; Wang, et al., 2015). However, it was unclear how N stabilizer and cropping sequence can affect soil pH, enzymatic activities, active microbial communities' structure, and abundance in podzolic soils under boreal climate. Therefore, we hypothesized that N stabilizer and cropping sequence will improve soil pH, enzymes activities, and active microbial community structure and abundance in podzolic soils under boreal climate. Specific objectives of the study were:

i. To determine the effect of N stabilizers and cropping sequence on soil pH, and enzymes activities in podzolic soil under boreal climate.
ii. To investigate the effect of N stabilizers and cropping sequence on active microbial community structure and abundance using PLFA method in podzolic soils under boreal climate.

3.3. Materials and Method

3.3.1 Experimental site and treatments

The details about experimental site, treatments, and design are given in section 2.3.1.

3.3.2. Soil sampling and analysis

Three soil samples (0-20 cm) were collected randomly from all plots after harvesting crop in 2020 and 2021 using a soil auger. Composite soil samples were prepared and put in Ziplock plastic bags transported to research laboratory at Grenfell Campus, Memorial University of Newfoundland. Soil samples were first sieved with 2 mm mesh and then divided in two subsamples. The first sample was stored at 4 °C prior to measuring enzyme activities. The second sample was stored at −20 °C to measure active microbial population. The soil pH was determined by extraction of 10 g air dry soil, using a 1:2 ratio of 20 mL of water, in 50 mL polypropylene tubes, using a pH metre (Mettler Toledo, Canada) (Scrimgeour, 2008). β-glucosidase and urease activities were assayed using kit according to the manufacturer specifications and protocols (Sigma Aldrich Canada). Briefly, 5 g soil was weighed, and 25 mL of buffer was added to it and was placed in the shaker for 1 h. Soil suspension was transferred into centrifuge tubes and centrifuged for 5 minutes at 4000 g then 0.7 µm glass-fiber filter used for filtration. Enzymatic activity of β-glucosidase and urease was determined through colorimetric method following protocol explained in technical bulletin of sigma Aldrich Canada using Biotek cytation 3 imaging reader.

3.3.3. Phospholipid fatty acids (PLFAs) analysis

The determination of PLFA was conducted using the procedures described by Folch et al. (1951) and Gómez-Brandón and Domínguez (2010). 4 g of soil was mixed with10 mL of chloroform-methanol (in a ratio of 2:1, volume to volume) in 20 mL glass vials used to extract fatty acids. Samples were analysed using Gas Chromatography-flame ionisation detection. There was a total of 45 PLFAs found, and out of them, 26 were employed to quantify the overall microbial biomass as mentioned in Table 3.1.

Table 3.1: Phospholipid fatty acids (PLFAs) as microbial biomarkers used to characterize the active microbial population adopted from Ali et al. (2019).

Organisms	**Biomarkers**	**References**
G^+	C14_0	(Sheng et al., 2012)
G^+	i-C15_0	(Wang et al., 2016; Zhang et al., 2016)
G^+	a-C15_0	(Wang et al., 2016; Zhang et al., 2016)
G^+	C15_0	(Huygens et al., 2011; Papatheodorou et al., 2012)
G^+	i-C16_0	(Wang et al., 2016; Zhang et al., 2016)
G^+	C16_0	(Kujur & Patel, 2014; Wu et al., 2015)
G^+	C16_1n-7	(Brockett et al., 2012; Wang et al., 2016)
G^+	i-C17_0	(Wang et al., 2016; Zhang et al., 2016)

G^+	C17_0	(Huygens et al., 2011; Papatheodorou et al., 2012)
G^+	C18_0	(Brockett et al., 2012; Wu et al., 2015)
G^+	C18_1n-9cis	(Brockett et al., 2012; Zhang et al., 2016)
G^-	2OH_C10_0	(Lasater et al., 2017)
G^-	2OH_C12_0	(Lasater et al., 2017)
G^-	C16_0	(Kujur & Patel, 2014; Wu et al., 2015)
G^-	C16_1n-7	(Brockett et al., 2012; Wang et al., 2016)
G^-	3OH_C12_0	(Kaur et al., 2005)
G^-	cycloC17_0	(Wang et al., 2016; Zhang et al., 2016)
G^-	C18_0	(Brockett et al., 2012; Wu et al., 2015)
G^-	C18_1n-9_trans	(Moreno et al., 2017)
G^-	C18_1n-9cis	(Brockett et al., 2012; Zhang et al., 2016)
G^-	3OH_C14_0	(Papatheodorou et al., 2012)
G^-	cycloC19_0	(Wang et al., 2016)
G^-	C14_1n_5	(Zhang et al., 2016)
G^-	C17_1n_7	(Gómez-Brandón & Domínguez, 2010)
F	C18_1n_9cis	(Brockett et al., 2012; Zhang et al., 2016)
F	C18_2n_6cis	(Joergensen & Potthoff, 2005; Zhang et al., 2016)

P	C20_0	(Schindlbacher et al., 2011)
E	C18_2n_6cis	(Joergensen & Potthoff, 2005; Zhang et al., 2016)

(Eukaryotes: E, Protozoa: P, Gram negative bacteria: G^-, Fungi: F, Gram positive bacteria: G^+)

3.3.4. Statistical analysis

Two-way analysis of variance (ANOVA) was conducted to assess effects of N sources and cropping sequence on soil pH, enzymatic activities, and microbial diversity using XLSTAT 2021.3.1(Lumivero, Denver, USA). Tukey's post hoc test was employed at the probability level of 0.05 to compare the treatment means. The software Sigma plot 15.0, developed by Systat Software Inc., was utilised for graph creation.

3.4. Results

3.4.1. Effect of N sources and cropping sequence on soil pH, urease and β Glucosidase enzyme activity after harvesting the crop in 2020 and 2021

Table 3.2: Two-way Analysis of variance (ANOVA) showing the effects of N sources, cropping sequence, and their interaction on soil pH and enzymatic activities after harvesting the crop in 2020.

Soil parameters	**Nitrogen Sources**	**Cropping Sequence**	**N × C**
pH	NS	NS	NS
Urease enzyme activity	NS	NS	NS
β Glucosidase enzyme activity	**	NS	NS

(**Significant at $p < 0.05$, NS: non-Significant, N x C: Nitrogen sources x Cropping Sequence)

Table 3.3: Two-way Analysis of variance (ANOVA) showing the effects of N sources, cropping sequence, and their interaction on soil pH and enzymatic activities after harvesting the crop in 2021.

Soil parameters	**Nitrogen Sources**	**Cropping Sequence**	**N × C**
pH	**	NS	NS
Urease enzyme activity	**	NS	NS
β Glucosidase enzyme activity	**	NS	NS

(**Significant at $p < 0.05$, NS: non-Significant, N x C: Nitrogen sources x Cropping Sequence)

3.4.2. Effect of N sources on soil pH, urease and β Glucosidase enzyme activity after harvesting the crop in 2020 and 2021

The ANOVA results revealed that N sources had significant effect on β glucosidase enzyme activity after harvesting the crop in 2020, however no significant influence was noted on soil pH and urease enzyme activity (Table 3.2). On the other hand, N sources demonstrated significant effect on soil pH and enzymatic activities (β glucosidase and urease enzymes) after harvesting the crop (Table 3.3) in 2021.

N sources had significant ($p < 0.000$) impact on soil pH after harvesting the crop in 2021 (Table 3.3). Application of UR and agrotain (AG) decreased the soil pH as compared to control and other N stabilizers eNtrench (EN) and superU(SU). Significantly higher soil pH (5.9) was observed in EN (N stabilizer) while lower (5.6) was recorded in urea split application (US), though statistically at par with UR and AG (Figure 3.1). Among N stabilizers EN and SU showed significantly higher soil pH, though statistically at par with control.

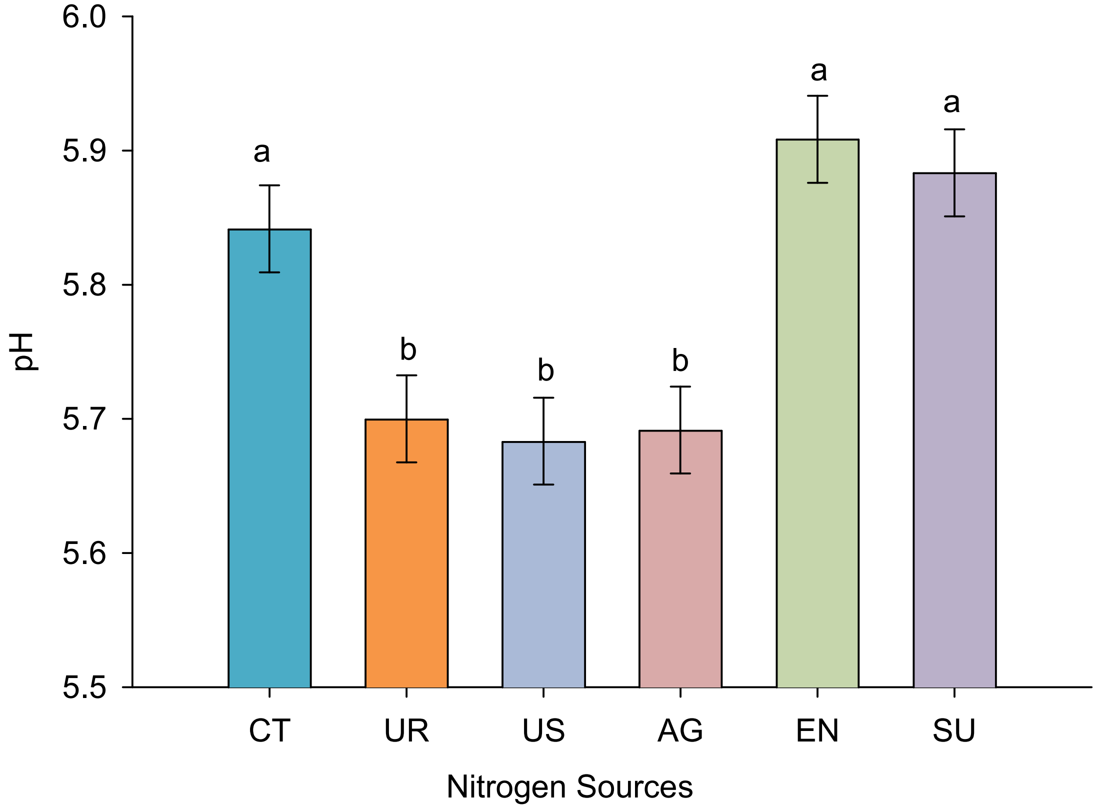

Figure 3.1: Effects of N sources on soil pH in podzolic soil in boreal climate. The presence of distinct letters on bars indicates statistically significant difference among the treatments and error bars show the standard error. CT: Control, UR: Urea, US: Urea split application, SU: Super-U (urease inhibitor + nitrification inhibitor), EN: eNtrench (nitrification inhibitor), and AG: Agrotain

(urease inhibitor).

N sources had significantly ($p < 0.000$) influenced soil β glucosidase enzyme activity after harvesting the crop in 2020 (Table 3.2). Application of AG, EN and SU (N stabilizers) exhibited lower β glucosidase enzyme activity while higher level was noted in US, UR, and control treatments (Figure 3.2a). AG, EN and SU application reduced β glucosidase enzyme activity by 10.53%, 8.5% and 8.6%, respectively compared to control. N stabilizers (AG, EN, SU) also reduced β glucosidase enzyme as compared to UR application after harvesting the crop in 2021 (Figure 3.2b) (Table 3.3). Application of UR, US, and control showed higher β glucosidase enzyme activity compared to EN, AG, SU (N stabilizers). Application of AG, EN, SU reduced β glucosidase enzyme activity by 11%, 10% and 12% compared to UR.

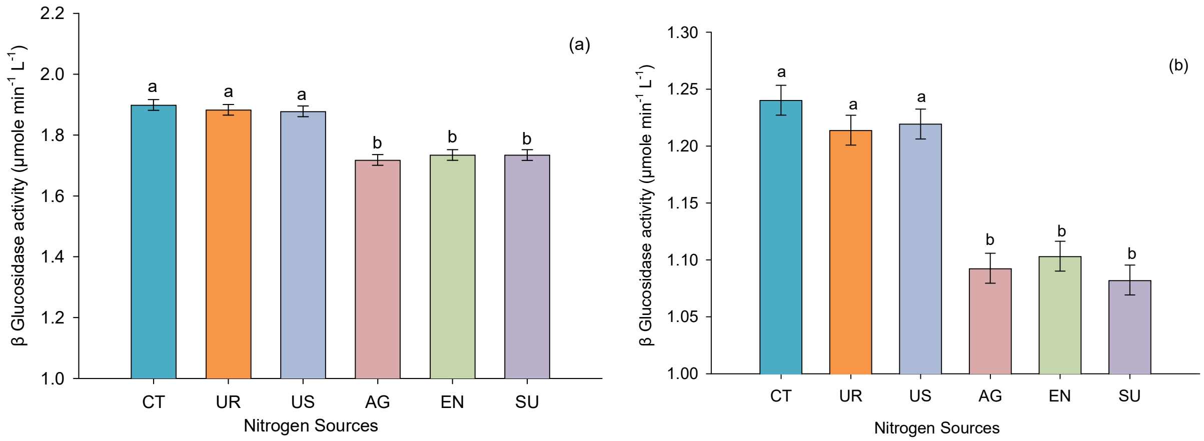

Figure 3.2: Effects of N sources on β glucosidase enzyme activity (a) after harvesting the crop in 2020, (b) after harvesting the crop in 2021 in podzolic soil in boreal climate. The presence of distinct letters on bars indicates statistically significant difference among the treatments and error bars show the standard error. CT: Control, UR: Urea, US: Urea split application, SU: Super-U

(urease inhibitor + nitrification inhibitor), EN: eNtrench (nitrification inhibitor), and AG: Agrotain (urease inhibitor).

Soil urease enzyme activity significantly ($p < 0.000$) influenced by N sources after harvesting the crop in 2021 (Table 3.3). The highest urease activity was observed in US application, though statistically at par with AG, EN, SU and UR applied treatments while lowest was recorded in control treatment (Figure 3.3). Application of urea (split application) recorded 1.6% more urease activity as compared to control treatment. Among N stabilizers, EN exhibited higher urease enzyme activity compared to AG and SU (Figure 3.3).

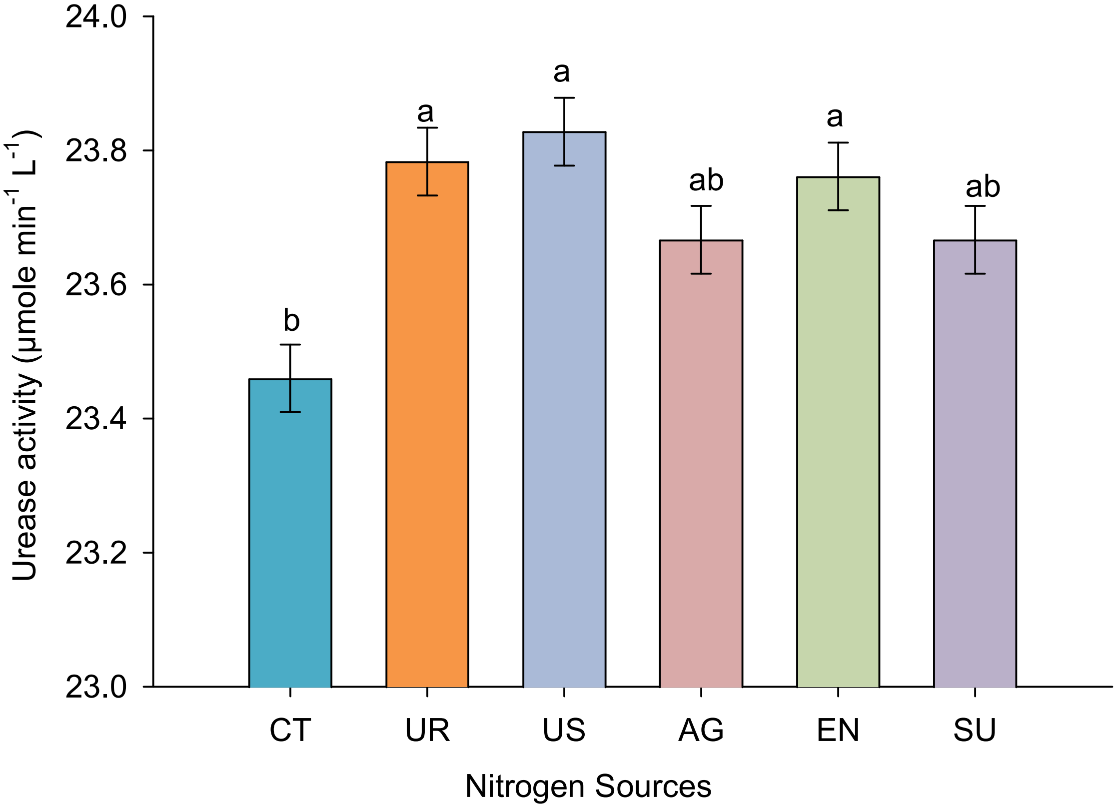

Figure 3.3: Effects N sources on soil urease enzyme activity on podzolic soil in boreal climate. The presence of distinct letters on bars indicates statistically significant difference among the treatments and error bars show the standard error. CT: Control, UR: Urea, US: Urea split application, SU: Super-U (urease inhibitor + nitrification inhibitor), EN: eNtrench (nitrification inhibitor), and AG: Agrotain (urease inhibitor).

3.4.3. Effect of N sources and cropping sequence on active soil microbial community structure and abundance after harvesting the crops in 2020 and 2021

Table 3.4: Two-way Analysis of variance (ANOVA) showing the effects of N sources, cropping

sequence, and their interaction on active soil microbial population after harvesting the crop in 2020.

Soil microbial Community	**Nitrogen Sources**	**Cropping Sequence**	**N × C**
G^+	NS	**	NS
G^-	NS	NS	NS
Fungi	NS	NS	NS
Protozoa	NS	NS	**
Eukaryotes	NS	NS	NS
Σ B-PLFAs	NS	**	NS
Σ PLFAs	NS	**	NS
G^+: G^-	NS	NS	NS
F: B	NS	**	NS

(*** Significant at $p < 0.001$, **Significant at $p < 0.05$, NS: non-Significant, N x C: Nitrogen sources x Cropping Sequence, F**:**B : fungi/bacteria ratio, G^+: G^-: gram positive bacteria/gram negative bacteria ratio, Σ B-PLFAs: Total bacterial phospholipid fatty acids, G^+: gram positive bacteria, , Σ PLFAs: Total phospholipids fatty acids, G^-: gram negative bacteria)

Table 3.5 Two-way Analysis of variance **(**ANOVA) shows the effects of N sources, cropping sequence, and their interaction on active soil microbial population after harvesting the crop in 2021.

Soil microbial Community	**Nitrogen Sources**	**Cropping Sequence**	**N × C**
G+	***	***	**

G-	**	**	**
Fungi	***	NS	NS
Protozoa	NS	NS	NS
Eukaryotes	NS	**	NS
Σ B-PLFAs	**	**	**
Σ PLFAs	**	**	**
G+: G-	NS	**	**
F: B	NS	NS	NS

(*** Significant at $p < 0.001$, **Significant at $p < 0.05$, NS: non-Significant, N x C: Nitrogen sources x Cropping Sequence, F:B : fungi/bacteria ratio, G^+: G^-: gram positive bacteria/gram negative bacteria ratio, Σ B-PLFAs: Total bacterial phospholipid fatty acids, G^+: gram positive bacteria, , Σ PLFAs: Total phospholipids fatty acids, G^-: gram negative bacteria)

3.4.4. Effect of N sources, cropping sequence and their interaction on soil microbial diversity after harvesting the crop in 2020

Statistical analysis demonstrated that N sources had no significant impact on active soil microbial population after harvesting the crop in 2020. However, cropping sequence significantly influenced G^+ bacteria, Σ B-PLFAs, Σ PLFAs and F:B whereas non-significant effect on G^- bacteria, fungi, G^+: G^-. The interactive effect of N sources and cropping sequence had significant effects on protozoa only, while exhibited non-significant effect on G^+ bacteria, G^- bacteria, fungi, Σ B-PLFAs, Σ PLFAs.

Cropping sequence had significantly ($p < 0.008$) influenced G^+ bacteria after harvesting the crop in 2020 (Table 3.4). The c-fb-c sequence showed higher G^+ bacteria (17.25 nmol g^{-1}) compared to c-w-c which produced the lowest G^+ bacteria (16.85 nmol g^{-1}) among all cropping sequence (Figure 3.4).

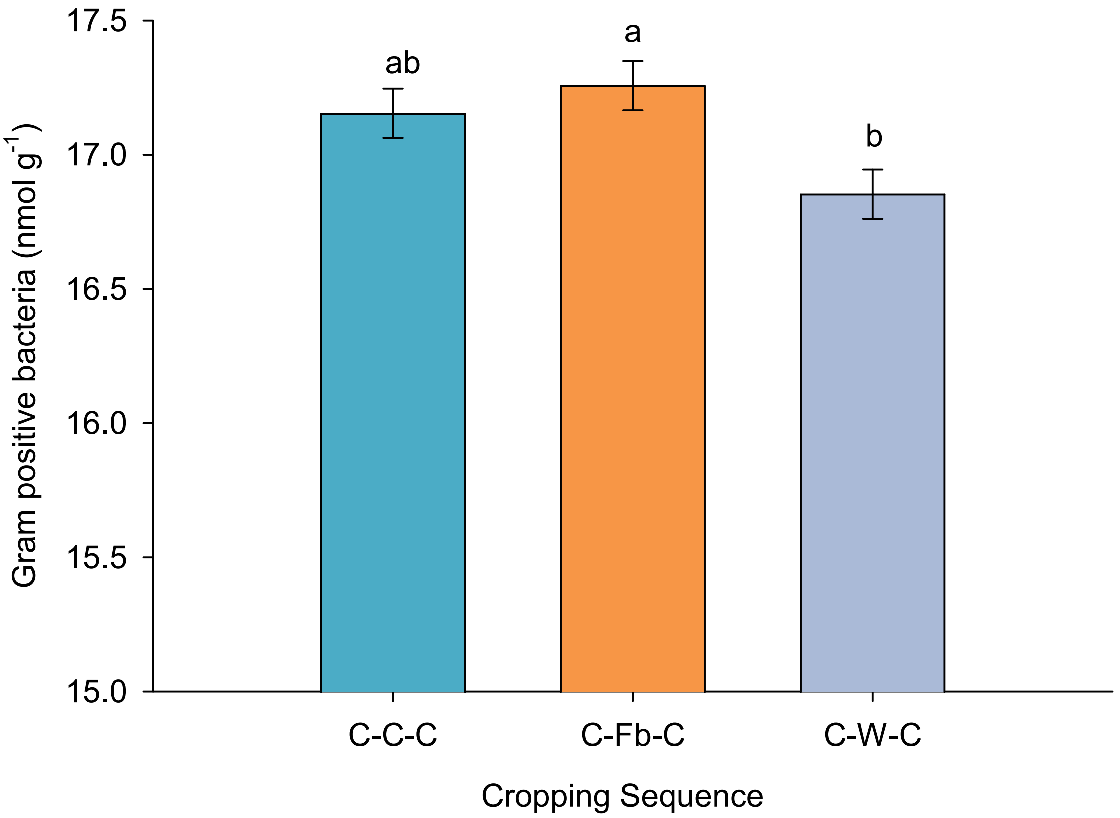

Figure 3.4: Effects of cropping sequence on gram positive bacteria in podzolic soil in boreal climate. The presence of distinct letters on bars indicates statistically significant difference among the treatments and error bars show the standard error. corn–corn–corn (c–c–c), corn–wheat–corn (c–w–c) and corn–faba bean–corn (c–fb–c).

Cropping sequence had significantly ($p < 0.014$) impacted Σ B-PLFAs after harvesting the crop in 2020 (Table 3.4). The c-fb-c sequence demonstrated higher Σ B-PLFAs (34.76 nmol g^{-1}) while lower (33.96 nmol g^{-1}) were recorded in c-w-c (Figure 3.5). The c-fb-c sequence exhibited 2.35% higher Σ B-PLFAs as compared to c-w-c cropping sequence.

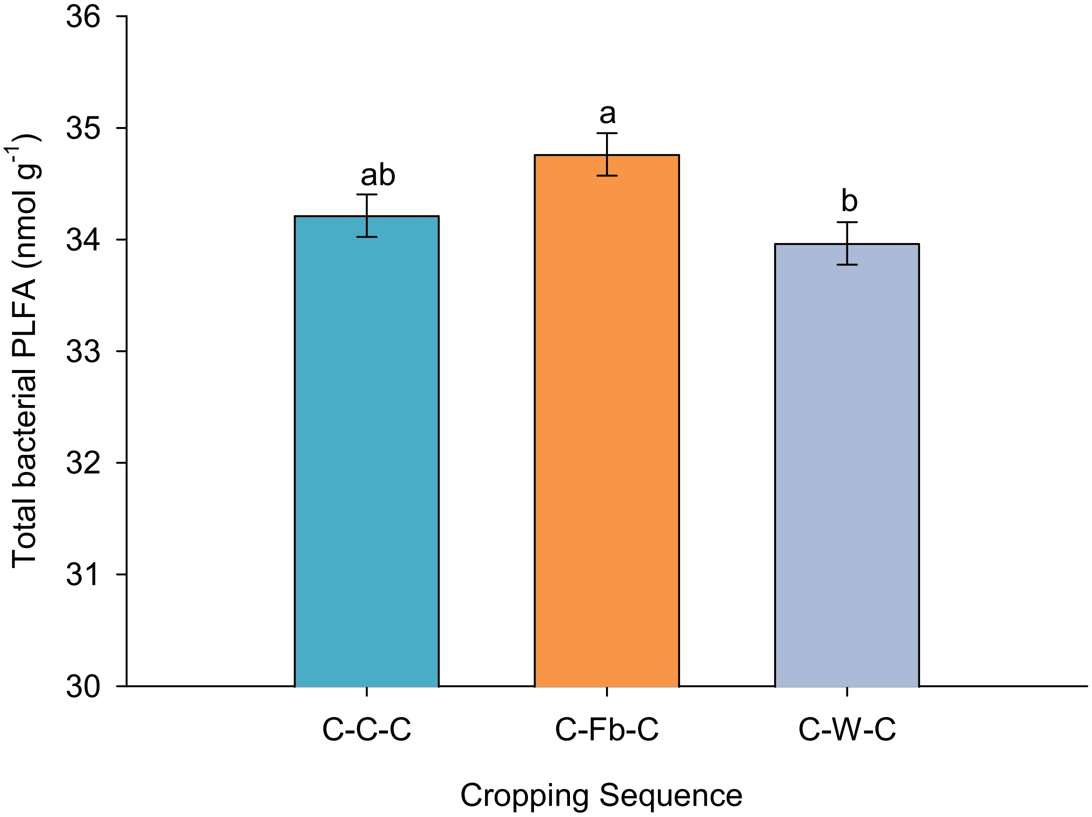

Figure 3.5: Effects of cropping sequence on total bacterial PLFA on podzols in boreal climate. The presence of distinct letters on bars indicates statistically significant difference among the treatments and error bars show the standard error. corn–corn–corn (c–c–c), corn–wheat–corn (c–w–c) and corn–faba bean–corn (c–fb–c).

Cropping sequence had significant ($p < 0.015$) effects on total PLFA after harvesting the crop in 2020 (Table 3.4). The c-fb-c sequence exhibited higher Σ PLFAs (38.31 nmol g^{-1}) while lower (37.47 nmol g^{-1}) were noted in c-w-c (Fig. 3.6). The c-fb-c sequence demonstrated 2.23% higher Σ PLFAs as compared to c-w-c cropping sequence.

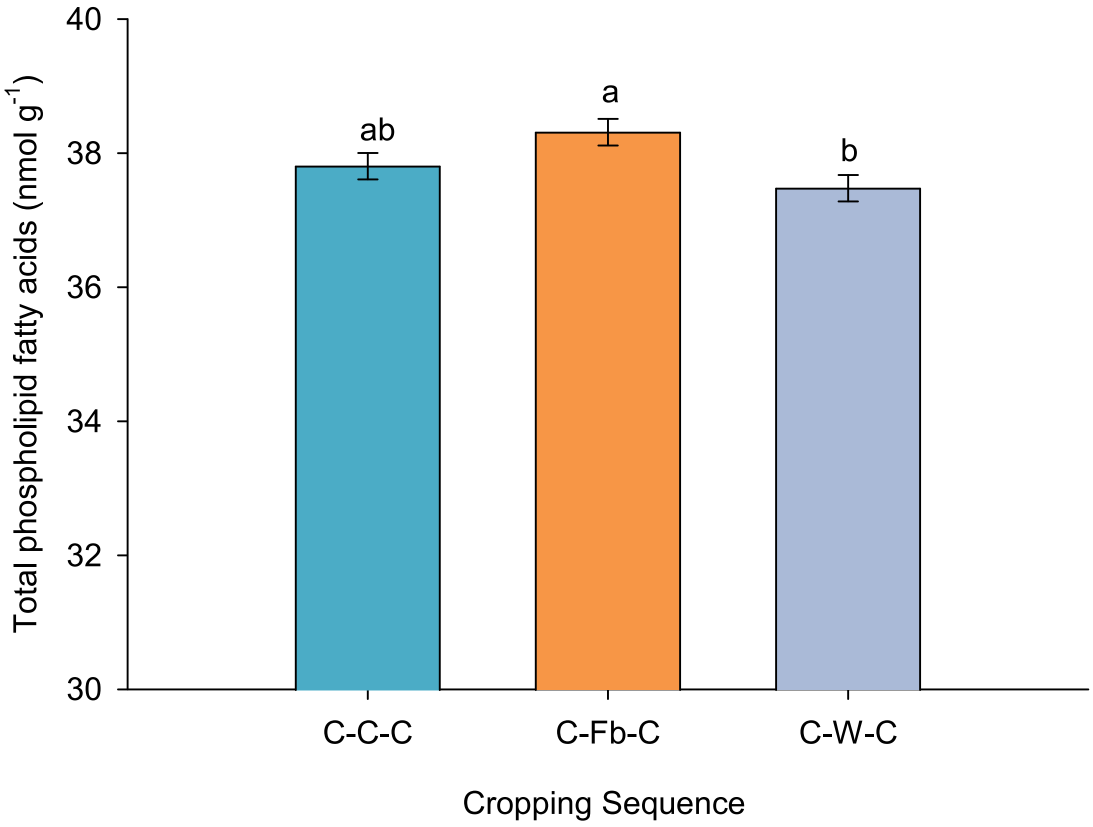

Figure 3.6: Effects of cropping sequence on total PLFA on podzols in boreal climate. The presence of distinct letters on bars indicates statistically significant difference among the treatments and error bars show the standard error. corn–corn–corn (c–c–c), corn–wheat–corn (c–w–c) and corn–faba bean–corn (c–fb–c).

N sources and cropping sequence interaction (N × cropping sequence) had significant ($p < 0.032$) effects on soil protozoa after harvesting the crop in 2020 (Table 3.4). Higher soil protozoa (0.603 nmol g^{-1}) were observed in c-c-c sequence with UR application while the lowest (0.546 nmol g^{-1}) was recorded in c-w-c cropping sequence with UR application (Figure 3.7). In c-c-c cropping sequence highest soil protozoa were noted in UR application, though statistically at par with US, EN and AG whereas, lowest was observed in control treatment. In c-fb-c cropping sequence, EN (N stabilizer) showed higher soil protozoa though statistically at par with US and AG application while lowest noted in control. UR and SU application were statistically non-significant with control treatment. In c-w-c cropping sequence the highest soil protozoa recorded in SU (N stabilizer) though statistically at par with control treatment while lowest was observed in UR application.

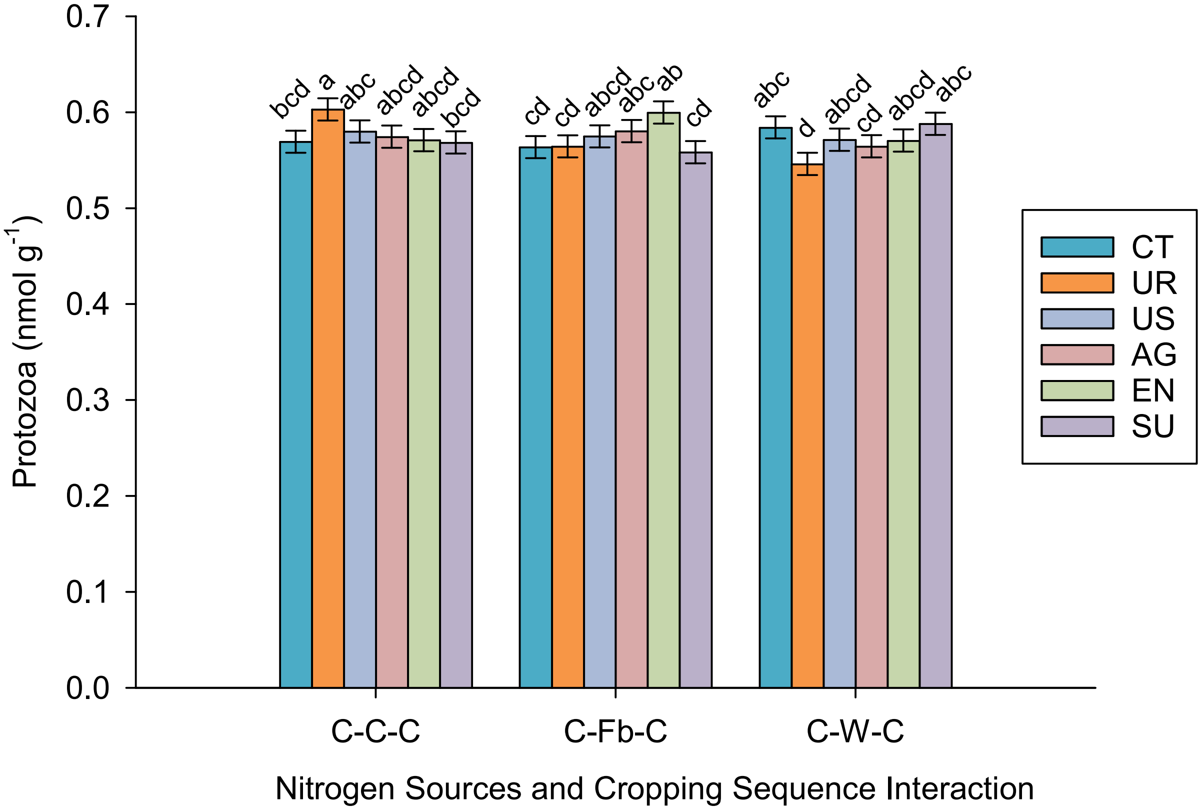

Figure 3.7: Interactive effect of nitrogen sources and cropping sequence on soil protozoa after three years cropping sequence (corn-corn-corn (c-c-c), corn-faba bean- corn (c-fb-c), corn-wheat-corn (c-w-c)) in podzolic soil in boreal climate. The presence of distinct letters on bars indicates statistically significant difference among the treatments and error bars show the standard error. CT: Control, UR: Urea, US: Urea split application, SU: Super-U (urease inhibitor + nitrification inhibitor), EN: eNtrench (nitrification inhibitor), and AG: Agrotain (urease inhibitor).

3.4.5. Effect of N sources, cropping sequence and their interaction on soil microbial diversity after harvesting the crop in 2021

The ANOVA results showed that N sources had significant impact on fungi, Σ B-PLFAs, G^+, Σ PLFAs, G^- whereas no significant influence on eukaryotes, protozoa, G^+: G^- and F:B after harvesting the crop in 2021 (Table 3.5). However, cropping sequence had significant effects on G^+, G^-, eukaryotes, Σ B-PLFAs, G^+: G^- and Σ PLFAs but no significant effect was observed in fungi, protozoa, and F: B. Interactive effect of N sources and cropping sequence was significant in G^+, G^-, Σ B-PLFAs, G^+: G^- and Σ PLFAs whereas non-significant effects on fungi, protozoa, F:B and eukaryotes.

Fungi had been significantly ($p < 0.001$) influenced by N sources after harvesting the crop in 2021 (Table 3.5). SuperU (N stabilizer) application demonstrated higher fungal community though statistically at par with other N stabilizers such as AG and EN treatments. The lowest fungal community was observed in split urea and UR treatments though statistically at par with control treatments (Figure 3.8). SuperU application enhanced 3.80% fungal population compared to UR application.

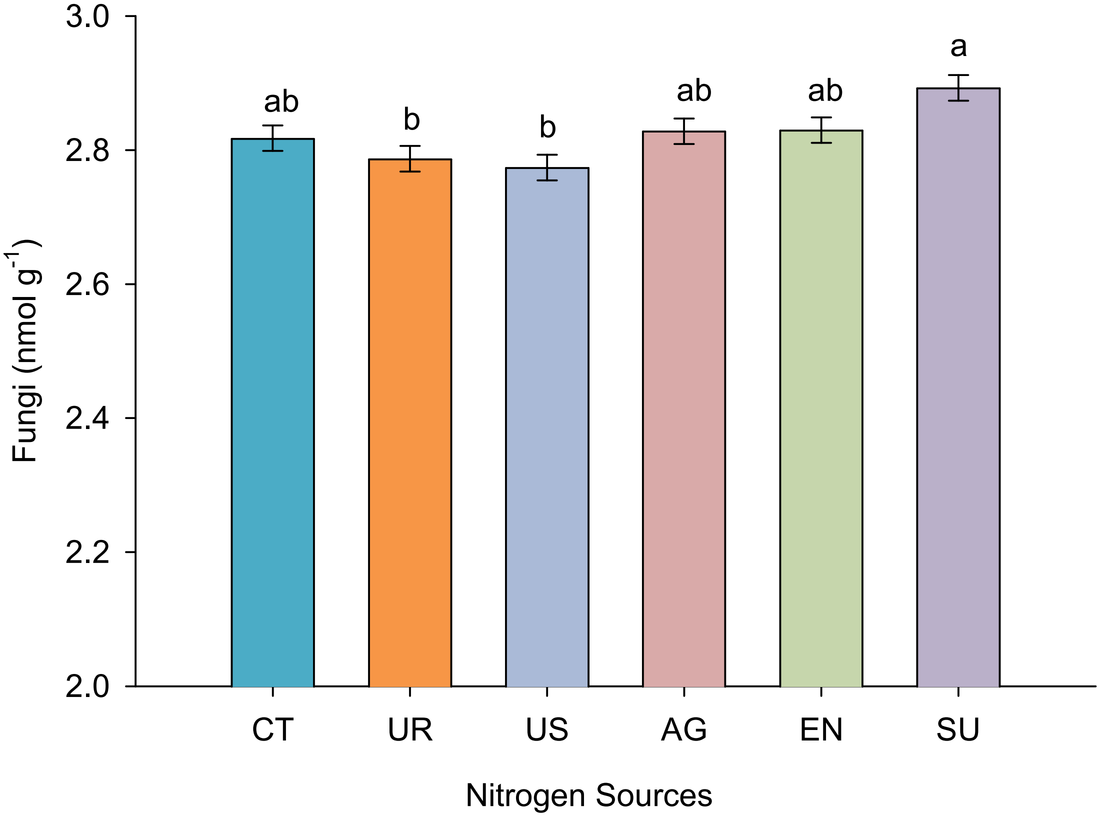

Figure 3.8: Effects of N sources on soil fungal population on podzolic soil in boreal climate. The presence of distinct letters on bars indicates statistically significant difference among the treatments and error bars show the standard error. CT: Control, UR: Urea, US: Urea split application, SU: Super-U (urease inhibitor + nitrification inhibitor), EN: eNtrench (nitrification inhibitor), and AG: Agrotain (urease inhibitor).

Cropping sequence had significantly ($p < 0.020$) impacted soil eukaryotes after harvesting the crop in 2021 (Table 3.5). The c-w-c cropping sequence demonstrated higher eukaryotic population while lower were recorded in c-fb-c (Figure 3.9). The c-w-c cropping sequence showed 3.52% more eukaryotic community as compared to c-fb-c cropping sequence.

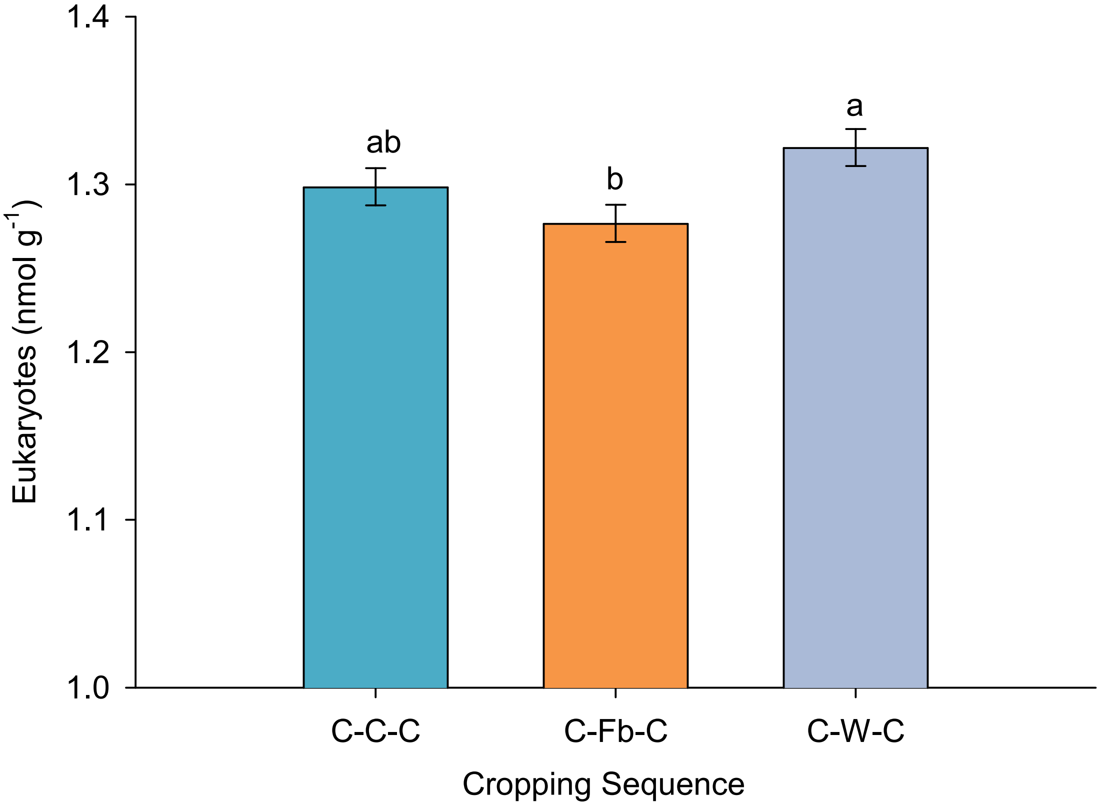

Figure 3.9: Effects of cropping sequence on eukaryotes on podzols in boreal climate. The presence of distinct letters on bars indicates statistically significant difference among the treatments and error bars show the standard error. corn–corn–corn (c–c–c), corn–wheat–corn (c–w–c) and corn–faba bean–corn (c–fb–c).

N sources and cropping sequence interaction had significant ($p < 0.022$) effects on soil G^+ bacteria after harvesting the crop in 2021 (Table 3.5). Higher soil G^+ bacterial population (18.72 nmol g^{-1}) was observed in c-w-c sequence with UR application while lowest (17.48 nmol g^{-1}) was recorded in c-c-c cropping sequence with UR application (Figure 3.10). In c-c-c cropping sequence, the highest soil G^+ population was noted in AG application though statistically at par with other N stabilizers (EN, SU) and US application whereas lowest was observed in UR application. In c-fb-c cropping sequence, SU (N stabilizer) showed higher soil G^+ population, however, statistically non-significant with other treatments including control and UR application (Figure 3.10). In c-w-c cropping sequence the highest soil G^+ population was recorded in UR though statistically at par with all N stabilizers, and US application while lowest was observed in control treatment (Figure 3.10).

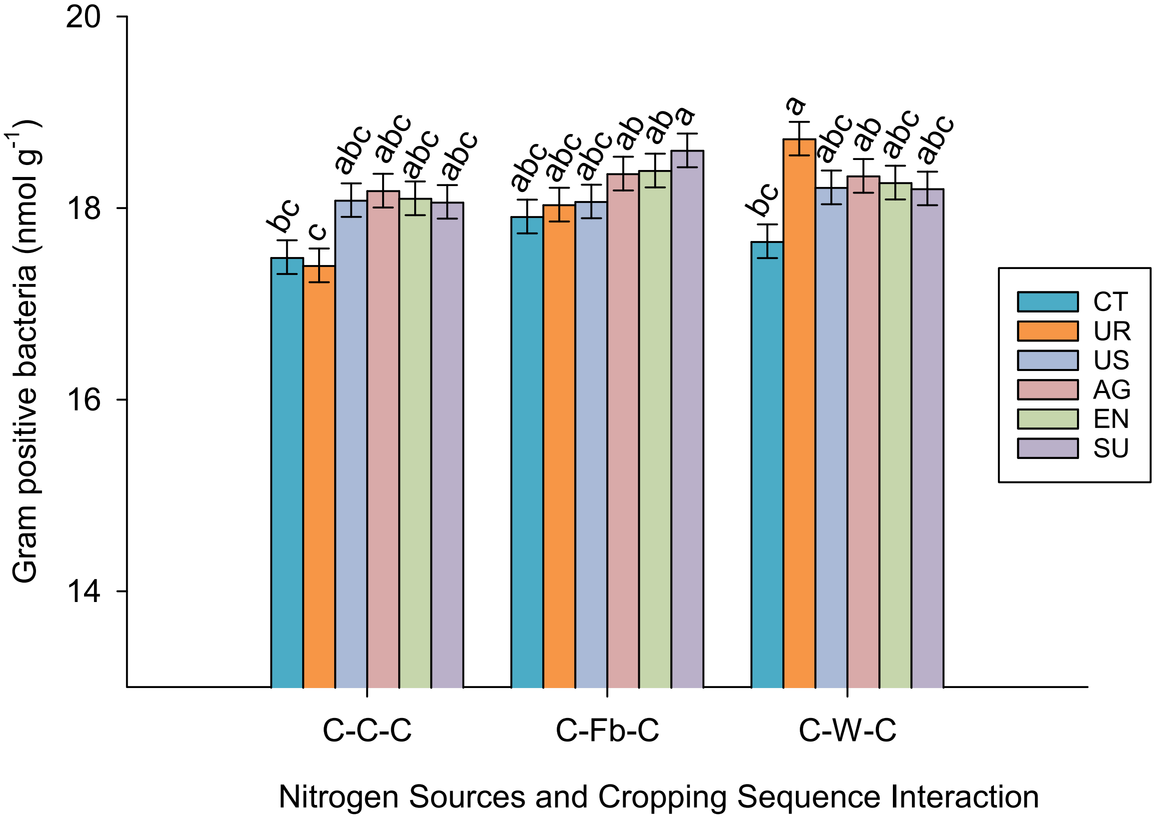

Figure 3.10: Interactive effect of nitrogen sources and cropping sequence on soil gram positive bacteria after three years cropping sequence (corn-corn-corn (c-c-c), corn-faba bean- corn (c-fb-c), corn-wheat-corn (c-w-c) on podzols in boreal climate. The presence of distinct letters on bars indicates statistically significant difference among the treatments and error bars show the standard

error. CT: Control, UR: Urea, US: Urea split application, SU: Super-U (urease inhibitor + nitrification inhibitor), EN: eNtrench (nitrification inhibitor), and AG: Agrotain (urease inhibitor).

N sources and cropping sequence interaction had significant (p < 0.006) impact on soil gram negative bacteria after harvesting the crop in 2021 (Table 3.5). Higher soil G^- bacterial community was observed in c-c-c cropping sequence with EN application (18.96 nmol g^{-1}) while lowest was recorded in control treatment (17.35 nmol g^{-1}) in c-fb-c sequence (Figure 3.11). There was no significant impact of N stabilizers or UR either full application or split application or control on gram negative bacteria within individual cropping sequence.

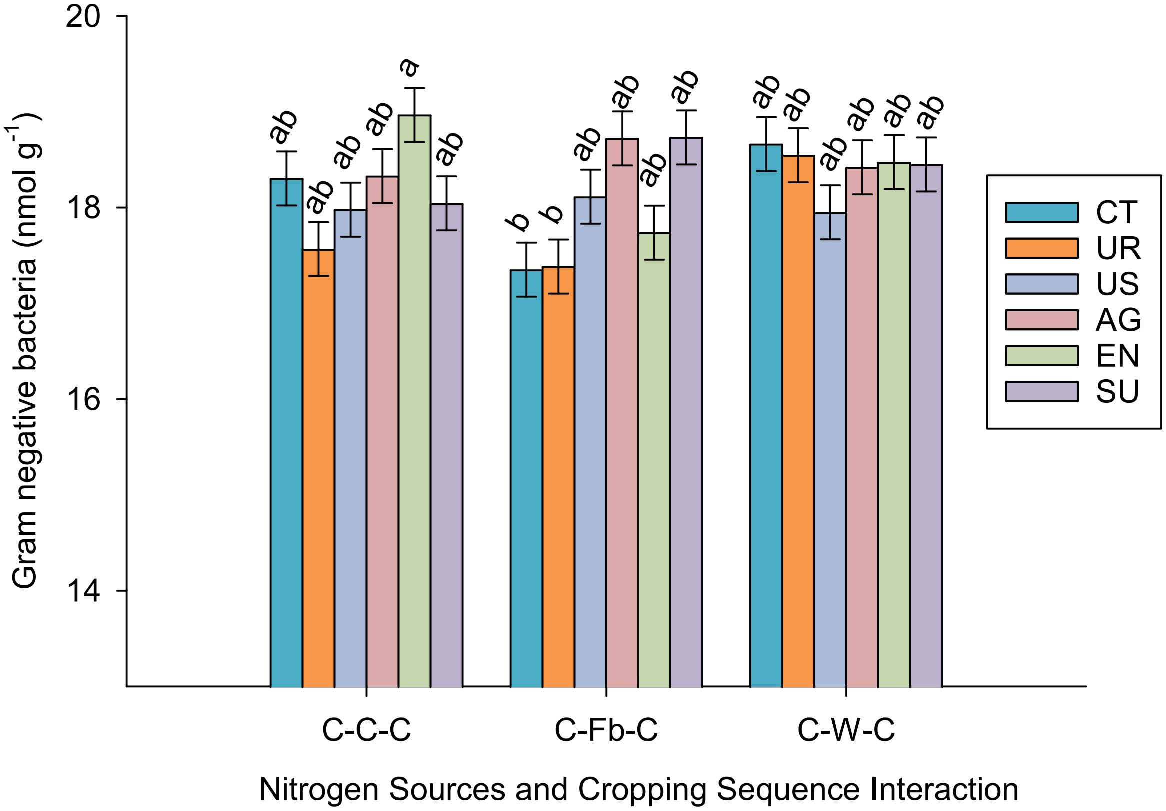

Figure 3.11: Interactive effect of nitrogen sources and cropping sequence on soil gram negative bacteria after three years cropping sequence (corn-corn-corn (c-c-c), corn-faba bean- corn (c-fb-

c), corn-wheat-corn (c-w-c) on podzols in boreal climate. The presence of distinct letters on bars indicates statistically significant difference among the treatments and error bars show the standard error. CT: Control, UR: Urea, US: Urea split application, SU: Super-U (urease inhibitor + nitrification inhibitor), EN: eNtrench (nitrification inhibitor), and AG: Agrotain (urease inhibitor).

N sources and cropping sequence interaction had significant ($p < 0.013$) effects on total bacterial PLFAs after harvesting the crop in 2021 (Table 6). A higher Σ B-PLFAs population (37.34 nmol g^{-1}) was observed in c-fb-c sequence with SuperU application while the lowest (34.97 nmol g^{-1}) was recorded in c-c-c sequence with UR application (Figure 3.12. In c-c-c cropping sequence, the highest soil Σ B-PLFAs population was noted in EN whereas the lowest was observed in UR application, though statistically at par with other N stabilizers, US, and control. In c-fb-c sequence, SU showed higher soil Σ B-PLFAs population while a lower was noted in control. The SU, other N stabilizers, UR and US were statistically non-significant with each other. There were no significant impacts of N stabilizers, UR application and control in c-w-c cropping sequence.

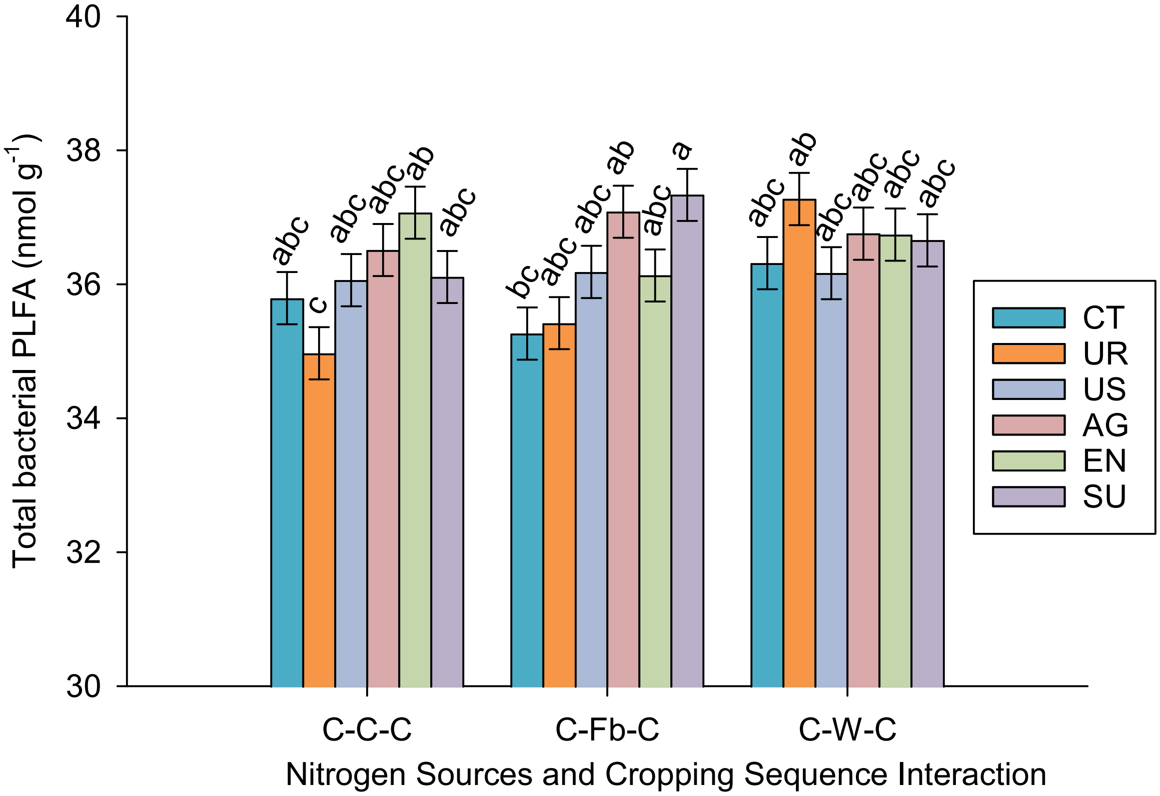

Figure 3.12: Interactive effect of nitrogen sources and cropping sequence on soil total bacterial PLFA after three years cropping sequence (corn-corn-corn (c-c-c), corn-faba bean- corn (c-fb-c), corn-wheat-corn (c-w-c) on podzols in boreal climate. The presence of distinct letters on bars indicates statistically significant difference among the treatments and error bars show the standard error. CT: Control, UR: Urea, US: Urea split application, SU: Super-U (urease inhibitor + nitrification inhibitor), EN: eNtrench (nitrification inhibitor), and AG: Agrotain (urease inhibitor).

N sources and cropping sequence interaction had significant ($p < 0.010$) influence on total PLFA after harvesting the crop in 2021 (Table 6). Higher Σ PLFAs community (42.32 nmol g^{-1}) were observed when SU was applied in c-fb-c sequence while the lowest (39.68 nmol g^{-1}) was recorded in c-c-c cropping sequence with UR application (Figure 3.13. In c-c-c cropping sequence EN produced higher Σ PLFAs and the lowest was observed in UR application, although SU, AG, split application, and control were statistically at par with UR application. In c-fb-c sequence, SU (N stabilizer) showed significantly higher soil Σ PLFAs while the lowest was noted in control though statistically at par with other N stabilizers, UR, and US application. There was no significant difference among N stabilizer, UR application and control in c-w-c cropping sequence.

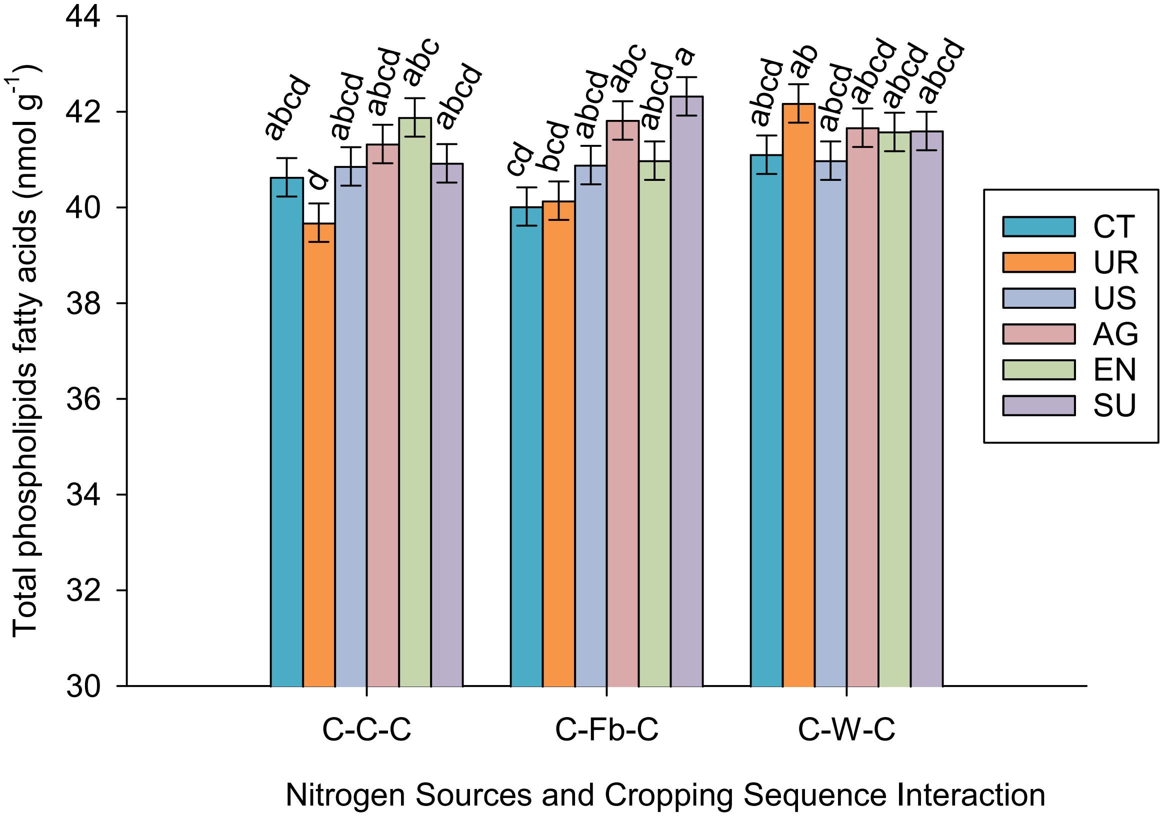

Figure 3.13: Interactive effect of N sources and cropping sequence on soil total PLFA after three years cropping sequence (corn-corn-corn (c-c-c), corn-faba bean- corn (c-fb-c), corn-wheat-corn (c-w-c) on podzols in boreal climate. The presence of distinct letters on bars indicates statistically

significant difference among the treatments and error bars show the standard error. CT: Control, UR: Urea, US: Urea split application, SU: Super-U (urease inhibitor + nitrification inhibitor), EN: eNtrench (nitrification inhibitor), and AG: Agrotain (urease inhibitor).

3.5. Discussion

Soil pH plays an important role in soil biogeochemical processes and known as master variable of soil due to its influence on nutrients availability, plant growth, crop yield, soil physical, biological and chemical properties (Brady & Weil, 1999). Nitrification is a significant N transformation process that holds environmental significance, like several biogeochemical processes, soil pH plays a significant role in controlling this process to a considerable degree (Zebarth et al., 2015). Nitrification is a biochemical process in which microorganisms catalyze the conversion of NH_4^+ to nitrate NO_3^-, the phenomenon often exhibits an upward trend as the soil pH increases, eventually reaching an optimal pH level (Kyveryga et al., 2004). In the current study, UR application and AG (N stabilizer) reduced the soil pH as compared to control and other N stabilizers (EN, SU). Decrease in pH with UR application can be attributed to the nitrification process in which H^+ are produced and then released into soil solution causing soil acidification (Aula et al., 2016). During the hydrolysis process, a single hydroxide ion (OH^-) is released, while in the process of nitrification, two H^+ are released. The effect of AG application was similar to the UR application which was not expected as AG (urease inhibitor) delays the UR hydrolysis process in soil (Manunza et al., 1999). This low soil pH with AG application could be due to high soil moisture conditions we observed in the experimental field. However, pH in soil treated with EN showed less acidification due to delayed ammonium oxidation as EN (NI) can inhibit first step of nitrification process by selectively targeting ammonia monooxygenase (AMO) enzyme (Arp et al., 2002). The findings of the present study align with the findings of previous research (Aula et al., 2016; Chen et al., 2019; Fu et al., 2020a), indicating that utilization of UR and UI effectively reduces soil pH. This reduction is attributed to the additional release of hydrogen ions (H^+) during the hydrolysis process of NH_4^+, which can replace base cations (such as Mg^{2+}, Ca^{2+}, Na^+, K^+) that are adsorbed onto soil surface. This displacement of base cations by NH_4^+ might result in their increased susceptibility to leaching from the soil. Consequently, this process diminishes the soil's capacity to resist acidification, thereby diminishing its buffering capacity against acidification (Matschonat & Matzner, 1996). Furthermore, the absorption of NH_4^+ by plant roots results in the release of H^+ into soil solutions, leading to acidification of soil (Smith & Read, 2008).

Soil enzymes, mostly excreted by soil microorganisms, serve as indicators of the microbial metabolic activities involved in nutrient cycling, and are highly responsive to environmental stress, making them valuable indicators of declining soil quality (Wang et al., 2011). The soil enzymes undergo substantial alterations in response to variations in SOM, the process of organic N mineralization in soil is primarily governed by the activities of extracellular enzymes (Tabatabai et al., 2010). The activities of urease enzymes in soil are of significant importance as they serve as catalysts for the conversion of UR, hydroxyurea, semicarbazid and dihydroxyurea into NH_3 and CO_2 (Kumar et al., 2022). As expected, AG and SU decreased the soil urease activity compared to UR in present study, this could be due to inhibition effect of NBPT which is a urease inhibitor present in AG and SU. The NBPT compound effectively inhibits the activity of the urease enzyme by binding to three active sites, this binding occurs through a tridentate bond, involving one oxygen and two nickel centers from carbamate bridge that connects the two metals. As a result of this binding, the likelihood of UR molecules reaching the nickel (Ni) atom is significantly reduced and UR hydrolysis was delayed (Manunza et al., 1999). In our findings, N stabilizers and UR application had statistically similar results in urease enzyme, this could be attributed to high moisture conditions in our field experiment that might have affected the efficiency of N stabilizers. Previous studies also demonstrated the effectiveness of NBPT in reducing the UR hydrolysis (Allende-Montalbán et al., 2021; Sravanthi et al., 2017; Tang et al., 2023; F. Xiao et al., 2022), urease inhibitor can delay the hydrolysis of UR by blocking active site of urease enzyme (Manunza et al., 1999) and thus decreasing the activity of urease enzyme in the soil.

The assessment of soil enzymatic activity related to the decomposition of SOM can serve as an early indicator of alteration in soil health resulting from modifications in soil management practices (Shukla & Varma, 2010). The enzyme β-glucosidase exhibits significant utility in the assessment of soil quality due to its pivotal role in the breakdown of cellulose and the cycling of SOM (Turner et al., 2002), and serve as an indicator of soils ability to retain and stabilize organic materials (Sherene, 2017). The β-glucosidase catalysis the hydrolysis of cellulase into glucose, is considered to be the step that limits the rate of cellulose degradation (Tabatabai, 1982), and this process plays a crucial role in determining the availability of C to soil microbes (Knight & Dick, 2004). In our study, AG, EN and SU (N stabilizers) significantly reduced the activity of β-glucosidase as compared to UR application and control. This could be attributed to higher NH_4^+–N in soil due to the inhibition of nitrification process by NIs. This higher NH_4^+–N in soil subsequently undergoes chemical reactions with SOM, leading to the formation of compounds that are not readily accessible to microorganisms (Zi et al., 2018). Another possible reason is soil acidification resulted due to N inhibitors, which is very harmful for enzymatic activities in soil, leading to reduction in β-glucosidase enzyme activity (Kang & Freeman, 1999). Previous studies reported decrease in β-glucosidase enzyme activity due to N application (Ramirez et al., 2012; Q. Zhang, W. Zhou, G. Liang, X. Wang, et al., 2015). Zhang et al. (2023) observed negative correlation between β-glucosidase enzyme activity and NH_4^+−N, indicating enzyme activity was inhibited by the presence of NH_4^+−N in soil. NH_4^+−N in the soil reacts with water and SOM resulted into formation of pyrroles and indoles compounds (Dail et al., 2001) which cannot be used by microorganisms (Sinsabaugh et al., 2009), leading to the reduction in microbial and enzymatic activities. Furthermore, the presence of NH_4^+−N frequently results in the displacement of salt-based ions (such as Ca^{2+}, Na^+, K^+, and Mg^{2+}) from the soil colloids surface, rendering them vulnerable to leaching and then uptake of NH_4^+−N by plants results in the release of H^+ ions into the soil solution, so inducing soil acidification (Kivlin & Treseder, 2014), which is unfavorable for the survival of microorganisms and cause reduction in enzymatic activities.

Soil microbial community plays an essential role in nutrient biogeochemical cycle, with particularly emphasis on N cycling and SOM decomposition (Cusack, Silver, Torn, Burton, et al., 2011). Specific ecological activities such as stabilization of SOM, decomposition, aggregate formation, and nutrient cycling are attributed to distinct microbial community (Dangi et al., 2018), and preservation of soil health and quality is reliant upon diversity of microorganisms (Janvier et al., 2007). The composition of microbial communities in the soil can undergo changes because of various factors, including the soil physicochemical properties, the chemical characteristics of root exudates, and the sources of fertilizer (P. Wang et al., 2017). Furthermore, soil microbial communities are sensitive to fertilizer application (Pan et al., 2014) and exhibits variation in response to different fertilizer management practices, particularly in relation to N fertilization (Zechmeister-Boltenstern et al., 2011). According to the findings by Ai et al. (2012), the application of synthetic N fertilizer resulted in a notable increase in fungal abundance and microbial biomass. Additionally, fertilizer management practices has resulted in modifications to both the quantity and structure of soil microbial communities (Q. Zhang, W. Zhou, G. Liang, X.

Wang, et al., 2015). In the current study, during 2020, N sources (N stabilizers, UR, US) had no significant effects on microbial community however cropping sequence had significant impact on bacterial community. The higher bacterial communities under cropping sequence could be due to secretion of root exudates, for instance release of organic acid, hormones and amino acids from root system might have enhanced the bacterial community in the soil (P. Wang et al., 2017). The potential underlying mechanisms in increasing the bacterial diversity may be attributed to physicochemical alterations in the soil resulting from crop diversification (Dias et al., 2015).

Cropping sequence provides diverse residual soil C due to plant litter and residual root exudates (Garbeva et al., 2004) which support growth of microbes and resulted in enhanced bacterial community. Addition of a leguminous crop in cropping sequence improved soil C pools which supports growth of microbiota, resulted in enhanced microbial diversity (Carranca et al., 2009). A meta-analysis conducted by (Venter et al., 2016) showed that grain-legume (rice-mung bean-maize-wheat) cropping sequence enhanced microbial richness. In the present study, during the 2021 growing season, N sources, cropping sequence and their interaction had significant effects on microbial population and abundance. This increase in microbial population and abundance can be attributed to diverse crop species which probably secreted secondary metabolites (Szoboszlay et al., 2015), and consequently promoted the growth of microbial communities. Additionally, N stabilizers application enhanced NH_4^+ availability and consequently higher below ground biomass which added more organic matter in the soil. The microbes use the energy through mineralization of SOM resulted in increased microbial community population and diversity. Another possible reason could be the soil enzymatic activity, which involved C, and N mineralization helped to improve soil microbial community structure and abundance observed in the present study. Prior studies demonstrated that soil C and pH are main factors affecting composition of soil microbial community (Wei et al., 2017; Q. Zhang, W. Zhou, G. Liang, X. Wang, et al., 2015). An acidic environment is more suitable for soil fungi growth (Rousk et al., 2011), for example, Zhao et al. (2014) reported that higher soil fungi was observed in lower soil pH . In our study, N sources had significant effect on soil fungi and this could be due to soil acidification caused by N fertilizer due to release of H^+ in soil solution The results of our research align with the studies reported by (Ai et al., 2012; Chu et al., 2007; Sileshi et al., 2008; Y. Wang et al., 2017) that N fertilizer or cropping sequence enhanced microbial community diversity due to release of secondary metabolites, secretion of root exudates and addition of C through plant roots.

3.6. Conclusion

This study showed that nitrogen (N) stabilizers had significant effect on soil pH, urease, and β-glucosidase enzyme activities. N stabilizers eNtrench (EN) and superU (SU) application decreased the soil pH however urea (UR) application and N stabilizer agrotain (AG) increased soil pH. Application of AG, EN and SU as N stabilizers reduced β glucosidase activity and split urea (US), and UR application increased β glucosidase activity during 2020 and 2021. UR application enhanced the urease enzyme activity, however AG and SU decreased the urease activity, though statistically at par with UR application. N stabilizers were statistically at par with UR, US application. Cropping sequence had no significant impact on soil pH, urease, and β-glucosidase enzyme activity after harvesting the crop in both 2020 and 2021. Cropping sequence significantly affected gram positive bacteria (G^+), total bacterial phospholipid fatty acids (Σ B-PLFAs) and total phospholipid fatty acids Σ PLFAs after harvesting the crop in 2020. The corn-faba bean-corn (c-fb-c) cropping sequence showed higher G^+ bacteria, Σ B-PLFAs and Σ PLFAs compared to corn-wheat-corn (c-w-c) which showed the lowest among all cropping sequence. In 2021, higher soil G^+ population was observed in c-w-c sequence with UR application while lower were recorded in UR application with c-c-c sequence. Higher soil gram negative bacteria (G^-)community was observed in corn-corn-corn (c-c-c) cropping sequence with EN application while the lowest was recorded in UR application and control treatment in c-fb-c sequence. The N stabilisers or UR, whether applied in full or split application, did not have a significant impact on G^- bacteria within individual cropping sequence. Higher Σ B-PLFAs and Σ PLFAs population were observed in c-fb-c sequence with SU application while the lowest was recorded in c-c-c sequence with UR application. Our study concluded that N stabilizers and cropping sequence have the potential to significantly affect soil biochemical attributes, active microbial community structures and abundance in podzolic soils under boreal climate. However, a long-term crop rotation trial and N stabilizers with different application rates is required to fully understand the effect of these management practices in improving soil biochemical attributes and microbial community diversity in podzolic soils under boreal climate.

3.7. References

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Chapter 4

4. General discussion and conclusion

Objectives of this research study were to examine the effect of nitrogen (N) stabilizers and cropping sequence on:

i. Soil carbon (C) and N fractions in podzol soils under boreal climate.
ii. Soil pH, and enzymes activities in podzolic soil under boreal climate.
iii. Active microbial population in podzolic soils under boreal climate.

Field experiments, which are detailed in Chapters 2 and 3, were carried out to achieve these objectives. Effects of N stabilizers and cropping sequence on soil C and N fractions in podzol soils under boreal climate were discussed in Chapter 2. Whereas effect of N stabilizers and cropping sequence on soil pH, enzymes activities, active microbial population in podzolic soils under boreal climate were described in Chapter 3.

4.1. Effect of N stabilizers and cropping sequence on soil C and N fractions in podzol soils under boreal climate

4.1.1. Effect of N stabilizers on soil C and N fractions in podzol soils under boreal climate

Soil C serves as a prominent indication of soil fertility, and the process of soil C sequestering plays a crucial role in mitigating greenhouse gas emissions (GHG) (Lal, 2004; Rodrigues et al., 2023). Therefore, monitoring soil C levels improves our knowledge of the C cycle's impact on reducing GHG emission, protecting biodiversity, enhance crop growth, food, and energy security. Soil C is affected by multiple factors, such as the quantity and quality of organic matter inputs, the activity of soil microorganisms, physico-chemical properties of the soil. (Wei et al., 2020). N stabilizers, EN (eNtrench) and SU (SuperU) produced higher soil organic C (SOC) compared to urea (UR) in our study. The higher SOC observed with the application of urease inhibitor (UI) and nitrification inhibitor (NI) can be attributed to the delayed hydrolysis of UR (Manunza et al., 1999), as well as the inhibition of nitrification. The N stabilizers reduce the activity of nitrobacteria and ammonia monooxygenase, resulting in decreased N losses. Additionally, they enhance N use efficiency and increase the input of fresh organic C through plant roots into the soil. N stabilizers (EN and SU) treatments increased belowground biomass of plants because of N addition owing to UI limit urease activity in soils, as a result, helped to stimulate the intake of root litter and improve the quality of litter when N was added (Aerts et al., 1995; Matsushima & Chang, 2007). This could potentially impact the process of litter decomposition (Knorr et al., 2005), which in turn can influence soil C storage. Application of split urea (US) is statistically at par with N stabilizers and UR. EN and SU stabilizers application enhanced SOC by 11% and 9% compared to UR, respectively. Our results are consistent with the results reported by (LeBauer & Treseder, 2008; Lu et al., 2021), they observed N fertilizer boosts litter production and root exudates, which in turn increases the organic matter that help build up the SOC.

N is necessary for the growth and productivity of crops. However, N deficiency is commonly observed in agricultural practises, primarily due to excessive input and a lower utilisation coefficient. As a result, a substantial amount of N fertiliser is lost through processes such as nitrate (NO^-_3) leaching, nitrous oxide (N_2O) emissions, and ammonia (NH_3) volatilization. In the present study after harvesting the crop in 2020, total soil N was significantly higher with SU application compared to control, though N stabilizers such as agrotain (AG), EN, SU and UR treatments were not statistically different from each other. UI delay UR hydrolysis in soils, which allowed urea to stay in the soil for a few weeks and NI inhibit nitrification process, which result in a reduction in the amount of N that is lost through leaching. Similar, trend was observed after harvesting crop in

2021, N stabilizers showed higher total soil N but statistically at par with UR application either US or full dose application at seeding. The N-(n-Butyl) thiophosphoric triamide (NBPT) inhibits three distinct catalytic sites within the urease enzyme, through a tridentate bond formation involving an oxygen atom and two nickel centres, resulting from the carbamate bridge that links the two metals. This prevents hydrolysis process, which in turn boosts the efficiency of N cycle (Cantarella et al., 2018). Another possible explanation for the increased total soil N content in N stabilized treatments could be attributed to the attachment of NI to the active sites of ammonia monooxygenase, this metallo-enzyme plays a crucial role in facilitating the initial stage of nitrification (Di & Cameron, 2002; Menneer et al., 2008). This inhibits the activity of ammonia oxidizers, which is necessary for the process of nitrification. The results of the current research align with those of prior studies that have reported N application increases soil NH_4^+ concentration due to delayed UR hydrolysis which enhanced soil N (Chen et al., 2019; Shen et al., 2016; Zhou et al., 2015). In our findings there was no significant difference between UR application and N stabilizers that might be due to the ineffectiveness of N stabilizers in high moisture levels which were observed in our field experiment. Matczuk and Siczek (2021) observed that UI efficiency was impaired due to high moisture content and temperature, while Adhikari et al. (2021) shown that the effectiveness of NI was influenced under similar soil conditions. Soil moisture can affect NIs efficiency by impacting rate of nitrification and denitrification (Dobbie & Smith, 2001).

An effective method for detecting early changes in soil organic matter (SOM) involves analysing the particle size of particulate organic matter (POM). This is because the sand-sized fraction (>53 μm) of SOM is more likely to undergo significant changes (Zeller & Dambrine, 2011). The POM is composed of C and N that are relatively easy to mineralize (Sequeira et al., 2011). In our study, AG (N stabilizer) exhibited higher particulate organic matter N (POM-N) compared to control

(CT), though statistically non-significant with other N stabilizers (EN and SU), UR, and US. This might be due to the application of N stabiliser leads to an increase availability of N in soil, which subsequently impacts growth of plant as well as the biomass of heterotrophic microorganisms and the activity of decomposition in the soil. Previous studies conducted by (Borges et al., 2019; Valdez et al., 2017) reported that N addition leads to a reduction in the CN ratio and an increase in crop-derived residues. This, in turn, may have a stimulating effect on the rate of decomposition of SOM mediated by microorganisms and can affect POM which is a vital component in the process of SOM turnover. The variations in POM-N are influenced by the interplay of plant biomass generation and microbial decomposition. The process of soil acidification caused by N addition can have the effect of inhibiting microbial decomposition which in turn, can restrict the conversion of plant litter into organo-mineral fraction and lead to an increase in POM (Ye et al., 2018).

4.1.2. Effect of cropping sequence on soil C and N fractions in podzol soils under boreal climate

Microbial biomass present in soil is of utmost importance in the preservation of soil fertility and is widely acknowledged as a biologically dynamic reservoir within soil systems (Y. Li et al., 2018). The significance of microbial biomass lies in its role in facilitating the transformation of soil organic and inorganic reservoirs, thereby exerting a crucial influence on the regulation of plant nutrient assimilation (Liang et al., 2011). In present study, cropping sequence had significantly affected microbial biomass C (MBC) and higher MBC (550 mg kg^{-1}) was recorded in c-w-c sequence. This can be attributed to lower priming effect of wheat residues due to reduction in basal mineralization of soil organic C (Kan et al., 2022). Plant species differ in the amount and quality of substrates secreted in litter and root exudates, which may have profound effects on soil quality and the microbial community. Our results align with studies conducted by Borase et al. (2020), Benbi et al. (2012) and Song et al. (2022)) who reported enhanced MBC due to quality and quantity of different crop residues and root exudates. Priming effect could have been more intense with high maize residue inputs as compared to wheat. Aromatic components like lignin are mostly responsible for the chemical recalcitrance of residue degradation (Schmatz et al., 2017). Lignin can only be broken down by white-rot fungus and requires strong oxidation agents for microbial decomposition. Maize residue and roots include a lot of lignin, their addition of C to soil organic matter reduced (Rasse et al., 2005).

Microbial biomass N (MBN), which is regarded as a biologically active N reservoir within soil (Treseder, 2008), plays an important role in facilitating transformation of soil organic and inorganic N pools, thereby regulating the uptake of plant nutrients (Zhou et al., 2017). In present study, soil MBN was found to be significantly impacted by the cropping sequence subsequent to the crop harvesting in the year 2020. The c-w-c sequence exhibited a higher MBN (60 mg kg^{-1}) compared to the c-fb-c sequence which produced lowest soil MBN (19.6 mg kg^{-1}). This cropping sequence was found to have a noteworthy impact on the soil MBN subsequent to the 2021 harvest. In contrast to the findings of the previous year 2020, it was observed that the c-fb-c cropping sequence exhibited a greater quantity of MBN (20.56 mg kg^{-1}) in comparison compared to c-w-c cropping sequence which showed lower MBN (15.11 mg kg^{-1}). There was no significant difference between c-fb-c and c-c-c cropping sequence on MBN. The observed phenomenon could potentially be attributed to enhanced residual root and litter variation within cropping sequences (Peralta et al., 2018). This, in turn, may serve to stimulate diversity of soil microbial community, ultimately promoting soil stability and enhancing resistance to environmental changes that were affecting the MBN (McDaniel et al., 2014). The results of our research align with the studies carried out by Fu et al. (2019) and Borase et al. (2020), who observed cropping sequence leads to higher MBN. This increase can be attributed to the greater diversity of residual roots and litter, which promotes a more diverse soil microbial population and enhances its growth efficiencies. Cropping sequences has been observed to have an impact on soil-borne microbial communities, resulting in an enhancement of bacterial diversity (Ceja-Navarro et al., 2010) which is deemed advantageous for the maintenance of MBN. This, in turn, can promote the proliferation and metabolic activity of bacteria, leading to increased diversity and biomass (Li et al., 2009).

To gain a deeper comprehension of the dynamics of SOM, it is crucial to assess the rapidly cycling SOM pools in various agricultural systems. Particulate organic matter C (POM-C), a labile fraction, is classed as an intermediate decomposable pool (Jichen Li et al., 2018). It represents a transitory pool in the continuous process of SOM decomposition (Smith et al., 2020). In present study, we observed that cropping sequence had significant impact on POM-C, with highest (16.47 g kg^{-1}) recorded in c-c-c cropping sequence. A possible reason for the higher POM-C in the c-c-c cropping sequence is the increased production of both below-ground and above-ground biomass. This leads to a greater amount of carbon input into the soil through increased root biomass production (King & Blesh, 2018). Additionally, there could be an increase in root exudation (Daly & Hernandez-Ramirez, 2020), which supplies energy and nutrients to soil microorganisms (Cates et al., 2019). The soil microbial population play a role in efficiently accumulating POM-C by modifying mineralization processes. The findings of our study align with previous research conducted by (Triberti et al., 2016), who observed diverse cropping sequences led to the accumulation of SOM due to increased supply of C from crop residues and root biomass. This is also consistent with the findings of Lorenz and Lal (2005) and Martens (2000), who reported that cereal roots with high C:N ratios, phenol, and lignin contents decompose gradually, resulting in increased POM-C under a c-c-c cropping sequence. The c-c-c cropping sequence showed an 12% increase in POM-C compared to the c-fb-c cropping sequence. There was no statistically significant difference between c-fb-c and c-w-c cropping sequence treatments on POM-C. Hence, it can be concluded that the cropping sequence brought alterations in the soil POM characteristics by means of the buildup of crop residue that is resistant to decomposition and organic matter that has low bioavailability.

4.2. Effect of N stabilizers and cropping sequence on soil pH, enzymes activities in podzolic soils under boreal climate

Soil pH plays an important role in soil biogeochemical processes and known as master variable of soil due to its influence on nutrients availability, plant growth, crop yield, soil physical, biological and chemical properties (Brady & Weil, 1999). In the present study, urea application and AG decrease soil pH as compared to other N stabilizers (EN, SU). Significantly higher pH (5.9) was observed in EN (N stabilizer) while lower (5.6) was recorded in US application, though statistically at par with UR and AG. Among N stabilizers EN and SU showed significantly higher soil pH, though statistically at par with control. Decrease in pH with UR application can be attributed to the nitrification process in which H^+ ions are produced and then released into soil solution causing soil acidification (Aula et al., 2016). During the hydrolysis process, a single hydroxide ion (OH^-) is released, while in the process of nitrification, two hydrogen ions (H^+) are released. The effect of AG application was similar to the UR application which was not expected as AG (urease inhibitor) delays the UR hydrolysis process in soil (Manunza et al., 1999). This low soil pH with AG application could be due to high soil moisture conditions we observed in our field. However, soil pH in EN treatments showing less acidification due to delayed ammonium oxidation as EN (NI) inhibit first step of nitrification process by selectively targeting ammonia monooxygenase (AMO) enzyme (Arp et al., 2002). The findings of present study align with the findings of previous research indicating that the utilization of urea and UI effectively reduces soil pH (Aula et al., 2016; Chen et al., 2019; Fu et al., 2020a).

Soil enzymes, serve as indicators of the microbial metabolic activities involved in nutrient cycling (Wang et al., 2011) and undergo substantial alterations in response to variations in SOM, the process of organic N mineralization in soil is primarily governed by the activities of extracellular enzymes (Tabatabai et al., 2010). As expected, AG and SU decreased the soil urease activity compared to urea in present study, this could be due to inhibition effect of NBPT. The NBPT compound effectively inhibits the activity of the urease enzyme by binding to three active sites, this binding occurs through a tridentate bond, involving two nickel centers and one atom of oxygen from carbamate bridge that connects two metals. As a result of this binding, the likelihood of UR molecules reaching the nickel atom is significantly reduced and urea hydrolysis was delayed (Manunza et al., 1999). Previous studies also demonstrated the effectiveness of NBPT in reducing the urea hydrolysis (Allende-Montalbán et al., 2021; Sravanthi et al., 2017; Tang et al., 2023; F. Xiao et al., 2022). UI delay the hydrolysis of UR by blocking active site of urease enzyme (Manunza et al., 1999) and thus decreasing the activity of urease enzyme in the soil. In our findings, N stabilizers and urea application were statistically non-significant in urease enzyme, this could be attributed to high moisture conditions in our field experiment that might have affected the efficiency of N stabilizers.

The enzyme β-glucosidase exhibits significant utility in the assessment of soil quality due to its pivotal role in the breakdown of cellulose and the cycling of SOM (Turner et al., 2002). The β-glucosidase catalysis the hydrolysis of cellulase into glucose, is considered to be the step that limits the rate of cellulose degradation (Tabatabai, 1982), and this process plays a crucial role in determining the availability of C to soil microbes (Knight & Dick, 2004). In our study, AG, EN and SU (N stabilizers) significantly reduced the activity of β-glucosidase as compared to UR application and CT. This could be attributed to higher NH^+_4-N in soil due to the inhibition of nitrification process by NI. This higher NH^+_4-N in soil subsequently undergoes chemical reactions with SOM, leading to the formation of pyrroles and indoles compounds (Dail et al., 2001) which cannot be used by microorganisms (Sinsabaugh et al., 2009), leading to the reduction in microbial and enzymatic activities (Zi et al., 2018). Previous studies reported decrease in β-glucosidase enzyme activity due to N application (Ramirez et al., 2012; Q. Zhang, W. Zhou, G. Liang, X. Wang, et al., 2015). Another possible reason is soil acidification resulted due to N inhibitors, which is very harmful for enzymatic activities in soil, leading to reduction in β-glucosidase enzyme activity (Kang & Freeman, 1999).

4.3. Effect of N stabilizers and cropping sequence on active microbial population in podzolic soils under boreal climate

Soil microbe plays an essential role in nutrient biogeochemical cycle, with particular emphasis on N cycling and SOM decomposition (Critter et al., 2004; Cusack, Silver, Torn, Burton, et al., 2011; Van Der Heijden et al., 2008). The composition of soil microbial community alters due to various factors, including the soil physio-chemical properties, the chemical characteristics of root exudates, and the sources of fertilizer (P. Wang et al., 2017). Notable alterations were detected in the rhizosphere microbial communities of two different maize genotypes. These changes were likely caused by the release of root exudates, such as amino acids, sugars, hormones, and organic acids. These exudates likely promoted the growth of bacteria in the soil rhizosphere and increased the availability of C (P. Wang et al., 2017).

Furthermore, soil microbial communities are sensitive to fertilizer application (Pan et al., 2014) and exhibits variation in response to different fertilizer management practices, particularly in relation to N fertilization (Zechmeister-Boltenstern et al., 2011). According to the findings by Ai et al. (2012), the application of synthetic N fertilizer resulted in a notable increase in fungal abundance and microbial biomass. Additionally, fertilizer management practices has resulted in modifications to both the quantity and structure of microbial communities (Q. Zhang, W. Zhou, G. Liang, X. Wang, et al., 2015). The cropping sequence has been identified as a highly beneficial practise that significantly impacts microbial diversity (Benitez et al., 2017; D'Acunto et al., 2018; Peralta et al., 2018). Phospholipid fatty acids (PLFAs) serve as prominent indicators of soil microbial biomarkers, revealing the viable constituents of soil microbial biomass. They offer more comprehensive insights into the active soil microbial community as compared to the culture approach (Liang et al., 2008; Yao et al., 2000). In the present study, during 2020, N sources (N stabilizers, urea, US) had no significant effects on microbial diversity, however cropping sequence had significant effects on bacterial community. The higher bacterial communities under cropping sequence could be due to secretion of root exudates, for instance release of organic acid, hormones and amino acids from root system might have enhanced the bacterial community in the soil (P. Wang et al., 2017).

The potential underlying mechanisms in increasing the bacterial diversity may be attributed to physico-chemical alterations in the soil resulting from crop diversification (Dias et al., 2015). Cropping sequence provides diverse residual soil C due to plant litter and residual root exudates (Garbeva et al., 2004) which support growth of microbes and resulted in enhanced bacterial community. Addition of a leguminous crop in cropping sequence improved soil C pools which supports growth of microbiota, resulted in enhanced microbial diversity (Carranca et al., 2009).

A recent study conducted by Hamel et al. (2018) found that incorporating a legume crop into the wheat rotation changed the bacterial and fungal community composition in the Canadian prairie

(Borrell et al., 2017). A meta-analysis conducted by (Venter et al., 2016) showed that grain-legume (rice-mung bean-maize-wheat) cropping sequence enhanced microbial richness. In the present study, during 2021 growing season, N sources, cropping sequence and their interaction had significantly impacted microbial population and abundance. This increase in microbial population and abundance can be attributed to diverse crop species which probably secreted secondary metabolites (Szoboszlay et al., 2015), and consequently promoted the growth of microbial communities. Additionally, N stabilizers application enhanced $NH^+{}_4$ availability and consequently higher below ground biomass which added more organic matter in the soil. The microbes use the energy through mineralization of SOM resulted in increased microbial community population and diversity. Another possible reason could be the soil enzymatic activity, which involved C, and N mineralization helped to improve soil microbial community observed in present study. Our findings are in line with research studies reported by (Ai et al., 2012; Chu et al., 2007; Sileshi et al., 2008; Y. Wang et al., 2017) observed that N fertilizer or cropping sequence enhanced microbial community diversity due to release of secondary metabolites, secretion of root exudates and addition of C through plant roots.

Fungi play a role in C and nutrients cycling in agroecosystems and are susceptible to fertiliser application (Jing Li et al., 2018). An acidic environment is more suitable for soil fungi growth (Rousk et al., 2011), for example, Zhao et al. (2014) reported that higher soil fungi was observed in lower soil pH . In our study N sources had significant effect on soil fungi and this could be due soil acidification caused by N fertilizer due to release of H^+ ion in soil solution. The results of the present research are consistent with Ai et al. (2018) and Cassman et al. (2016), who reported that cropping sequence has a notable impact on the soil fungus communities. Plant species and genotypes typically secrete organic acids, carbohydrates, aromatic compounds, lipids, amino acids, and enzymes, from their roots. These substances promote the growth of inactive microbial species (Li et al., 2014; Szoboszlay et al., 2015).

4.4. References

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