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Contrasting Nitrogen Sources Impact Nitrogen Use Efficiency and Soil Health Under Silage Corn Production in a Semi-Arid Environment

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CONTRASTING NITROGEN SOURCES IMPACT NITROGEN USE EFFICIENCY AND SOIL HEALTH UNDER SILAGE CORN PRODUCTION IN A SEMI-ARID ENVIRONMENT

by

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ABSTRACT

Contrasting Nitrogen Sources Impact Nitrogen Use Efficiency and Soil Health under Silage Corn Production in a Semi-Arid Environment

by

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Silage corn production challenges sustainable intensification and soil health in semi-arid environments because the entire aboveground biomass is harvested and removed from the field. An irrigated silage corn field study was conducted over a decade comparing nitrogen fertility sources using a complete randomized block design with four treatments: control with no nitrogen fertilizer (control), low ammonium sulfate at 112 kg N ha^{-1} (AS100), high ammonium sulfate at 224 kg N ha^{-1} (AS200), and steer manure compost at 224 kg total N ha^{-1} (compost). Research focused on the impact of these contrasting nitrogen sources on silage corn production, nitrogen use efficiency (NUE), and soil health indicators.

Variable responses in yield, NUE, and soil health indicators across years emphasized the importance of multi-season studies. Yield under compost treatment exhibited a notable 41% increase compared to control but was approximately 31% lower than the average yield under AS100 and AS200 treatments. AS100 achieved a yield comparable to AS200 and demonstrated higher NUE, challenging conventional belief that increased nitrogen application rate ensures maximum yield and profitability.

Despite lower yield and NUE, the compost significantly increased STN and SOC by 23%. Multiple soil health indicators including mineralizable nitrogen (N_0), mineralizable carbon (C_0), autoclaved-citrate extractable protein, water-extractable organic nitrogen, permanganate-oxidizable carbon, and N-acetylglucosaminidase and beta-glucosidase enzyme activities were substantially increased by compost. Strong positive correlations were found between soil health carbon and nitrogen indicators, emphasizing interactions between soil carbon and nitrogen and the responsiveness of active organic matter pools and enzyme activities.

In summary, multiple-season studies are crucial for understanding carbon and nitrogen dynamics and soil health. Ammonium sulfate fertilizers proved effective in achieving higher silage corn yields and NUE compared to compost treatment. In contrast, compost demonstrated significant enhancement of soil C and N and their indicators, highlighting benefits for overall soil health. The positive shifts in soil health indicators underscore the advantages of combining compost with commercial fertilizers for improved fertility and sustainable soil management. Farmers are encouraged to adopt a balanced approach, incorporating compost alongside commercial fertilizers and implementing soil health practices for sustainable silage corn systems.

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PUBLIC ABSTRACT

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Yield under compost treatment exhibited a notable 41% increase compared to control but was approximately 31% lower than the average yield under AS100 and AS200 treatments. AS100 achieved a yield comparable to AS200 and demonstrated higher NUE, challenging conventional belief that increased nitrogen application rate ensures maximum yield and profitability. Despite lower yield and NUE, the compost significantly enhanced soil health indicators such as STN, carbon, soil N and C mineralization, soil protein, soil water extractable organic N and C, and soil enzymes.

In summary, multiple-season studies are crucial for understanding carbon and nitrogen dynamics and soil health. Ammonium sulfate fertilizers proved effective in

achieving higher silage corn yields and NUE compared to compost treatment. In contrast, compost demonstrated significant enhancement of soil C and N and their indicators, highlighting benefits for overall soil health. The positive shifts in soil health indicators underscore the advantages of combining compost with commercial fertilizers for improved fertility and sustainable soil management. Farmers are encouraged to adopt a balanced approach, incorporating compost alongside commercial fertilizers and implementing soil health practices for sustainable silage corn systems.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Literature Review

Land degradation is a major concern for world food security and escalation of poverty, especially in the developing world (Badapalli et al., 2023; Rodgers et al., 2021). The amount of arable land necessary to produce agricultural production to meet the demands of the rapidly increasing global population is declining at a concerning rate. Factors that contribute into the declination of soil fertility in numerous regions across the globe are non-sustainable land use practices such as intensive farming, excessive grazing, fertilization and pesticides, salinization, climate change, and natural disaster (Badapalli et al., 2023; Forni et al., 2016; Goswami & Deka, 2020; Gupta, 2019; Rodgers et al., 2021). Experts estimate that this decline varies from less than 1 billion hectares to over 6 billion hectares globally (Gibbs & Salmon, 2015). This rate is concerning because when soil fertility declines, it will lead to high input costs, declines in agricultural productivity, biodiversity loss, food insecurity, and farmland abandonment (Badapalli et al., 2023; Rodgers et al., 2021).

Arid and semi-arid regions play an important role in ensuring the world food security (Ayangbenro & Babalola, 2021). Unfortunately, these regions are considered to be prone to land degradation (Badapalli et al., 2023; Reynolds et al., 2011). Arid and semi-arid lands regions cover approximately 45.4% of the Earth's land area, spanning 66.7 million square kilometers, and are home to around 2 billion people (Pinheiro Junior et al., 2019; Prăvălie, 2016). Growing condition in those regions face many challenges due to the short growing season, limited water resources, soil nutrient limitation, and salinity problems (Ayangbenro & Babalola, 2021; Creswell et al., 1993; Idowu et al., 2000). Despite the challenges, these regions are important sources of food and fiber production globally (International Union for Conservation of Nature, 2019; United Nation Convention to Combat Desertification, 2019).

Soil health and sustainable agriculture are closely associated (Tahat et al., 2020). Sustainable agriculture is a farming approach that seeks to protect the environment, enhance natural resource conservation, minimize the use of nonrenewable resources while satisfying human food and fiber needs and sustaining the economic viability of the farm operation (National Agricultural Library, 2023). Sustainable agriculture aims to meet the current requirements of society's food and textiles demands while preserving the resources for future generations (Feenstra, 2021; Lichtfouse et al., 2009). Soil health is defined as "the capacity of a specific type of soil to operate, within the confines of natural or managed ecosystems, to sustain plant and animal productivity, uphold or improve water and air quality, and promote human health and habitation" (Karlen et al., 1997; Wienhold et al., 2004). Soil health may also be defined as the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans (Yost et al. 2019).

Farming practices, including cultivation, irrigation, fertilization, and pest management can profoundly affect overall soil health (Farmers.gov, 2022). Soil health assessment serves as a crucial tool for evaluating the impact of farming practices on soil health. It provides farmers, ranchers, and land managers with insights into how their practices influence soil health on their land (Idowu et al., 2019).

Fertilizers are characterized as any substance, either organic or inorganic, occurring naturally or produced synthetically, that provide the essential chemical elements for plant growth (Kiiski et al., 2016). Organic fertilizers are composed of plant or animal-derived substance such as animal manure, composted organic materials, and the by-products of other living organism (El-Haggar, 2007; Wei et al., 2020). Chemical or synthetic fertilizers contain compounds which are artificially created and designed to provide plants with the essential nutrients in a readily available form. Those substances consist of the concentrations of essential nutrients for plant growth, such as nitrogen, phosphorus, potassium, sulfur, and occasionally micronutrients, either alone or in combination (Pahalvi et al., 2021). Fertilization practices significantly influence soil conditions. Several studies have shown that organic fertilizers have the potential to enhance soil health, while inorganic fertilizers tend to decrease it (Montgomery & Biklé, 2021; Noor et al., 2020; Thaler et al., 2021). The growing demand and limited land availability have led to the increased use of chemical fertilizers in higher amounts for maximum productivity and as a form of insurance (Ali et al., 2021; Raun & Schepers, 2015). This practice has the potential to harm soil health and the environment because excessive N fertilization can lead to greenhouse gas emissions and water pollution (Aranguren et al., 2018; Cordero et al., 2019). Approximately 50% of the N fertilizer applied to cropping systems may be lost to the environment in the forms of ammonia (NH_3), nitrate (NO_3^-), and nitrous oxide (N_2O) (Coskun et al., 2017). Due to immediate need for the economic survival, farmers may overlook environmental pollution caused by excessive use of N (Drury et al., 1996; Yadav et al., 2017).

In contrast to inorganic fertilizers, many studies demonstrate that organic fertilizers contribute to improved soil structure, increased organic matter content, and enhanced microbial diversity, all necessary for long-term soil health (Dincă et al., 2022; Montgomery & Biklé, 2021; Noor et al., 2020; Wang et al., 2023). High organic matter and sound soil structure prevent erosion, runoff, and enhance soil resilience to climate change (April, 2019; Girija Veni et al., 2020).

In addition to fertilization, cropping systems such as monoculture also exert a significant influence on soil health (Farmers.gov, 2022; Yang et al., 2020). Corn silage is essential in animal feed production, particularly for dairy cows and fattening cattle, due to its high energy content (Bates, 1998). One of the obstacles faced by farmers growing corn silage is finding the optimal fertilizer application rate to reach desired crop yields without compromising soil health and environmental sustainability.

Nitrogen use efficiency (NUE) is used an essential tool for evaluating N fertilizer management plans (Beatty & Wong, 2017; Congreves et al., 2021). This assessment aims to optimize N input use, produce economically viable crop yields, and reduce N loss to the environment (Fixen et al., 2015). NUE alone cannot directly quantify nutrient loss, as some nutrients may remain in the soil (Fixen et al., 2015). A low NUE does not necessarily indicate harm to the environment, nor does a high NUE automatically guarantee environmental friendliness. NUE is influenced by various factors, including soil and climate conditions, as well as different farm management practices (Fixen et al., 2015).

The global demand for N fertilizer has surged, rising from 110 million tons in 2015 to approximately 119 million tons in 2020 (FAO, 2020). While maintaining yield, even a 1% increase in NUE could lead to substantial savings of up to $23 million in N fertilizer costs (Johnson & Raun, 2003). A more substantial 20% improvement in NUE has the potential to save over $4.7 billion annually (Raun & Johnson, 1999). As the result, enhancing NUE not only boosts crop yields but also upholds environmental quality, reduces production expenses, and ensures sufficient profit margins for farmers (Fageria & Baligar, 2005; Hirel et al., 2007).

The benchmarks for NUE differ between corn grain and corn silage (Augarten et al., 2019). Discussions about NUE often pertain to corn grain systems, and these values should not serve as the benchmark for assessing NUE of corn silage. This distinction arises from the fact that corn silage production results in the removal of more N compared to corn grain. Corn silage harvest involves the removal of the entire aboveground biomass, whereas corn grain production removes the grain, leaving the stalks in the field to decompose (Malone et al., 2019; Sawyer, 2007). Given these variances in N removal rates, it is imperative to evaluate the NUE of corn silage recognizing its unique attributes. The limited available data on NUE for corn silage production underscores the need for additional research in this area. More research and study on this subject will be helpful for assessing the sustainability of corn silage management systems.

The study of NUE and corn silage yield is important. However, it is crucial to conduct a study on soil health within that system, as monoculture of silage practices for multiple years can lead to soil erosion and degradation particularly if soil conservation measures are not integrated into production systems (Ramirez et al., 2023; Roth & Heinrichs, 2001; Siller et al., 2016). Silage production involves the removal of the majority of above ground biomass during harvest, this is residue essential for safeguarding the soil from erosion and maintaining soil organic matter (Blanco-Canqui & Lal, 2009; Stella et al., 2019). The removal of crop residue results in the soil surface being susceptible to erosive forces, including water and wind erosion, thus elevating the chances of soil loss (Klopp & Blanco-Canqui, 2022).

Carbon and N transformations play key roles in soil organic matter formation and decomposition, N cycle, and food web maintenance (FAO, 2021). N is vital for the growth and productivity of plants and all living creatures (Govindasamy et al., 2023; Walworth, 2013). Adequate N supply can promote robust crop development; conversely, inadequate N supply may cause yield reduction and nutrient disparities (Goulding et al., 2008). Additionally, improper N fertilizer management can cause detrimental effects on water quality and aquatic ecosystems due to N leaching and run off (Goulding et al., 2008; Govindasamy et al., 2023).

There have been various methods have been proposed for assessing soil health related to N, focusing on organic N pools, N-related processes, and proxies for these pools and processes (Grandy et al., 2022; Liptzin et al., 2023). These indicators such as potential mineralizable N, soil total nitrogen (STN), water-extractable organic nitrogen (WEON), autoclave citrate-extractable soil protein (ACE soil protein), N-acetyl-β-D-glucosaminidase activity (NAGase), and inorganic N (ammonium-N +nitrate-N) provide valuable insights into N status and dynamics in soils (Cappellazzi & Morgan, 2021; Liptzin et al., 2023). The purpose of these assessments is to evaluate the long-term ability of the soil to supply N, in contrast to conventional soil fertility tests that only provide a single snapshot of available N (Grandy et al., 2022; Liptzin et al., 2023).

Soil potential mineralizable N is an indicator used to model the release of organic N to the available pool, which correlates highly with the release of mineral N during long-term incubations (Keeney & Bremner, 1967). ACE protein is an indicator for a variety of protein-like substances in soil organic matter that may become available for plant and microbial uptake (Van Es et al., 2020). WEON represents the pool of organic N that is available to microbes and easily broken down into the form that plant can used (Bellows et al., 2020; Haney et al., 2012, 2018). NAGase, (EC 3.2.1.30) is one of the enzymes that plays an important role in N cycling (Cappellazzi & Morgan, 2021; Ekenler & Tabatabai, 2004). NAGase measurement represent the potential enzyme activity within the soil responsible for catalyzing the final step in the degradation of chitin (Ekenler & Tabatabai, 2004). All these N indicators contribute to the assessment of soil health status and N availability to plants under different agricultural practices.

Soil has the capacity to store carbon in organic matter, which is closely tied to soil quality and productivity (Anderson et al., 2022; Klopp et al., 2023). The United States Department of Agriculture (USDA NRCS, 2019) recommends soil organic C, C mineralization, water-extractable organic C (WOEC), permanganate-oxidizable C (POXC), and β-glucosidase enzyme activity (BG) as important soil health indicators of the C and energy available to soil microbial communities. These C indicators are sensitive to management practices and their analysis is rapid and cost-effective, which is an important criteria for soil health evaluation tools.

Carbon mineralization is a pivotal component of the terrestrial C cycle, representing the processes by which microorganisms break down organic substances assimilating some into microbial biomass while releasing carbon dioxide and nutrients

(Guo et al., 2019). This process facilitates the release of essential nutrients such as N for plant uptake. The relationship between C mineralization and nutrient release serves as a critical determinant in sustaining soil fertility and promoting robust plant growth (Gan et al., 2020; Guo et al., 2019; Jonasson et al., 1999). POXC, also known as active carbon, serves as an indicator of a crucial nutrient source for microorganisms. This labile C fraction undergoes oxidation when exposed to a potassium permanganate solution (Culman et al., 2012; USDA, 2014; Weil et al., 2003). WEOC measures a readily available C pool, which reflects a substrate pool that is easily accessible to microorganisms (Haney et al., 2012; USDA NRCS, 2019). It is one of the most sensitive indicators for tracking changes in the labile C pool. In addition, WEOC plays important roles in transformations of SOM and N mineralization (Haney et al., 2008, 2012, 2018; Sun et al., 2017). BG enzyme (EC 3.2. 1.21) is a key player in the C cycle involved in the degradation of cellulose. Its primary function is to catalyze the hydrolysis of cellobiose, a disaccharide, into glucose molecules (Adetunji et al., 2017; Almeida et al., 2015). It acts as a key enzyme responsible for releasing labile carbon and energy sources essential for soil microorganisms. BG offers an early indication of shifts in soil organic C levels and turnover rates rate (Adetunji et al., 2017; Deng & Popova, 2015).

Previous studies have emphasized the importance of conducting a comprehensive study that considers both NUE and soil health indicators, particularly N and C indicators (Wade et al., 2021; Yuan et al., 2023). NUE is utilized as a benchmark to gauge the effectiveness of N management strategies, with the objective of minimizing nutrient losses and mitigating the adverse environmental consequences linked to excessive fertilizer usage (Congreves et al., 2021; Galloway et al., 2014). The use of N indicators is vital in evaluating the N levels and changes in soil, emphasizing the significance of implementing appropriate management practices to mitigate the risk of environmental pollution (Hossen et al., 2021; Liptzin et al., 2023). Simultaneously, C indicators gauge soil organic matter levels, influencing soil structure and fertility (Billings et al., 2021; Liptzin et al., 2022; Rice et al., 2005). The interaction and dynamic relationship between N and C play a crucial role in several important soil functions, such as nutrient cycling, microbial activity, and the overall health of the soil. Therefore, the research on NUE and soil health indicators is crucial for resource conservation and climate resilience, as well as mitigating nitrogen's environmental impact and promoting sustainable agriculture.

Research Needs

The success of farming practice in semi-arid and arid regions plays an important role in ensuring global food security, despite distinct challenges arising from limited nutrient availability and precipitation (Ayangbenro & Babalola, 2021). However, the majority of research related to soil health and corn silage production are conducted in regions including the Midwest, Southeast, Northeast, and Pacific regions of the USA (Chu et al., 2019; Fine et al., 2017; Nunes et al., 2018, 2020a, 2020b, 2021; Singh et al., 2020; Yost et al., 2018). More research is needed on soil health and crop production response to fertilization in arid and semi-arid regions.

Research Questions

1. What is the comparative impact of contrasting N sources on corn silage yield, plant nitrogen uptake, nitrogen use efficiency, and soil total nitrogen under semi-arid conditions in northern Utah, USA?
2. How do contrasting nitrogen sources impact soil health N indicators and their interactions during corn silage production across multiple seasons, and what are the relationships between these soil N indicators within a corn silage system under varying N sources across different growing seasons?
3. What are the effects of contrasting N fertilizers on a variety of soil health carbon indicators, and how do these indicators relate to each other within a corn silage system under contrasting N sources across multiple growing seasons? Additionally, how do soil carbon and nitrogen indicators interconnect in response to different N sources?

Objectives and Hypothesis

This study examines the effects of contrasting N sources on nitrogen use efficiency (NUE) and soil health under silage corn production in the semi-arid environment of northern Utah USA. We examine the effects of contrasting N sources on NUE, soil health N and C indicators, and the relationship between these indicators. There are three main objectives for this research.

Objective 1: To assess and compare the contrasting effects of contrasting nitrogen sources on corn silage yield, plant nitrogen uptake, nitrogen use efficiency, and soil total nitrogen in the semi-arid conditions of northern Utah, USA (Chapter II).

Hypothesis 1: There are significant differences in corn silage yield, plant nitrogen uptake, nitrogen use efficiency, and soil total nitrogen among the different contrasting nitrogen sources under the semi-arid conditions of northern Utah, USA.

Objective 2: To comprehensively assess the influence of contrasting N sources on soil health N indicators and their dynamic interactions throughout the duration of corn silage production across multiple growing seasons, while simultaneously investigating the intricate relationships between these soil N indicators within a corn silage system under contrasting N sources across different seasons (Chapter III).

Hypothesis 2: Contrasting N sources have a significant impact on soil N indicators and their interactions during corn silage production across multiple seasons, and distinct relationships exist between these soil N indicators within a corn silage system under varying nitrogen sources across different growing seasons.

Objective 3: To investigate the impacts of contrasting N fertilizers on a range of soil carbon indicators and their interrelationships within a corn silage system under contrasting N sources across multiple growing seasons, with a specific focus on understanding the connections between soil C and N indicators in response to different N sources (Chapter IV).

Hypothesis 3: Contrasting N fertilizers have a significant impact on various soil carbon indicators, leading to distinct relationships among these indicators within a corn silage system under varying N sources across multiple growing seasons. Additionally, soil health C and N indicators exhibit significant interconnections in response to contrasting N sources.

The impact of field experiments reaches far beyond what is typically associated with academic research. Especially, long-term field studies provide insights directly applicable to challenges faced by farmers in semi-arid and arid regions (Chu et al., 2019; Johnston& Poulton, 2018). The results from this study serve as valuable knowledge for researchers, agricultural institutions, and, most importantly, for the farmers themselves. By addressing the gaps in the existing literature and conducting dedicated, extended field studies in these challenging regions, we can develop practical solutions and strategies that have a direct and positive impact on agriculture in arid and semi-arid environments (Johnston & Poulton, 2018).

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CHAPTER II

NITROGEN USE EFFICIENCY OF SILAGE CORN WITH CONTRASTING NITROGEN FERTILITY SOURCES

Abstract

The increasing demand for food production and the need to reduce excess reactive nitrogen in the environment pose a dual challenge for agriculture. A field study was conducted in northern Utah USA from 2012 to 2021 to examine the effect of nitrogen fertility sources on plant nitrogen uptake, corn silage yield and soil total nitrogen (STN) under semi-arid conditions. The experiment followed a randomized complete block design with four treatments: no nitrogen control (control), low ammonium sulfate (AS100) at a rate of 112 kg N ha^{-1} year^{-1}, high ammonium sulfate (AS200) at a rate of 224 kg N ha^{-1} year^{-1}, and steer manure compost (compost) at 224 kg total N $\mathrm{ha}^{-1}\mathrm{year}^{-1}$. Nitrogen use efficiency (NUE) was interpreted through multiple metrics, including uptake efficiency (UE), agronomic efficiency (AE), partial factor productivity (PFP), and partial nutrient balance (PNB). Yield under compost treatment exhibited a notable 41% increase compared to control; however, it was approximately 31% lower than the average yield under AS100 and AS200 treatments. Compost treatment increased STN by 23.1% compared to treatments. Yields and UE under AS100 and AS200 were comparable. However, AS100 treatment outperformed the AS200 treatment because of higher values of AE, PFP and PNB. The AS100 treatment, with yields not significantly different from AS200, exhibited superior NUE. Yield and NUE under compost treatment were lower than other treatments, but it had the advantage of increased STN. Therefore, to maintain soil health, farmers might consider combining

compost with commercial fertilizers for N fertility and practicing good soil health practices such as crop rotation or cover crops.

Introduction

Agricultural intensification has increased crop production in response to world hunger. For the past 50 years, farmers around the world have used synthetic fertilizers to increase crop yields, sometimes over-fertilizing, as a form of insurance or because of public policies subsidizing fertilizer costs (Li et al., 2013; Scholz & Geissler, 2018; Wang et al., 2023). Growers may be less concerned about the indirect costs of environmental pollution from excessive nitrogen (N) application due to short-term goals of economic survival (Yadav et al., 2017).

Corn silage is a high-energy feed for dairy cows and fattening cattle (Bates, 1998). As the result, the acreage and production of corn silage have experienced significant growth in the Pacific Northwest (PNW) because of the livestock industry (Brown et al., 2010). In addition to its nutritional benefits, there are other benefits to growing corn silage such as an opportunity for the salvage of stressed or damaged corn fields (Roth & Heinrichs, 2001). However, the practices of corn silage production also come with some disadvantages. Transporting and selling silage over long distances is a challenge. Another challenge is that this practice has a high risk of contributing to soil erosion and soil degradation. During harvesting, the majority of aboveground biomass is removed which results in leaving the minimal crop residue left on the field. This practice can be detrimental for the maintenance of soil organic matter because crop residues play an important role in protecting the soil from erosion and maintaining soil organic matter (Blanco-Canqui & Lal, 2009; Stella et al., 2019).

Corn silage growers face the dual challenge of increasing yields without compromising soil health and environmental quality. Fertilizer is one of the largest expenses for farmers. They often increase fertilizer application to achieve the target yield (Sheriff, 2005). However, this practice does not guarantee higher profits. Additionally, excessive N application may have negative impacts on soil health and the environment. Deteriorated soil quality and polluted environment may jeopardize the farmers' businesses and the community. Therefore, it is important for farmer to adopt practices that increase nitrogen use efficiency (NUE) so that N inputs matches the crop demand without exceeding crop needs (Curtin et al., 2017).

Nitrogen use efficiency (NUE) is defined as the ratio of crop N uptake to total N input (EU Nitrogen Expert Panel, 2015). Managing N to achieve the target yield with minimal N loss to the environment is challenging (Beegle, 2017). Therefore, assessment of NUE is a valuable procedure for evaluating N fertilizer management practices. The goal of assessing NUE is to increase the use and uptake of N inputs, while achieving an economically viable yield and reducing the loss of N to the environment (Congreves et al., 2021). Low NUE does not necessarily imply environmental degradation, and a higher NUE does not guarantee harmless N management. NUE does not directly measure nutrient loss as some nutrients may be retained in the soil (Fixen et al., 2015). Diverse factors, such as soil and climatic conditions, coupled with farm management practices, intricately influence NUE (Beegle, 2017; Fixen et al., 2015).

Nitrogen use efficiency (NUE) can be assessed by using different metrics such as partial factor productivity (PFP), agronomic efficiency (AE), partial nutrient balance (PNB) and uptake efficiency (UE) (Augarten et al., 2019; Fixen et al., 2015). Uptake efficiency (UE) demonstrates apparent N recovery in response to N fertilization (Augarten et al., 2019; Congreves et al., 2021; Fixen et al., 2015). Agronomic Efficiency (AE) is used to address the question of how much productivity improvement was gained by a unit of N input (Augarten et al., 2019; Fixen et al., 2015). Partial factor productivity (PFP) is used to evaluate the productivity of the cropping system compared to N application (Augarten et al., 2019). Partial nutrient balance (PNB) is used to calculate how much N is being taken out of the system compared to how much was added (Augarten et al., 2019).

NUE is often discussed regarding the corn grain system, and these values should not be used as the benchmark for the NUE of corn silage. More N is removed with corn silage production than for corn grain since the entire aboveground biomass is removed at harvest; while corn grain production only removes the grain, leaving the stalks in the field to decompose. Because of these differences in N removal rates, the NUE of corn silage should be assessed independently of the NUE for grain production (Brown et al., 2010). Sparse data on NUE for corn silage production suggests that additional research on NUE of corn silage will be helpful for assessing the sustainability of corn silage management systems.

A field experiment was established at the Greenville Research farm at Utah State University (USU) in 2011 to study the effect of different N fertilizer amounts and sources on corn silage. The objective of this experiment was to investigate the impact of compost fertilizer on corn silage yield, nitrogen uptake, nitrogen use efficiency and soil total nitrogen (STN), and to compare the effects of compost fertilizer with those of ammonium sulfate fertilizer. The other goal of this study was to determine which type of fertilizer provides the greatest benefit for farmers. In related studies, additional factors were investigated focused on other aspects of the soil N cycle and microbial communities (Ouyang, 2016; Ouyang & Norton, 2020). In the current study, parameters collected and analyzed were yield of corn silage, N uptake of aboveground plant biomass, STN, and indicators of NUE, including UE, AE, PFP, and PNB. By analyzing these factors, we expected to provide insight into the performance of different fertilizers and their potential to improve sustainability in agriculture.

Materials and Methods

Site description and experimental design

The site is located at the USU Greenville Research Farm (41°45'56.6"N 111°48'52.2"W) in North Logan, Utah. The soil is a highly calcareous Millville silt loam (coarse-silty, carbonatic, mesic Typic Haploxeroll) with a pH of 8.2 (1:2 soil: water). The plots were established in 2011 to investigate N cycling and different N transformations under contrasting N management, as outlined in previous studies (Kakkar, 2017; Ouyang, 2016). Before, the field was utilized for conventional cultivation of small grains, involving an annual application of 70 kg of N per hectare in the form of urea. The experimental design in this study was a randomized complete block design (RCBD) with four N fertility source treatments and four replications, totaling 16 plots (Figure A1). Each plot measured 9.1 m in length and 3.8 m in width. Treatments were assigned to the same plots each year. The treatments include a no N control (control), low ammonium sulfate at 112 kg N $ha^{-1}year^{-1}$ (AS100), high ammonium sulfate at 224 kg N $ha^{-1}year^{-1}$, (AS200), and steer manure compost at 224 total kg N $ha^{-1}year^{-1}$ (compost). Compost was obtained commercially and consisted of composted steer manure, slaughter by-products and woodchips (Miller companies LLC, Hyrum, Utah). Compost nitrogen and dry matter content were determined yearly, and these parameters were used to apply the desired total nitrogen rate of 224 kg TN $ha^{-1}year^{-1}$, equivalent to 14.4 ± 1.8 metric ton of dried weight compost $ha^{-1}year^{-1}$. Silage corn has been planted every May from 2012 until 2021 except for 2017 when a cover crop of vetch was grown.

Field operations

During early spring of each year pre-plant soil samples were collected from each plot using a Giddings probe with two cores per plot at depths of 0-15 cm, 15-30 cm and 30-60 cm. Soil was weighed, sieved (2 mm) and air-dried before analysis for available P and K. To meet the crop requirement of P and K, fertilization for P and K in each plot was carried out according to the recommendations outlined in the Utah Fertilizer Guide for silage corn (James & Topper, 1993). The fertilizer applications and compost amendments took place in early May of each year. N, P, K fertilizers were applied to the field using an Edge Guard mini push broadcast spreader (The Scotts Company LLC. USA). For compost treatment, the amendment was applied manually and subsequently, bow rakes were utilized to evenly distribute the fertilizers and compost amendments within individual plots. Following this, the amendments were incorporated into the soil through tillage within one day of application.

After the amendments were added and incorporated, the seedbed was prepared, and seed (DEKALB® Corn Hybrids (glyphosate tolerant) were planted with a row spacing of 76 cm. Within each block, approximately 4 rows of silage corn were planted at a density of 50,000 plants per hectare using a John Deere planter. Throughout the growing season, an overhead sprinkler irrigation system was used to apply water on a weekly basis as required and as available. To control weed growth, Killzall herbicide, containing 41% glyphosate and diluted to a concentration of 18.7 g L^{-1} with water, was applied at a rate of 1.12 kg 1.12 kg $^{-1}$. This application was done once via broadcast before the corn reached a height of 30 inches.

Plant and soil analysis

To analyze soil total nitrogen (STN), topsoil samples were manually collected every year from 2012-2021 in August from the 0-15 cm layer (four cores per plot) using Slide Hammer soil probe. The soil samples were sieved through a 2 mm mesh, air-dried, and then a subsample was finely ground to pass through a 0.25 mm sieve (equivalent to a 60-mesh) for TN analysis using dry combustion (Bremner, 1996) with a PrimacsSN (Skalar, Inc. GA, USA).

For leaf tissue N analysis, samples of the corn ear leaf were collected approximately 80 days after planting each year. Four corn leaves from each row, located in the middles of the plots, were harvested. In total, eight leaves were sampled per plot. Leaves were dried at 60°C to constant weight, followed by grinding using a Wiley Mill. Subsequently, the subsample was further ground to achieve a particle size equivalent 0.25 mm (60 mesh). Once the silage reached maturity in late September, aboveground plant material from the inner two rows of each plot, covering a distance of 3 meters, was harvested using machetes. Plant counts and fresh wet weight were recorded for each row per plot. The harvested corn was subsequently dried at 60°C for approximately one week, and its dry weight was determined. The dried stalks were then coarsely chopped, and a subsample was finely ground using a Willey Mill. The subsamples were then finely ground with a rolling ball mill to 0.05 mm sieve before TN analysis by combustion (PrimacsSN Skalar, Inc., GA, USA).

Nitrogen use efficiency (NUE)

Uptake efficiency (UE), agronomic efficiency (AE), partial factor productivity (PFP), partial nutrient balance (PNB) are important metrics for interpreting NUE. The equations of NUE is adapted from previous studies (Augarten et al., 2019; Raun et al., 2002). The metrics and their equations are shown Table 2.1.

Data analysis

The parameters in this study included leaf nitrogen (N) content, dry matter yield, nitrogen uptake at harvest, nitrogen use efficiency (NUE) indicators (including uptake efficiency (UE), agronomic efficiency (AE), partial factor productivity (PFP), and partial nutrient balance (PNB)), and STN content collected annually from the years 2012 to 2021. There was no data collected during 2017 due to the planting and management of a cover crop of hairy vetch.

For each year within the study duration, we performed an analysis of variance (ANOVA) to assess the impact of different fertilizer sources on the above-mentioned parameters. The PROC MIXED procedure available in SAS® OnDemand was utilized. Our examination focused on the significant differences among the treatment groups at each year. Mean differences were considered significant at $p \leq 0.05$.

To gain a comprehensive understanding of the overall treatment effects across the study years, we employed repeated measures analysis of variance (ANOVA) using the PROC MIXED procedure. In this analysis, year was considered a fixed and repeated effect. Blocks and interactions with treatment were considered as random effects. Several covariance structures were evaluated, and the compound symmetry (CS) covariance structure was used because it had the lowest Akaike Information Criteria or best fit. The mean separations were conducted at $p < 0.05$ using Tukey's test. To ensure the validity of our statistical tests, we assessed the normality of residuals using the UNIVARIATE procedure in SAS. Additionally, we generated scatterplots of residuals against predicted values to ascertain the presence of common variance. These steps were undertaken to verify that the assumptions underlying our statistical analyses were met.

Results

Yield

Contrasting N sources showed inconsistent effects on corn silage yield from 2012 to 2021 (Figure 2.1). In 2012, the compost treatment displayed the lowest yield, whereas the yield differences between AS200 and AS100, in comparison to the control treatment, showed no significant differences. N immobilization may have occurred in the compost treatment during 2012. During this period, soil microbes compete with the growing crop for available nitrogen (N), potentially suppressing crop growth and consequently lowering yield (Keena et al., 2022). However, in 2013, 2014, 2018, and 2021, the yield of compost treatments was significantly higher than that of the control. From 2012 to 2021, the corn silage yields with the AS200 treatment (224 kg ha^{-1}) were not significantly different from those with the AS100 treatment (112 kg ha^{-1}), except for 2020. In some years, the yield of corn silage with the ammonium sulfate treatment was comparable to that with the compost treatment (2013, 2016, and 2018), while in other years, the ammonium sulfate treatments yielded more than the compost treatment (2012, 2014, 2020, and 2021) (Figure 2.1).

There was a considerable yield variation ranging from 2-20, 5-24, 8-24, and 8-29 Mg ha^{-1} for the control, compost, AS100, and AS200 treatments, respectively from 2012 to 2021 (Figure 2.1). These results demonstrate that the yield of corn silage is influenced by the growing season (Biswas & Ma, 2016), leading to inconsistency in determining the effect of fertilizers on yield. To address this, repeated measures analysis was employed to examine the effects of fertilizers on yield. This approach enables the detection of treatment differences in datasets collected over multiple years in agronomic field trials (Pagliari et al., 2022).

The impact of N fertilizer treatments on yields was significant based on yield estimates from repeated measures analysis for 2012-2021. The response of corn silage yield to N fertilizer treatments followed this order: AS200 and AS100 yielded the highest, followed by compost, and then the control treatment. The estimated yields from repeated measure for control, compost, AS100, and AS200 were 7.9, 11.1, 14.9, and 17.2 Mg ha^{-1} , respectively (Figure 2.3). While the compost treatment significantly increased yield by 3.21 Mg ha^{-1} (40.5%) compared to the control treatment, it still yielded 3.74 Mg ha^{-1} (25.5%) and 6.12 Mg ha^{-1} (35.51%) less than the AS100 and AS200 treatments, respectively.

Nitrogen uptake

The findings of the current study indicate that the uptake of N from the compost treatment was significantly higher than that of the control treatment only in the year 2014 (Figure 2.2). Nitrogen uptake under compost treatment tended to be lower than that of ammonium sulfate treatment; however, the significant difference was only detected in 2013. Nitrogen uptake under AS200 was significantly higher than that under AS100, with the exception of 2013, 2018, and 2021. This inconsistent response across years makes it challenging to determine the true effect of fertilizer treatments on N uptake (Figure 2.2).

The results obtained from repeated measures analysis revealed that the average estimates of N uptake by corn silage were 50.1, 80.1, 114.9, and 177.2 kg of N ha^{-1} for the control, compost, AS100, and AS200 treatments, respectively (Figure 2.3). Our finding agreed with previous studies that have stated that N uptake increases with higher rates of fertilizer (Amado et al., 2013; Biswas & Ma, 2016; Davies et al., 2020). However, a higher amount of N uptake may not necessarily result in an increase in biomass production (Anas et al., 2020).

Corn leaf N content

From 2012 to 2021, N concentration in ear leaves collected at 80 days revealed that the control treatment had a concentration of 1.37%, while compost, AS100, and AS200 treatments had concentrations of 1.53%, 1.81%, and 2.36%, respectively (Figure 2.4). Compared to the control treatment, compost, AS100, and AS200 treatments increased leaf N concentrations by 11.7%, 32.1%, and 72.3%, respectively. AS200 treatment resulted in the highest N concentration in leaves, followed by AS100, while the

N concentration in leaves under the control treatment was not significantly different from that of the compost treatment (Figure 2.4).

The result from this study showed that source of fertilizer significantly affected N concentration of 80-day leaves which agrees with the other study (Ziadi et al., 2009). The observed N concentrations in the corn ear leaves of this study were found to be inadequate when compared to the recommendations from the University of Wisconsin study. For instance, we observed the N content in the leaves under the AS200 treatment appears to be on the borderline of being sufficient, despite receiving a significant amount of N fertilizer. According to research from the University of Wisconsin, the N concentration in the ear leaves should fall within the range of 2.5% to 3.33% to meet the criteria for sufficiency (University of Wisconsin, 2016). Many factors can affect the N concentration in leaves. Those factors are the developmental stage of the leaves, changes in the proportion of structural leaf tissue as newer leaves attain larger sizes, and the gradual shading caused by more recently emerged leaves (Lemaire & Gastal, 1997; Ziadi et al., 2009). Besides the factors mentioned previously, other variables such as location, species, time and management practices, and climatic conditions can also impact the N levels in the leaf (Schulte & Kelling, 1914; University of Wisconsin, 2016). Therefore, it can be challenging to compare the N concentrations observed in our study with those of other investigations.

Nitrogen use efficiency (NUE) indicators responses

Uptake efficiency (UE) is a measure of the proportion of nitrogen applied that is taken up by the entire crop (Augarten et al., 2019). The response of UE to different treatments varied from year to year. For instance, in 2012, the UE under the compost treatment was negative because the dry matter yield was lower than that of the control treatment (Table A1 and Figure 2.1). In 2013, the UE under the compost and AS200 treatments were significantly lower than AS100. In 2014, 2016, 2019, and 2020, the UE under the compost treatment was significantly lower than UE under the AS100 and AS200 treatments. However, in 2018, differences in UE among the treatments could not be detected (Table A1).

The result from repeated measure estimated from 2012 to 2019 showed that estimate of value of UE from compost, AS100, and AS200 treatments were 13.4%, 57.8%, and 56.7%, respectively (Figure 2.5 A). From this result, it indicated that AS100 and AS200 treatment performed better than compost in terms of UE response. However, the values of UE under the AS200 and AS100 treatments were not significantly different (Figure 2.5 A).

According to the NUE benchmarking for corn silage, an uptake efficiency (UE) value higher than 50% is considered a high uptake efficiency (Augarten et al., 2019). In our study, the AS100 and AS200 treatments showed high UE values, while the compost treatment was classified as very inefficient (UE<30%).

Agronomic efficiency (AE)

Agronomic efficiency (AE) is commonly used to address the question of how much productivity is improved by application of a unit of N (Augarten et al., 2019; Černý et al., 2012). In 2013, the AE value under compost and AS200 treatments did not show a significant difference and was significantly lower than that observed under the AS100 treatment. However, in both 2014 and 2020, the AE showed a clear and significant response to N fertilizer, with the AS100 treatment producing the highest value, followed by the AS200 treatment and then the compost treatment. In 2021, the value of AE under AS100 and AS200 treatments were comparable and significantly higher than that observed in the compost treatment (refer to Table A1). From 2015 to 2019, the N fertilizer treatment did not have a significant impact on AE. These variations and inconsistencies in the AE response to N treatment suggest that seasonal conditions influence corn silage AE (Hlisnikovský et al., 2020).

Results from repeated measures for 2012 and 2021 show that the estimated mean of AE for AS100, AS200, and compost were 62.1, 41.7 and 14.4, respectively (Figure 2.5 B). This suggests that the higher application of the AS fertilizer resulted in a decrease in AE (Amado et al., 2013; Boulelouah et al., 2022). The decrease in AE under compost reflects the lack of available N in this organic material or the slow release of available N from compost fertilizer.

Partial factor productivity (PFP)

Partial factor productivity (PFP) is commonly used to assess the productivity of a cropping system in relation to its N application (Augarten et al., 2019). In this study, the numerical value of PFP was highest for the AS100 treatment (Table A1). However, at a significance level of $p \leq 0.05$, the PFP values showed inconsistency across growing seasons. Specifically, the PFP value of AS100 was the highest in all growing seasons except for 2019, when the PFP values for AS100 and AS200 were not significantly different. The PFP values for compost and AS200 were comparable from 2012 to 2019. In 2020 and 2021, the PFP values for AS100 were the highest, followed by AS200 and compost.

Repeated measures analysis demonstrated that the PFP values for AS100, AS200, and compost were 132.56, 76.9, and 49.6, respectively (Figure 2.5 C). According to corn silage benchmark efficiency ranges from the study of Augarten et al., (2019), the PFP value of AS100 was the highest and within the range of high efficiency (PFP > 108), while the PFP values under AS200 and compost were in the low efficiency range (PFP > 81). These findings agreed that the PFP decreased with an increasing rate of AS fertilizer (Amado et al., 2013; Chen et al., 2018).

Partial nutrient balance (PNB)

Partial nutrient balance (PNB) allows an estimate of how much N is taken up from the system compared to how much is applied (Augarten et al., 2019). Its value is interpreted based on whether the value is greater than or less than 1.0 (Augarten et al., 2019; Fixen et al., 2015). AS100 treatment produced high PNB values in 2013 and 2018 (Table A1). However, over the years, PNB under AS100 were insignificantly different from AS200 in 2012, 2014-2016 and 2019-2021. Compost treatment had the lowest PNB values, except for 2015 and 2016, which were not significantly different from those of AS100 and AS200 (Table A1).

Figure 2.5 D displays the PNB values obtained from repeated measures analysis, which indicates that AS100, AS200, and compost treatments resulted in PNB values of 1.03, 0.79, and 0.36, respectively. As per the classification proposed by Augarten et al. (2019), AS100, AS200, and compost treatments exhibited mid, low, and very low N use efficiency, respectively. While the AS200 treatment resulted in a higher PNB value than the compost treatment, this increase was not statistically significant when compared to the AS100 treatment.

Soil total nitrogen (STN)

The results from this study showed that the STN response to fertilizer treatments varied depending on the growing season (Figure 2.6). Compost treatment had the highest STN content in 2013, 2014, and 2021. Control treatment exhibited the lowest STN content in 2013 and 2014, and AS100 had the lowest STN in 2021. In the remaining years, N fertilization treatments did not significantly affect STN, although the STN levels under compost treatment were numerically than the other treatments. STN is generally not sensitive to management practices, and it may take time to observe the impact of management practices on changes in STN (Hurisso et al., 2018). Long-term studies are necessary to fully understand the impact of N fertilizer treatments on STN.

Based on the repeated measures analysis from 2012-2021, it was found that the STN content of 1.28 g kg^{-1} was highest under the compost treatment, which was significantly greater than under the control treatment (1.05 g kg^{-1}), AS100 (1.01 g kg^{-1}), and AS200 (1.06 g kg^{-1}) treatments (Figure 2.7). The STN under the control treatment was not significantly different from that under the AS100 and AS200 treatments. The results of this study showed a significant increase of about 0.24 g kg^{-1} (23.1%) in STN levels in soils treated with compost compared to other treatments (Figure 2.7). Earlier studies on the same plots investigating various aspects of the soil nitrogen cycle also showed that compost treatment enhanced the diversity of microbial communities and promoted N mineralization compared to AS fertilizer treatments (Ouyang, 2016; Ouyang & Norton, 2020). This study suggests that the long-term use of compost amendments increases STN, unlike the use of ammonium sulfate fertilizers.

Discussions

Contrasting N sources effects on yield of corn silage

In this study, the result showed that yield of corn silage was improved by application of compost and ammonium sulfate fertilizer (Figure 2.4). There was no significant difference in corn yield between the AS100 treatment, which received 112 kg of N ha^{-1}, and the AS200 treatment, which received 224 kg of N ha^{-1} (Figure 2.3). Several studies have suggested that applying fertilizer rates ranging from 0 to 101 kg of N ha^{-1} can increase corn yield, but this increase levels off at 101 kg of N ha^{-1} (Biswas & Ma, 2016; Hejazi & Soleymani, 2014; McSwiney & Robertson, 2005). For sustainable maize production on volcanic soil in Bea Cameroon, an N fertilization rate between 50 and 100 kg of N ha^{-1} is considered optimal (Ngosong et al., 2019). However, in the midwestern United States, optimizing N rates for maximum ecosystem value requires an N rate of about 156 kg of N ha^{-1} (Ewing & Runck, 2015).

The yield under compost treatment demonstrated a significant increase relative to the control, yet remained lower than the average yield observed under AS100 and AS200 (Figure 2.3). This finding was aligned with the previous studies (Chivenge et al., 2011; Seufert et al., 2012; Wei et al., 2016). An integrated analysis of long-term experiments conducted by Wei et al., (2016) indicated that despite the application of organic amendments over a decade, organic amendment still produced lower yield in comparison to chemical fertilizer. They effectiveness of organic amendments in increasing yield is contingent upon several factors, including the quality of organic resources, soil fertility status, farming system, management practices, and site characteristics (Chivenge et al., 2011; Seufert et al., 2012; Wei et al., 2016).

Available N is the major factor that affects crop yield (Berry et al., 2002). Numerous studies have substantiated those organic amendments, such as compost, animal manure, or cover crops, slowly release N that is available to plants, yet they do not provide an adequate N supply to meet the demands of crops during the peak of the growing season (Berry et al., 2002; Pang & Letey, 2000; Seufert et al., 2012). Therefore, a farming system that exclusively relied on organic amendment has the potential to substantially increase yield, as long as there is a substantial quantity of them accessible. Otherwise, this system may fail to generate enough yield to satisfy food demand (Chivenge et al., 2011; Seufert et al., 2012; Wei et al., 2016).

It is important to note that N rate is not the only factor affecting corn yield (Mangan et al., 2016). Other factors, such as rainfall, irrigation, soil texture and quality, farming management practices, planting date, and environmental conditions throughout the growing season, also significantly affect corn yield variability (Chivenge et al., 2011; Hlisnikovský et al., 2020; Seufert et al., 2012; Wei et al., 2016).

Contrasting N sources effects on nitrogen use efficiency (NUE) indicators

In the study, the AS100 treatment showed the highest PNB value, slightly exceeding 1. This increase in PNB above 1, as observed in Augarten et al.'s research (2019), indicates potential soil organic matter mining, where more N is removed in the crop than applied. However, it's noteworthy that the PNB value for AS100 remains within the acceptable range of high low-to-mid use efficiency ($0.92 < PNB < 1.08$). In contrast, the PNB value for AS200 treatment ($PNB=0.79$) falls within the range of low use efficiency, indicating that more N is being applied than removed by the crop (Augarten et al., 2019). A PNB value less than 1 signifies N surplus and can lead to potential nitrogen losses such as volatilization and leaching (Andrews et al., 2018; Fageria & Baligar, 2005). Therefore, reductions in N application may be necessary. The compost treatment had an extremely low PNB value (PNB=0.38) indicating that a considerable amount of N was being retained in the soil but unavailable for plant uptake due to slow N mineralization or even immobilization (Andrews et al., 2018; Fageria & Baligar, 2005).

Yield and nitrogen use efficiency under compost treatments

Our study revealed that the compost treatment resulted in a higher yield than the control treatment, although it was still lower than the ammonium sulfate treatments. This contrasts with the findings of Lin et al. (2022), who observed that the yield of corn under the compost treatment was comparatively close to that of the control treatment (Lin et al., 2022). The duration of the experiment can affect the accuracy of the results, and in this regard, the study conducted by Lin et al. (2022) spanned only two growing seasons. In contrast, our study continued for nine years (2012-2021), providing more comprehensive data to evaluate the impact of different fertilizers on crop yield. The limited duration of Lin et al.’s (2022) experiment may have contributed to the absence of significant differences in yield between the organic fertilizer and control treatments reported in their study. It is well-known that the yield of corn can be influenced by the growing season (Biswas & Ma, 2016), and the response to nitrogen fertilizer treatments can also vary from year to year. These factors could explain why Lin et al. (2022)'s results differ from ours and highlight the importance of conducting long-term experiments to account for variability in crop growth and nutrient uptake over time.

In the current study, the compost treatment did not result in an improvement in nitrogen use efficiency (NUE) compared to the ammonium sulfate treatment. This observation is consistent with the findings of Lin et al. (2012), who reported lower NUE of corn under organic fertilizers compared to chemical fertilizers. Despite using the same quantity of STN in the compost and AS200 treatments, not all of the STN in the compost was readily available for plant uptake, which explains the lack of improvement in NUE and lower yield. Compost is considered a slow-release fertilizer that gradually releases plant-available nutrients over time. Sullivan et al. (2018) reported that within the first year of application, plant-available nitrogen (N) released from compost was less than 10% of its total N content (Sullivan et al., 2018). Other nutrients in compost may become available over years, although at a slower rate (Mangan et al., 2013). However, insufficient N supply from compost can lead to a decrease in crop yield, N uptake, and NUE.

Contrasting N sources effects on soil total N (STN)

Maintaining optimal STN levels is critical for preserving soil quality, enhancing crop productivity, and ensuring environmental sustainability (Al-Kaisi et al., 2005; Li et al., 2022). A decrease in STN can adversely affect soil fertility, nutrient availability, and overall productivity (Gray & Morant, 2003). Thus, the long-term application of organic fertilizers can improve nutrient use efficiency by enhancing soil fertility and organic matter, which can lead to higher crop yields (Hua et al., 2020).

The application of compost significantly increased soil nitrogen, but the yield, nitrogen uptake, and nitrogen use efficiency were lower compared to the use of ammonium sulfate. Similarly, research conducted by Gao (2020), demonstrated that compost fertilizer enhanced STN levels while commercial fertilizer did not (Gao et al., 2022). This finding is in line with Steiner et al. (2007), which found that organic fertilizers improve soil fertility but do not sustain crop productivity (Steiner et al., 2007). Numerous studies have shown that incorporating both organic and inorganic fertilizers increases yield, STN, and NUE (Ding et al., 2018; Gao et al., 2022; Li et al., 2012). Therefore, farmers may want to consider both methods for silage corn production.

The results of this study indicate that both fertilizer treatment and seasonal weather conditions have a significant impact on the STN, yield, and nitrogen use efficiency (NUE) of corn silage. This is consistent with previous research reported in previous studies (Baker & Capel, 2011; Biswas & Ma, 2016; Hlisnikovský et al., 2020). STN is generally not sensitive to management practices, and it may take time to observe the impact of management practices on changes in STN (Hurisso et al., 2018). Therefore, conducting long-term field trials is crucial and urgently needed for fertilization trials, as it can be used to verify whether proper practical application is conducive to sustainable agricultural development (Körschens, 2006).

Conclusions

The response of yield, nitrogen use efficiency (NUE), and total soil nitrogen (STN) to N fertilizer treatments was inconsistent in this study. Therefore, conducting field experiments over a long period of time is crucial to assess the effects of fertilization on crop yield and NUE. Nitrogen uptake in response to fertilization followed this order: AS200 > AS100 > compost > control. Yield and uptake efficiency were highest with AS100 and AS200 treatments, followed by compost and then the control. The yield under the compost treatment was 40.75% higher than the control. The order of agronomic efficiency (AE) and PFP response to nitrogen fertilizer treatment was AS100, AS200, and compost treatment. The PNB levels under the AS200 treatment were in the low-efficiency range (PNB > 1), indicating an excess of nitrogen, which could result in nitrogen loss through volatilization or leaching.

Compost fertilizer was applied in this experiment at a rate of 224 kg of total nitrogen per hectare, equivalent to the nitrogen content in AS200. However, not all of the total nitrogen is readily available for plant uptake, which can lead to reduced crop yields and lower NUE. Consequently, the yield and NUE were low under the compost treatment compared to the ammonium sulfate treatments. Although the compost treatment was the least efficient in terms of yield, uptake, and NUE, it improved STN by 23.1% compared to other treatments, which plays a crucial role in the sustainability of soil quality, crop production, and environmental quality. In contrast, the ammonium sulfate treatment had a higher yield and nitrogen use efficiency, but it had no effect on STN.

In summary, the AS100 treatment produce the yield that were not significantly different from those of AS200. However, it outperformed the AS200 treatment in terms of NUE. Farmers may consider that increasing nitrogen fertilizer rates may not guarantee maximum yield and profit. The compost treatment did not provide enough nitrogen supply to meet crop demand, resulting in lower yield and NUE, but it had the advantage of improving STN. Therefore, to maintain soil health, farmers might consider mixing compost with commercial fertilizers and practicing good soil health practices such as crop rotation or cover crops.

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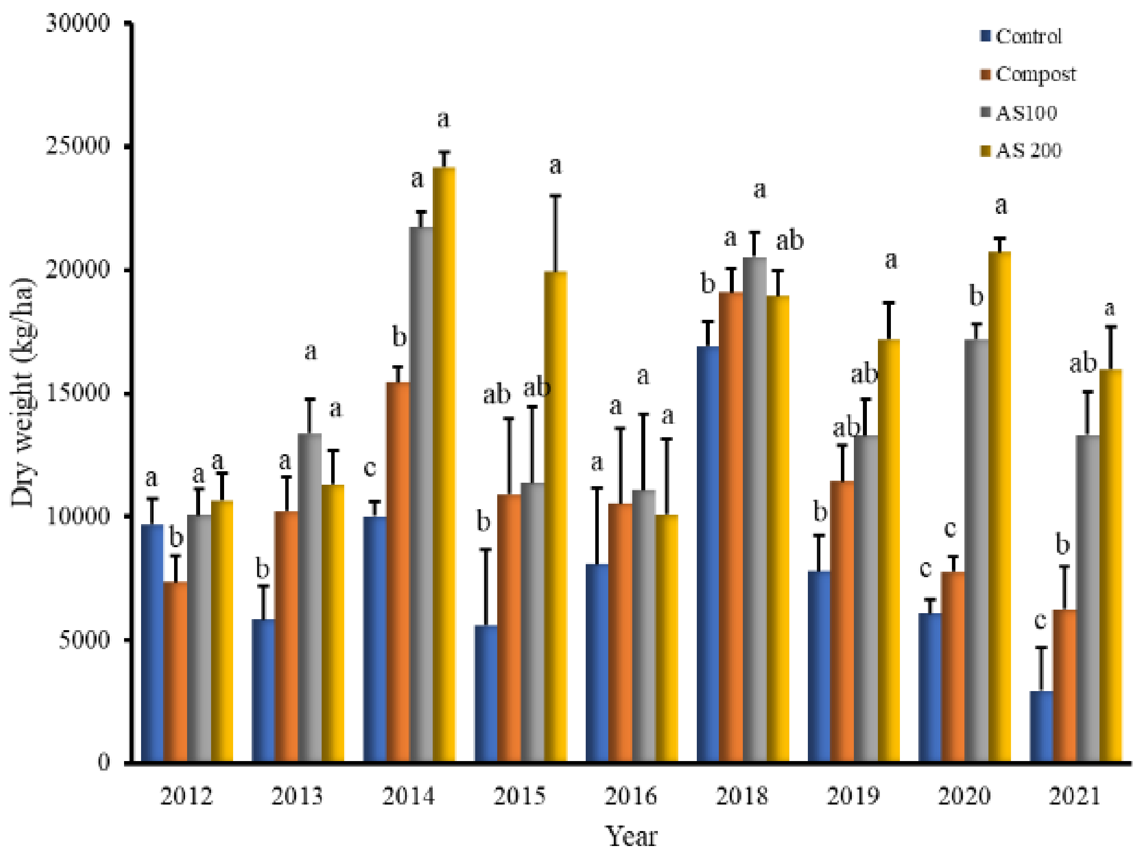
Figures and tables

Figure 2. 1.

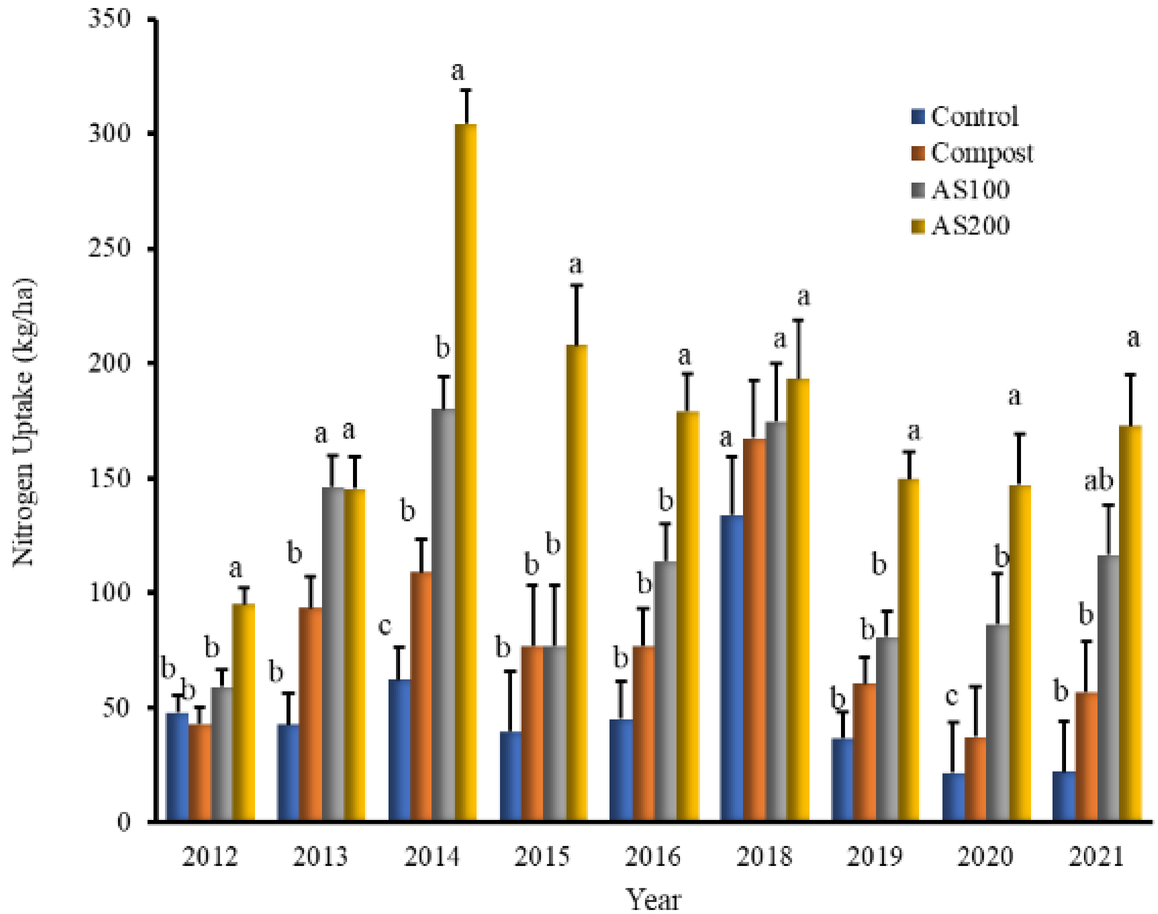
Yield of corn silage at harvest during various times of harvesting



Note. Yield of corn silage was anlayzed from years 2012 to 2021. Error bars represent standard errors (n = 4). Different lowercases above the bars indicate a significant difference within each year (p ≤0.05).

Figure 2. 2.

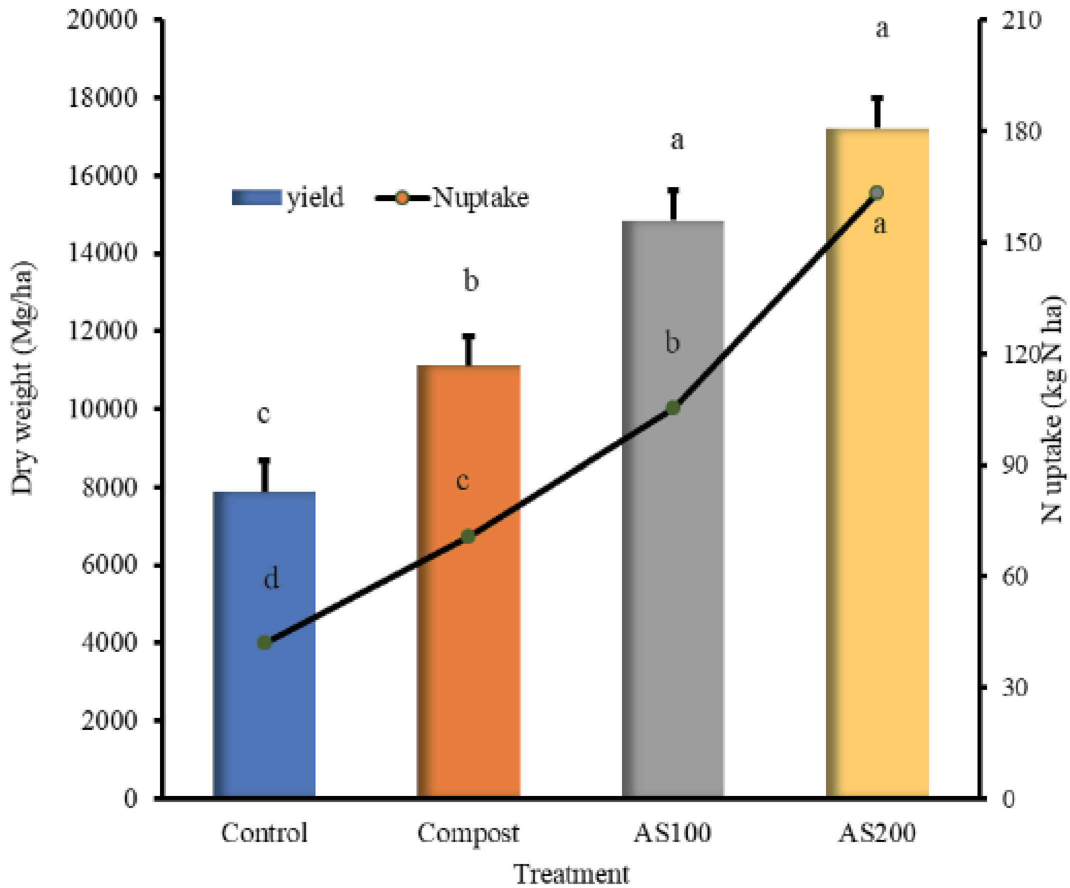
Nitrogen uptake of silage corn aboveground at harvest from 2012 to 2021



Note. Nitrogen uptake of silage corn aboveground at harvest was anlayzed from year 2012s to 2021. Error bars represent standard errors (n = 4). Different lowercases above the bars indicate a significant difference within each year ($p \leq 0.05$).

Figure 2. 3.

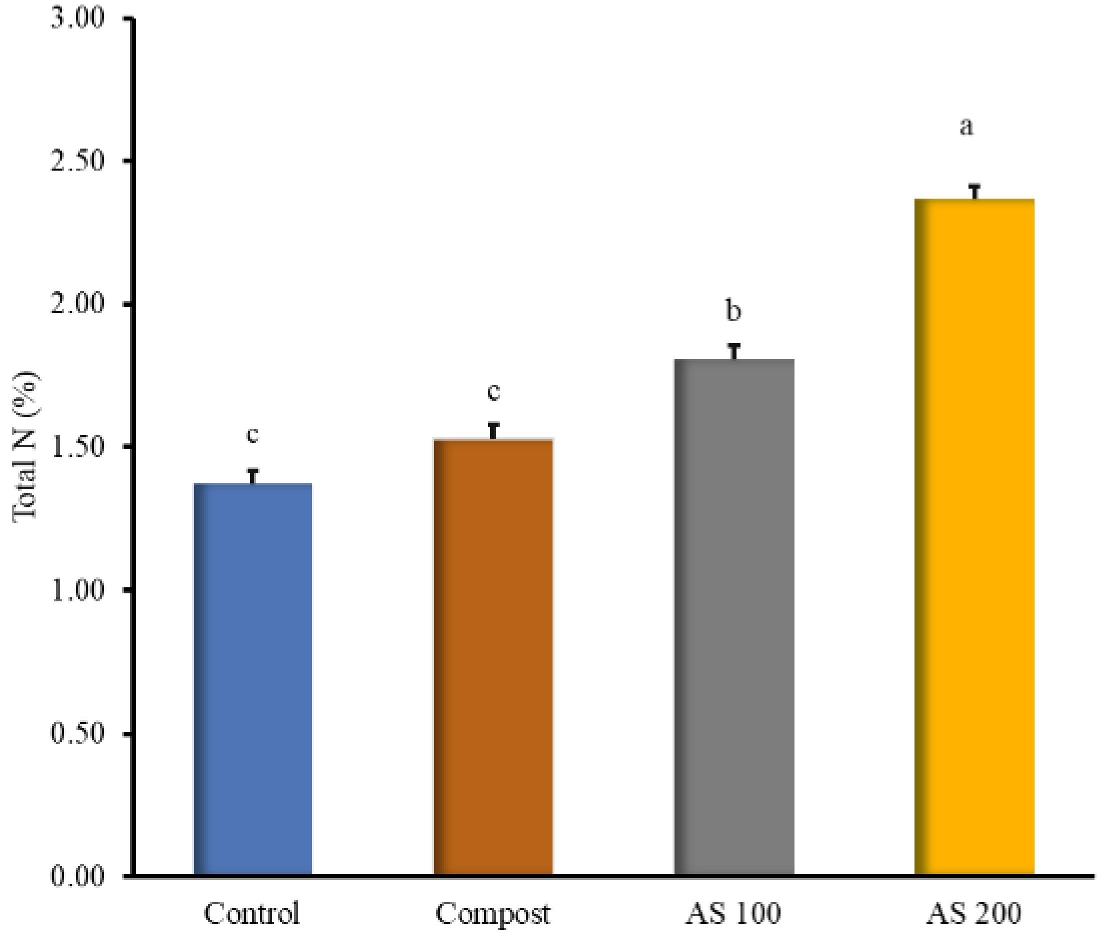
Effects of contrasting nitrogen sources on yield and N uptake in silage corn



Note. Repeated measures were employed to analysis the impacts of effects of contrasting N sources yield and N uptake in silage corn calculated from 2012-2021. Error bars represent standard errors (n = 36). Different lowercases above the bars indicate a significant difference by treatment (p ≤0.05).

Figure 2. 4.

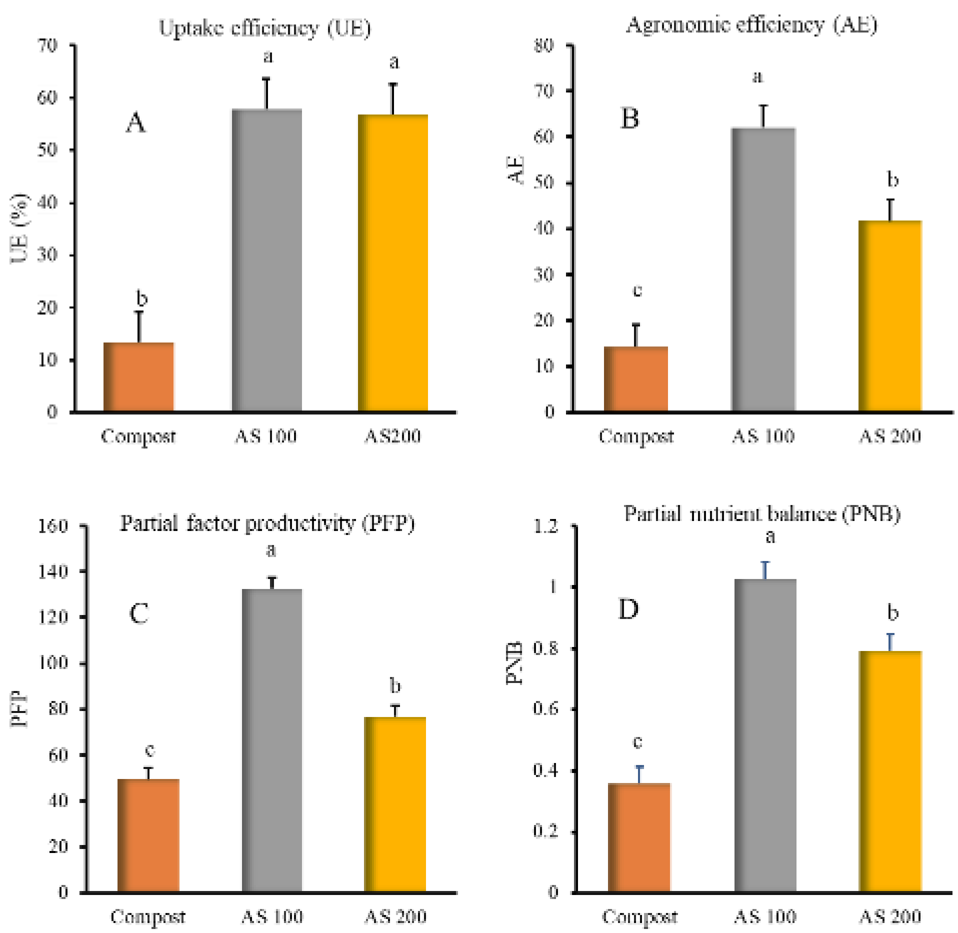
Effects of contrasting N sources on total N content of the 80-day corn ear leaf in silage corn



Note. Repeated measures were employed to analysis the effects of contrasting N sources on total N content of the 80-day corn ear leaf in silage corn, calculated from 2012 to 2021. Error bars represent standard (n = 36). Different lowercases above the bars indicate a significant difference by treatment ($p \leq 0.05$).

Figure 2. 5.

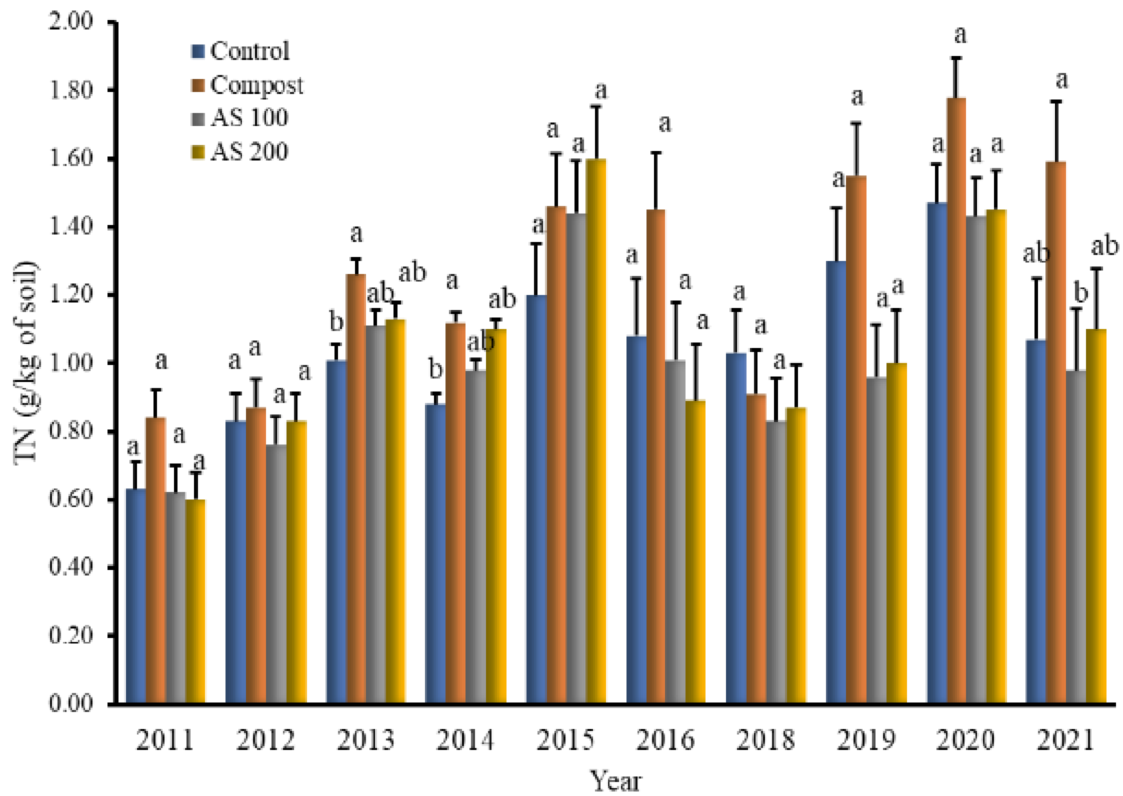
Effect of contrasting N sources on nitrogen use efficiency (NUE) indicators of corn silage



Note. Repeated measures were employed to analysis the effects of contrasting N sources on NUE indicators: A) Uptake efficiency (UE), B) Agronomic efficiency (AE), C) Partial factor productivity (PFP), and D) Partial nutrient balance (PNB). The NUE data were collected from 2012-2021. Error bars represent standard errors (n = 27). Different lowercases above the bars indicate a significant difference ($p \leq 0.05$).

Figure 2. 6.

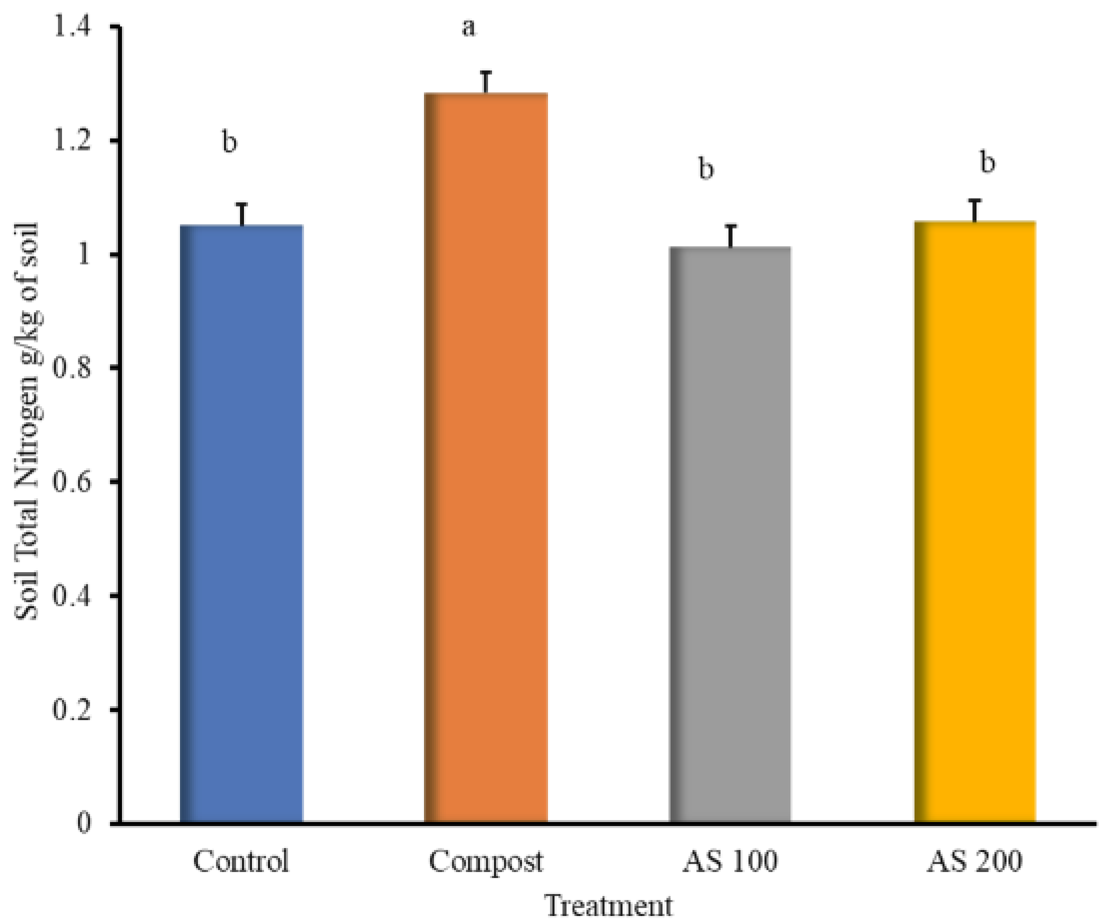
Soil total nitrogen (STN) content



Note. STN analysis was conducted on soil samples collected in August, at a depth of 0-15 cm, from a corn silage field spanning the years 2011 to 2021. Error bars represent standard errors (n = 4). Different lowercases above the bars indicate a significant difference within each year (p ≤0.05).

Figure 2. 7.

Effects of Contrasting Nitrogen Sources on Soil Total Nitrogen (STN)



Note. Repeated measures were employed to analysis the impact of contrasting N sources on STN. Soil samples were collected in August at a depth of 0-15 cm from 2011 to 2021. Error bars represent standard errors (n = 40). Different lowercases above the bars indicate a significant difference among treatments (p ≤0.05).

Table 2. 1.

Nitrogen use efficiency metrics

Trait	Description	Equation	Unit
UE	Uptake efficiency	($Nuptake_N$ – Nuptake0) / FN	%
AE	Agronomic efficiency	(YN-Y0) / FN	
PFP	Partial factor productivity	Y / FN	
PNB	Partial nutrient balance	Nuptake / FN	

NUptake = the total N uptake in aboveground biomass from N fertilizer treatment; NUptake0 = the total N uptake in aboveground biomass in plot that received no N fertilizer; YN = the yield of corn silage from the treatment, which received the N fertilizer; Y0 = the control treatment which received no N fertilizer; Y = the yield of crop; NUptake = total nitrogen uptake in above ground biomass with nutrient applied; FN is amount of fertilizer N applied.

CHAPTER III

EVALUATING SOIL NITROGEN INDICATORS FOR CORN SILAGE PRODUCTION WITH CONTRASTING NITROGEN SOURCES IN A SEMI-ARID ENVIRONMENT

Abstract

Agriculture faces the challenge of optimizing nitrogen (N) use for crop yield and environmental sustainability. A silage corn field study spanning from 2012 to 2021 in northern Utah, USA, evaluated the impacts of contrasting N sources on soil N indicators and their interrelationships in response to contrasting sources for N fertility. The experimental design was a randomized complete block design with four treatments: a control with no N fertilizer applied (control), low ammonium sulfate (AS100) at a rate of 112 kg N ha^{-1} $year^{-1}$, high ammonium sulfate (AS200) at a rate of 224 kg N ha^{-1} $year^{-1}$, and steer manure compost (compost) at 224 kg total N $ha^{-1}year^{-1}$. The results showed that compost increased soil total N, potential mineralizable N, autoclaved citrate-extractable protein (ACE protein), N-acetyl-β-D-glucosaminidase (NAGase) activity, and water-extractable organic N (WEON), as compared to other treatments. N indicators showed high levels in the topsoil (0-15cm) and decreased with soil depth. Pearson correlation analysis revealed a moderate to strong linear relationship among most N indicators, except for inorganic N. However, these relationships were influenced by seasonal variations and specific fertilizer treatments. The relationship between STN and associated N indicators is significantly and positively influenced only by the compost treatment. The result from this study highlights the importance of organic amendments, such as compost, in elevating soil N levels and enzyme activity. Expanding research across

diverse settings and larger scales is crucial for deepening our understanding of complex interactions between soil properties and management strategies.

Introduction

Healthy soil functions as a vibrant, living environment that provides numerous ecosystem benefits. These include maintaining water quality, supporting plant growth, regulating the recycling of soil nutrients and organic matter decomposition, and removing greenhouse gases from the atmosphere (Tahat et al., 2020). Soil health is intrinsically linked to sustainable agriculture, with soil microbial diversity and activity serving as the main component of soil health. Among the many factors influencing soil health, N plays a pivotal role due to its central role in plant nutrition and crop yield especially in the context of agricultural practices (Anas et al., 2020; Ribaudo et al., 2011). However, the excessive application of N fertilizers can have adverse effects on soil health and the environment, including soil acidification, nutrient imbalances, and the release of greenhouse gases (Singh, 2018). To mitigate these issues and promote sustainable agriculture, there is a growing need to assess the impact of different N sources on soil health through the evaluation of N related soil health indicators.

Within the context of soil health, there are several indicators that are employed to evaluate the organic N pools, N-related processes, or proxies for these pools and processes. The underlying goal of these indicators is to determine the soil capacity to continuously provide N, rather than solely focusing on a single moment's measurement of bioavailable N, as is the case with conventional soil fertility tests for inorganic N (Grandy et al., 2022; Liptzin et al., 2023). The objective, which is guided by soil health principles emphasizing the minimization of soil disturbance and the maximization of plant cover, is to retain N within both living and non-living biomass. This approach effectively increases the reservoir of organic N, which has the potential to be mineralized. As a result, various assessment methods have been proposed to offer valuable insights into a soil capacity for N cycling and its overall health (Liptzin et al., 2023). The indicators such as potentially mineralizable N, soil total N (STN), water-extractable organic N (WEON), autoclave citrate-extractable soil protein (ACE soil protein), N-acetyl-β-D-glucosaminidase activity (NAGase), and inorganic N (ammonium-N +nitrate-N) offer valuable information about the status and dynamics of N in soils (Cappellazzi & Morgan, 2021; Liptzin et al., 2023).

STN reflects the pool of soil N which encompasses both organic and inorganic forms (Cappellazzi & Morgan, 2021; Hurisso et al., 2018). This measure is a key indicator of soil fertility and overall quality within agricultural ecosystems (Al-Kaisi et al., 2005; Li et al., 2022). Furthermore, STN emerges as a significant macro-nutrient in soil health evaluation, directly influencing crop yield (Hossen et al., 2021). In addition, STN can serve as an indicator for assessing the quantity of N available for mineralization, particularly when considering broader spatial scales (Liptzin et al., 2023). A reduction in STN levels is associated with a decrease in soil fertility, nutrient availability, soil permeability, and overall soil productivity (Gray & Morant, 2003). Recognizing the distribution of STN and its intricate associations with other soil factors holds paramount significance for the sustainable management of land use. This knowledge provides a foundational basis for agricultural measurement and management (Gray & Morant, 2003; Lai et al., 2022; Sainju et al., 2022).

Nitrogen mineralization potential is a biological method to estimate the capacity of soil to provide available N from soil organic matter for crop production (USDA

NRCS, 2014). This method is based on laboratory incubation study of N released under optimum and constant environmental conditions. In 1972, Stanford & Smith proposed the concept of potential N mineralization, which estimates the potential future availability of soil N (Stanford & Smith, 1972). The amount of released N in soil during a specified period (7 to 210 days) is defined as soil N mineralization (Keeney, 1983). Potential N mineralization is derived by fitting measured inorganic N concentration into a first-order kinetic model (Stanford & Smith, 1972). Long-term aerobic incubation is considered an accurate method to predict the potential supply of N over a growing season. However, this method is expensive, time-consuming, and laborious, and is not suitable for high-throughput laboratories (Hurisso et al., 2018; USDA NRCS, 2019). Although this measurement is not typically included in widely adopted soil health assessments, it has been specifically selected by a panel of scientists involved in advising the North American Project to Evaluate Soil Health Measurements (NAPESH) project as a standardized means to estimate mineralizable N (Cappellazzi & Morgan, 2021).

The Soil Health Institute has identified soil protein as one of the most effective soil health indicators of bioavailable N. Proteins and related peptides represents the largest pool of organic N in the soil (Nannipieri & Eldor, 2009), which may mineralize and become availableto plants (Geisseler & Horwath, 2009; Hurisso et al., 2018). Wright & Upadhyaya, (1999) developed a procedure for extracting soil proteins that relies on a neutral sodium citrate buffer solution, which is the commonly employed method for extracting "glomalin," a protein that is believed to be produced in significant amounts by arbuscular mycorrhizal fungi. Until this point, glomalin-related soil protein (GRSP) has been defined primarily by the methods used for its extraction and the assays employed for its detection. A fundamental assumption underlying this definition is that the extraction process eliminates the majority of soil proteins that are not heat-stable, leaving behind only glomalin (Rillig et al., 2013; Wright & Upadhyaya, 1999). However, this assumption has been disproven (Hurisso et al., 2018). According to their research, they demonstrated that this extraction method can be applied to a range of proteins, not limited to glomalin protein alone (Hurisso et al., 2018; Purin & Rillig, 2007; Rillig et al., 2013; Rosier et al., 2006; Schindler et al., 2007).

In their 2018 study, Hurisso et al. recommend discontinuing the use of terms such as 'glomalin,' 'easily extractable glomalin (EEG),' and 'glomalin-related soil protein (GRSP).' Instead, they propose adopting more comprehensive terms such as 'soil protein' or 'autoclaved citrate-extractable protein (ACE protein) (Hurisso et al., 2018). The reason for this suggestion is that the earlier terminology is inappropriate and can be misleading. Instead of considering the proteins extracted through this method as solely representative of glomalin, they should be viewed as indicative of a broader pool of soil proteins. This broader pool of proteins has the potential to indicate the primary pool of organically bound N in the soil, which in turn can be considered as potentially available organic N and a reflection of overall soil health (Hurisso et al., 2018).

ACE soil protein stands as a significant soil health indicator, quantifying the presence of protein-like substances within soil organic materials. This measurement provides valuable insights into the potential availability of organic N for plants and microorganisms, underlining its importance in assessing soil fertility and ecosystem vitality (Moebius-Clune et al., 2016; USDA NRCS, 2019; van Es et al., 2020). Moreover, ACE soil protein is sensitive to management practices such as tillage and crop production

(Borie et al., 2006; Emran et al., 2012; Hurisso et al., 2018; Liebig et al., 2006; Moebius-Clune et al., 2017; Nichols & Millar, 2013; Wright et al., 2007). In addition, ACE soil protein correlates strongly with corn yield, carbon mineralization, total organic matter, and aggregate stability (Roper et al., 2017; Wright & Upadhyaya, 1996, 1999). Recognizing its importance, the Cornell Soil Health Laboratory includes ACE soil protein analysis as one of their comprehensive assessments of soil health (CASH), further emphasizing its role as a biological indicator (Fine et al., 2017; Moebius-Clune et al., 2016).

N-acetyl-β-D-glucosaminidase (NAGase, EC 3.2.1.30) is one of the enzymes that plays an important role in N assessment (Cappellazzi & Morgan, 2021; Ekenler & Tabatabai, 2004). NAGase measurement represent the potential enzyme activity within the soil responsible for catalyzing the final step in the degradation of chitin (Ekenler & Tabatabai, 2004). Chitin, as a significant structural component, is abundantly found in the cell walls of fungi and the exoskeletons of arthropods, making it a prominent nitrogenous molecule in soil ecosystems (Ekenler & Tabatabai, 2004; Parham & Deng, 2000).

Ekenler and Tabatabai (2004) found that NAGase levels are sensitive to various management practices and cropping systems and demonstrates significant correlations with key soil parameters such as organic carbon, STN, inorganic N, microbial biomass carbon, and N mineralization (Ekenler & Tabatabai, 2004). Recognizing its importance, the NAGase enzyme is among the four enzyme measurements recommended by the USDA-NRCS Soil Health Division for monitoring soil health (USDA NRCS, 2019).

Water extractable organic nitrogen (WEON) represents the pool of organic N that is available to soil microbes and easily broken down by microbes into the form that plants can use (Bellows et al., 2020; Haney et al., 2012, 2018). It is a key indicator of soil water extractable organic matter WEOM (He et al., 2017). WEOM represents a primary source of carbon and energy for soil microorganisms, playing a central role in governing the carbon cycle and related nutrient cycles (Wang et al., 2021).

WEON is also sensitive to management practices (Chantigny, 2003; He et al., 2017). This heightened responsiveness to soil management which establishes it as a valuable indicator for evaluating the impact of agricultural and soil management practices on soil health and fertility. Furthermore, WEON demonstrates meaningful correlations with other essential soil health indicators, including soil carbon levels, potential mineralizable N, STN content, and the activity of NAGase enzyme (Cappellazzi & Morgan, 2021; Das et al., 2023; Geisseler et al., 2019; Hurisso et al., 2018; Liptzin et al., 2023).

WEON is a key component of soil organic matter that serves as an indicator of soil health and nutrient availability. Its responsiveness to management practices and interactions with other soil indicators make it a valuable tool for assessing and improving soil fertility and overall ecosystem health. Consequently, it has been used as a N indicator for soil health in many studies (Cappellazzi & Morgan, 2021; Chu et al., 2019; Das et al., 2023; Liptzin et al., 2023).

Limited studies have utilized these measurements to assess soil N cycling within the context of corn silage systems in semi-arid environments like Utah. Various factors, including soil type, climate, farming practices, and different agricultural settings, may influence these indicators and their interrelationship. Comprehending the intricate relationships among these N indicators may yield insights into crucial N cycling processes. It can also shed light on how different N sources, including organic and inorganic fertilizers, impact soil N dynamics. This knowledge is pivotal for crafting sustainable N management strategies that strike a balance between maximizing crop productivity and ensuring environmental stewardship. Such strategies are crucial for maintaining agricultural sustainability in regions characterized by semi-arid conditions like Utah.

The aim of this study is to assess the effects of different N sources on soil N indicators and their interactions during corn silage production across multiple seasons. Our first objective was to analyze the impact of contrasting N fertilizers on a suite of soil N indicators. The second objective is to explore the relationships among these soil N indicators and assess their responses within a corn silage system under different N sources across multiple growing seasons.

This research provides valuable insights into the sustainable management of N in agriculture. It helps bridge the gap between soil health, N indicators, and agricultural practices, facilitating more informed and environmentally responsible approaches to N use in farming.

Materials and Methods

Site description and experimental design

The site was located at the USU Greenville Research Farm (41°45'56.6"N 111°48'52.2"W), in North Logan, Utah. The soil is a highly calcareous Millville silt loam (coarse-silty, carbonatic, mesic Typic Haploxeroll) with a pH of 8.2 (1:2 soil: water). The plots were established in 2011 to investigate N cycling and different N transformations under contrasting N management, as outlined in previous (Kakkar, 2017; Ouyang, 2016). Before, the field was utilized for conventional cultivation of small grains, involving an annual application of 70 kg of N per hectare in the form of urea. The experimental design in this study was a randomized complete block design (RCBD) with four N treatments and four replications, totaling 16 plots (Figure A1). The treatments include a no N control (control), low ammonium sulfate at 112 kg N $ha^{-1}year^{-1}$ (AS100), high ammonium sulfate at 224 kg N $ha^{-1}year^{-1}$, (AS200), and steer manure compost at 224 total kg N $ha^{-1}year^{-1}$ (compost). Commercially sourced compost in this study was composed of composted steer manure, slaughter by-products, and woodchips (Miller companies LLC, Hyrum Utah). Compost nitrogen and dry matter content were determined yearly (Table B.1), and these parameters were used to apply the desired total nitrogen rate of 224 kg TN $ha^{-1}year^{-1}$, equivalent to 14.4 ± 1.8 metric ton of dried weight compost $ha^{-1}year^{-1}$. Each plot measured 9.1 m in length and 3.8 m in width, with a 1.2 m alley between each block and a 4.6 m alley between each plot within a block. Silage corn has been planted every May from 2012 through 2021 with the exception of 2017 when a cover crop of vetch was grown.

Field experiments

During early spring of each year pre-plant soil samples were collected from each plot using a Giddings probe with two cores per plot at depths of 0-15 cm, 15-30 cm and 30-60 cm. Soil was weighed, sieved (2 mm) and air-dried before analysis for available P and K. To meet the crop requirement of P and K, fertilization for P and K in each plot was carried out according to the recommendations outlined in the Utah Fertilizer Guide for silage corn (James & Topper, 1993). The fertilizer applications and compost amendments took place in early May of each year. The fertilizer applications and compost amendments occurred in early May each year. N, P, K fertilizers were applied to the field using an edge guard mini push broadcast spreader . For compost treatment, the amendment was applied manually by shovel. Subsequently, bow rakes were utilized to evenly distribute the fertilizers and compost amendments within individual plots. Following this, the amendments were incorporated into the soil through tillage within one day of application.

After the amendments were added and incorporated, the seedbed was prepared, and seed DEKALB® Corn Hybrids (glyphosate tolerant) were planted with a row spacing of 76 cm. Within each block, approximately 4 rows of silage corn were planted at a density of 50,000 plants per hectare using a John Deere planter. Throughout the growing season, an overhead sprinkler irrigation system was used to apply water on a weekly basis as required and as available. To control weed growth, Killzall herbicide, containing 41% glyphosate and diluted to a concentration of 18.7 g L^{-1} with water, was applied at a rate of 1.12 kg ha^{-1}. This application was done once via broadcast before the corn reached a height of 30 inches.

Soil analysis

For this study soil sampling was conducted over multiple seasons from 2019 to 2021. In May, soil samples were taken at depths of 0-15 cm, 15-30 cm, and 30-60 cm. In August, samples were collected at depths of 0-15 cm and 0-30 cm, while in November, samples were collected at depths of 0-15 cm, 15-30 cm, 30-60 cm, and 60-90 cm.

Sampling methods varied: in May and November, two soil cores were collected using a truck-mounted soil sampler from the middle of each plot (Giddings Machine Co.

Windsor CO, USA). In August, soil sampling was conducted using stainless steel soil sampler probes and a slide hammer soil probe. During August sampling, two soil cores were collected from each plot, with one taken from the middle of the corn plants and the other from between the rows of corn.

Upon collection, the soil samples were promptly transported to the laboratory. There, soil gravimetric moisture content was determined, and soils extracted with 2 M KCl solution to determine inorganic N. The resulting extracts were preserved at -20°C until analysis for ammonium-N and nitrite-N +nitrate-N, using microplate colorimetric methods adopted from previous studies (Doane & Horwáth, 2003; Verdouw et al., 1978).

Subsequently, the remaining soil was meticulously mixed and passed through a 2mm sieve before undergoing air-drying. After air-drying, a portion of the soil sample was finely ground to pass through a 0.25 mm (60 mesh) sieve for total N analysis by combustion (Bremner, 2018), using the PrimacsSN for total N (Skalar, Inc. GA, USA).

Potential Soil Nitrogen Mineralization by Long-Term Incubation

The assessment of potential N mineralization involved an 84-day laboratory incubation. The laboratory incubations were done on field replicated soils and reflect the cumulative effects of repeated treatments of the soil over nine years. The soil samples were obtained from 0-15 cm depth in August 2019. Two large soil cores were taken from each of 16 plots (0-15 cm depth), one in the corn plant row and the other from between rows.

The two soil cores from each plot were composited and thoroughly mixed, sieved to 2mm, adjusted to 18% moisture content, and subsamples weighing 10 g (o.d. equivalent) were placed into plastic cups for incubation. Each individual plot was represented by duplicate samples (from the 16 plots 4 blocks with 4 treatments). Samples were taken at 0, 7, 14, 21, 35, 49, 70, and 84 days. Sample Day 0 subsamples were extracted immediately with 50 ml 2M KCl to determine the amount of inorganic N present at the start of incubation (Day 0). The additional cups were placed inside quart mason jars, and one ml of deionized water was added to the bottom to avoid loss of soil moisture. These jars were then sealed using jar lids and rings. The lids for day 84 sampling were provided with septa for gas sampling. Time was noted for the start of incubation and all the jars were incubated in the dark at 25^0C. Headspace gas samples were taken at each timepoint from the Day 84 jars and then all jars were aerated and additional water added to the jars as needed. Each soil subsample was extracted with 50 ml of 2M KCl. The filtrate was collected and frozen at -40^0C for subsequent inorganic N analysis. The analysis of ammonium, and nitrate-N+ nitrite-N, levels were performed using a flow injection analyzer, (QuikChem 8500, methods 12-107-06-1-A and 12-107-04-1-J (Lachat Instrument, Loveland, CO). Gas samples were analyzed for $CO_{2\ by}$ gas chromatography with a thermal conductivity detector with helium carrier gas and 20 mL min^{-1} flow rate as described previously (Ouyang 2016) .

Net nitrogen mineralization =

$(NH_4^+ - N + NO_3^- - N)$ at day 84 - $(NH_4^+ - N + NO_3^- - N)$ at Day 0 of incubation (Hart et al., 1994). Net N mineralized from the organic fertilizer or material was determined and fit to first-order kinetics to determine the rate of mineralization (k) and the pool of mineralizable N (N_0) (Stanford & Smith, 1972). The fit of mineralized N to a first order kinetic model was assessed.

First-order reaction kinetics: $\mathrm{Nt} = \mathrm{N_0}(1 - \mathrm{e}^{-\mathrm{kt}})$

- Nt= Nitrogen mineralized evolved at time t (mg kg^{-1}soil)
- $\mathrm{N_0}$= Potential mineralizable N (mg kg^{-1}soil)
- k= the rate constant (d^{-1})
- t= the incubation time in days

N-acetyl-β-D-glucosaminidase (NAGase, EC 3.2. 1.30)

The NAGase activity assay was adapted from previous studies (Parham & Deng, 2000). Air-dried soil (0.5 g) was mixed with 0.5 mL of 10.0 mM p-nitrophenyl-N-acetyl-β-D-glucosaminide substrate in a 100 mM acetate buffer at pH 5.5. The sample was incubated for 1 hour at 37°C. Absorbance at 400 nm was measured in microtiter plate cells using a SpectroMax® M2/M2e microplate spectrometer.

Autoclaved-citrate extractable protein (ACE protein)

Soil autoclaved-citrate extractable (ACE) protein was determined on air-dry samples in accordance with previously described protocols (Schindelbeck et al., 2016; USDA NRCS, 2019). The extraction procedure was based on the work of Wright and Upadhyaya (1999) with modifications as proposed by Hurisso et al., (2018). Air-dried soil samples, 2 g in weight, were mixed with 24 ml of 20mM sodium citrate solution adjusted to pH 7. The mixture was then subjected to autoclaving at 121°C for 30 minutes using the liquid cycle. After autoclaving, the solution was clarified by centrifugation at 10,000 × g for 3 minutes. A small quantity of the resulting supernatant was transferred to a 96-well cell culture plate and reacted with the bicinchoninic acid (BCA) protein reagent (Bioscience®). The absorbance of the reaction was measured at 562 nm using a SpectroMax® M2/M2e microplate spectrometer, with measurements conducted in microtiter plates.

Water extractable organic nitrogen (WEON)

The analysis of WEON followed established protocols outlined (Haney et al., 2012; Jones & Willett, 2006). In this procedure, soil samples were initially mixed with distilled water, using 6 grams of soil with 30 ml of DI water. Subsequently, the mixture underwent clarification via centrifugation at 6000 rpm (3,600 × g) for 8 minutes to isolate the dissolved organic N. Next, the extractant was filtered through a 0.45 µm PES filter into sterile tubes. Finally, water extractable nitrogen (WEN) was analyzed using a Shimadzu TOC-L/TNM-L operations as suggested by the manufacturer. Water-extractable organic N (WEON) was calculated by subtracting inorganic N content from WEN.

Data analysis

Statistical analysis was conducted using SAS OnDemand for Academics, which can be accessed at https://www.sas.com/en_us/software/on-demand-for-academics.html. The dataset comprised soil N indicator data from 2019 to 2021. Initially, a two-way ANOVA was employed to assess the impact of annual treatments on each soil N index (STN, ACE protein, WEON, Inorganic N, and NAGase). All statistical analyses were conducted with a confidence level of 95% ($P \leq 0.05$). Subsequently, repeated measures analysis was performed to evaluate the overall treatment effects on soil N indicators across the years 2019 to 2021. To investigate the strength of the relationship of soil N metrics, Pearson correlation was employed. This analysis was executed using PROC CORR to explore the degree of association among soil N indicators. Furthermore, a comprehensive statistical analysis was conducted to investigate the relationship between STN and several response variables, including ACE protein, NAGase, and WEON. To achieve this, the PROC MIXED procedure in SAS OnDemand was selected due to its suitability for modeling mixed-effects data structures, which were inherent in our dataset. This modeling approach was particularly relevant because the data spanned multiple years and encompassed the influence of various treatments, both of which could potentially impact the relationship between STN and the response variables.

Results

Soil total nitrogen (STN)

The results showed that the STN response to fertilizer treatments varied depending on the growing season in the current study (Figure 3.1). Notably, the compost treatment had the highest STN content in 2013, 2014, and 2021, whereas the control treatment exhibited the lowest STN content in 2013 and 2014, and AS100 had the lowest STN in 2021. In the remaining years, different N sources treatments did not significantly affect STN, although the compost treatment had numerically higher STN levels than the other treatments. Nevertheless, it is worth noticing that STN is generally not sensitive to management practices, and it may take several seasons to observe the impact of management practices on changes in STN (Hurisso et al., 2018). Long-term studies are necessary to fully understand the impact of N fertilizer treatments on STN.

Expanding on this comprehensive investigation, we collected soil samples at a depth of 0-15 cm during three pivotal time points: May (pre-planting), August (mid-growing season), and November (post-harvest) throughout the years 2019 to 2021. Our investigation revealed that the STN content exhibited notable temporal fluctuations during different phases of the growing season. Specifically, STN displayed significant responses to N treatments when soil sampling took place in August 2013, 2014, and 2021

(Figure 3.1). The estimated values for STN in May, August, and November were found to be 1.46 ± 0.06, 1.37 ± 0.07, and 1.16 ± 0.08 g kg^{-1} of soil, respectively, as illustrated in Figure 3.2.

Over a comprehensive period from 2012 to 2021, repeated measures analysis highlighted that the compost treatment yielded the highest STN content of 1.28 g kg^{-1} , significantly surpassing the control treatment (1.05 g kg^{-1}), AS100 (1.01 g kg^{-1}), and AS200 (1.06 g kg^{-1}) treatments (Figure 3.3). The STN under the control treatment was not significantly different from that under the AS100 and AS200 treatments. Notably, the control treatment did not significantly differ from the AS100 and AS200 treatments. These results underscore a substantial increase of approximately 23.1% in STN levels within compost-treated soils compared to other treatments (Figure 3.3).

Moreover, we assessed STN variations at different soil depths. STN at depth 0-15 cm, 15-30, 30-60 and 60-90cm were 1.15, 0.94, 0.67, and 0.45 ± 0.065 mg kg^{-1} of soil, respectively (Figure 3.4). The STN concentration was 18.26%, 41.74%, and 60.87% at depths of 15-30 cm, 30-60 cm, and 60-90 cm respectively, lower than the concentration at 0-15 cm depth. Previous studies have shown that STN increased in the topsoil and decreased with soil depth (Reddy et al., 2003; Toosi et al., 2012; Xu et al., 2020; Zhang et al., 2022). Our results also showed that contrasting N sources did not exert significant effects on STN below 15 cm depth.

Soil nitrogen mineralization

In this experiment, the cumulative N mineralization data for all treatments was fit to the first-order kinetic model : $\mathrm{Nt} = \mathrm{No}\left(1 - \mathrm{e}^{-\mathrm{kt}}\right)$. Overall, the cumulative N (Nt) increased with increasing time of incubation, consistent with previous studies (Cordovil et al., 2005; Fu et al., 2021; Yampracha, 2018) (Figure 3.5). The mineralization rate was intense at the beginning, followed by a slower rate after day 22, similar to previous studies (Cordovil et al., 2005; El Gharous et al., 1990; Stanford & Smith, 1972).

On day 14, net N mineralization values for compost treatment were the lowest with some samples still undergoing immobilization (Table 3.1). The net N mineralization at day 84 was 16.6, 17.7, 11.0, and 13.9 ± 2.02 mg kg^{-1} of soil for control, compost, AS100 and AS200 treatment, respectively (Table 3.1). There was no signficant impacts of N source treatment on net mineralization at day 84 (Table 3.1).

The rate constant of decomposition (k) in this incubation study was 0.0286 ±0.004 per day. Treatment did not have significant impacts on k. The potential mineralizable N (N_0) levels in the control, compost, AS100, and AS200 treatments were 19.5 ± 2.9, 33.4 ± 19.9, 18.1 ± 1.8, and 18.5 ± 1.7 mg kg^{-1} of soil, respectively (Table 3.2). The N_0 value in compost-amended soil was approximately 1.79 time higher than the values observed in the other treatments but with high standard error (Table 3.2). In summary, compost exhibited a trend of increasing the N_0 but had a lower k value. On the other hand, ammonium fertilizer application resulted in the highest k value but had no impact on N_0. Compost treated soils had the highest variability.

Autoclaved citrate-extractable soil protein (ACE soil protein)

In this study, we access the ACE soil protein content at a depth of 0-15 cm from 2019 to 2021. The average estimated amount of ACE was 5.39 ± 1.62 mg N $g^{-1}$of soil. This finding falls within the category of low soil protein levels as determined by a comprehensive assessment of soil health (Moebius-Clune et al., 2017).

Beyond this initial finding, we conducted a more in-depth investigation into how soil ACE protein content responded to various N treatments at different sampling times. The variations in ACE protein content were found to be contingent on the timing of soil sampling. The ACE protein content ranged from 3.18 to 11.9 ± 1.53mg N $g^{-1}$of soil (Figure 3.6) across the months of May, August, and November. Notably, our results indicated a gradual increase in ACE protein content over the course of the growing seasons which aligns with findings previously reported by Huang et al., (2022) (Figure 3.7).

The estimate means of ACE soil protein, obtained from repeated measurements, under control, compost, AS100 and AS200 treatment were 4.90, 6.76, 4.90, and 4.93 mg $g^{-1}$of soil (Figure 3.8). Notably, the ACE protein content was significantly higher in the compost treatment, showing an increase of approximately 36.7% compared to the average ACE protein content in the other treatments. Conversely, for both ammonium sulfate treatments, ACE protein levels did not significantly differ from those in the control treatment.

To delve deeper into the relationship between ACE protein content and STN within the soil, the soil samples collected from 2019 to 2021 at depth 0-15 cm were used to analyze this relationship. To calculate the ACE protein-N content, we used the conversion factor 6.25 under the assumption that protein contains 16% N, as proposed by Jones, (1941). The estimate STN and ACE protein-N were 1.35± 0.03 and 0.87 ± 0.26 mg N $g^{-1}$of soil, respectively (Table 3.3). As a result of the analysis, it was found that ACE protein-N content in the soil was 0.87 ± 0.26 mg N $g^{-1}$of soil, constituting 64% of the STN content, similar to findings from a previous study (Geisseler et al., 2019).

ACE protein content determined using soil samples collected in November 2019 at depths 0-15cm, 15-30cm, 30-60cm and 60-90 cm were 4.2, 2.79, 1.52, and 1.48 mg N g^{-1} of soil, respectively (Figure 3.9). We observed a significant decrease in ACE protein content in comparison to the 0-15 cm depth, with reductions of approximately 33.6% at 15-30 cm, 63.81% at 30-60 cm, and 64.8% at 60-90 cm.

Water extractable organic nitrogen (WEON)

In this study, we analyzed WEON in soil samples collected from 2019 to 2021 at a depth of 0-15 cm. The concentration of WEON ranged from 5.53 to 29.8 mg kg^{-1} of soil, with an average of 12.6 ± 4.20 mg kg^{-1} of soil.

The concentration of WEON exhibited high variability in response to N fertilizer sources, primarily due to the timing of soil sampling (Figure 3.10). In the year 2019, the WEON concentration was not significantly affected by treatments, despite observing the higher amount of WEON under the compost treatment. Subsequently, we noted significant increases in WEON under the compost treatment from 2020 until August 2021. However, in November 2021, no significant response of WEON to treatment was observed (Figure 3.10).

The estimated concentration of WEON before planting in May measured 11.6 ± 0.3 mg kg^{-1} of soil. During the growing season in August, it increased to 14.4 ± 0.3 mg kg^{-1} of soil and then decreased to 11.1 ± 0.3 mg kg^{-1} of soil in November (Figure 3.11). The concentration of WEON exhibited variability and was not significantly different by time in the growing season.

The study involved conducting repeated measurements on soil samples that were collected between 2019 and 2021 at a depth of 0-15 cm. The results of these measurements showed that different N sources treatments had a significant impact on the WEON concentration. The WEON amounts for the control, compost, AS100, and AS200 treatments were 11.9, 15.9, 12.7, and 12.2 ± 0.58 mg kg^{-1} of soil (Figure 3.12). The compost treatment increased WEON by approximately 29.6% compared to the average of other treatments.

The vertical distribution of WEON concentration across different soil depths was analyzed using soil samples from November 2019. The results showed that WEON at depths of 0-15, 15-30, 30-60, and 60-90 cm were 9.85, 6.53, 4.69, and 3.02 ± 0.2 mg $kg^{-1}$of soil respectively (Figure 3.13). The WEON content at depths of 15-30, 30-60, and 60-90 cm were less by approximately 33.7%, 54.4%, and 69.4%, respectively, compared to the WEON concentration at the 0-15 cm depth.

Inorganic nitrogen (ammonium + nitrate)

In this study, we examined the average estimate of inorganic N collected from 2019 to 2021 at a depth of 0-15 cm, which was found to be 1.63 ± 1.39 mg N kg^{-1}soil (Table 3.4). Notably, the concentration of nitrate (0.97 ± 1.29 mg N kg^{-1}soil) in the soil was higher than that of ammonium (0.73 ± 0.48 mg N kg^{-1}soil), consistent with previous studies (Kaboneka et al., 2008; Liang & MacKenzie, 2011; Ma et al., 1999; Ouyang et al., 2017). The estimated values of inorganic N in May, August, and November were 1.48 ± 0.14, 1.57 ± 0.14, and 1.83 ± 0.21 mg N kg^{-1}soil, respectively (Figure 3.14).

From 2019 to 2021, the repeated measure analysis at a depth of 0-15 cm demonstrated the amount of inorganic N under control, compost, AS100 and AS200 were 1.11, 1.41, 1.84, and 1.90 ±0.2 mg N kg^{-1} of soil, respectively. Among these treatments, the highest inorganic N levels were observed in the AS200 treatment, which were not significantly different from the AS100 treatment, while the lowest inorganic N was found in the control treatment (Figure 3.15).

Furthermore, our study assessed the distribution of inorganic N at different soil depths based on data collected in November 2019. The results demonstrated variations in inorganic N content across different depths. Specifically, the inorganic N accumulation at 0-15, 15-30, 30-60, and 60-90 cm depths were 1.48, 1.17, 0.57, and 0.42 ± 0.18 mg N kg^{-1}soil, respectively (Figure 3.16). When compared to the 0-15 cm depth, deeper layers exhibited substantial decreases in inorganic N content, including a 14.2% reduction at 15-30 cm, a 61.5% decrease at 30-60 cm, and a substantial 71.6% decrease at 60-90 cm. These findings underscore the pronounced decline in inorganic N levels in deeper soils.

N-acetyl-β-D-glucosaminidase (NAGase)

In this study, we accessed the impacts of the contrasting N sources on NAGase activity using the soil collected from 2019 to 2021 at depth 0-15 cm. The NAGase activity exhibited inconsistent responses, with variations observed based on the date of sample collection (Figure 3.17). For example, only in May and August 2020 did the compost treatment exhibit significantly higher NAGase activity compared to the other treatments. In November 2019, N sources did not have any significant impact on NAGase activity. In August 2019 and May 2021, NAGase activity under compost were not significantly different from AS200 and control treatment, but it was higher than AS100 treatments. In August 2021 and November 2021, NAGase activity under compost was not significantly different from the AS200 and AS100 treatments, but it was higher than control treatments. These results indicate that NAGase activity responded differently to N source treatments depending on the sampling date, which is consistent with findings from other studies (Allison et al., 2008; Iamjud et al., 2022).

Despite the inconsistent response of NAGase activity to N treatments at different times of soil sampling, the estimate value of NAGase activity from multiple soil sampling occasions, as illustrated in Figure 3.18, revealed no significant difference in NAGase activity among pre-planting (May), mid-growing season (August), and post-harvesting (November) samplings.

According to the results of the repeated measure analysis, the overall estimated mean of NAGase activity from samples collected at a depth of 0-15 cm between the years 2019 and 2021, under control, compost, AS100, and AS200 treatments, were determined to be 51.8, 66.5, 50.8, and 51.2 g p-nitrophenol kg^{-1}soil h^{-1}, respectively (Figure 3.19). The analysis detected the significant impacts of compost on increasing NAGase but ammonium sulfate treatments did not. The percentage increase in NAGase under compost treatment, compared to the average of the other treatments, was approximately 29.7%.

The assessment of NAGase activity in soil samples obtained in November 2019 demonstrated a decrease in NAGase activity corresponding to increasing soil depth (Figure 3.20). NAGase activity at depth 0-15, 15-30, 30-60 and 60-90 cm were 52.6, 17.3, 7.6, and 5.5 g p-nitrophenol kg^{-1}soil h^{-1}, respectively. The NAGase activity decreased by approximately 67.07% from 0-15 cm to 15-30 cm, by approximately 85.56% from 0-15 cm to 30-60 cm, and by approximately 89.47% from 0-15 cm to 60-90 cm.

Relationships among soil N indicators

Pearson correlation

In our comprehensive study conducted over a three-year period (2019 to 2021), soil samples were systematically collected during the months of May, August, and November at a depth of 0-15cm. As a consequence of this extended duration, the Pearson correlation analysis unveiled notable variations in the relationships among key soil N indicators, specifically STN, ACE protein, NAGase, WEON, and inorganic N. These findings are presented in detail in the as Table B3.

To mitigate the variation across years, in this study, we computed the averages of these sample dates within each year. This averaging process improved the correlation between soil indicators, facilitating a more stable assessment of their relationship. Significant correlations between ACE and STN ($r = 0.46$, $p < 0.001$), STN and WEON ($r = 0.43$, $p =0.002$), and STN and NAGase ($r = 0.41$, $p < 0.004$) were observed. A notably strong correlation was found between ACE and WEON ($r = 0.87$, $p < 0.0001$), and a strong correlation was evident between ACE and NAGase ($r = 0.63$, $p < 0.0001$). Additionally, a moderate correlation was identified between NAGase and WEON ($r = 0.40$, $p < 0.0046$) (Table 3.5).

Effects of treatment on the relationship between STN and other soil N indicators:

In our study, we initially employed Pearson correlation analysis to investigate the relationships between various soil N indicators gaining insights into how these indicators are related. However, given the multi-year nature of our experiment, which involved different treatments and replication across four blocks, we recognized the need for a more comprehensive analysis. To achieve this, we utilized a mixed-effects model approach through the PROC MIXED procedure to examine the relationship between STN with soil

N indicators such as ACE protein, NAGase, and WEON and other various factors, treatment, and year. This analytical approach allowed us to account for both fixed and random effects, providing a comprehensive understanding of the data.

Our specific focus in this analysis was to understand the impact of two key factors, treatment and year, on the relationships between our chosen dependent variables, namely ACE protein,WEON, and NAGase, with respect to the predictor variable STN. Additionally, we considered the influence of the 'block' variable as a random effect, recognizing its potential contribution to the observed variability in our data. The use of PROC MIXED not only enables us to explore the effects of these factors on our dependent variables but also provides valuable statistical output and insights that aid in interpretation.

A. Impact of treatments on the relationship between STN and ACE Protein

The mixed-effects model analysis revealed that the relationship between STN and ACE protein was influenced by both treatment and year (see Table B4). Significant interactions were observed between STN and treatment, with the Compost treatment demonstrating high statistical significance ($p < 0.0001$). Specifically, the coefficient estimates for the interaction between STN and the Compost treatment level was 0.7729 ($p \leq 0.0001$), indicating that a one-unit increase in STN under the Compost treatment condition, corresponded to an approximate 0.7729-unit increase in ACE protein levels, while controlling for other variables (see Table B4).

Conversely, the interaction effects between STN and the AS 100 and AS 200 treatment levels were not statistically significant. This suggests that there is insufficient evidence to conclude that the relationship between STN and ACE protein levels differs significantly for the AS 100 and AS 200 treatments compared to the baseline level.

B. Impact of treatments on the relationship between STN and WEON

The relationship between STN and WEON is significantly influenced by the treatment applied (see Table B5). Notably, only the interaction with the Compost treatment level demonstrates high statistical significance ($p < 0.0001$).

The coefficient estimates for the interaction between STN and the Compost treatment level is 0.002474 within the Compost treatment condition. This suggests that, under the Compost treatment, a one-unit increase in the STN variable corresponds to an approximate 0.002474 unit increase in the response variable WEON assuming all other factors remain constant (see Table B5).

In contrast, the interaction effects between STN and both the AS100 and AS200 treatments are not statistically significant (Table B5). This implies that there is no strong evidence to conclude that the relationship between STN and WEON differs significantly for the AS100 and AS200 treatments compared to the baseline.

C. Impact of treatments on the relationship between STN and NAGase activity

Our analysis highlights the substantial influence of treatment on the relationship between NAGase activity and STN. Notably, the interaction term STN*Treatment Compost exhibited statistical significance with a coefficient of 0.006098 (see Table B6). This result suggests that a one-unit increase in STN under the Compost treatment condition corresponds to an approximate 0.006098-unit increase in NAGase activity, while controlling for other variables. In contrast, the AS100 and AS200 treatments do not significantly alter the relationship between STN and NAGase. Therefore, this finding indicates that compost treatment has a positive and significant impact on the relationship between STN and NAGase activity (see Table B6).

Discussion

In this study, we assessed the effects of contrasting N sources on a range of soil N indicators during corn silage production across multiple seasons and years of repeated treatments. Then we explored the relationships among these soil N indicators and assessed these by correlation and mixed effects modeling. Overall, the contrasting N sources significantly impacted the N indicators and their relationships with each other.

Contrasting N sources effects on soil total N (STN)

STN, in general, tends to be less responsive to immediate management practices, and the effects of such practices on STN may require extended observation periods to become evident (Hurisso et al., 2018). The results from this study underscored the variable response of STN to fertilizer treatments, which is contingent upon the specific growing season under investigation. These fluctuations in STN levels, influenced by seasonal variations and their intricate interactions with the timing of soil amendments (Hurisso et al., 2018; Turner et al., 2015), posed challenges in discerning the impacts of fertilizers on STN. Therefore, conducting long-term studies becomes imperative for gaining a comprehensive understanding of how N fertilizer treatments influence STN.

Repeated measurements in this experiment (2011-2021) revealed that the compost treatment led to an approximately 23% increase in STN compared to other treatments. In contrast, the ammonium sulfate treatment did not yield similar improvements, consistent with findings from other studies (Gao et al., 2022; Steiner et al., 2007). Earlier studies on the same plots investigating various aspects of the soil N cycle also showed that compost treatment enhanced the diversity of microbial communities and promoted N mineralization compared to AS fertilizer treatments (Ouyang, 2016; Ouyang & Norton, 2020).

Maintaining optimal STN levels plays an important role in preserving soil quality, enhancing crop productivity, and ensuring environmental sustainability (Al-Kaisi et al., 2005; Li et al., 2022). A decrease in STN can adversely affect soil fertility, nutrient availability, and overall productivity (Gray & Morant, 2003). Thus, the long-term application of organic fertilizers can increase soil fertility and organic matter, which may lead to higher crop yields (Hua et al., 2020).

Contrasting N sources effects on N mineralization

The potential mineralizable nitrogen (N_0) value in compost-amended soil was 33.4 ± 19.9, approximately 1.79 time higher than the values observed in the other treatments but with high variability (Table 3.2), rendering results statistically insignificant In our study, immobilization occured in compost treated soils from the initial date of incubation until day 7 and a few samples were still immobilizing N at day 14 (Figure 3.5) Our observation of an initial immobilization of N during incubation is consistent with previous studies (Cabrera et al., 2005; Cassity-Duffey et al., 2020; Cassity-Duffey et al., 2018; Mukai & Oyanagi, 2019). Previous studies have found that compost treated soils exhibit net N immobilization from the initial date until day 14 (Cabrera et al., 2005; Gale et al., 2006) or until day 28 of incubations (Mukai & Oyanagi, 2019).

The constant rates of decomposition (k) for the compost treatment was 0.009 ± 0.007, per day (Table 3.2). The value of *k* in the compost treatment in our study falls within the range reported in previous studies (Gale et al., 2006; Hadas & Portnoy, 1994; N'dayegamiye et al., 1997). Its value was was lower than that observed in the ammonium sulfate treatments, consistent with findings from earlier research (Cabrera et al., 2005; Sharifi et al., 2014). This finding suggests that the organic N present in the compost was more stable and resistant to mineralization than ammonium sulfate treatments (Cabrera et al., 2005; Gil et al., 2011; Hadas & Portnoy, 1994).

The value of N_0 in this study was lower than those in previous studies (Cassity-Duffey et al., 2020; Sharifi et al., 2014). In the study of Cassity-Duffey et al., (2020), their soil were collected from an organic farm with intensive practice of crop rotations and the additon of organic matter. That practice improves soil organic matter, and enhance microbial activity resulting in in in a higher value of N_0 (Cassity-Duffey et al., 2020). The N_0 in our study was also lower compared to N_0 from Sharifi et al. (2014) who used cow manure with wheat straw bedding. They explained the higher N_0 value was higher than the other due to the higher inherent soil organic N at their site and the cummulative effect of long term application of the organic amendents (Sharifi et al., 2014). They also mentioned that the variations in compost composition highlights the fundamental problem that composts lack a specific definition as a product, even across batches from the same supplier with cattle manure as a primary element (Hadas & Portnoy, 1994; Lazicki et al., 2020; Sharifi et al., 2014).

The process of N mineralization of SOM can be influenced by the compost C:N ratio (Calderón et al., 2005; Gale et al., 2006; Geisseler et al., 2021; Harmsen &

Kolenbrander, 2015; Myrold & Bottomley, 2015; Paul, 2007). The critical C:N ratio for determining organic N immobilization and/or mineralization varies with materials and then changes after soil incorporation. The composition of bedding materials, such as wood chips or straw, and the choice of green waste or manure sources are known to significantly impact the levels of organic matter, C, and N content within compost (Geisseler et al., 2021; Hadas & Portnoy, 1994; Harmsen & Kolenbrander, 2015; Myrold & Bottomley, 2015; Sharifi et al., 2014). In additon to C:N ratio, there are many factors that can affect mineralization processes such as soil moisture, aeration, temperature, type and rate of organic ammendment application, the amount and type of crop residues remaining in the soil, as well as other physical, chemical, and biochemical factors that affect soil N dynamics (Deans et al., 1986; Robertson & Groffman, 2015).

Our findings corroborate previous research, demonstrating that cropping systems incorporating compost amendments experienced a significant enhancement in N_0 (Heisey et al., 2022; Ippolito et al., 2021; Mahal et al., 2018; Sharifi et al., 2014). However, the application of inorganic N fertilizer did not result in a significant improvement in N_0 levels when compared to the control treatment (Mahal et al., 2018; Sharifi et al., 2014).

Contrasting N sources effects on autoclaved citrate extractable soil protein (ACE protein)

In this study we observed the level of ACE increased gradually over the season which agrees with the study of Huang et al., (2022). The accumulation of ACE soil protein was highest at the surface soil and gradually decreased with increasing soil depth as expected (Cissé et al., 2023; Wang et al., 2017).

Contrasting N sources had significant impacts on ACE protein content. Compost treatment increased ACE protein by approximately 36.7% compared to the other treatments. In addition, under the assumption that protein contains 16% N, ACE protein-N accounted for 64% of STN. However, the level of ACE protein-N content varied and was affected by the N treatments and seasonal timing. The value of ACE protein-N from repeated measure under control, compost, AS100 and AS200 treatments were 0.81, 1.1, 0.79, and 0.78 ± 0.87 mg N g^{-1} of soil. The ACE protein-N in the compost treatment increased by approximately 38.56% compared to the average of the other treatments. It is important to notice that the pool of ACE protein-N is highly variable (Mattila et al., 2023). It can be influenced by many factors such as STN content, soil texture, geographic location, and soil color during extraction (Geisseler et al., 2019; Mattila et al., 2023). Numerous studies have demonstrated a strong relationship between ACE protein levels and various management practices (Borie et al., 2006; Emran et al., 2012; Hurisso et al., 2018; Liebig et al., 2006; Moebius-Clune et al., 2017; Nichols & Millar, 2013; Wright et al., 2007).

For the ammonium sulfate treatments, ACE protein levels did not significantly differ from those in the control treatment. These findings lend support to the theory that the addition of organic amendments, such as compost, effectively enhances soil protein (Borie et al., 2006; Emran et al., 2012; Hurisso et al., 2018; Liebig et al., 2006; Moebius-Clune et al., 2017; Nichols & Millar, 2013; Wright et al., 2007).

Contrasting N sources effects on water extractable organic nitrogen (WEON)

WEON levels varied according to the time of soil sampling. WEON concentration at different times during the growing season were influenced by seasonal variations, management practices, and soil types (Chahalid & Van Eerd, 2020; Embacher et al., 2007; He et al., 2017; Yokobe et al., 2018; Zhang et al., 2015).

The compost treatment increased WEON concentrations while ammonium sulfate treatments did not. The compost treatment increased WEON by approximately 29.6% compared to the average of other treatments. This result demonstrates that organic fertilizer had a notable impact on elevating WEON, while inorganic fertilizer had no significant impact on WEON, aligning with findings from other studies (Das et al., 2023; He et al., 2017).

The concentration of WEON decreased with increasing soil depth which reflects the decreasing availability of WEON as soil depth increases, aligning with findings from previous studies (Embacher et al., 2007; Zhang et al., 2011; Zhang et al., 2022). However, other studies have reported insignificant variations in WEON distribution at different soil depths (Embacher et al., 2008; Toosi et al., 2012). The variability in concentrations of WEON across soil depth can be influenced by site and/or soil characteristics, season, cropping systems, fertilizer management, and/or climate (Chahalid & Van Eerd, 2020; Embacher et al., 2007; He et al., 2017; Yokobe et al., 2018; Zhang et al., 2015).

Contrasting N sources effects on inorganic N

In this study, inorganic N varied according to the soil depth. The inorganic N accumulations at the topsoil and decreases with deeper soil profile. This observation is in line with previous investigations which reported an accumulation of inorganic nitrogen in the topsoil and a decline in concentration as soil depth increases (Ouyang et al 2017; Hirsh & Weil, 2019; Sainju et al., 2019; Xu et al., 2020; Zhang et al., 2022).

The levels of N in soil are dynamic and influenced by various factors (Mahal et al., 2019). It is noteworthy that only a small percentage of soil N exists in an inorganic state, and that the majority of inorganic N in soil is water-soluble, enabling easy translocation. The lower concentration of ammonium in the soil compared to nitrate is likely due to the rapid nitrification (Camberato & Nielsen, 2017; Huffman, 1989; Varvel & Peterson, 1990).

Various factors, including time, temperature, soil type, N sources, application rates, and methods of applying fertilizer, can influence the rate at which ammonium is converted into nitrate-N (Norton and Ouyang 2019; Camberato & Nielsen, 2017). In addition to N transformation, ammonium is taken up by plants, utilized by soil microbes, immobilized in humus, or absorbed into clay minerals (Harmsen & Kolenbrander, 2015). Furthermore, inorganic N in the soil can undergo losses by volatilization and denitrification, which can occur rapidly (Li et al., 2022).

Inorganic N in the soil is considered to be ephemeral (Hurisso et al., 2018). Given the highly dynamic and responsive nature of inorganic N in the soil, taking a single measurement at a specific point in time provides only a snapshot of its content. To gain a more comprehensive understanding of the soil N dynamics, continuous monitoring and measurements over time are essential (Harmsen & Kolenbrander, 2015). This helps researchers and practitioners track how inorganic N levels change in response to various factors, enabling better management of soil nutrient resources in agriculture and ecosystem studies.

Contrasting N sources effects on N-acetyl-β-D-glucosaminidase (NAGase)

NAGase activity was observed to have inconsistent responses at different time of soil sampling.and did not show significant differences between pre-planting (May), mid-growing season (August), and post-harvesting (November) samplings (Figure 3.9). This finding disagreed with the results Wallenstein et al. (2009) who observed seasonal variations in NAGase activity. In their research, NAGase activity was high in May but steadily declined through the summer into winter. The discrepancies between our study and theirs may be attributed to differences in environmental conditions, vegetation types, and temperature variations. Enzyme pools such as NAGase are known to be sensitive to numerous factors, including climate, temperature, and vegetation types (Vourlitis et al., 2021; Wallenstein et al., 2009).

In our study, we observed significant increases in NAGase activity under the compost similar to others (Das et al., 2023; Jiang et al., 2022; Yanagi & Shindo, 2016). Meanwhile, we did not observesignificant impacts of AS fertilizer on NAGase activity which is in contrast to a previous study. Zhang et al. (2016) found high urea application rates (224 and 392 kg N ha^{-1} yr^{-1}) led to a decrease in NAGase activity by 4-42% in response to N addition. These differences highlight the mixed responses of this enzyme to N applications as noted by Vourlitis et al. (2021). The variability in soil enzyme activity is influenced by factors such as the N fertilizer source, the duration and magnitude of N application, and variables including soil moisture, temperature, and soil pH (Jian et al., 2016; Turner, 2010; Ullah et al., 2019; Vourlitis et al., 2021).

Correlation

The significant variation in soil indicator correlations can be attributed to the timing of soil sample collection. Several studies, including Omer et al. (2018) and Sherbine et al. (2023), have noted that seasonal changes have an impact on soil N indicators, leading to variability in measurements taken at different sampling dates (Omer et al., 2018; Sherbine et al., 2023). Additionally, soil indicators can be influenced by various management practices, further contributing to this variation (Omer et al., 2018). This variability underscores the potential effects on the strength of soil indicators. As a response, Omer et al. (2018) suggested the importance of regular sampling throughout the year to ensure consistent interpretation of directional changes in soil quality indicators.

The N indicators exhibited moderate to strong linear relationships with each other, with the exception of the inorganic N indicator. These correlation strengths fell within the ranges reported in previous studies (Cappellazzi & Morgan, 2021; Das et al., 2023; Geisseler et al., 2019; Hurisso et al., 2018; Liptzin et al., 2023). The variability in the strength of correlation between the N indicators was influenced by multiple factors. Notably, level of STN played a significant role in shaping the correlation strength between these indicators, as observed in Geisseler et al. (2019). In their research, a moderate correlation between ACE and STN ($r = 0.52$, $p \leq 0.0001$) was noted in soils with low STN levels. Conversely, they found that the correlation strength between ACE and STN increased significantly to a very high level ($r = 0.98$, $p < 0.0001$) when the soil exhibited a high STN content. This finding aligns with similar results from Das et al. (2023), where the STN level was 11.61 g kg^{-1}, a range consistent with the study by Geisseler et al. (2019). In Das et al.'s study (2023), a very high correlation (0.85, $p <$

0.001) was observed between STN and ACE, reinforcing the influence of soil STN on these correlations (Das et al., 2023). Additionally, other factors contributing to correlation variability include cropping systems, farming and management practices, soil type (Cappellazzi & Morgan, 2021; Geisseler et al., 2019; Liptzin et al., 2022, 2023) as well as sample size and data variability (Goodwin & Leech, 2010). These multifaceted factors collectively influence the observed strength of correlations among N indicators in soil studies.

This study highlights the significance of treatment interactions with STN in influencing the relationships with soil indicators, particularly emphasizing the substantial impact of the compost treatment on these interactions compared to other treatments. While our study has provided valuable insights into the relationship between N indicators and soil properties, further investigation on a larger scale and across various contexts is needed to validate and expand upon our findings. These limitations underscore the ongoing need for robust research in this field to inform sustainable agricultural practices effectively.

Conclusions

We investigated the dynamic responses of various soil N indicators to different N sources under corn silage production within a semi-arid environment. Results showed that levels of N indicators fluctuated over the growing season, reflecting the interplay of biological, environmental, and management factors. The indicators showed the highest accumulation of N occurring in the topsoil (0-15cm) and decreasing with soil depth.

Our findings revealed that compost application increased the potential mineralizable N pool (N_0) but simultaneously led to a lower rate of mineralization (k). In contrast, ammonium sulfate treatments amplified the k value without significantly affecting N_0. This suggests that compost enriched the stability of organic N, whereas ammonium sulfate accelerated its mineralization.

Furthermore, under compost treatment, significant improvement was observed across various N indicators, except for inorganic N. STN levels increased by 23.1%, ACE protein exhibited an approximate 36.7% increase, NAGase activity saw an increase of about 29.7 %, and WEON showed an increase of approximately 29.6%, all in comparison to other treatments. Notably, inorganic N was high under ammonium sulfate treatments, attributed to ammonium applications surpassing plant uptake capacity.

N indicators displayed moderate to strong linear relationships with each other, suggesting a clear interconnection between these variables. These relationships were found to be influenced by seasonal variations and the specific fertilizer treatments applied. Notably, when compost was applied as a fertilizer, it resulted in a significant increase in STN. This increase was accompanied by elevated levels of N indicators, such as ACE protein and NAGase activity. Conversely, the use of ammonium sulfate as a N source did not have any effects on either STN levels or the associated N indicators.

In summary, our study demonstrated the variable impacts of contrasting N sources on soil N indicators. Our findings illustrated the significance of organic amendments, such as compost, in elevating STN levels and enzyme activity. The chemical fertilizer, ammonium sulfate, did not improve soil health indicators. Our research advances our understanding of soil N dynamics, offering essential insights for optimizing agricultural practices. To bolster these findings, future investigations should explore these relationships in various settings and on a broader scale, ensuring that sustainable agricultural practices are founded on robust research and informed decision-making.

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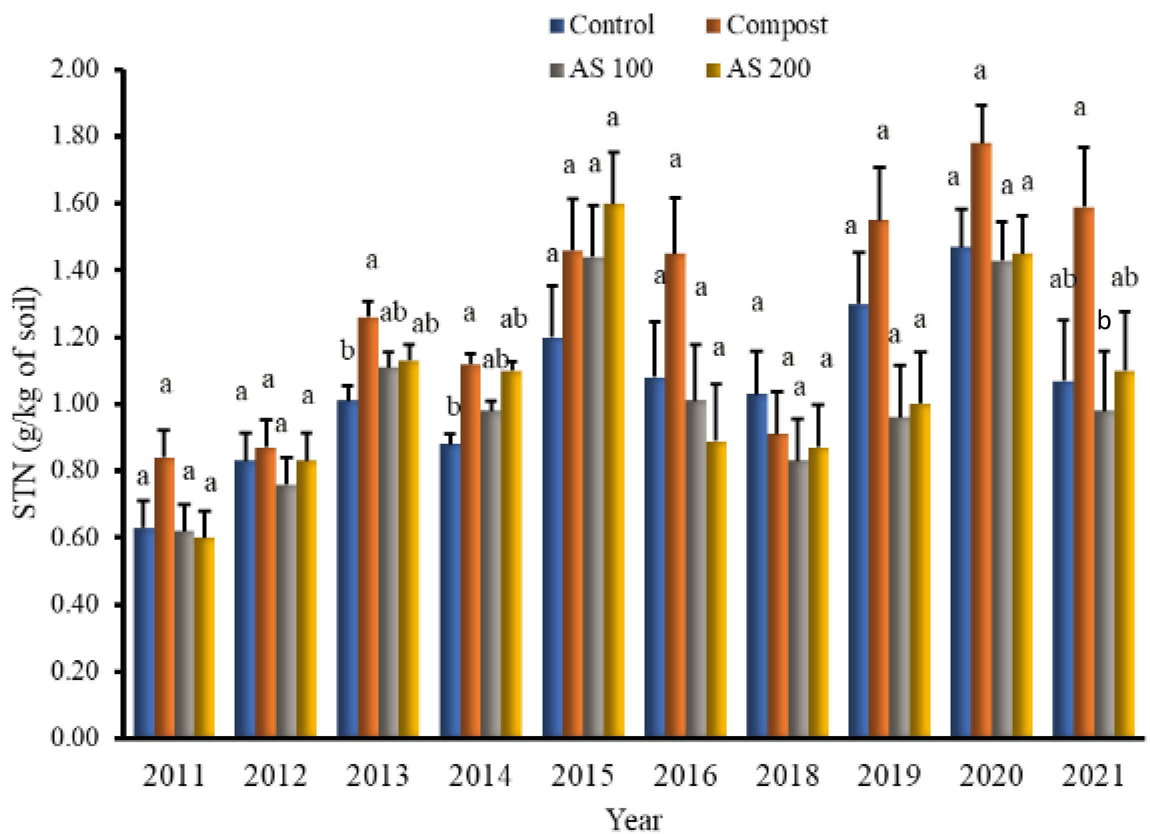
Figures and Tables

Figure 3. 1.

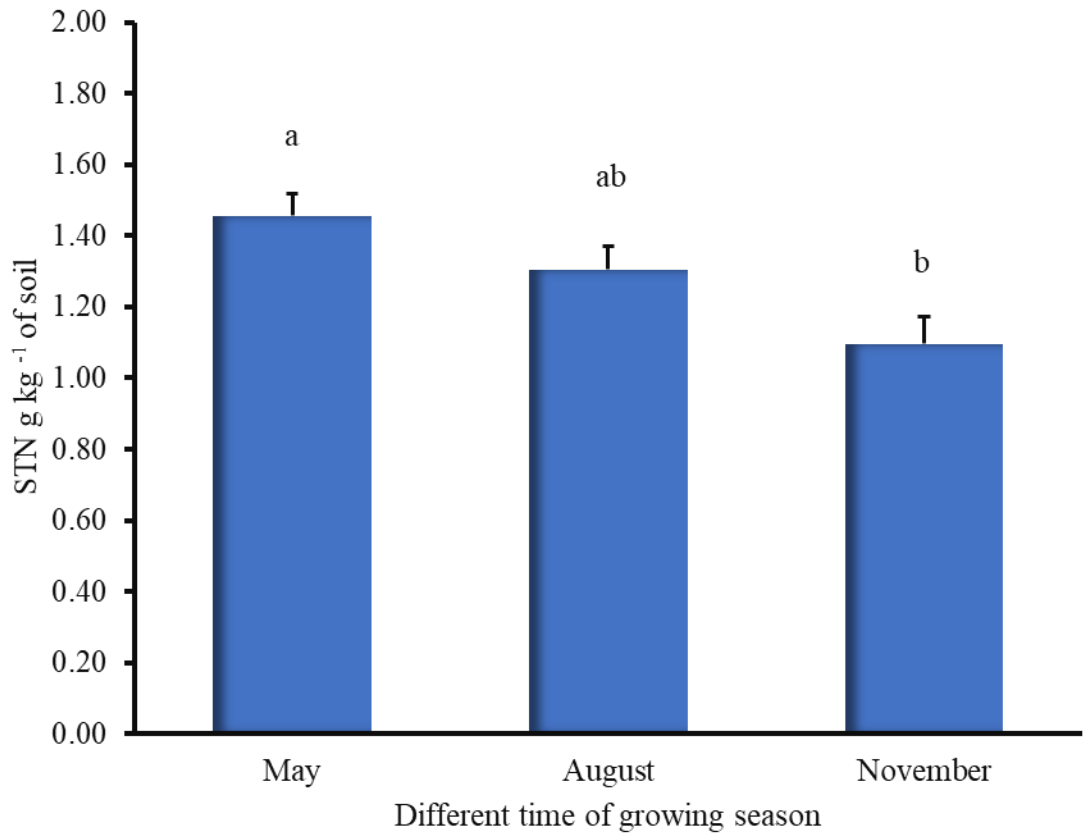
Soil Total Nitrogen (STN) Content in Soil at different dates of soil sample collection



Note. STN analysis was conducted on soil samples collected in August, at a depth of 0-15 cm, from a corn silage field spanning the years 2011 to 2021. Error bars represent standard errors (n = 4). Different lowercases above the bars indicate a significant difference within each year (p ≤0.05).

Figure 3. 2.

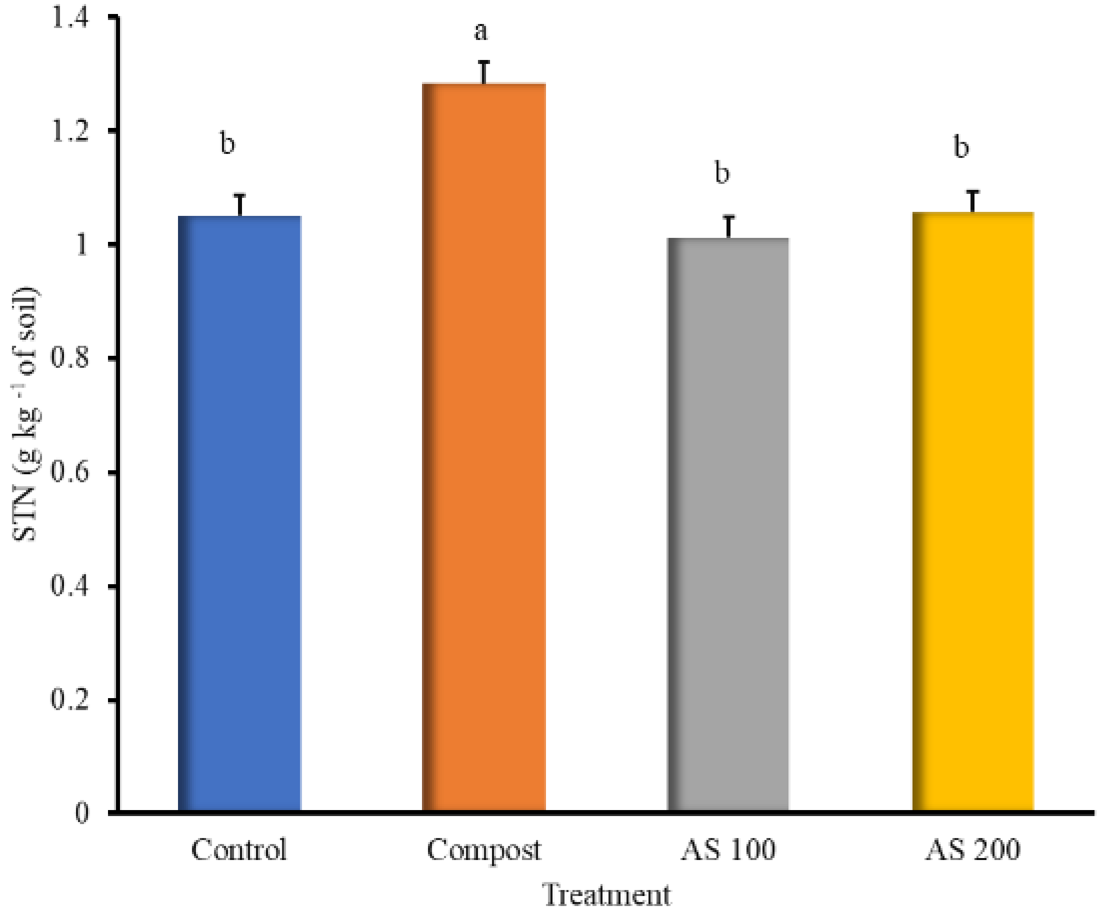
Soil Total Nitrogen (STN) at different times of growing season



Note. Repeated measure was employed to analyze the seasonal variation in STN (May, August, and November). Soil samples were collected at a depth 0-15 cm from 2019 to 2021. Error bars represent standard errors (n = 12). Different lowercases above the bars indicate significant differences among seasons ($p < 0.05$).

Figure 3. 3.

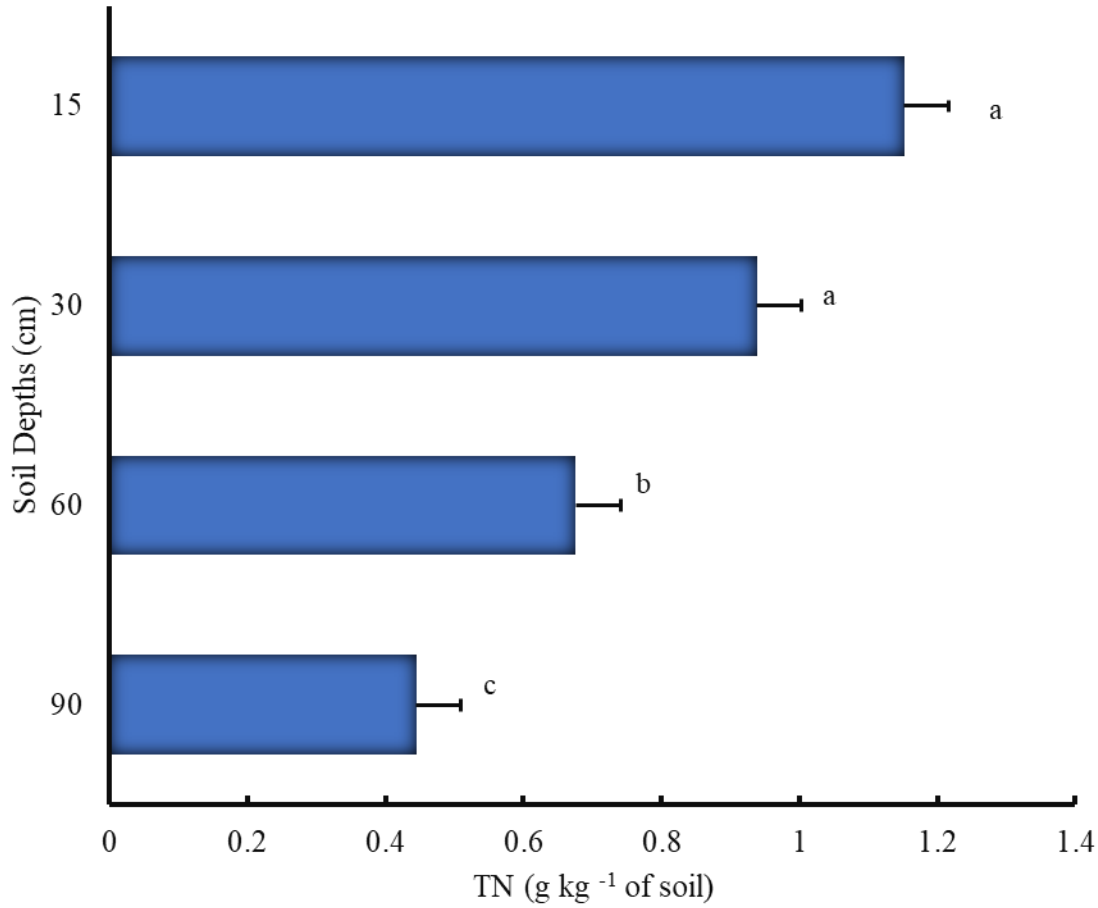
Effects of contrasting N sources on soil total nitrogen (STN)



Note. Repeated measures were employed to analysis the effects of contrasting N sources on STN over a decade of application. Soil samples were collected in August at a depth of 0-15 cm spanning the years 2011 to 2021. Error bars represent standard errors (n = 40). Different lowercases above the bars indicate a significant difference among treatments ($p \leq 0.05$).

Figure 3. 4.

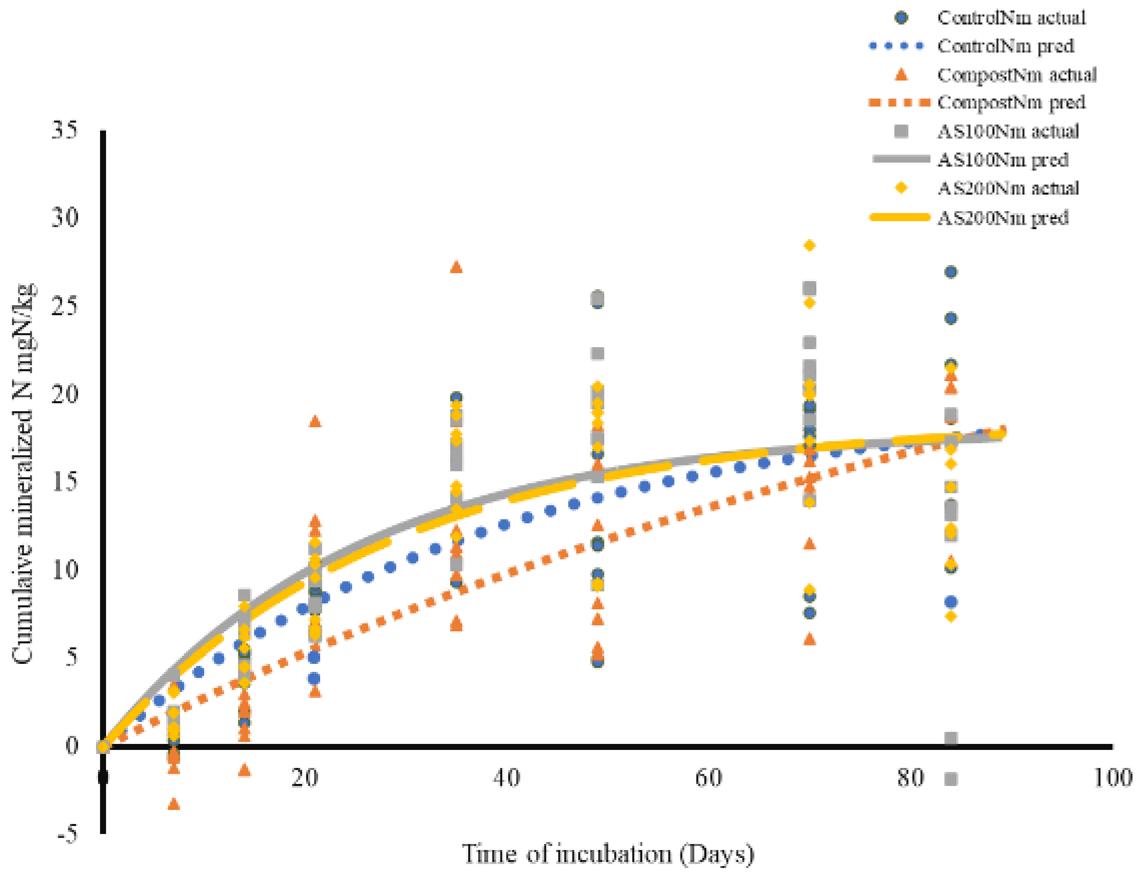
Soil total nitrogen (STN) by soil depth



Note. Measurement of STN at various soil depths across treatments (0-15 cm, 15-30 cm, 30-60 cm, and 60-90 cm) in November 2019. Error Bars reflect standard errors (n=16). Lowercase letters above the bars indicate significance between depths (p ≤0.05).

Figure 3. 5.

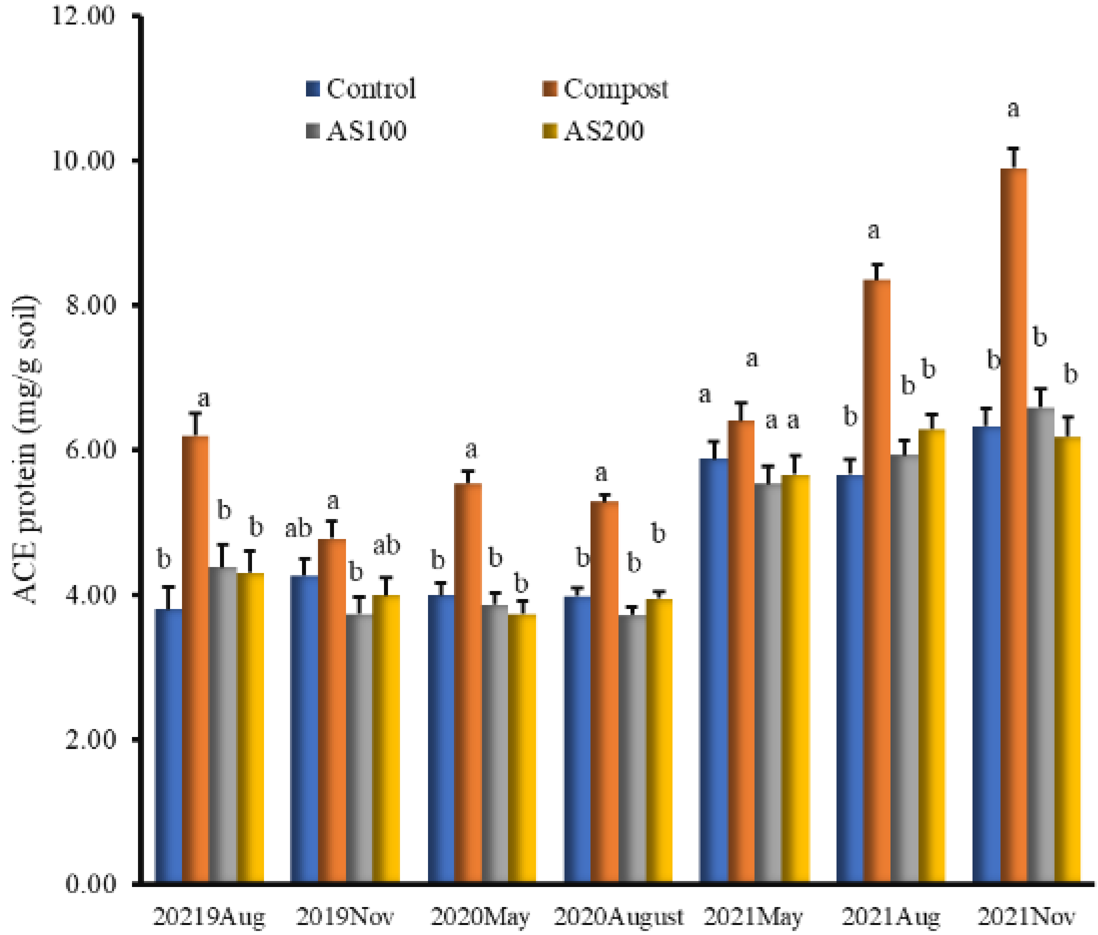
Cumulative net mineralized N (Nt) with a first-order model from a long-term incubation of 84 days



Note. Cumulative N mineralization in soil from an aerobic 84-days incubation of soils from August 2019 soil sampling at 0-15 cm soil depth. Individual treatments were fit to the first order equation $Nt = N_0(1 - e^{-kt})$. A non-linear least-squares approach was used to estimate the N_0 and k parameters. The treatments are Control, Compost and AS100 and AS200. For statistics on the model goodness of fit (R^2), N_0 and k, see table 4.1.

Figure 3. 6.

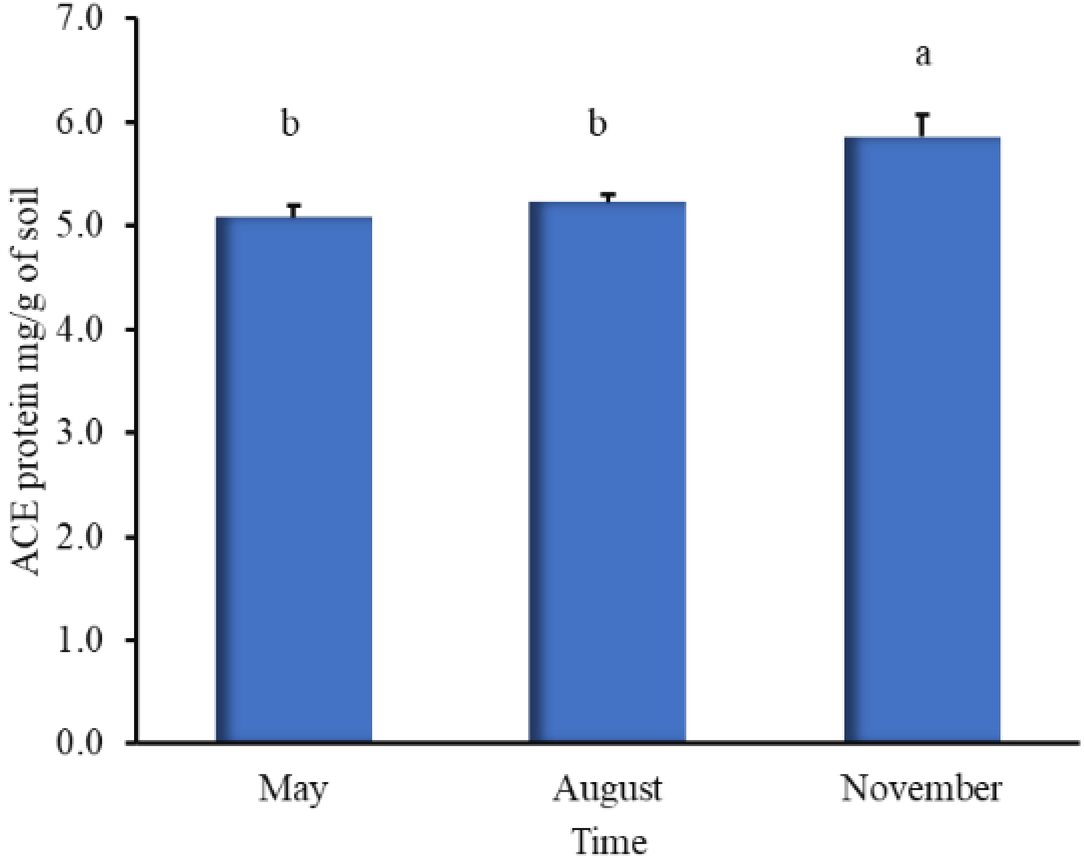
Autoclaved citrate- extractable protein (ACE protein) at different dates of soil sample collection



Note. ACE Protein analysis was conducted on soil samples collected at a depth of 0-15 cm from corn silage field spanning the years 2019 to 2021. Error bars reflect standard errors (n = 4), with lowercase letters above the bars signifying significant differences (p ≤0.05) with each sampling.

Figure 3. 7.

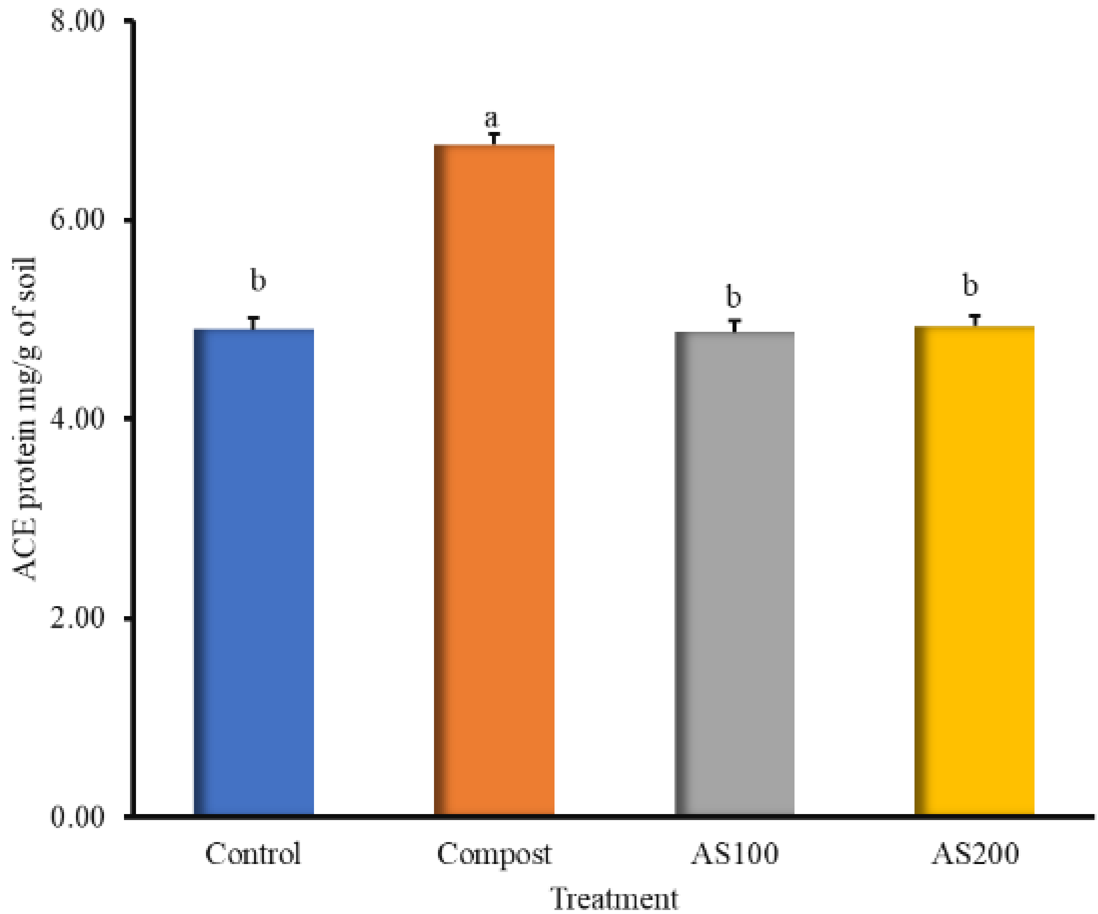
Autoclaved-Citrate Extractable Protein (ACE Protein) at different times of growing season



Note. Repeated measure was employed to analyze ACE Protein at different times of growing season (May, August, and November). Soil samples were collected at a depth of 0-15 cm from 2019 to 2021. Error bars represent standard errors (n = 12). Different lowercases above the bars indicate as significant difference among seasons ($p \leq 0.05$).

Figure 3. 8.

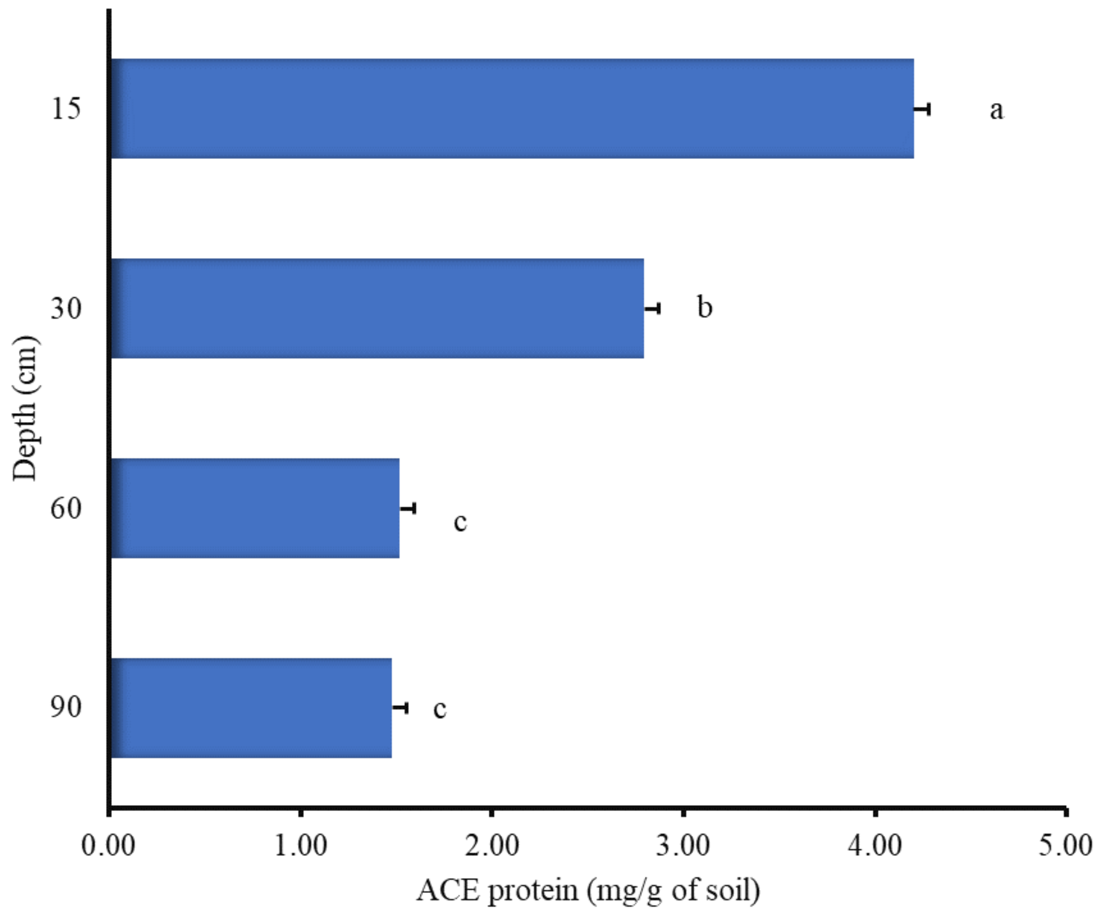
Effects of contrasting N sources on autoclaved citrate-extractable protein (ACE Protein)



Note: Repeated measures were employed to analyze the effects of contrasting N sources on soil ACE protein. Soil samples were collected at a depth of 0-15 cm from 2019 to 2021. Error bars represent standard (n=28). Different lowercases above the bars indicate a significant difference by treatment ($p \leq 0.05$).

Figure 3. 9.

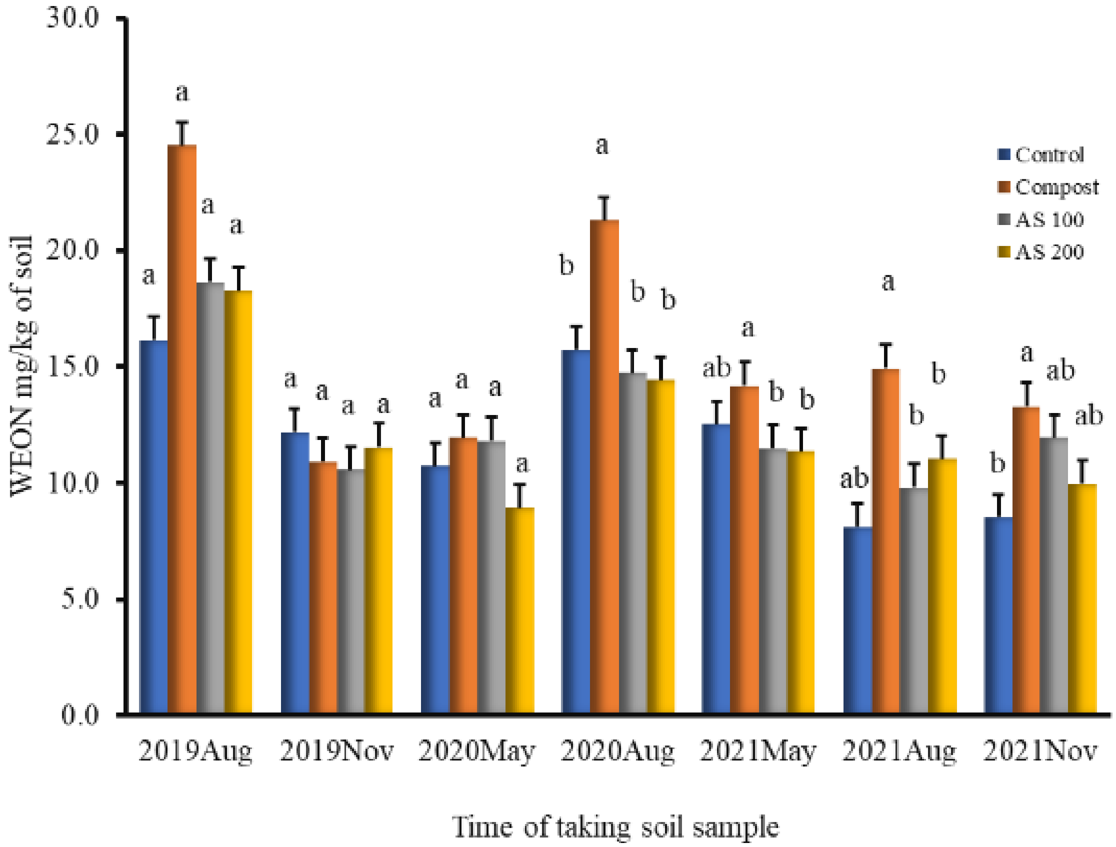
Autoclaved-Citrate Extractable Protein (ACE Protein) at different soil depths



Note. Measurement of soil ACE protein measured at various soil depths across treatments (0-15 cm, 15-30 cm, 30-60 cm, and 60-90 cm) in November 2019. Error bars reflect standard errors (n=16). Lowercase letters above the bars indicate significance at the specified depth ($p \leq 0.05$).

Figure 3. 10.

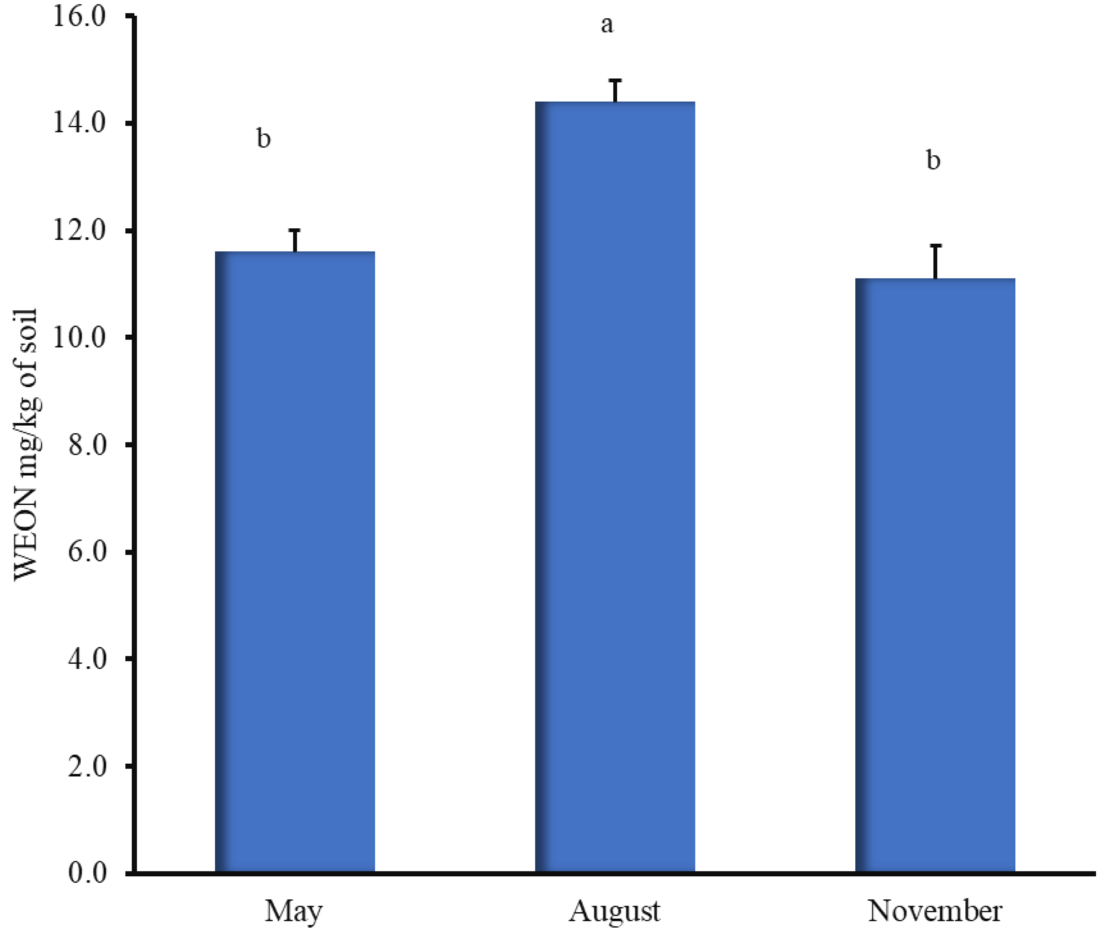
Water extractable organic nitrogen (WEON) at different dates of soil sample collection



Note. WEON analysis was conducted on soil samples collected at a depth of 0-15 cm, from corn silage field spanning the years 2019 to 2021. Error bars reflect standard errors (n = 4), with lowercase letters above the bars signifying significant differences within sampling date ($p \leq 0.05$)

Figure 3. 11.

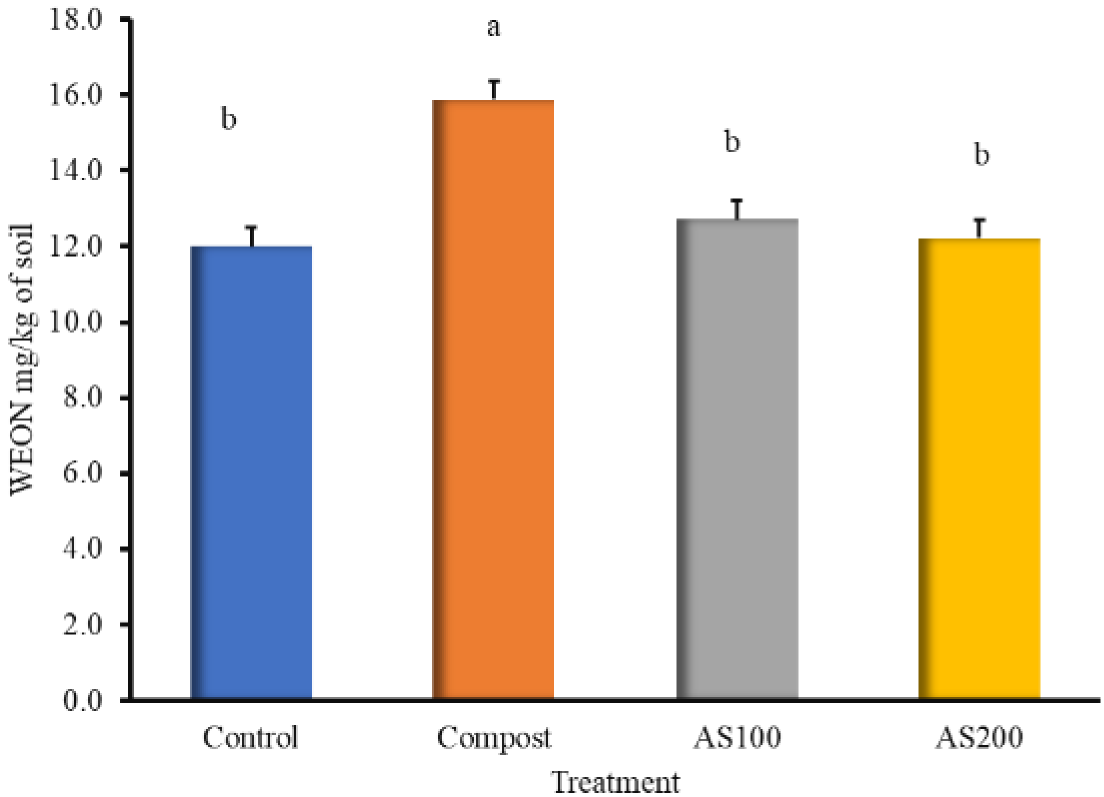
Water extractable organic N (WEON) at different times of growing season



Note. Repeated measure was employed to analyze WEON at different times of growing season (May, August and November). Soil samples were collected at a depth 0-15 cm from 2019 to 2021. Error bars represent standard errors (n = 12). Different lowercases above the bars indicate as significant difference among seasons ($p \leq 0.05$).

Figure 3. 12.

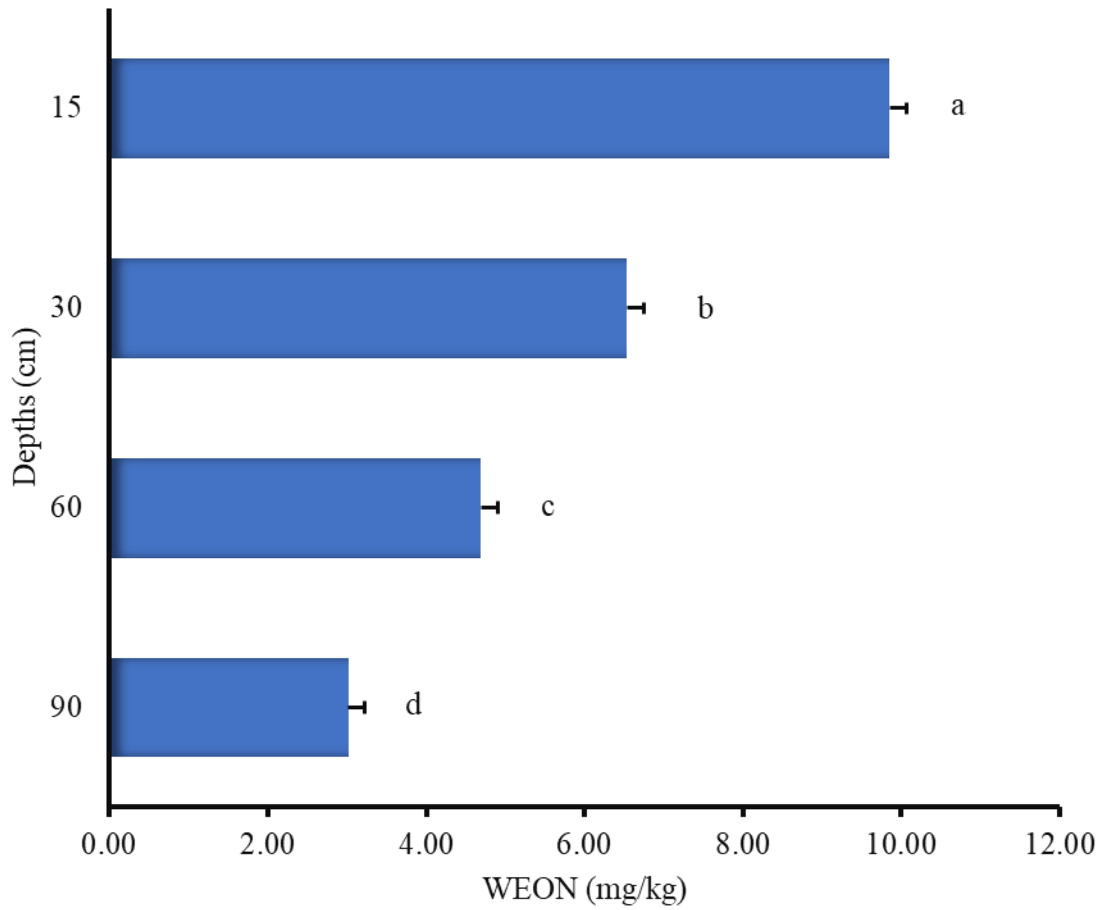
Effects of contrasting N sources on water extractable organic nitrogen (WEON)



Note. Repeated measures were employed to analysis the effects of contrasting N sources on WEON. Soil samples were collected at a depth of 0-15 cm from 2019 to 2021. Error bars represent standard error (n=28). Different lowercases above the bars indicate a significant difference by treatment ($p \leq 0.05$).

Figure 3. 13.

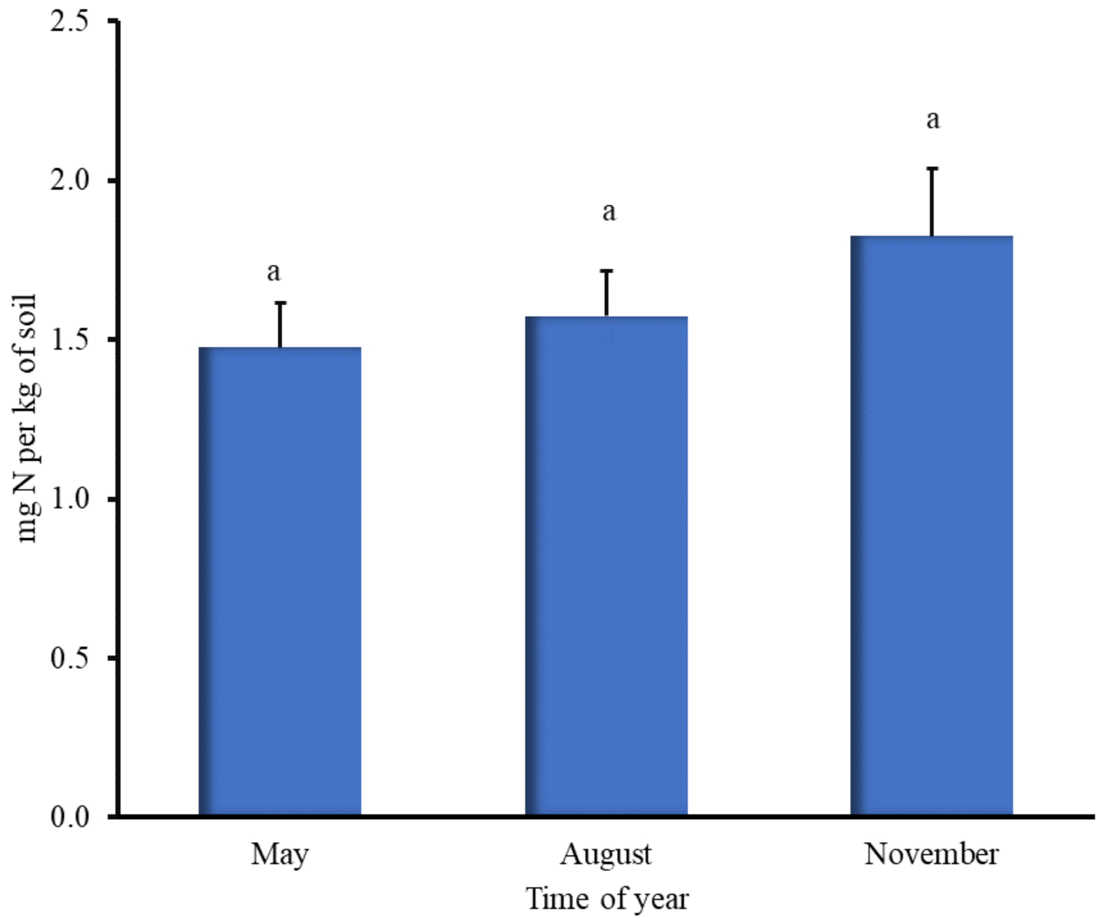
Water extractable organic nitrogen (WEON) at different soil depths



Note. Measurement of WEON at various soil depths across treatments (0-15 cm, 15-30 cm, 30-60 cm, and 60-90 cm) in November 2019. Error bars reflect standard errors (n=16). Lowercase letters above the bars indicate significance at the specified depth ($p \leq 0.05$).

Figure 3. 14.

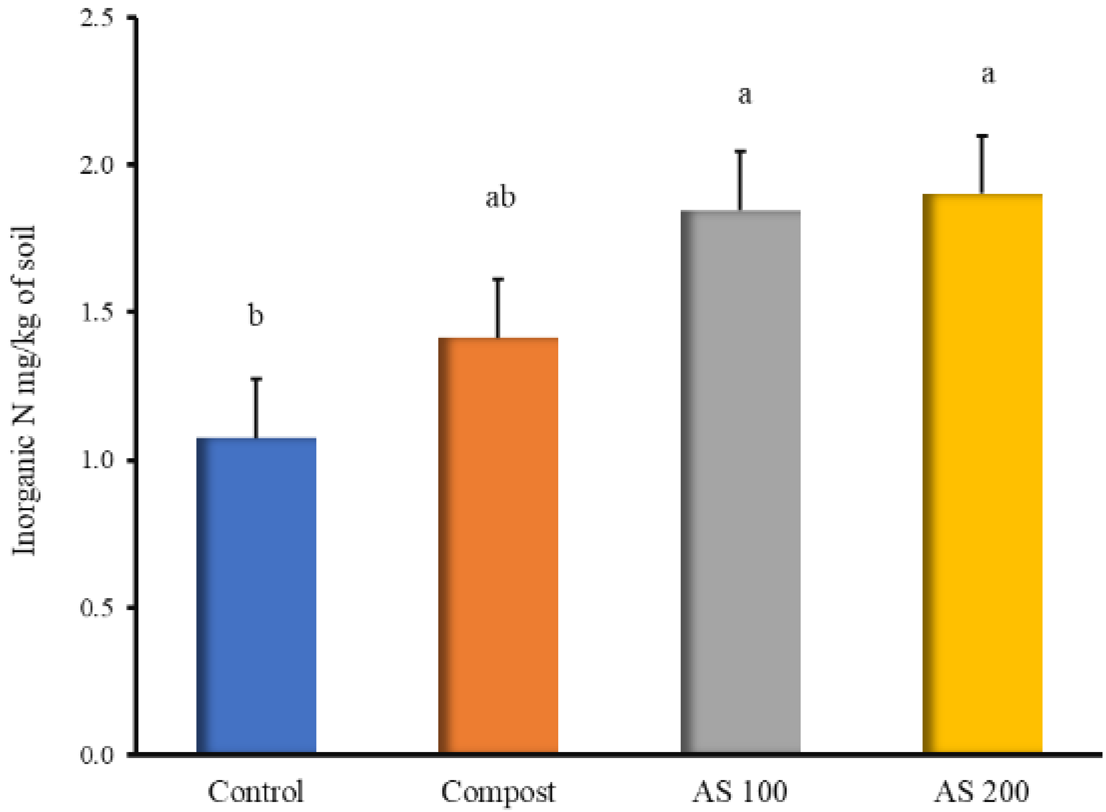
Inorganic N at different times of growing season



Note . Repeated measure was employed to analyze inorganic N at different times of growing season (May, August, and November). Soil samples were collected at a depth 0-15 cm from 2019 to 2021. Error bars represent standard errors (n = 12). Different lowercases above the bars indicate as significant difference among seasons (p ≤0.05).

Figure 3. 15.

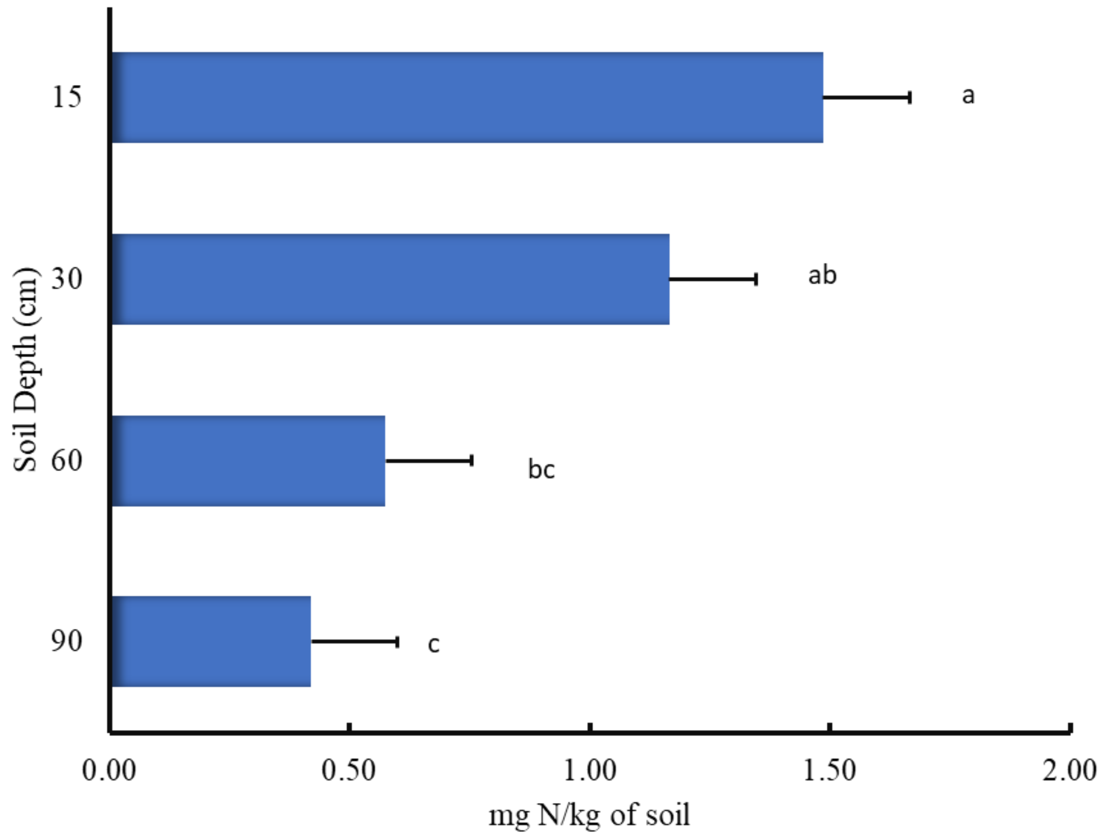
Effects of contrasting N sources on inorganic N



Note: Repeated measures were employed to analysis the effects of contrasting N sources on inorganic N. Soil samples were collected at a depth of 0-15 cm spanning the years 2019 to 2021. Error bars represent standard errors (n = 28). Different lowercases above the bars indicate a significant difference among treatments ($p \leq 0.05$).

Figure 3. 16.

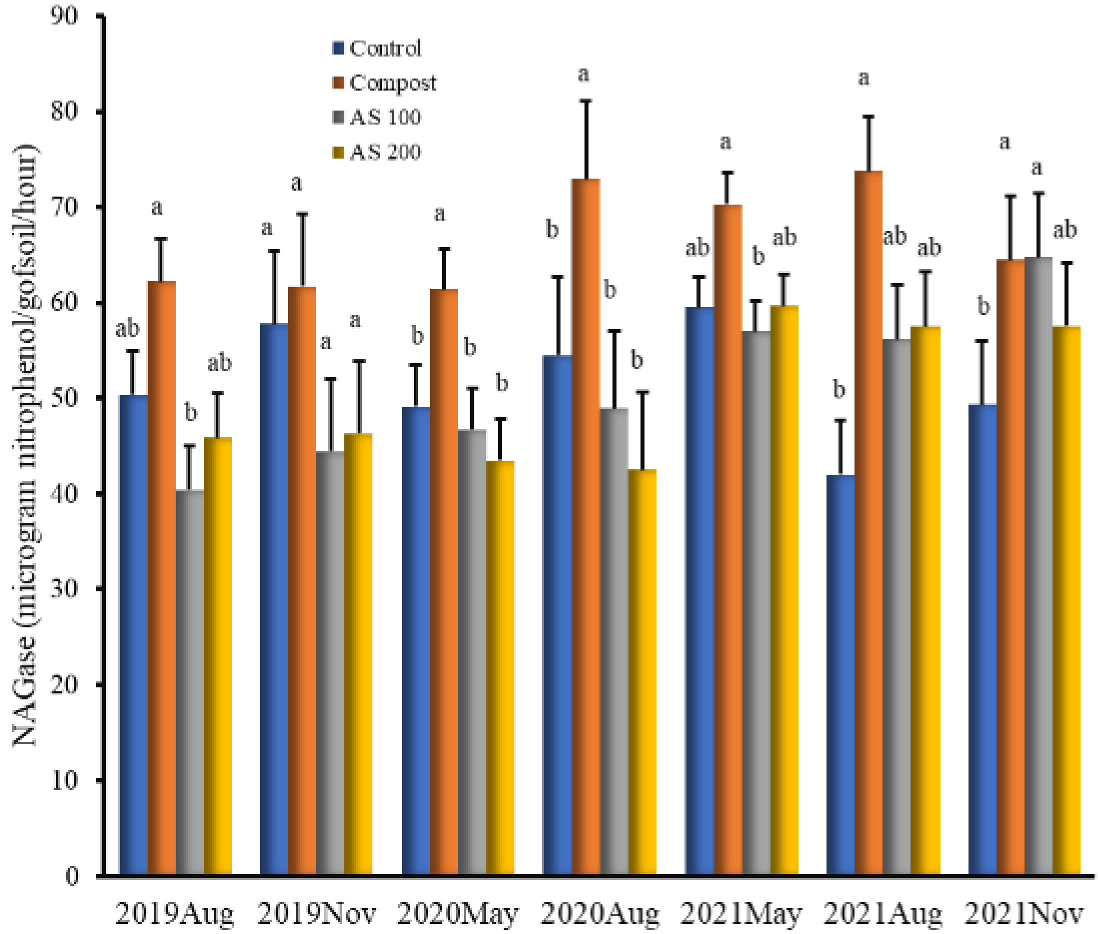
Inorganic N in November 2019 at different soil depths



Note: Measurement of inorganic N at various soil depths across treatments (0-15 cm, 15-30 cm, 30-60 cm, and 60-90 cm) in November 2019. Error bars reflect standard errors (n=4). Lowercase letters above the bars indicate significance between the specified depths ($p \leq 0.05$).

Figure 3. 17.

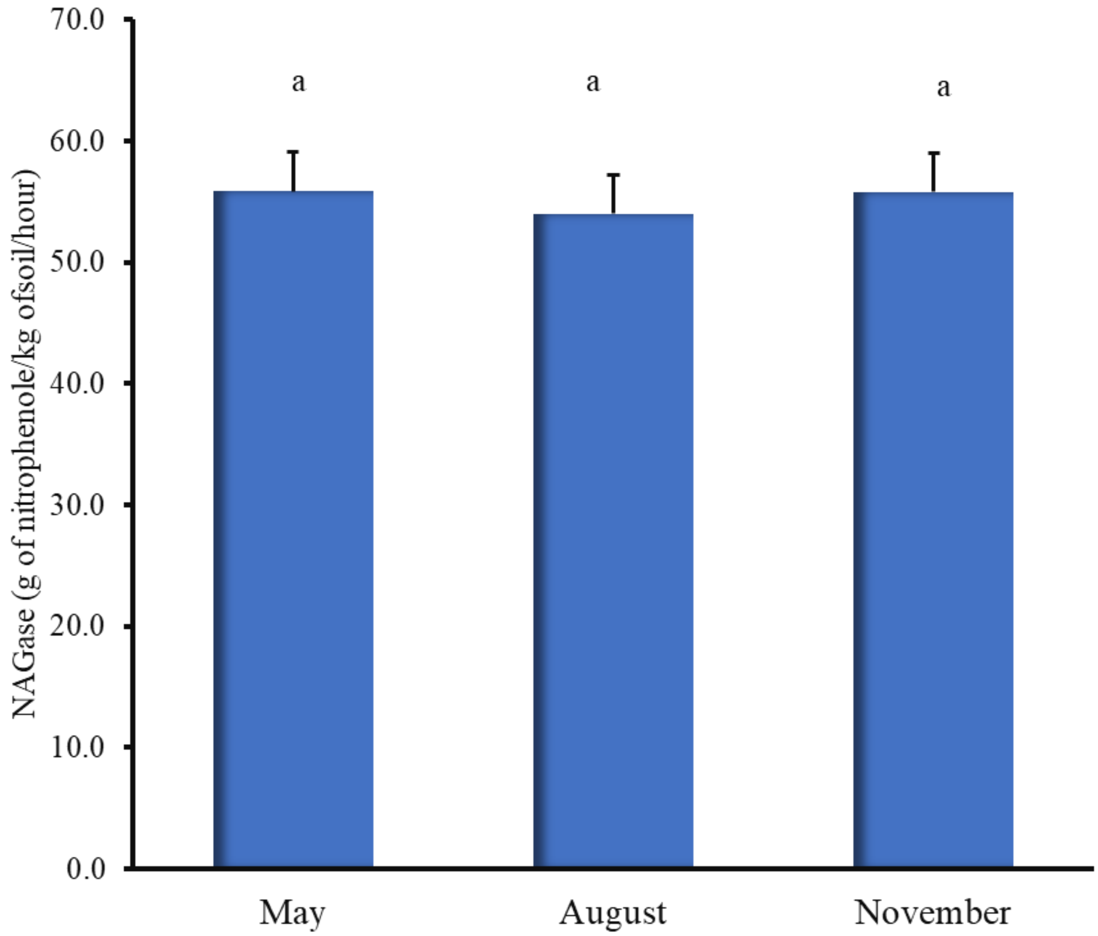
N-acetyl-beta-D-glucosaminidase activity (NAGase) at different dates of soil sample collection



Note: NAGase analysis was conducted on soil samples collected at a depth of 0-15 cm, from a corn silage field spanning the years 2019 to 2021. Error bars represent standard errors (n = 4). Different lowercases above the bars indicate a significant difference within date of sample collection (p ≤0.05).

Figure 3. 18.

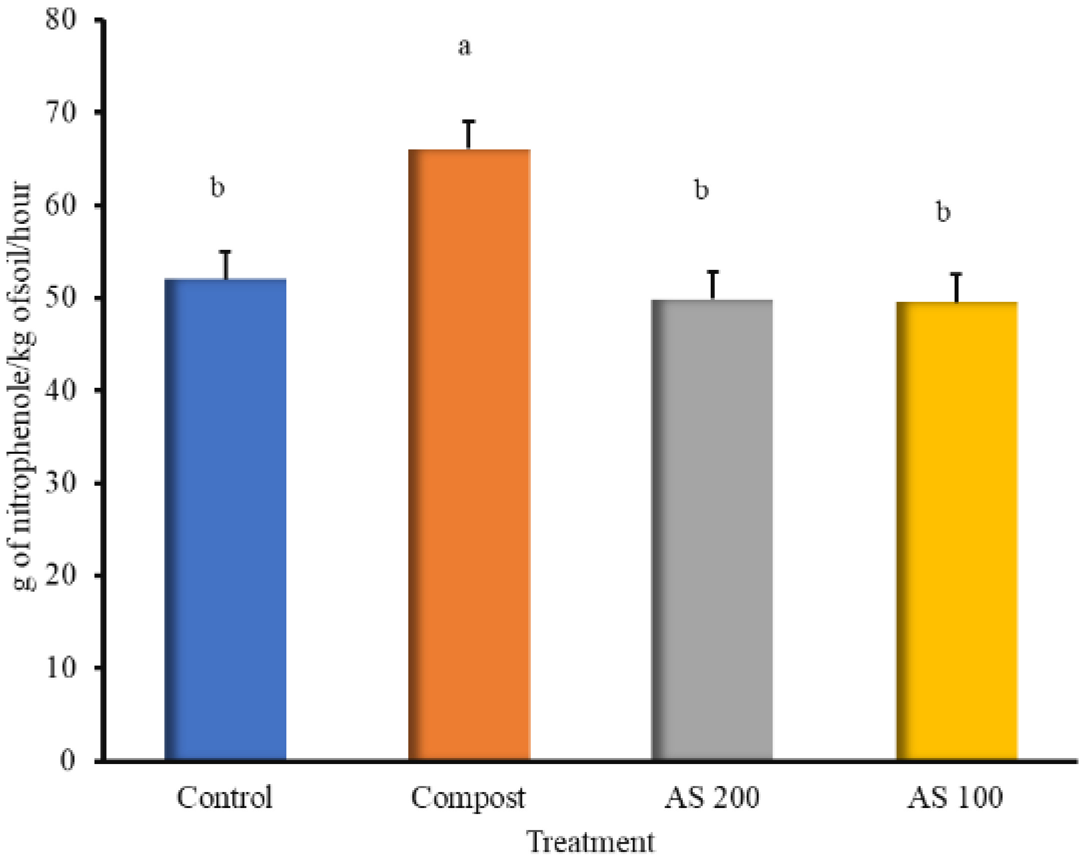
N-acetyl-β-D-glucosaminidase (NAGase) at different times of growing season



Note: Repeated measure was employed to analyze NAGase at different times of growing season (May, August, and November). Soil samples were collected at a depth 0-15 cm from 2019 to 2021. Error bars represent standard errors (n = 12). Different lowercases above the bars indicate as significant difference among seasons ($p \leq 0.05$).

Figure 3. 19.

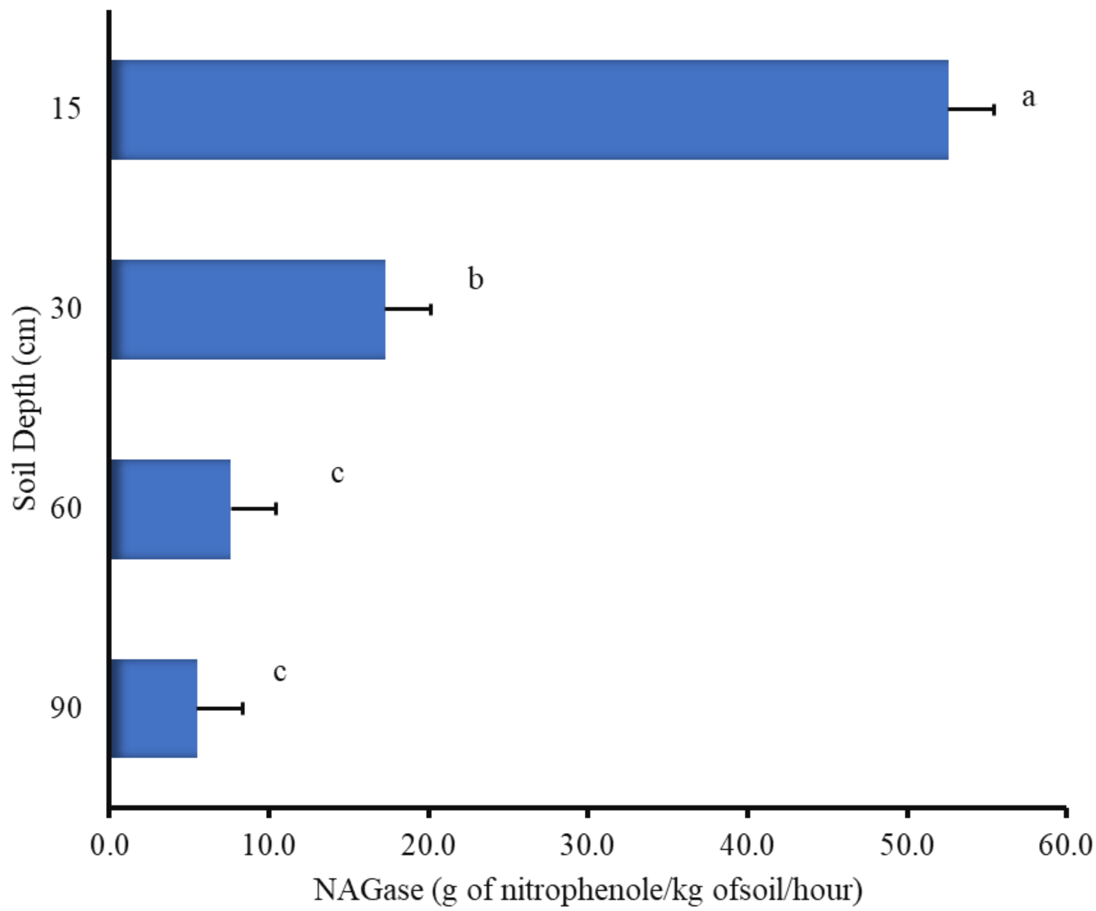
Effects of contrasting N source treatment on N-acetyl-β-D-glucosaminidase activity (NAGase)



Note: Repeated measures were employed to analysis the effects of contrasting N sources on NAGase over a decade of application. Soil samples were collected at a depth of 0-15 cm spanning the years 2019 to 2021. Error bars represent standard errors (n = 28). Different lowercases above the bars indicate a significant difference among treatments ($p < 0.05$).

Figure 3. 20.

N-acetyl-β-D-glucosaminidase (NAGase) at different soil depths



Note. Measurement of NAGase at various soil depths across treatments (0-15 cm, 15-30 cm, 30-60 cm, and 60-90 cm) in November 2019. Bars reflect standard errors (n=16). Lowercase letters above the bars indicate significance between the specified depths ($p \leq 0.05$).

Table 3. 1.

Net N mineralization (NetMin) at 14-day incubation (NetMin14) and 84-day incubation (NetMin84)

	NetMin14	**NetMin84**
Control	4.09 ± 0.63 a	16.6 ± 2.03 a
Compost	1.07 ± 0.63 b	17.7 ± 2.03 a
AS100	5.79 ± 0.63 a	11.0 ± 2.03 a
AS200	5.34 ± 0.63 a	13.9 ± 2.03 a

Unit is mg/kg soil

Note. The net mineralization was calculated from the 84-day incubation of soil samples taken in August 2019 from 0-15 cm depth. Different lowercases indicate a significant difference by treatment ($p \leq 0.05$).

Table 3. 2.

Potential mineralizable N (No), and N mineralization rate constant (k) from 84 days incubation.

	No	**k**	**R^2**
Control	19.4 ±2.9	0.03 ±0.008	0.56
Compost	33.3±19.9	0.009 ±0.007	0.62
AS100	18.0±1.8	0.034 ± 0.008	0.59
AS200	18.5±1.7	0.04 ±0.008	0.57

Note. Nitrogen mineralization potential (N_0, mg kg^{-1}) and rate constant (k, day^{-1}) were estimated with SAS OnDemand using a nonlinear least square curve-fitting technique (Proc NLIN, method Marquardt). This regression analysis assumed that N mineralization was a first order reaction $Nt = N_0(1 - e^{-kt})$. This model is run without considering the block effect. The incubated soil was sampled in August 2019 from 0-15 cm depth.

Table 3. 3.

Autoclave citrate extractable (ACE protein) and soil total N (STN)

Treatment	STN	ACE protein	ACE protein-N content
cm	mg N/g of soil	Protein mg/g of soil	mg N/ g of soil
Control	1.34 b	4.99 b	0.81 b
Compost	1.65 a	6.76 a	1.09 a
AS 100	1.19 b	4.94 b	0.80 b
AS 200	1.22 b	4.88 b	0.79 b
Average	1.35	5.39	0.87
Standard Error	0.05	0.12	0.02

Note. Analysis of STN and ACE Protein (0-15cm) from soil samples collected at a depth of 0-15 cm, spanning years 2019 to 2021. To calculate the ACE protein-N content, we used the conversion factor 6.25 under the assumption that protein contains 16% N, as proposed by Jones, (1941). Lowercase letters signify significant differences ($p \leq 0.05$).

Table 3. 4.

The mean and standard deviation (Std Dev) of inorganic N, ammonium-N, and nitrate-N.

Variable	Mean	Std Dev
Nitrate-N	0.97	1.29
Ammonium-N	0.77	0.67
Inorganic N	1.63	1.39

Note. Mean and Std Dev of inorganic N, ammonium-N, and nitrate-N were calculated across various treatments and across soil sampling time. Soil samples were collected at a depth of 0-15 cm from 2019 to 2021.

Table 3. 5

Pearson correlation matrix for N indicators.

	STN	***WEON***	***ACE***	***NAGase***	***Inorganic N***
STN	1.00				
WEON	0.43**	1.00			
ACE	0.46**	0.87***	1.00		
NAGase	0.41**	0.40**	0.63***	1.00	
Inorganic N	0.19	-0.2	-0.10	0.10	1.00

*Abbreviations: ACE, autoclavable citrate extractable protein; NAGase, N-acetyl β-D-glucosaminidase; STN, total soil nitrogen; WEON, water-extractable organic nitrogen. Inorganic N, inorganic N (ammonium+nitrate) *p≤0.05, **p≤0.01, ***≤0.001*

Note. Pearson correlation is calculated across various treatments and the time of soil sample collection. Soil samples were collected at depths of 0-15 cm from 2019-2021.

CHAPTER IV

EVALUATING THE EFFECTS OF CONTRASTING NITROGEN SOURCES ON SOIL HEALTH CARBON INDICATORS AND THE CARBON-NITROGEN RELATIONSHIP UNDER CORN SILAGE PRODUCTION

Abstract

Agricultural practices and the sustainability of our food production systems are intrinsically linked to the health and quality of the underlying soil. A comprehensive silage corn field study spanning nearly a decade from 2012 to 2021 was conducted in the semi-arid region of northern Utah, USA. The experimental design was a randomized complete block design with four treatments: a control with no nitrogen fertilizer applied (control), low ammonium sulfate (AS100) at 112 kg N $\mathrm{ha^{-1}\ year^{-1}}$, high ammonium sulfate (AS200) at 224 kg N $\mathrm{ha^{-1}\ year^{-1}}$, and steer manure compost (compost) at 224 kg total N $\mathrm{ha^{-1} year^{-1}}$. This study investigates the impact of different nitrogen (N) fertility sources on soil carbon (C) indicators and their association with soil health C indicators in a semi-arid corn silage production system. Compost treatment significantly increased cumulative C mineralization, C mineralization rates, and potential mineralizable carbon (C_0). Compost treatment increased total carbon (4.63%), soil organic carbon (23.04%), permanganate-oxidizable C (19.9%), water-extractable organic C (35.2%), and β-glucosidase enzyme activity (10.3%) compared to other treatments. Carbon indicators exhibited higher concentrations in the uppermost soil layer (0-15 cm) attributed to organic matter input, increased microbial activity, and conditions for organic C accumulation. Positive correlations were found between soil health C and N indicators, emphasizing interactions between soil C and N and the responsiveness of active organic

matter pools and enzyme activities. Positive shifts in soil health indicators underscore the advantages of combining compost with commercial fertilizers for improved fertility and sustainable soil management.

Introduction

The level of soil organic carbon (SOC) plays a significant role in soil health which is vital in determining crop productivity and environmental sustainability (Liptzin et al., 2022; Yang et al., 2020). SOC provides energy and nutrients for microbes, enhances soil aggregation, improves structural resilience, fosters microbial activity, enhances water retention, and protects against soil erosion (Anderson et al., 2022). SOC contains distinct pools with different chemical compositions and stages of decomposition (Bongiorno et al., 2019; Deb et al., 2015). SOC, the C stored in soil organic matter, is derived from plant and animal residues decomposition, root exudates, microorganisms and soil biota (Nelson & Sommers, 1996).

The measurement of SOC plays a crucial role in determining soil quality and productivity. However, SOC is not sensitive to management practices on short timescales (Awale et al., 2017; Cookson et al., 2008; West & Post, 2002). It often takes many years of soil analysis to see significant changes in this pool (Ghimire et al., 2014). Due to this challenge, labile C indicators have gained significant attention as they are more sensitive to management practices within shorter timeframes and therefore cost-effective. The labile C pool, the easily decomposable SOM pool, is the main food source for microorganisms and plays an important role in nutrient cycling, especially the turnover of SOM (Awale et al., 2017; Culman et al., 2013; Hoyle et al., 2006; Wander, 2004; Weil et al., 2003). As the result, the United States Department of Agriculture recommends soil measurements such as short-term C mineralization, water-extractable organic carbon (WEOC), permanganate-oxidizable C (POXC), and β-glucosidase enzyme activity (BG) as the indicators for estimating soil labile C (USDA NRCS, 2019). These measurements are used to predict the C and energy available to soil microbial communities and reflect soil health.

Carbon mineralization

Decomposition of organic matter in soil are affected by the available of N and C (Qiao et al., 2016). If the C:N ratio of organic matter is high, above 20-30, decomposition slows down and the material can remain in the soil for a long time (Geisseler et al., 2021; Harmsen & Kolenbrander, 2015; Myrold & Bottomley, 2015; Paul, 2007). On the other hand, with a low C:N ratio, decomposition accelerates and excess N is released, making it accessible to plants and other organisms (Diaz & Presley, 2019; Hoorman & Islam, 2010).

Carbon mineralization is a key component of the terrestrial C cycle, representing the process by which microorganisms break down organic substances into inorganic compounds through microbial degradation releasing carbon dioxide through respiration (Dai et al., 2017; Guo et al., 2019b). Soil potentially mineralizable organic C (C_0), also known as biodegradable C, is a measure of the total amount of organic matter within soil that can be rapidly decomposed by microorganisms (Guo et al., 2019b; Ribeiro et al., 2010). Laboratory incubations measuring respiration have been used to estimate the potentially mineralizable C pool (C_0). Predicting the C_0 pool allows researchers to monitor the decomposition of organic matter in a controlled environment (Busby et al., 2007; Calderón et al., 2004; Flavel & Murphy, 2006; Ribeiro et al., 2010). The actual value of CO_2 released is collected during the experiment and a first-order kinetic model is used to fit the experimental data. This model provides the estimate value of C_0 and first-order decomposition rate constant (k) (Alvarez & Alvarez, 2000; Beraud et al., 2005; De Neve et al., 2003; Fernández et al., 2007; Glaser et al., 2001; Ribeiro et al., 2010; Stanford & Smith, 1972).

Long term incubation is considered an accurate method to estimate C_0 pool. However, this method comes with certain drawbacks, including high costs, time-intensive procedures, and high labor demands, rendering them less suitable for high-throughput laboratory settings (Hurisso et al., 2018; USDA NRCS, 2019). While this measurement is not commonly included in widely used soil health assessments, many studies have utilized this approach to assess the effects of different farming practices on agricultural soils (Bernal et al., 1998; Fernández et al., 2007; Ghimire et al., 2019; Guo et al., 2023; Kaur et al., 2019). This is because it allows researchers to gain a more comprehensive understanding of SOC mineralization patterns under different land use practices (Guo et al., 2023).

Permanganate-oxidizable carbon (POXC)

Permanganate-Oxidizable Carbon (POXC), also known as active carbon, serves as an indicator of C availability to microorganisms. The labile carbon C fraction of SOM undergoes oxidation when exposed to a potassium permanganate solution (Culman et al., 2012; USDA, 2014; Weil et al., 2003). POXC or Active C originates from various sources, including fresh organic material, microbial biomass within the soil, particulate organic matter, and metabolized organic compounds such as carbohydrates (sugars) and proteins (amino acids) (USDA, 2014). The quantification of active C plays a pivotal role in assessing the dynamic labile C pool, reflecting practices that can either foster the accumulation and/or stabilization of organic matter, thus contributing to processes like C sequestration (Calderón et al., 2017; Hurisso et al., 2016). In addition, POXC has a strong positive relationship with different soil health indicators such as SOC, microbial activity including microbial biomass carbon, dehydrogenase activity and soil respiration (Duval et al., 2018; Lucas & Weil, 2012; Mandal, et al., 2011; USDA, 2014).

Water-extractable organic carbon (WEOC)

Water-extractable organic matter (WEOM) represents a soluble fraction of organic matter obtained through the agitation of soil samples with aqueous solutions under controlled laboratory conditions (Chantigny, 2003). It represents the most active and mobile portion of SOM (Sun et al., 2017) and plays a crucial role in the biochemical C cycle and significantly influences the regulation of microbial communities (Bausenwein et al., 2008; Chantigny, 2003). Despite WEOM constituting a small portion of soil organic matter (SOM), it exerts a substantial impact on various ecological processes in the soil (Sun et al., 2017).

Water extractable organic C (WEOC) is the C content of WEOM (Zhang et al., 2011). Unlike SOC, WEOC represents the readily available C pool, reflecting the energy and substrates that are more easily accessible to microorganisms (Haney et al., 2012; USDA NRCS, 2019). It is one of the most sensitive indicators for tracking changes in the labile pool (Haney et al., 2008, 2012, 2018; Sun et al., 2017). In addition, WEOC plays an important role in transformation of SOM and nutrient cycling, such as N mineralization.

Beta-glucosidase enzyme activity

Enzymes are is a valuable indicators of soil health because they are the mediators of decomposition of SOM and the transformations of both C and N in the soil (Bausenwein et al., 2008; Dick, 1994; Tabatabai, 1994; Yang et al., 2012). Beta-glucosidase (BG) (EC 3.2.1.21) enzyme is a key player in the C cycle, specifically involved in the degradation of cellulose and related molecules (Adetunji et al., 2017; Almeida et al., 2015). BG serves as a crucial enzyme that releases labile carbon and energy sources necessary for soil microorganisms, providing an early indication of changes in soil organic C (Adetunji et al., 2017; Deng & Popova, 2015). In addition, many studies use this enzyme for estimating labile C because it is sensitive to management practices, and the analysis procedure is both practical and straightforward (Adetunji et al., 2017; Almeida et al., 2015; USDA NRCS, 2019).

The measurement of soil C and N levels is not only essential for assessing soil health, but it also provides valuable insights into soil fertility, nutrient cycling, and the overall availability of essential nutrients required for the healthy growth of plants (Tong et al., 2023). For this reason, it is essential to consider the relationship between soil C and N when evaluating the impact of agricultural practices on soil health (Liptzin et al., 2023).

There are still limited studies on assessing soil C cycling in response to different N sources within the context of corn silage systems in semi-arid environments like Utah. Various factors, including soil type, climate, farming practices, and different agricultural settings, may influence these indicators and their interrelationships.

Our first objective is to assess the effects of various N fertility sources on a range of soil C indicators. The second objective involves exploring the relationships among these C indicators and evaluating their responses within a corn silage system under different N sources across multiple growing seasons. Our final objective is to examine the connections between soil health C and N indicators in response to different N sources. For instance, SOC serves as a crucial indicator for assessing the effects of farming practices on soil health. Nevertheless, SOC might exhibit insensitivity to immediate management practices, requiring an extended period to observe impacts. By exploring the correlation between SOC and other soil C indicators that are more rapidly responsive to management practices, there is potential for these alternative indicators to substitute for the analysis of SOC. This research assists in navigating the delicate balance between agricultural productivity and the long-term health of our soils and ecosystems.

Materials and Methods

Site description and experimental design

The research site used in this study is located at the USU Greenville Research Farm (41°45’56.6”N 111°48’52.2”W), in North Logan, Utah. The soil is a highly calcareous Millville silt loam (coarse-silty, carbonatic, mesic Typic Haploxeroll) with a pH of 8.2 (1:2 soil: water). The plots were established in 2011 to investigate N cycling and different N transformations under contrasting N management, as outlined in previous studies (Kakkar, 2017; Ouyang, 2016). Before, the field was utilized for conventional cultivation of small grains, involving an annual application of 70 kg of N per hectare in the form of urea. The experiment design in this study is randomized complete block design (RCBD) with four N treatments and four replications, totaling 16 plots (Figure A1). The treatments include a no N control (control), low ammonium sulfate at 112 kg N $ha^{-1}year^{-1}$ (AS100), high ammonium sulfate at 224 kg N $ha^{-1}year^{-1}$, (AS200), and steer manure compost at 224 total kg N $ha^{-1}year^{-1}$ (compost). Commercially sourced compost in this study was composed of composted steer manure, slaughter by-products, and woodchips (Miller companies LLC, Hyrum Utah. Compost nitrogen and dry matter content were determined yearly (Table B.1), and these parameters were used to apply the desired total nitrogen rate of 224 kg TN $ha^{-1}year^{-1}$, equivalent to 14.4 ± 1.8 metric ton of dried weight compost $ha^{-1}year^{-1}$. Each plot measures 9.1 m in length and 3.8 m in width, with a 1.2 m alley between each block and a 4.6 m alley between each plot within a block. Silage corn was planted every May since 2012 with the exception of 2017 when a cover crop of vetch was grown.

Field experiments

During spring of each year pre-plant soil samples were collected (May samples) from each plot using a Giddings probe with two cores per plot at depths of 0-15 cm, 15-30 cm and 30-60 cm. Soil was weighed, sieved (2 mm), gravimetric moisture determined, and air-dried before analysis for available P and K. Fertilization for P and K to meet the crop requirement was carried out according to the recommendations outlined in the Utah Fertilizer Guide for silage corn (James & Topper, 1993). The fertilizer applications and compost amendments took place in early May of each year. N, P, K fertilizers were applied to the field using an Edge guard mini push broadcast spreader (The Scotts Company LLC. USA). For compost treatment, the amendment was applied manually by buckets and shovel. Subsequently, bow rakes were utilized to evenly distribute the fertilizers and compost amendments within individual plots. Following this, the amendments were incorporated into the soil through tillage up to a depth of 30 cm within one day of application.

After the amendments were added and incorporated, the seedbed was prepared, and seed DEKALB® Corn Hybrids (glyphosate tolerant) were planted with a row spacing of 76 cm. Within each block, approximately 4 rows of silage corn were planted at a density of 50,000 plants per hectare using a John Deere planter. Throughout the growing season, an overhead sprinkler irrigation system was used to apply water on a weekly basis as required and as available. To control weed growth glyphosate herbicide, (Killzall, Fertilome, USA) containing 41% glyphosate and diluted to a concentration of 18.7 g L^{-1} with water) was applied at a rate of 1.12 kg ha^{-1}. This application was done once via broadcast before the corn reached a height of 30 inches.

Soil analysis

For this study soil sampling was conducted over multiple seasons from 2019 to 2021. In May, soil samples were taken at depths of 0-15 cm, 15-30 cm, and 30-60 cm. In August, samples were collected at depths of 0-15 cm and 0-30 cm, while in November, samples were collected at depths of 0-15 cm, 15-30 cm, 30-60 cm, and 60-90 cm.

Sampling methods varied: in May and November, two soil cores were collected using a truck-mounted soil sampler from the middle of each plot (Giddings Machine Co. Windsor CO, USA). In August, soil sampling was conducted using stainless steel soil sampler probes and a slide hammer soil probe. During August sampling, two soil cores were collected from each plot, with one taken in the row between corn plants and the other from between the rows of corn. These were collected into 1 bag and then samples were promptly transported to the laboratory. There, total soil weight was recorded and gravimetric soil moisture content was determined.

Subsequently, the remaining soil was meticulously mixed and passed through a 2 mm sieve before air-drying. After air-drying, a portion of the soil sample was finely ground with a ball mill to pass 0.25 mm (60 mesh) sieve for total C analysis using dry combustion using the Primacs SLC, (Skalar, Inc, GA, USA). The soil bulk density was determined utilizing the core method, which involved dividing the calculated dry weight of soil core by the volume of the core.

Long Term Potential Carbon Mineralization

The assessment of potential C mineralization involved an 84-day laboratory incubation. The laboratory incubations were done on field replicated soils and reflect the cumulative effects of repeated treatments of the soil over nine years. The soil samples were obtained from 0-15 cm depth in August 2019. Two large soil cores were taken from each of 16 plots (0-15 cm depth), one in the corn plant row and the other from between rows.

The two soil cores from each plot were composited and thoroughly mixed, sieved to 2mm, adjusted to 18% moisture content, and subsamples weighing 10 g (o.d. equivalent) were placed into plastic cups for incubation. Each individual plot was represented by duplicate samples (from the 16 plots 4 blocks with 4 treatments). The cups were placed inside quart mason jars, and one ml of deionized water was added to the bottom to avoid loss of soil moisture. These jars were then sealed using jar lids and rings. The lids for day 84 sampling were provided with septa for gas sampling. Time was noted for the start of incubation and all the jars were incubated in the dark at 25^0C. Headspace gas samples were taken by syringe from the jars with septa at 2, 7, 14, 21, 35, 49, 70, and 84 days. The first sampling is at 2 days to prevent oxygen depletion in the early very rapid respiration phase after mixing and wetting. A 10 mL syringe was used to sample the headspace, removing a 6 mL gas sample that was injected into a sealed evacuated sample vial, over pressurizing the vial. All jars were aerated after sampling, additional water added as needed, and then resealed for further incubation. A 1-ml sample from the vial was injected and analyzed by gas chromatography with helium carrier gas at a 20 mL min^{-1} flow rate with a thermal conductivity detector as described previously (Ouyang 2016) . This approach allowed us to assess potential C mineralization over time.

The cumulative amount of SOC mineralization refers to the total amount of soil CO_2-C released from the beginning of incubation time till the sampling day and then until the end of the incubation (84 day). The first order kinetic model was used to describe the C mineralization process (Ribeiro et al., 2010; Stanford & Smith, 1972).

$$C_t = C_0(1 - e^{-kt})$$

Where C_t is the measured cumulative carbon mineralization in mg C kg^{-1} of soil after time t, C_0 is the soil potential mineralizable organic C in mg C kg^{-1} of soil; k is the rate constant of C mineralization, d^{-1}; t is the time (days) of incubation. A non-linear least-squares approach was used to estimate the C_0 and k parameters.

Soil Organic Carbon

Soil organic carbon determinations were complicated in this highly calcareous soil so that methods for soil carbon analysis were adapted to improve their accuracy. SOC analysis was conducted using a loss on ignition method adapted from procedures outlined in previous work (Bojko & Kabala, 2014; Hoogsteen et al., 2015; Wotherspoon et al., 2015). Two subsamples are analyzed, with one set being assessed for total carbon (C) and the other for inorganic carbon. Basically, 150-200 mg of the finely ground soil is weighed into a crucible cup and combusted at 1050 ºC to determine the total carbon content, as specified by the manufacturer (PrimacsSLC, Skalar, Inc, GA, USA). From the same sample vial, another 150-200 mg of the finely ground sample is weighed into a separate quartz crucible cup, which is then placed in a muffle furnace at 550ºC for 5 hours to combust and eliminate organic C. This sample is subsequently analyzed for total C using the PrimacsSLC equipment as above. The assumption here is that all soil organic matter is removed at temperatures below or equal to 550°C (Hoogsteen et al., 2015). SOC concentration is then determined by difference between the total C and total inorganic C in the combusted sample for each sample individually (Tabatabai & Bremner 1970).

Beta-glucosidase (BG)

The BG enzyme assay is adapted from USDA, (2019) and Eivazi & Tabatabai, (1988). To perform the assay, 0.5 g of air-dried soil is mixed with a solution containing 1 ml of p-nitrophenyl-β-D-glucopyranoside (0.05M) and 2 ml of start buffer (MUB, pH 6.0). The mixture is then incubated for 1 hour at 37°C. To stop the reaction, 0.5 ml of 0.5M $CaCl_2$ and 2 ml of 0.1 M THAM (pH 12.0) are added. The absorbance is measured at 405 nm.

Permanganate-oxidizable C (POXC)

POXC, also known as Active Carbon, was extracted, and analyzed following the procedure of Weil et al. (2003) with some modifications. In summary, 2.5 g of air-dried soil was placed in a polypropylene tube. Then, 18 ml of demineralized water and 2 ml of 0.2 M K_2MnO_4 were added. The tube was shaken for 2 minutes at 120 rpm and left undisturbed on a lab bench for 8 minutes to complete the oxidation reaction. Afterward, 0.5 ml of the solution was transferred to another tube containing 49.5 ml of sterile deionized water to halt the reaction. The absorbance (Abs) of each sample was measured at 550 nm.

POXC was calculated according to Weil et al., (2003):

$$\mathrm{POXC\ (mg\ kg^{-1})} = [0.02\ \mathrm{mol\ L^{-1}} - (\mathrm{a} + \mathrm{b} * \mathrm{Abs})] * (900\ \mathrm{mg\ C\ mol^{-1}})(0.02\ \mathrm{L\ solution\ Wt^{-1}})$$

where $0.02\ mol\ L^{-1}$ is the concentration of the K_2MnO_4 solution, *a* is the intercept and *b* is the slope of the standard calibration curve, 9000 mg is the amount of carbon oxidized by 1 mol of MnO_4 changing from Mn^{+7} to Mn^{+4} , 0.02 L is the volume of the K_2MnO_4 reacting with the samples, and Wt is the mass of soil in kg used for the reaction.

Water extractable organic carbon (WEOC)

WEOC was analyzed based on methods from previous studies (Haney et al., 2012; Jones & Willett, 2006). Initially, 6 grams of soil were mixed with 30 ml of deionized water. The mixture was then clarified by centrifugation at 6000 rpm for 8 minutes to isolate water extractable (dissolved) C. Next, the extractant was filtered through a 0.45 µm PES filter. Water extractable total carbon (WETC) and water extractable inorganic carbon (WEIC) were analyzed using a Shimadzu TOC-L/TNM-L system (Shimadzu, Kyoto Prefecture, Japan) following the manufacturer's recommendations. WEOC was calculated by subtracting WEIC from WETC.

Data analysis

Statistical analysis was conducted using SAS OnDemand for Academics, which can be accessed at https://www.sas.com/en_us/software/on-demand-for-academics.html. The dataset comprises soil carbon indicators data from 2019 to 2021. Initially, a two-way ANOVA was employed to assess the impact of treatments on each soil C index (TC, SIC, SOC, POXC, WEOC and BG) at different time of soil collection. All statistical analyses were conducted with a confidence level of 95% ($P \leq 0.05$). Subsequently, repeated measures analysis was performed to evaluate the overall treatment effects on those soil C metrics across the years 2019 to 2021.

Soil potential mineralized organic carbon (C_0, mg kg^{-1}) and rate constant (k, day^{-1}) were estimated with SAS OnDemand using a nonlinear least square curve-fitting technique (Proc NLIN, method Marquardt). This regression analysis assumed that C mineralization was a first order reaction $Ct = C_0(1 - e^{-kt})$.

To investigate the strength of the relationship of soil C metrics, Pearson's correlation was employed. This analysis was executed using PROC CORR to explore the degree of association between soil carbon indicators. Furthermore, a comprehensive statistical analysis was conducted to investigate the relationship between SOC and several response variables, including POXC, WEOC, and BG. To achieve this, the PROC MIXED procedure in SAS OnDemand was selected due to its suitability for modeling mixed-effects data structures, which were inherent in our dataset. This modeling approach was particularly relevant because the data spanned multiple years and encompassed the influence of various treatments, both of which could potentially impact the relationship between SOC and the response variables. The analysis was to understand the impact of two key factors, treatment and year, on the relationships between our chosen dependent variables, namely POXC, WEOC, and BG, with respect to the predictor variable SOC. Additionally, we considered the influence of the block variable as a random effect, recognizing its potential contribution to the observed variability in our data.

Results

Soil carbon

In this study, we assessed the effect of different N sources on soil C content at a depth of 0-15 cm from 2019 to 2021. TC is a measure of the sum of both organic and inorganic forms of carbon in the soil. TC ranged from 76.6 to 65.2, with an average of 70.9 ± 2.41 g kg^{-1} of soil. The variation of SOC was from 32.6 to 12.2 with average of 19.4 ±3.71 g kg^{-1} of soil. Soil inorganic carbon (SIC) varied from 57.2 to 42.0, with an average of 51.5 ±2.85 g kg^{-1} of soil. The mean estimate of SOC in May (pre planting), during growing season (August) and after harvesting (November) were 19.3, 18.2, and 21.5 ±1.06, g C kg^{-1} of soil, respectively (Figure 4.1). The highest SOC was in November and lowest in August.

The results from the repeated measures analysis showed that the TC and SOC at a depth of 0-15 cm were found to be significantly influenced by the different N source treatments from 2019 to 2021, as depicted in Figure 4.2. However, the SIC levels remained unaffected by these treatments. Total C under control, compost, AS100 and AS200 are 69.6, 73.1, 70.5, and 69.6 ±0.68 g kg^{-1} of soil , respectively. SOC under control, compost, AS100 and AS200 are 17.4, 21.9, 17.9, 18.0 ±0.97 g kg^{-1} of soil, respectively (Figure 4. 3). The compost treatment shows a significant increase in both TC and SOC with approximately a 4.63% increase in TC and a 23.04% increase in SOC, compared to the average of the other treatments.

In November 2019, we collected soil samples to determine the soil C content (TC, SOC, and SIC) at different depths. In this study, TC in 0-15cm, 15-30cm, 30-60cm and 60-90cm were 69.6, 71.0, 76.0, and 79. 6 g kg^{-1}, respectively (Figure 4.3). Total C at depth 60-90 cm is highest, followed by TC in depth 30-60 cm. The results of the study indicated that there was a significant difference in TC values at different depths. Specifically, TC values increased by approximately 2.01% at 15-30cm, 9.20% at 30-60cm, and 14.37% at 60-90 cm when compared to the 0-15cm depth. These findings highlight the depth-dependent nature of carbon distribution in the soil.

In this study, SIC in soil depth 0-15 cm, 15-30 cm, 30-60 cm and 60-90 cm are 51.5, 50.7, 61.9, and 73 g kg^{-1}, respectively (Figure 4. 3). Specifically, SIC content exhibited approximately 0.99% increase at 15-30cm, 23.31% increase at 30-60cm, and a notable 46.82% increase at 60-90cm when compared to the 0-15cm depth. This finding suggested that SIC increases with increasing soil depth as expected.

The SOC content is also significantly different due to the soil depth profile. The SOC in depth 0-15cm, 15-30cm, 30-60 and 60-90cm are 19.5, 18.6, 14.1, and 5.9 g kg^{-1}, respectively (Figure 4.3). The SOC content in the depth of 0-15cm is not significantly different from the SOC content in the depth of 15-30cm. However, both of these depths show significantly higher SOC content compared to the depths of 30-60cm and 60-90cm, as shown in Figure 4.3. At the depth of 15-30cm, there was an estimated decrease of approximately 4.62%, followed by a significant decrease of 27.7% at 30-60cm, and a remarkable decrease of 69.7% at 60-90cm, in comparison to the SOC content at the 0-15cm depth.

Soil bulk density

Contrasting N sources did not have significant impacts on soil bulk density at different soil depths. Bulk density at depth 0-15 cm was significantly higher than the bulk density at deeper soil depths. Soil bulk density at depth 0-15 cm, 15-30 cm, 30-60 cm, and 60-90 cm were 1.33, 1.20, 1.45, and 1.14 ±0.03 g cm^{-3}, respectively (Table C1).

Carbon mineralization

Carbon mineralization is a complicated, microbially driven process influenced by environmental factors. In our study, we conducted an 84-day incubation to comprehensively explore this process and generate data for quantifying the effects of different N sources on C mineralization, particularly the potential for C mineralization and its rate. However, we acknowledge that 84-day incubations can be labor-intensive and costly. Consequently, many researchers have conducted short-term C mineralization studies with varied incubation durations. In our research, we opted for a shorter 14-day incubation period. This choice is supported by findings from Stanford et al. (1974), which demonstrated that it is possible to reliably estimate potential mineralizable N (N_0) from the quantities of nitrogen mineralized during 2-week incubations following preliminary 1 to 2-week incubations, using a first-order equation. This suggests the potential for predicting C_0 (soil potential mineralized organic carbon) and k (the rate constant of mineralization) from a 2-week incubation period as well. Our study builds upon this foundation, aiming to better understand and model the C mineralization process.

Long term carbon mineralization (84 days of incubation)

In our study, the cumulative C mineralization data for all the treatment fit well to the first order kinetic model: $\mathrm{Ct} = \mathrm{C_0}(1 - \mathrm{e}^{-\mathrm{kt}})$. The cumulative mineralized C at day 2 was 15.5 mg $\mathrm{C\,kg^{-1}}$of soil and increased to 177.7 mg $\mathrm{C\,kg^{-1}}$of soil at day 84 of incubation (Figure 4.4). The cumulative mineralized C estimates for the period spanning from day 0 to day 84 of incubation for control, compost, AS100 and AS200 were 92.8, 159.8, 85.2, and 72.8 ±10.5 mg $\mathrm{C\,kg^{-1}}$of soil, respectively. The application of ammonium sulfate fertilizer (AS100 and AS200) did not have significant impacts on the cumulative mineralized carbon when compared to the control treatment. The soil treated repeatedly with compost exhibited the highest cumulative mineralized C in the incubation study.

On day 2 of incubation, the carbon mineralization rate was notably high at 7.7 $\mathrm{mg\,kg^{-1}day^{-1}}$ (Figure 4.5). However, by day 84, this rate had decreased to 0.88 $\mathrm{mg\,kg^{-1}day^{-1}}$, marking an approximately 88.6% reduction from the initial rate. The estimated C mineralization rate 84 days incubation using repeated measure showed that the Control, Compost, AS100 and AS200 were 2.83, 4.81,2.66, and 2.21 ±0.34 $\mathrm{mg\,C\,kg^{-1}day^{-1}}$. The rate of C mineralization observed in the compost treatment was approximately 87.2% higher than the average of the other treatments (Control, AS100, and AS200).

The modeled $\mathrm{C_0}$ from 84 days of incubation under Control, Compost, AS100 and AS200 treatments were 158.0, 311.7, 161.5, and 138.7 ± 23.1 mg $\mathrm{C\,kg^{-1}}$of soil, respectively (Table 4.1). Compost treatment had the highest $\mathrm{C_0}$ while $\mathrm{C_0}$ under ammonium sulfate treatments (AS100 and AS200) were not significantly different than control (Table 4.1). The rate constant of C mineralization (*k*) was not affected by the treatment. The average *k* value for this experiment was 0.03 per day.

Short-term carbon mineralization (14 days incubation)

In this study, data obtained from the two-week incubation were used to estimate C mineralization, and the results were fitted to the first-order equation $\mathrm{Ct14} = \mathrm{C}_{014}(1 - \mathrm{e}^{-\mathrm{k14t}})$. The estimate value of C_{014} for the control, compost, AS100, and AS200 treatments were as follows: 81.1, 205.0, 73.8, and 63.4 ±16.6 mg kg^{-1} of soil, respectively (Table 4.1).

In this study, we observed the rate constants of decomposition (k14) as follows: 0.10, 0.06, 0.12, and 0.10 ±0.02 per day for the control, compost, AS100, and AS200 treatments, respectively. Interestingly, the treatments did not exert a significant influence on the value of *k*. This lack of significant effect may be attributed to the relatively small sample size and the considerable variability in the data.

The Pearson correlation analysis revealed significant and moderately strong correlations between the C_0 determined from the 14-day incubation and C_0 from the 84-day incubation (r = 0.74) (Table 4.2). Additionally, the rate of C mineralization at day 14 displayed correlations with both the rate of C mineralization at day 84 (r = 0.50) and cumulative C mineralized at day 84 (r = 0.81) (see Table C2 for details).

In this study, the potential mineralizable C obtained from the short-term incubation exhibited correlations with various soil parameters, including soil TC, soil total N (STN), soil ACE protein, NAGase enzymes, water-extractable nitrogen and -carbon (see Table C3 for details).

Permanganate-Oxidizable Carbon (POXC)

Permanganate oxidizable carbon (POXC) was analyzed from soil collected at depth 0-15 from 2019 to 2021. At each date of taking sample, the POXC from compost treated soil was significantly higher compared to other treatment except in August 2019, and May 2021. The average POXC in this experiment is 721.6 ± 113.8 $\mathrm{mg\ POXC\ kg^{-1}}$soil with the maximum value of 1197.1 and minimum value of 575.9 $\mathrm{mg\ POXC\ kg^{-1}}$soil (Figure 4.6).

Repeated measure of the POXC showed the estimate average value of POXC under control, compost, AS100 and AS200 were 693.0, 824.2, 673.4, and 695.8 ±19.0 $\mathrm{mg\ POXC\ kg^{-1}}$soil, respectively (Figure 4.7). Compost significantly increased the POXC but the ammonium sulfate treatments did not affect POXC compared to control treatment. POXC was increased by approximately 19.9 % under compost compared to the average of the other treatments.

The level of POXC at different depths was analyzed using the soil samples collected from November 2019. The level of POXC varies according to soil depth. The POXC at 0-15, 15-30, 30-60, and 60-90 cm were 814.3, 677.2, 470.8, 376.6 ±41.8 $\mathrm{mg\ POXC\ kg^{-1}}$soil, respectively (Figure 4.8). At depths 15-30 cm, 30-60 cm, and 60-90 cm, the POXC content was 16.8%, 43.2%, and 53.7% lower than at a depth of 0-15 cm.

Water extractable organic carbon (WEOC)

The WEOC was analyzed using the soil samples collected in spring (May) before planting, during the summer growing season (August), and in the fall (November) after harvesting, spanning the period from 2019 to 2021. The level of WEOC exhibited variation, ranging from 93.4 to 238.9 $\mathrm{mg\ kg^{-1}}$of soil, with an average WEOC value of

136.28 ±30.2 mg kg^{-1} of soil. The response of WEOC to various fertilizer treatments varied over different time points when soil samples were taken (Figure 4.9). In most instances, the compost treatment led to an increase in WEOC levels. However, there were exceptions, notably in November 2019 and May 2021, where no significant difference was observed between the compost and control treatments. Although the timing of sample collection had an impact on the WEOC levels, there were no significant differences in WEOC levels between spring, summer, and autumn when all years were considered together (Figure 4.10).

The WEOC repeated measure for soil collected at depth 0-15cm from 2019 to 2021 demonstrated that the control, compost, AS100, and AS200 treatments had values of 132.7, 173.6, 130.1, and 122.3 ± 3.58 mg kg^{-1} of soil, respectively (Figure 4.11). Compost treatment significantly increased the WEOC, but ammonium sulfate treatments did not. The percentage increase in WEOC under the compost treatment compared to the average of the other treatments (control, AS100, and AS200) was approximately 35.2%.

WEOC also was analyzed at different soil depths, using soil samples collected in November 2019. WEOC accumulated at the topsoil and decreased deeper in the soil profile. The level of WEOC at 0-15, 15-30, 30-60 and 60-90 cm were 142.6, 96.6,73.9, and 53.9 ±3.42 mg kg^{-1} of soil, respectively (Figure 4.12). The WEOC content was 32.4% lower at a depth of 15-30 cm, 48.1% lower at a depth of 30-60 cm, and 62.2% lower at a depth beyond 60 cm, all in comparison to the soil depth of 0-15 cm.

β-glucosidase (BG) enzyme activity

In this study, we conducted an analysis of BG in soil samples collected at a depth of 0-15 cm over the period spanning from 2019 to 2021. The value of activity of BG was observed to flucuate over time (Figure 4.13). Specifically, BG activity did not show a significant response to treatment from August 2019 until August 2020. However, by August 2021, the treatment began to affect BG activity, with significantly higher levels observed under compost and both ammonium sulfate treatments when compared to the control treatment (Figure 4.13). Across different time sample collection, the average BG activity in May, August, and November were measured at 192.4, 199.1, and 182.9 ± 8.7 $\text{mg PNP kg}^{-1}\text{of soil hour}^{-1}$ respectively (Figure 4.14).

Results of the repeated measures analysis on soil samples obtained at a depth of 0-15 cm between 2019 and 2021 demonstrated that BG activity varied under control, compost, AS100, and AS200 treatments, measuring 177.3, 207.8, 201.7, 185.5 ±8.8 $\text{mg PNP kg}^{-1}\text{of soil hour}^{-1}$, respectively (Figure 4.15). The BG activity was significantly higher in the compost treatment compared to the control treatment.

BG activity was also analyzed at different soil depths, using soil samples collected in November 2019. BG activity was measured at depths of 0-15 cm, 15-30 cm, 30-60 cm, and 60-90 cm, resulting in values of 187.5, 49.9, 24.5, and 18.6 ± 6.8 $\text{mg PNP kg}^{-1}\text{of soil hour}^{-1}$, respectively (Figure 4.16). The BG activity at depths of 15-30 cm, 30-60 cm, and 60-90 cm was 73.3%, 86.9%, and 90.1% lower, respectively, compared to the activity at a depth of 0-15 cm.

Relationship between soil carbon and nitrogen indicators

Pearson correlation between soil carbon indicators

In our extensive three-year study spanning from 2019 to 2021, we gathered soil samples during the months of May, August, and November. This extended duration allowed us to conduct a thorough Pearson correlation analysis, revealing significant fluctuations in the relationships between key soil carbon indicators. Specifically, we observed variations in the correlations between SOC and other soil carbon indicators such as POXC, WEOC, and BG, which ranged from very low to high. The relationship between TC and SOC consistently remained very high, irrespective of the timing of soil sampling (Table C4).

To address the variability observed across years, we employed a year-wise averaging approach in this study. This process entailed computing the averages of the soil samples collected within each year. This strategy yielded notable improvements in the correlations between soil indicators, enhancing the overall stability of the assessment of their relationships.

Soil TC and SOC are postively correlated with all the soil C indicators (WEOC, POXC, and BG) (Table 4.3). Soil TC is strongly correlated with SOC and POXC ($r=0.7$, $p\leq0.001$). SOC was also strongly correlated with WEOC ($r=0.7$, $p\leq0.0001$). For all other pairs of indicators in this experiment, except BG and POXC, the correlations ranged from 0.5 to 0.8 which agreed with the finding from previous study (Liptzin et al., 2022) (Table 4.3).

Pearson correlation between soil carbon and soil nitrogen indicators

The relationships among various soil C and N parameters were examined in this study. Soil C, represented by both TC and SOC, exhibited strong positive correlations with STN, with correlation coefficients of 0.7 and 0.6, respectively ($p < 0.0001$ for both) (Table 4.3). Additionally, SOC displayed strong positive associations with ACE protein and WEON, with correlation coefficients of 0.8 and 0.7, respectively ($p < 0.0001$ for both). In contrast, SOC showed a moderate positive correlation with NAGase, with an r-value of 0.4 ($p < 0.006$). This result showed that soil carbon was positively and strongly correlated with the soil health N indicators TN, ACE protein, and WEON, but not NAGase, which exhibited a moderate correlation (Table 4.3).

STN shows a moderate correlation with POXC ($r = 0.5$, $p < 0.0002$) and a strong correlation with WEOC ($r = 0.6$, $p < 0.0001$). However, there is no significant correlation between STN and BG (Table 4.3).

Water-extractable organic nitrogen (WEON) and WEOC exhibit a strong positive correlation ($r = 0.8$, $p < 0.0001$), and both are strongly correlated with SOC ($r = 0.7$, $p < 0.0001$). Furthermore, WEOC and WEON are positively correlated with STN. In contrast, no significant correlation was observed between BG and NAGase in this study (Table 4.3).

Effects of treatment on the relationship between soil organic C (SOC) and other soil c indicators

In our study, we conducted a mixed-effects model analysis using the PROC MIXED procedure to examine the relationship between SOC with soil C indicators such as POXC, WEOC, and BG and other various factors, including treatment, and year.

A. Impact of treatments on the relationship between SOC and POXC

The mixed-effects model analysis revealed that the relationship between SOC and POXC was influenced by N treatment (see Table C5). Significant interactions were observed between SOC and treatment, with the Compost treatment demonstrating exceptional statistical significance ($p \leq 0.0001$). Specifically, the coefficient estimates for the interaction between SOC and the Compost treatment level was 0.005302 ($p \leq 0.0001$), indicating that a one-unit increase in SOC under the Compost treatment condition, corresponded to an approximate 0.005302-unit increase in POXC levels, while controlling for other variables (see Table C5).

Conversely, the interaction effects between SOC and the AS100 and AS200 treatment levels were not statistically significant ($p > 0.05$). This suggests that there is insufficient evidence to conclude that the relationship between SOC and POXC levels differs significantly for the AS100 and AS200 treatments compared to the baseline level.

B. Impact of treatments on the relationship between SOC and WEOC

The relationship between SOC and WEOC is significantly influenced by the treatment (see Table C6). Notably, only the interaction with the Compost treatment demonstrates statistical significance ($p< 0.0001$).

The coefficient estimates for the interaction between SOC and the Compost treatment level is 0.001994 within the Compost treatment condition. This suggests that, under the Compost treatment, a one-unit increase in the SOC variable corresponds to an approximate 0.001994-unit increase in the response variable WEOC assuming all other factors remain constant (see Table C6).

In contrast, the interaction effects between SOC and both the AS100 and AS200 treatments are not statistically significant (see Table C6). This implies that there is no strong evidence to conclude that the relationship between SOC and WEOC differs significantly for the AS100 and AS200 treatments compared to the baseline.

C. Impact of treatments on the relationship between SOC and BG

The analysis suggests that the effects of different treatments on the relationship between SOC and BG activity is not statistically significant. Specifically, the interaction effects between SOC and different treatments (AS100, AS200, Compost, and Control) do not exhibit significant differences, as indicated by their p-values, which are all above the significance level ($p > 0.05$) (see Table C7). Furthermore, the Type 3 test of fixed effects for the SOC*Treatment" interaction is not significant ($F = 1.62$, $p = 0.1997$). These results collectively indicate that, within the scope of this analysis, there is no strong evidence to suggest that the different treatments have a significant impact on the relationship between SOC and BG activity (see Table C7).

Discussion

Contrasting N sources effects on Soil carbon

The contrasting N sources had significant impacts on soil C. Our research demonstrated that the application of compost led to a notable increase in TC and SOC. Specifically, SOC experienced a remarkable 23.04% rise under the Compost treatment in comparison to the average of the other treatments (Fig 4.3). This observation aligns with previous studies that have also shown a significant enhancement in SOC with the use of organic fertilizers such as compost (Allam et al., 2022; Das et al., 2023; Gross & Glaser, 2021; Rambaut et al., 2022). In contrast, the application of inorganic fertilizers like ammonium sulfate did not have any discernible impact on TC or SOC (Rambaut et al., 2022).

In addition to the influence of N-source on SOC, the study also revealed that SOC is impacted by seasonal changes, a finding consistent with other studies (Babur et al., 2020; Liu et al., 2019; Wu et al., 2021). This study observed the highest mean of SOC level was in November and lowest in August (Figure 4.2). During November and May, the SOC may have increased due to lower temperatures that slowed down the mineralization of SOC (Wu et al., 2021; Yu et al., 2022). Multiple studies have shown that soil C levels are influenced by a variety of factors, including seasonal fluctuations, land use, and climate variables (Babur & Dindaroglu, 2020; Hobley & Wilson, 2016; Rolando et al., 2021).

For the vertical distribution of soil C, we found that soil C level was significantly different at different soil depths (Figure 4.3). In this study, we observed TC increases as soil depth increased, attributed to the significant accumulation of SIC deeper in the soil profile (Figure 4.3). The analysis of SIC distribution in soil profiles shows significant increases in SIC as depth increases which agrees with the previous observations especially in arid and semi-arid environments (Dold et al., 2021; Du & Gao, 2020; Guo et al., 2016; Naorem et al., 2022; Sharififar et al., 2023).

Soil inorganic C is considered as the primary carbon reservoir in arid and semi-arid regions, primarily due to the substantial accumulations of carbonates (Bai et al., 2017). This phenomenon is fostered by the favorable conditions found in dryland ecosystems, which promote calcification and the formation of secondary carbonates (Guo et al., 2016; Naorem et al., 2022; Pilli et al., 2023).

The storage and distribution of SIC in dryland soils constitute a complex process that involves a myriad of interactions among various factors. These factors encompass climate, geological processes, land use patterns, agricultural management practices, irrigation, soil types, inherent soil characteristics, soil-related biological factors, soil moisture levels, salinity, and soil temperature (Bai et al., 2017; Naorem et al., 2022;

Wang et al., 2023). Besides that, the quantity and quality of irrigation water applied in dry regions directly influences the depth of water infiltration, subsequently affecting the rate of inorganic carbon transport within the soil profile. However, there are limitations on how deeply soil water can penetrate, potentially impeding the downward movement of inorganic C (Bai et al., 2017; Naorem et al., 2022). This outcome may significantly impact the temporal and spatial variations in the distribution of inorganic C content within the soil profile.

Although SIC was observed to increase with greater soil depth, the concentration of SOC exhibited a decrease as soil depth increases (Figure 4.3). These patterns align with findings from various studies (Dold et al., 2021; Du & Gao, 2020; Ghosh et al., 2018; Guo et al., 2016; Henneron et al., 2022; Kramer & Gleixner, 2008; Rolando et al., 2021). The study of Rolando et al., (2021) documented that the greatest SOC concentrations are found in the A horizon (0-30 cm) because organic matter inputs and biological activity are generally the highest close to the surface.

The higher concentration of SOC in the topsoil, compared to deeper layers, can be attributed to various factors, including contributions from plant roots and litter, as well as active interactions among plants, microbes, and the soil (Alhassan et al., 2018; Chen et al., 2017; De Deyn et al., 2008; Jobbágy & Jackson, 2001; Qi et al., 2021). This elevation in SOC levels in the topsoil not only fosters greater biological activity but also bolsters the soil's resilience in the face of extreme weather conditions (Chellappa et al., 2021). It is important to note, however, that the distribution of SOC within different soil profiles is primarily influenced by site-specific factors, notably bulk density, soil type, land use, tillage and climate conditions (Hobley & Wilson, 2016).

Contrasting N sources effects on Carbon Mineralization

In this study, long-term incubation study showed that only compost amendments notably enhanced the release of CO_2-C. We observed that the rate of C mineralization exhibited an initial rapid phase, followed by a gradual decrease over time until it approached a stable state (Figure 4.5). Previous studies also found similar trends (Coban et al., 2016; Grunwald et al., 2016; Guo et al., 2019a; Guo et al., 2019b). Respiration of C in the early stages of incubation is dominated by release from an active pool of the most accessible and easily decomposable organic substrates leading to rapid decomposition until rates decline over time as the active pool diminishes (Nagy et al., 2018). The exhaustion of readily decomposable substrates is one key reason for the declining mineralization rates (Guo et al., 2019b; Nagy et al., 2018).

Using compost as a N source increased C_0, indicating increased availability of organic matter for microbial breakdown. Previous studies have also shown the advantages of using organic fertilizers like compost to increase C_0 (Autret et al., 2020; Carpenter-Boggs et al., 2000; Guo et al., 2019b; Kalala et al., 2020). Importantly, this process also facilitates the release of essential nutrients, rendering them readily accessible for plant growth (Bot & Benites, 2005; Wang et al., 2023).

We observed that the *k* value did not show significant differences among the treatments (Table 4.1). This finding contrasts somewhat with the results of other studies conducted in humid areas, which showed that organic amendments lead to higher values of *k* than inorganic fertilizers (Cheng et al., 2016; Guo et al., 2019a). It is important to note that the decomposition of organic matter can be influenced by numerous factors, including the native SOC content and moisture levels (Kaur et al., 2023; Zhang & Zhou, 2018). Additionally, *k* values can be affected by various factors such as the type and application rate of fertilizers, farming practices (including residue type and placement), and land use management patterns (Datta et al., 2019; Duan et al., 2023; Toh et al., 2020).

The mineralization of organic materials is a complex process influenced by various factors, including the type of organic fertilizer used, the quantity and characteristics of added organic materials, soil processes, environmental conditions, and crop management practices (Cai et al., 2016; Hossain et al., 2017; Kalala et al., 2020; Zhang et al., 2018). Therefore, reproducing similar data across different studies is challenging due to the substantial variations in experimental setups and environmental conditions (Hossain et al., 2017). These differences in research conditions can significantly influence the outcomes and make direct comparisons between studies less straightforward.

In our experiment, the 14-day short-term incubation was fit to a first order equation as proposed by previous studies (Stanford et al., 1974; Stanford & Smith, 1972). Therefore, this approach enables us to estimate two critical parameters: the carbon mineralizable pool ($C_0 14$) and rate constant of decomposition (*k14*).

The estimate value of $C_0 14$ from this experiment was 105.8 ± 66.5 mg kg^{-1} of soil, showing a range from a minimum of 50.6 mg kg^{-1} to a maximum of 268.2 mg kg^{-1} of soil. The estimated value of *k14* from this experiment was 0.1 ± 0.04 per day which exhibited a range from a minimum of 0.03 to a maximum of 0.17 per day. These value falls within the ranges reported in other short-term carbon mineralization studies (Reddy et al., 1982; Riffaldi et al., 1996). Further research with a larger dataset may provide more insights into the potential impact of fertilizer treatments on the value of *k14 and* $C_0 14$.

We found a strong correlation between C_0 values obtained through short-term incubation and those from the long-term incubation (84 days) (Table C2). Moreover, this $C_0 14$ values displayed positive associations with various soil health indicators within the soil N and C pool such as soil total N, soil ACE protein, NAGase enzymes, water extractable organic N and C (Table C3). These findings align with previous studies that have also identified relationships between short-term C mineralization and the carbon and N pools, as well as microbial activity (Riffaldi et al., 1996).

These initial findings hold promise for potentially substituting other indicators for the resource-intensive long-term incubation method. However, it is essential to acknowledge that our research is at a preliminary stage and requires further validation through extensive testing across a broader spectrum of conditions. This would involve diverse treatments, farming practices, soil types, and environmental contexts to ensure the reliability and applicability of our results.

Contrasting N sources effects on Permanganate-Oxidizable Carbon (POXC)

The value POXC was within the range of other studies (Hurisso et al., 2016; Sepahvand & Feizian, 2016; Wade et al., 2020). The level of POXC was different according to sampling time which agreed with previous studies (Figure 4.6) (Ginakes et al., 2020; Martin & Sprunger, 2022). The environmental condition such as temperature and precipitation, farming system, and cropping system lead to the POXC variation within a year (Dahal et al., 2020; Ginakes et al., 2020; Martin & Sprunger, 2022).

In this study, we found compost increased the POXC level in soil and the ammonium sulfate treatment did not. This finding agreed with other studies (Du et al., 2022; Mpeketula & Snapp, 2019; Tong et al., 2020). The POXC levels decline with the increasing soil depth (Figure 4.8) (Kumar et al., 2014; Mpeketula & Snapp, 2019; Oliveira et al., 2022; Wang et al., 2017). The POXC levels at depths below 15 cm was not affected by treatment which align with previous study (Mpeketula & Snapp, 2019).

Contrasting N sources effects on Water Extractable Organic Carbon (WEOC)

In this experiment, we observed that the value of WEOC fluctuated over time (Figure 4.9). This observation aligns with the findings of several other studies (Grebliunas et al., 2016; He et al., 2017; Zhang et al., 2020). The concentration of WEOC is higher in the topsoil and lower in deeper soil layers (Figure 4.12). This pattern is consistent with the results of various studies (Hamkalo & Bedernichek, 2014; Zhang et al., 2020).

Contrary to the findings of previous studies, the findings of our study revealed no significant variations in WEOC concentrations at specific time points - prior to planting in May, during the season in August, and post-harvest in November (Figure 4.10) (Embacher et al., 2007; Petraityte et al., 2022; Praise et al., 2020). This disparity in findings prompts us to consider several factors that may contribute to the variation in results between studies.

The variability in WEOC during the growing season is influenced by several factors, including N fertilizer rates, meteorological conditions of the year, land management practices, timing of soil sample collection, temperature regimes, and the form of fertilizers (Grebliunas et al., 2016; Liu et al., 2019; Petraityte et al., 2022; Praise et al., 2020). Seasonal trends in WEOC concentrations have been shown to correlate with soil temperature, water content, and weather conditions, albeit with variations observed across different years (Campbell et al., 1999). Moreover, Petraityte et al. (2022) highlighted the significance of the interaction between fertilizer type and the timing of application on WEOC outcomes (Petraityte et al., 2022).

In this study, we observed that Compost treatment increased WEOC, which aligns with previous research indicating the positive effect of organic fertilizer on WEOC (Marinari et al., 2010; Zsolnay & Görlitz, 1994). Our study also showed that ammonium sulfate fertilizer did not affect the WEOC compared to control treatment which agrees with previous studies (Rochette & Gregorich, 1998; Zsolnay & Görlitz, 1994). Meanwhile, other studies found that inorganic fertilizer decreases the WEOC especially with the higher application rates more than 180 kg N ha^{-1} (Chantigny et al., 1999; Liang et al., 1997).

WEOC serves as a source of nutrients and microbial substrates, (Pinsonneault et al., 2016; Zhang et al., 2020; Zhou et al., 2013). Many studies have indicated that the dynamics of WEOC are influenced by various factors, including vegetation types, soil temperature, moisture levels, SOC concentration, C:N ratio, and soil organic N (Wang & Wang, 2007; Zhang et al., 2020; Zhou et al., 2012).

However, it is essential to recognize that factors like regional climate variations, variations in sampling dates, and human management practices can influence soil organic matter dynamics. These factors introduce significant uncertainties, and more research is needed to better understand the processes that govern WEOC dynamics in soils across diverse ecosystems (Zhang et al., 2020).

Contrasting N sources effects on β-glucosidase enzyme

Our study showed an average of BG activity of 193.3 ±40.7 mg PNP $\mathrm{kg}^{-1}$of soil hour^{-1}. This value of BG activity was within the range of BG activities found other studies (Acosta-Martínez et al., 2003; Stott et al., 2010). Activity of BG in response to N treatmentments was inconsistent across the time of soil sampling (Figure 4.13) which agreed with other studies (Davies et al., 2022; Martín-Lammerding et al., 2015; Piotrowska & Koper, 2010; Tyler, 2020). Although BG was affected by the time of taking of the samples, it is interesting to note that BG activity did not show significant differences during different periods of the year specifically, before planting (May), during planting (August), and after harvesting (November) (Figure 4.14).

The level BG activity during the growing season is subject to multiple influencing factors. Seasonal variations from year to year, sampling time, location, and various management practices have all been identified as key factors affecting BG activity (Davies et al., 2022; Mariscal-Sancho et al., 2018). These findings emphasize the complex interplay of environmental and management variables in shaping the dynamics of BG activity in soil during the growing season. The findings from our study revealed that compost significantly increased BG activity being compared to Control. BG activity under AS100 and AS200 showed no significant difference compared to the Compost treatments, and these two treatments were not significantly different from the Control treatment. The responses of BG activity under N fertilization have been investigated in various studies, and the results generally exhibit variations in both direction and magnitude across these studies (Jian et al., 2016; Kracmarova et al., 2020).

Many studies have demonstrated the positive impacts of N fertilizers on increasing BG activity (Ajwa et al., 1999; Crecchio et al., 2004; Geisseler & Scow, 2014; Jian et al., 2016; Piotrowska & Koper, 2010). However, some studies have found that BG activity remained relatively constant after N fertilization (Davies et al., 2022; Kracmarova et al., 2020; Zeglin et al., 2007), while some show BG activity decreased as a result of N fertilization (Zhang et al., 2015).

In our investigation, we observed a consistent decline in BG activity with increasing soil depth (Figure 4.16), which is in line with the results of multiple prior studies (Acosta-Martínez et al., 2003; Deng & Tabatabai, 1996; Tiwari et al., 2019; Xiao-Chang & Qin, 2006). The decrease in BG activity was particularly rapid, as activity at 60 cm depth was only 10% of that found in the surface layer (0-15 cm), which supports the findings of Xiao-Chang & Qin (2006). The primary reason behind this decline is the strong dependence of BG activity on substrate availability. Microorganisms responsible for producing this enzyme are primarily active in the upper soil layers (Tiwari et al., 2019; Xiao-Chang & Qin, 2006). Decreases in various enzyme activities within the soil profile have been documented in different soil types, and these changes often correlate with reductions in organic C content (Acosta-Martínez et al., 2003).

BG activity has been thought to be one of the most sensitive indicators of soil quality (Kracmarova et al., 2020). However, the relationship between N fertilizer and enzyme activity is complex, involving intricate interactions with other nutrients and microbial processes within the soil (Adetunji et al., 2017). The response of BG enzyme activity to N fertilizer is highly variable and depends on key factors such as the type of fertilizer, application rate, specific soil characteristics, management regime, and prevailing environmental conditions (Adetunji et al., 2017; Kracmarova et al., 2020; Mariscal-Sancho et al., 2018; Tavali, 2021; Tiwari et al., 2019) .

In short, the application of organic fertilizer serves a dual purpose by not only supplying a valuable carbon source but also a diverse array of essential nutrients crucial for microbial growth and diversity. Additionally, it contributes to the overall enhancement of soil conditions and the increase in soil organic matter content. These combined effects are of paramount importance for the long-term sustainability of global agriculture (Assefa & Tadesse, 2019; Bot & Benites, 2005; Wang et al., 2023).

Contrasting N sources effects on Correlation between C indicators

In this study, we found that TC and SOC were positively correlated with other soil C indicators such as WEOC, POXC and BG which agreed with the studies of Das et al., 2023; Liptzin et al., 2022. In additon, this study also found positive correlations between SOC and POXC and WEOC which agreed with previous studies (Bagnall et al., 2023; Culman et al., 2012; Das et al., 2023; Liptzin et al., 2022; Sainju et al., 2022). POXC and WEOC represent labile fraction of SOM which can be used as indicators of microbially active C (Bongiorno et al., 2019; Das et al., 2023; Emran et al., 2020). Therefore, high positive correlation of SOC and POXC and WEOC suggest higher labile fractions of SOC and an active microbial community. This maintains soil health and productivity of the farming system (Das et al., 2023).

Our study found positive correlation between BG and SOC which aligned with many studies (Acosta-Martínez et al., 2003; Das et al., 2023; Elvazrt & Tabatabai, 1990;

Lemanowicz et al., 2023; Liptzin et al., 2022; Stott et al., 2010; Zhang et al., 2015). β-glucosidase activity plays a crucial role in the degradation of cellulose, a major component of plant material (Deng & Tabatabai, 1996; Fansler et al., 2005). For instant, soils amended with lower C:N crop residue favor BG activity. This results in quick organic matter decomposition and nutrient release (Adetunji et al., 2017). Soils abundant in organic C tend to support a more diverse and active microbial community leading to increased BG activity (Chellappa et al., 2021; Deng & Tabatabai, 1996; Oldfield et al., 2018).

However, there are some studies that did not detect the correlation between BG and soil C (Bandick & Dick, 1999; Green et al., 2007; Sainju et al., 2022; Shao et al., 2015; Tian et al., 2010). Bandick and Dick (1999) conducted a study on the impact of different management practices on enzyme activities. Their study showed that while BG activity showed a positive correlation with soil C in experiments under a winter wheat ± summer fallow, no such correlation was observed in continuous fescue and four winter cover crop treatments in annual rotation with a summer vegetable crop (Bandick & Dick, 1999). This suggested that BG activity exhibits varying behaviors depending on the farming system (Bandick & Dick, 1999; Tian et al., 2010). Sainju et al. (2022) found a correlation between BG activity and SOC in Froid, MT, but this correlation was not observed in Sidney, MT. These differences in correlation can be attributed to variations in farming practices, soil characteristics, and geographical location, all of which may impact the relationship between these two indicators (Sainju et al., 2022). In addition to farming practices, BG activity has been shown to be influenced by various factors, including climatic regions, diverse soil types, and soil textures (Bandick & Dick, 1999; Lagomarsino et al., 2009; Stott et al., 2010; Tian et al., 2010).

The correlations between SOC and various C indicators reveal essential aspects of soil health and ecosystem functioning. The positive associations observed between SOC and POXC and WEOC signify a strong link between different C fractions, indicating the presence of a dynamic, microbial-driven C cycle (Das et al., 2023). Higher SOC levels correspond to elevated labile carbon fractions, suggesting an active microbial community and a propensity for increased C turnover. Furthermore, the positive correlation between SOC and BG activity underscores the role of microorganisms in organic matter decomposition, emphasizing the significance of microbial activity in C cycling (Chellappa et al., 2021; Deng & Tabatabai, 1996; Oldfield et al., 2018). Notably, the strength of these correlations is not universally consistent and can be influenced by local conditions, farming practices, and soil types.

Contrasting N sources effects on Correlation between C and N indicators

SOC exhibits a positive correlation with STN, a finding supported by multiple studies (Das et al., 2023; Liptzin et al., 2023; Qi et al., 2020; Sainju et al., 2022). Nitrogen plays a pivotal role in shaping organic C cycling and sequestration (Meng et al., 2022). Notably, higher levels of SOC enhance the soil's capacity to retain N, as observed previously (Wibowo & Kasno, 2021). The strong correlation between STN and SOC is attributed to their shared role as integral components of soil organic matter (Meng et al., 2022; Tong et al., 2023).

In this study, we found that SOC and WEON are postively correlated (Braos et al., 2023; Cappellazzi & Morgan, 2021; Das et al., 2023; Haney et al., 2012; Sainju et al., 2022). The strength of this correlation can be influenced by the specific cropping system employed (Cappellazzi & Morgan, 2021).

Both WEOC and WEON are correlated (Sequeira et al., 2011; Xu et al., 2013). These components are part of the larger SOC pool (Haney et al., 2012). They are the critical component used by soil microoorganisms that drive the nutrient cycling system (Haney et al., 2012). Elevated soil microbial activity corresponds to increased degradation of organic compounds, leading to the production of smaller molecules, such as those found in WEOC and WEON, which are readily metabolized by soil microorganisms (Braos et al., 2023; Sun et al., 2015).

In this study, a positive relationship between WEON and WEOC with TN and soil SOC was evident, consistent with the findings of Das et al. (2023). These bioavailable C and N fractions hold particular significance in shaping the structure of the microbial community (Zhang et al., 2011; Sun et al., 2017). Increasing STN and SOC could have positive effects on augmenting the levels of WEOC and WEON, which is corroborated by the data obtained in this investigation.

In this study, SOC is positively correlated with ACE protein which agrees with many previous findings (Cappellazzi & Morgan, 2021; Cissé et al., 2020; Sainju et al., 2022; Zhang et al., 2017). Numerous studies have consistently shown that ACE protein is associated with the accumulation of SOC and may maintain the stability of the soil C pool (Irving et al., 2021; Wang et al., 2017; Zhang et al., 2017). Moreover, another study found higher levels of SOC are often accompanied by elevated soil protein content (Li et al., 2020). From those studies, it is no surprise that we found that soil protein is related with SOC as it represents a substantial pool of soil organic matter (Edu, 2017). However, the strength of their correlation coefficients may vary depending on the specific cropping system, as found in the study by Cappellazzi & Morgan (2021).

In our study, we observed that BG was not correlated with STN, which aligns with the findings of other studies (Burket & Dick, 1998; Sainju et al., 2022). However, there are other studies that reported a positive correlation between BG and STN (Liu et al., 2022; Piotrowska & Koper, 2010; Salazar et al., 2011; Turner et al., 2002). Soils amended with crop residue with a lower C:N ratio tends to enhance the activity of BG which leading to faster organic matter decomposition and nutrient release (Adetunji et al., 2017). Furthermore, high N availability can increase the microbial demand for C, thereby inducing the production of glucosidases (Asmar et al., 1994; Uwituze et al., 2022).

Nevertheless, the relationship between BG and STN is intricate and subject to multiple influencing factors. These factors encompass soil characteristics, location, and farming practices, all of which can impact the correlation between BG and STN (Sainju et al., 2022; Salazar et al., 2011). Moreover, the research by Sainju et al. (2022) suggests that variations in farming practices and geographic locations may have a favorable influence on strengthening the correlation between these indicators of soil health.

In our study, NAGase shows a positive correlation with SOC, aligning with the findings of previous studies (Cappellazzi & Morgan, 2021; Das et al., 2023; Fansler et al., 2005; Sainju et al., 2022). NAGase is an extracellular soil enzyme that plays a pivotal role in the decomposition of soil organic matter and nutrient cycling (Uwituze et al., 2022). This is due to NAGase's involvement in the processes that convert chitin into amino sugars, which serve as a major source of easily mineralizable C and N in soils (Acosta-Martínez et al., 2007; Luo et al., 2017; Uwituze et al., 2022). Notably, Cappellazzi & Morgan (2021) found that the correlation between NAGase and SOC was weak under row crop practices but became moderately correlated under perennial cropping systems. The variation in the strength of their relationship highlights the influence of the specific cropping systems on this relationship.

In our study, no significant correlation was found between NAGase and BG, a finding consistent with other studies (Lagomarsino et al., 2009; Zhang et al., 2023). However, it is important to note that some studies, including those by Das et al. (2023) and Liptzin et al. (2023), have reported a significant positive relationship between BG and NAGase. While BG and NAGase activities are typically associated with C and N cycle, respectively, and respond to different factors affecting substrate availability, they tend to exhibit a correlation when studied across multiple sites or when management practices vary within a specific site (Liptzin et al., 2023).

The positive correlation between soil C and N indicates the pivotal role of N in shaping organic C cycling and sequestration. Higher SOC levels enhance the soil capacity to retain N, highlighting their shared role as integral components of soil organic matter (Meng et al., 2022; Tong et al., 2023). Furthermore, SOC shows a positive correlation with WEOC, emphasizing the impact of cropping systems on the strength of this relationship. Additionally, the relationships between WEOC and WEON highlight their role as critical components of the SOM pool, driven by soil microorganisms involved in nutrient cycling (Haney et al., 2012).

The positive relationships between WEON, WEOC, STN, and SOC suggests that increasing STN and SOC can positively influence the levels of these bioavailable C and N fractions. The positive correlation between SOC and ACE protein suggests that soil protein plays a significant role in SOM and therefore enhancing soil quality. While the correlation between BG and STN is subject to multiple influencing factors, the relationship between NAGase and SOC underscores the enzyme role in the decomposition of organic matter and nutrient cycling, influenced by factors such as farming practices and cropping systems.

Our findings provide an improved understanding of the interconnectedness of various C and N pools and enzymes, shedding light on the dynamic soil processes in semi-arid corn silage production systems and offering valuable insights for sustainable soil management practices. In addition, the observed correlations and their complexities underscore the multifaceted nature of soil health, which is influenced by a combination of factors, including soil properties, agricultural practices, and environmental conditions. While our study has provided valuable insights into the relationship between C and N indicators, further investigation on a larger scale and across various contexts is needed to validate and expand upon our findings and their implications. These limitations underscore the ongoing need for robust research in this field to inform sustainable agricultural practices effectively.

Conclusions

We conducted a thorough investigation evaluating the impact of contrasting N sources on soil carbon soil health indicators and carbon-nitrogen relationships across multiple growing seasons in corn silage production.

The distribution of C indicators exhibited variation in relation to soil depth. Most soil C indicators, including SOC, POXC, WEOC, and BG activity, showed higher concentrations in the uppermost soil layer (0-15 cm) and gradually decreased with increasing soil depth. This pattern is attributed to the influx of organic matter, elevated microbial activity, and the presence of favorable conditions for organic carbon accumulation within the topsoil. In contrast, soil TC levels increased as soil depth increased, primarily due to the higher content of SIC deeper in the soil profile. Notably, in semi-arid and arid environments, SIC levels were observed to increase with greater soil depth.

The compost treatment led to higher TC and SOC levels compared to other treatments, with approximately a 4.63% increase in TC and a significant 23 % increase in SOC. Unlike TC and SOC, SIC was not significantly affected by fertilizer treatments. Furthermore, the compost treatment exhibited a positive influence on other carbon indicators, including as POXC, WEOC and BG. Specifically, POXC levels increased by approximately 19.9 %, WEOC showed a substantial boost of roughly 35.24%, and BG activity witnessed an increase of approximately 10.27% when compared to the average values recorded in the other treatments.

The carbon mineralization data fit well to the first-order kinetic model. Notably, the compost treatment exerted a substantial influence, resulting in the highest levels of cumulative mineralized carbon, higher carbon mineralization rates, and potential mineralizable carbon (C_0). Specifically, the value C_0 under the compost treatment exhibited an impressive increase of approximately 103.8 % when compared to average of the other treatments.

SOC displayed moderate to strong positive correlations with various carbon indicators (POXC, WEOC, and BG). These correlations affirm the intricate relationships among these variables and underscore the pivotal role of microorganisms in carbon cycling. In addition, we found a strong, positive relationship between SOC with soil N indicators such as STN, ACE protein and WEON. STN also exhibited a moderate correlation with POXC and WEOC.

In summary, our study has uncovered the diverse impacts of contrasting nitrogen sources on soil C indicators and on their correlations with N indicators. There was a positive influence of the compost treatment on various soil carbon parameters and enzyme activity, highlighting its potential for enhancing soil health and promoting sustainable agricultural practices. Conversely, chemical fertilizers like ammonium sulfate did not significantly enhance soil health indicators although they were the key to silage crop yields. Our research contributes to a deeper understanding of soil carbon dynamics and their interplay with nitrogen, which is essential for efficient nutrient management, improved soil health, increased crop yields, and sustainable agricultural practices. This knowledge empowers farmers to make informed decisions while reducing the environmental footprint of agriculture. To strengthen these findings, future investigations should further explore these relationships across diverse settings and on a larger scale.

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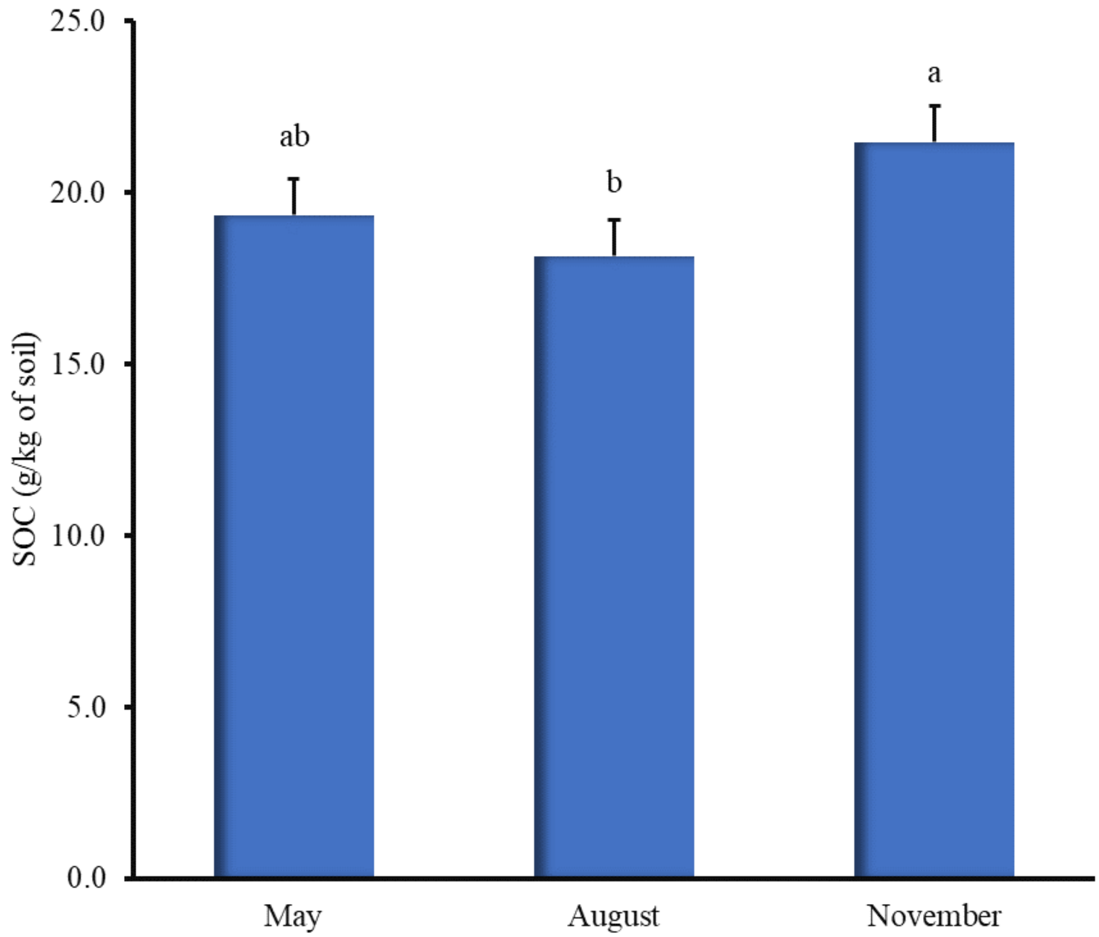
Figure and table

Figure 4. 1.

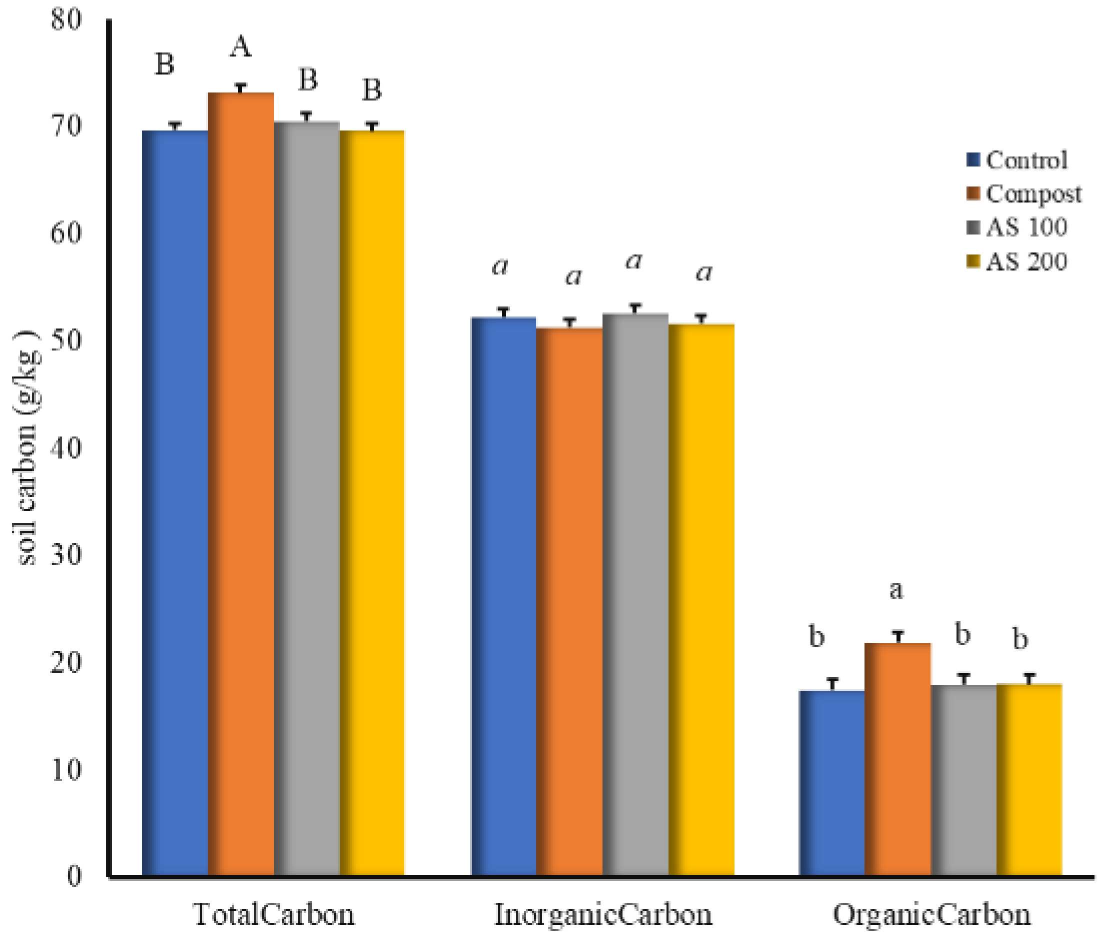
Soil organic carbon (SOC) at different times of growing season



Note. Repeated measure was employed to analyze SOC at different times of growing season (May, August, and November). Soil samples were collected at a depth 0-15 cm from 2019 to 2021. Error bars represent standard errors (n = 16). Different lowercases above the bars indicate as significant difference among seasons ($p \leq 0.05$).

Figure 4. 2.

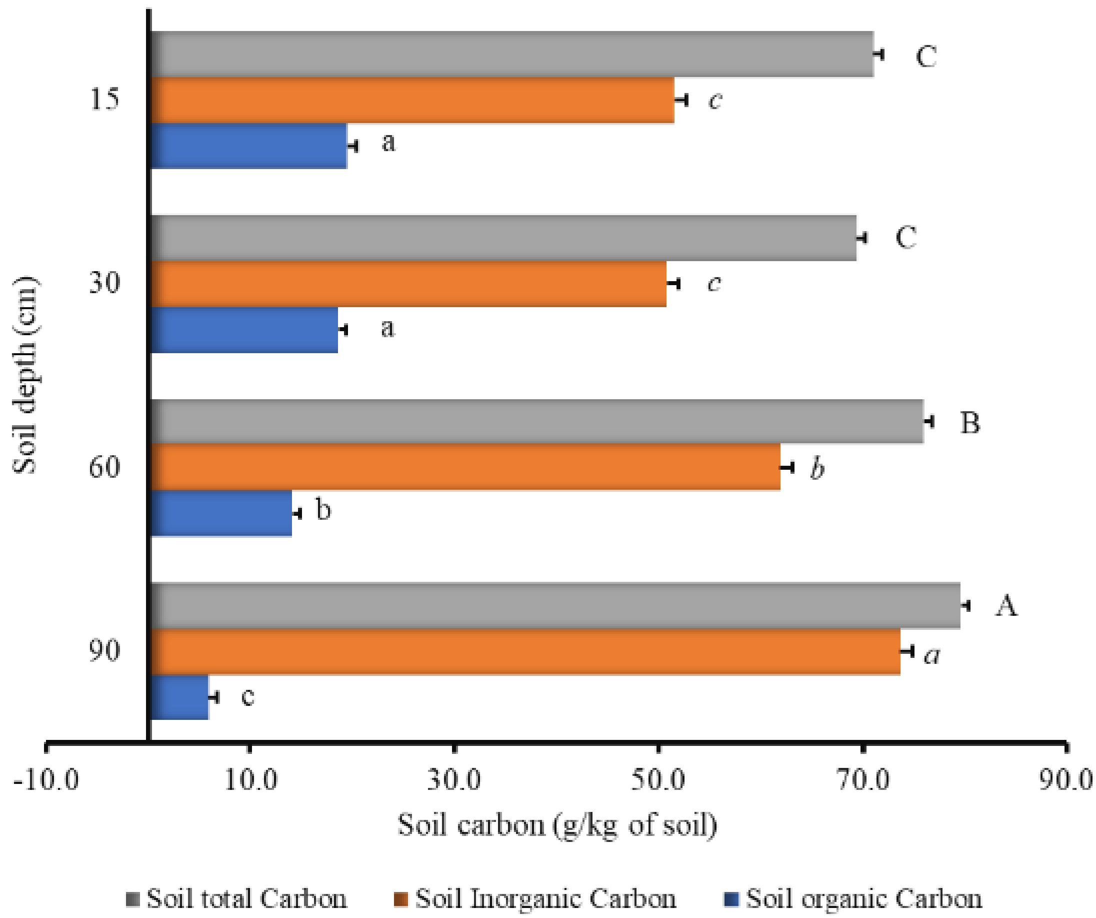
Effects of contrasting N sources on soil carbon pools



Note. A repeated measures analysis was conducted to analyze the effects of contrasting N sources on soil carbon. Soil samples were collected at a depth of 0-15 cm from 2019 to 2021. Error bars on the graph represent standard errors (n = 28). In the results, uppercase letters (A, B, C) are used to indicate statistically significant differences in TC due to the treatments. Lowercase letters (a, b, c) represent statistically significant differences in SOC, and italicized letters (*a*, *b*, *c*) signify statistically significant differences in SIC. All significance levels are set at $p \leq 0.05$.

Figure 4. 3.

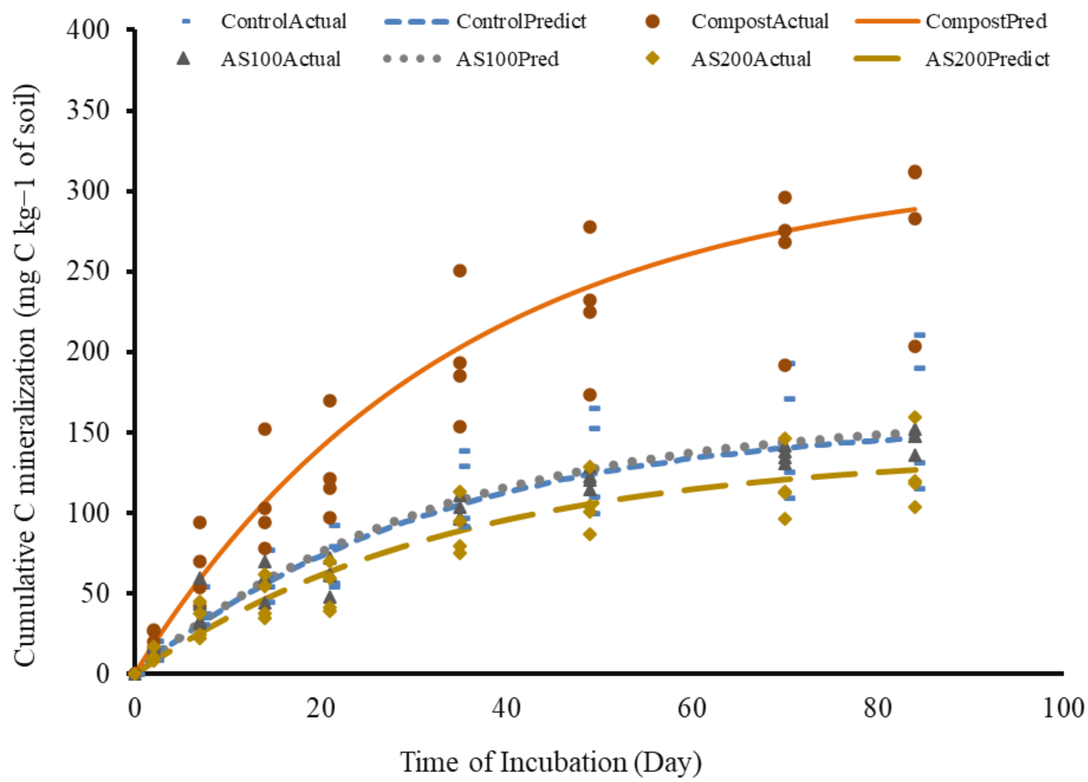
Soil carbon at different soil depths



Note. Measurement of soil carbon at different soil depths (0-15 cm, 15-30 cm, 30-60 cm, and 60-90 cm) in November 2019. In the results, uppercase letters (A, B, C) are used to indicate statistically significant differences in total C (TC) due to the treatments. Lowercase letters (a, b, c) represent statistically significant differences in soil organic C (SOC), and italicized letters (*a*, *b*, *c*) signify statistically significant differences in soil inorgnanic C (SIC). Error bars on the graph represent standard errors (n = 16). All significance levels are set at $p \leq 0.05$.

Figure 4. 4.

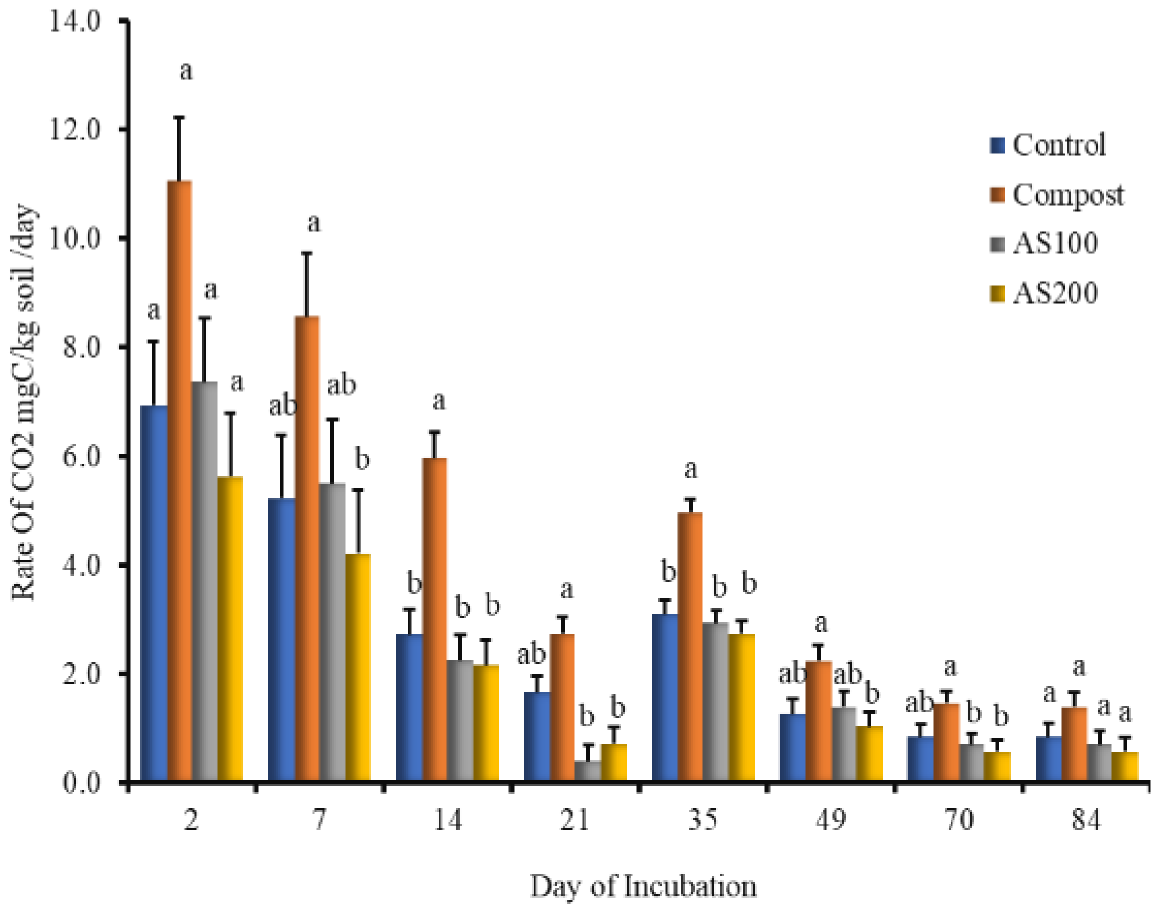
Cumulative C mineralization (Ct) with a first-order model from a long-term incubation of 84 days



Note. Cumulative C mineralization (Ct) in soil (0-15 cm soil depth) from an aerobic 84-days incubation from August 2019. Individual treatments were fit to the first order equation $\mathrm{Ct} = \mathrm{C_0}(1 - \mathrm{e}^{-\mathrm{kt}})$. A non-linear least-squares approach was used to estimate the $\mathrm{C_0}$ and k parameters. The treatments are Control, Compost and AS100 and AS200. For the measure of the goodness of fit of a model (R^2), $\mathrm{C_0}$ and k, see table 4.1.

Figure 4. 5.

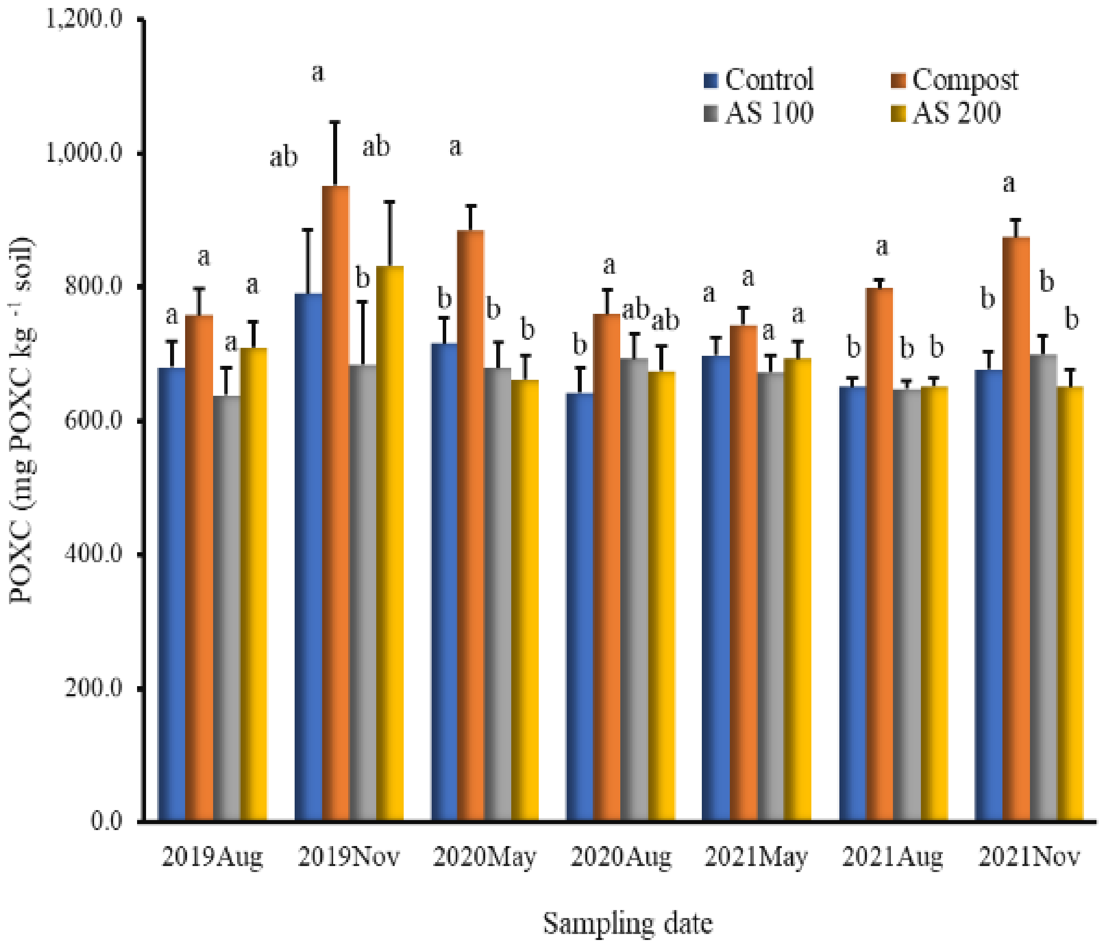
Rate of carbon mineralization at different dates of incubation



Note. Rate of carbon mineralization from 84-day incubation. Soil samples were taken in August 2019 from 0-15 cm depth. Different lowercases above the bars indicate a significant difference by treatment within sampling date of incubation time ($p \leq 0.05$).

Figure 4. 6.

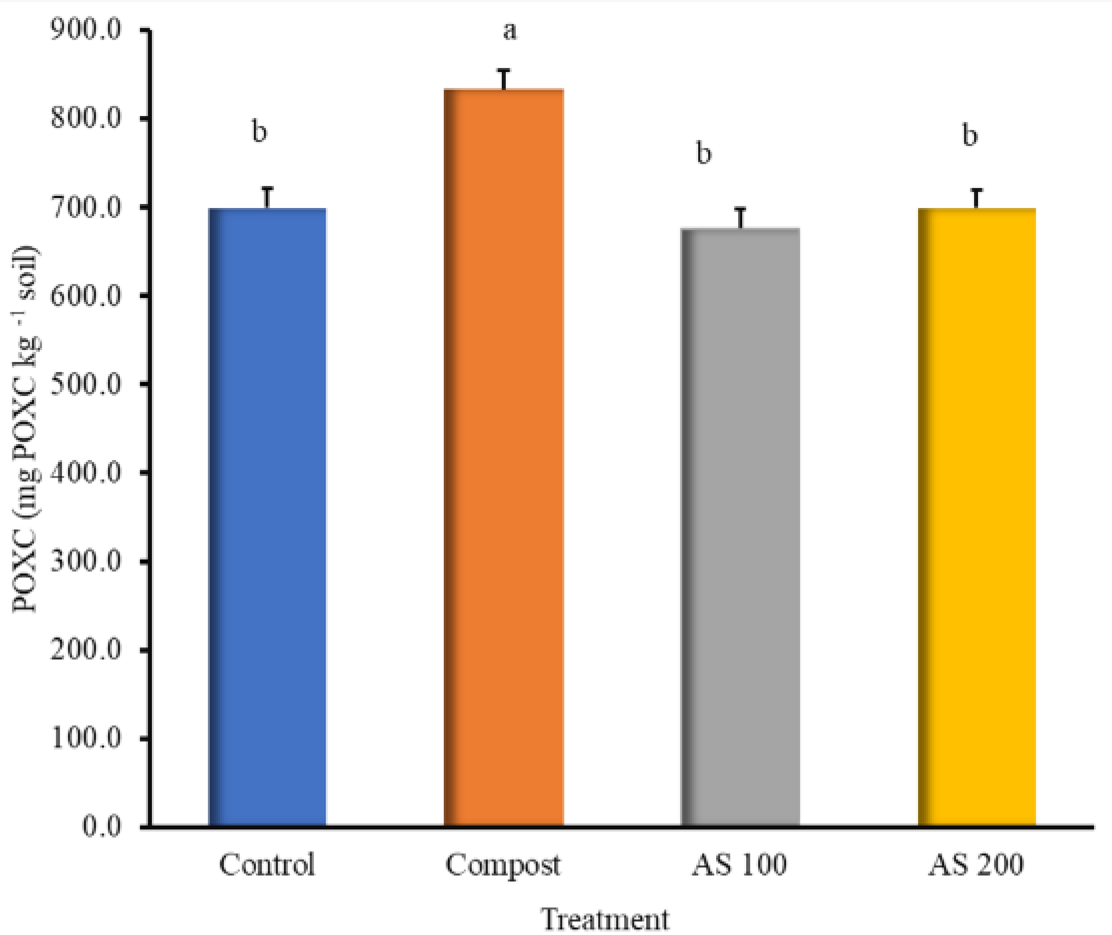
Permanganate oxidizable carbon (POXC) at different dates of soil sample collection



Note. POXC analysis was conducted on soil samples collected at a depth of 0-15 cm, from a corn silage field spanning the years 2011 to 2021. Error bars represent standard errors (n = 4). Different lowercases above the bars indicate a significant difference within date of soil sample collection ($p \leq 0.05$).

Figure 4. 7.

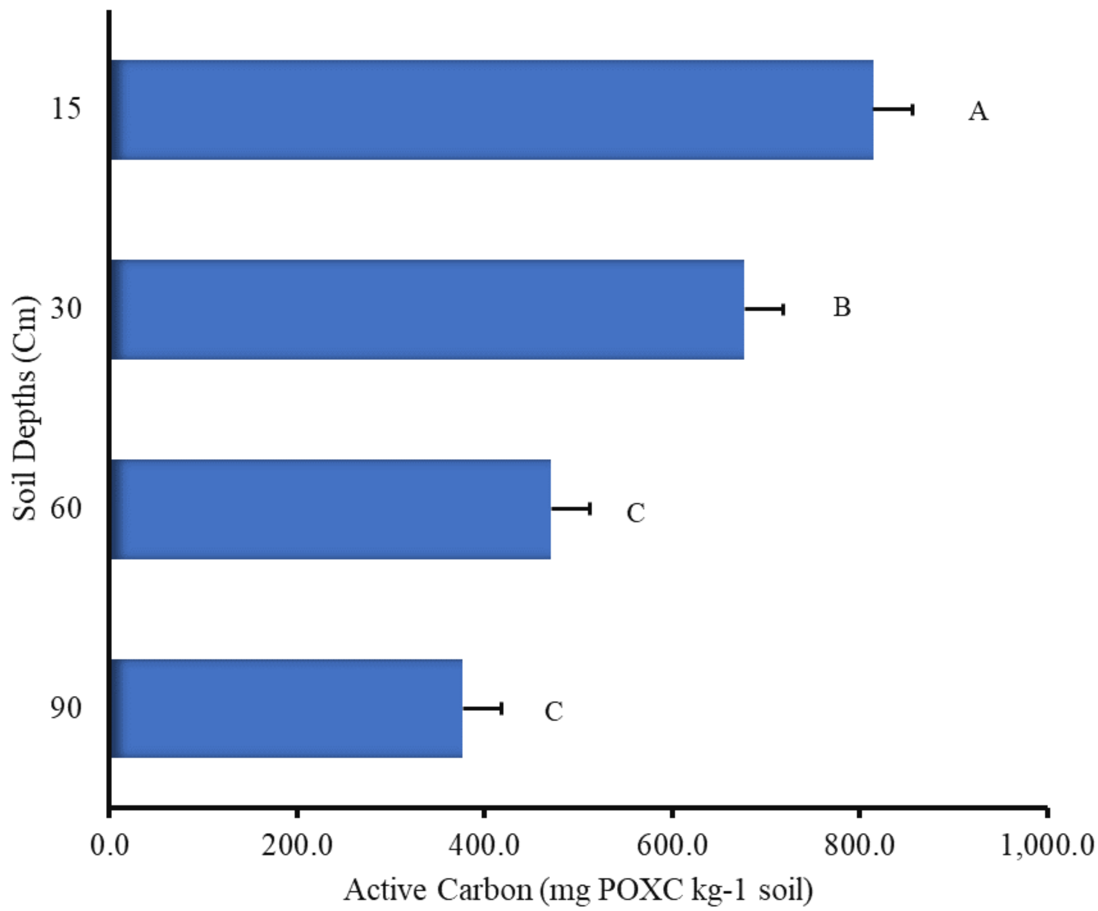
Effects of contrasting N sources on soil permanganate-oxidizable carbon (POXC)



Note. Repeated measure was employed to analysis the effects of contrasting N sources on POXC. Soil samples were collected in at a depth of 0-15 cm spanning the years 2019 to 2021. Error bars represent standard errors (n = 28). Different lowercases above the bars indicate a significant difference among treatments (p ≤0.05).

Figure 4. 8.

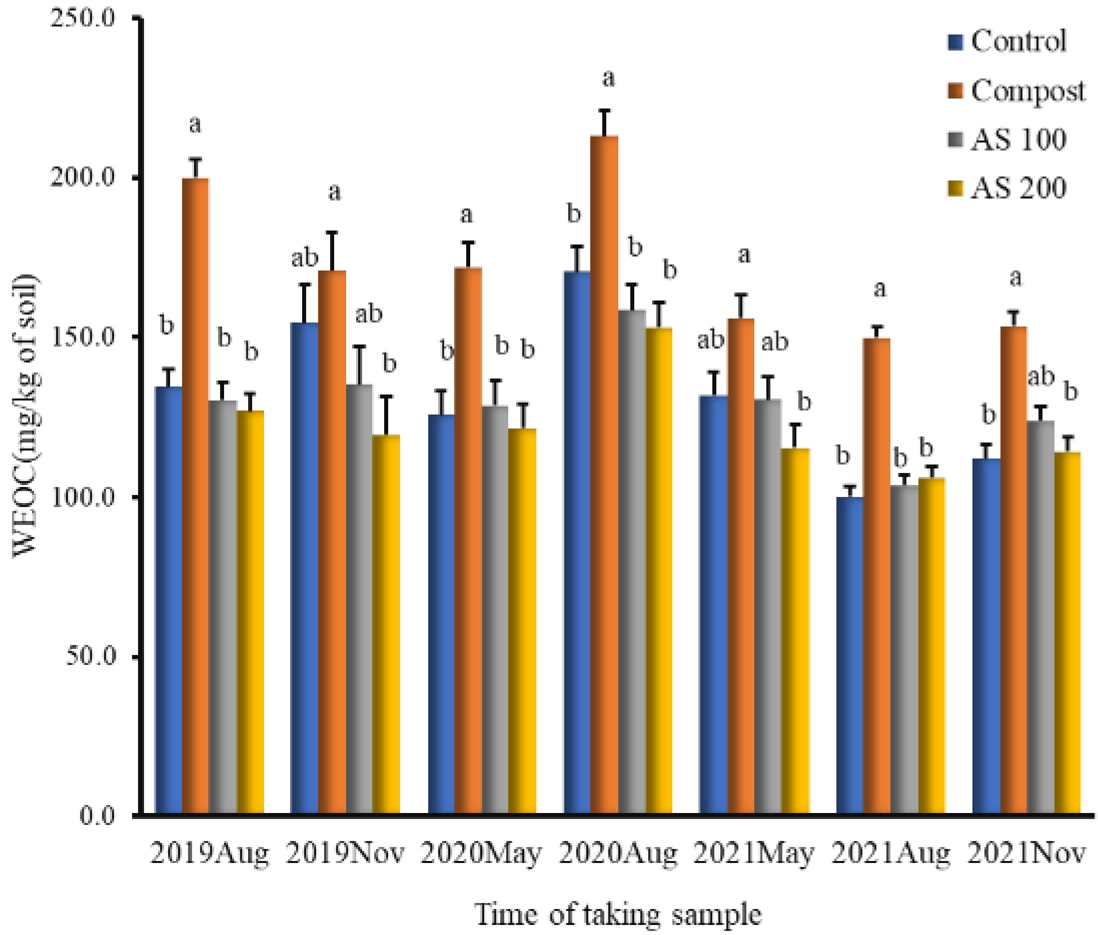
Permanganate-oxidizable carbon (POXC) at different soil depths



Note. Measurement of POXC at various soil depths across treatments (0-15 cm, 15-30 cm, 30-60 cm, and 60-90 cm) in November 2019. Bars reflect standard errors (n=4). Lowercase letters above the bars indicate significance at the specified depth ($p \leq 0.05$).

Figure 4. 9.

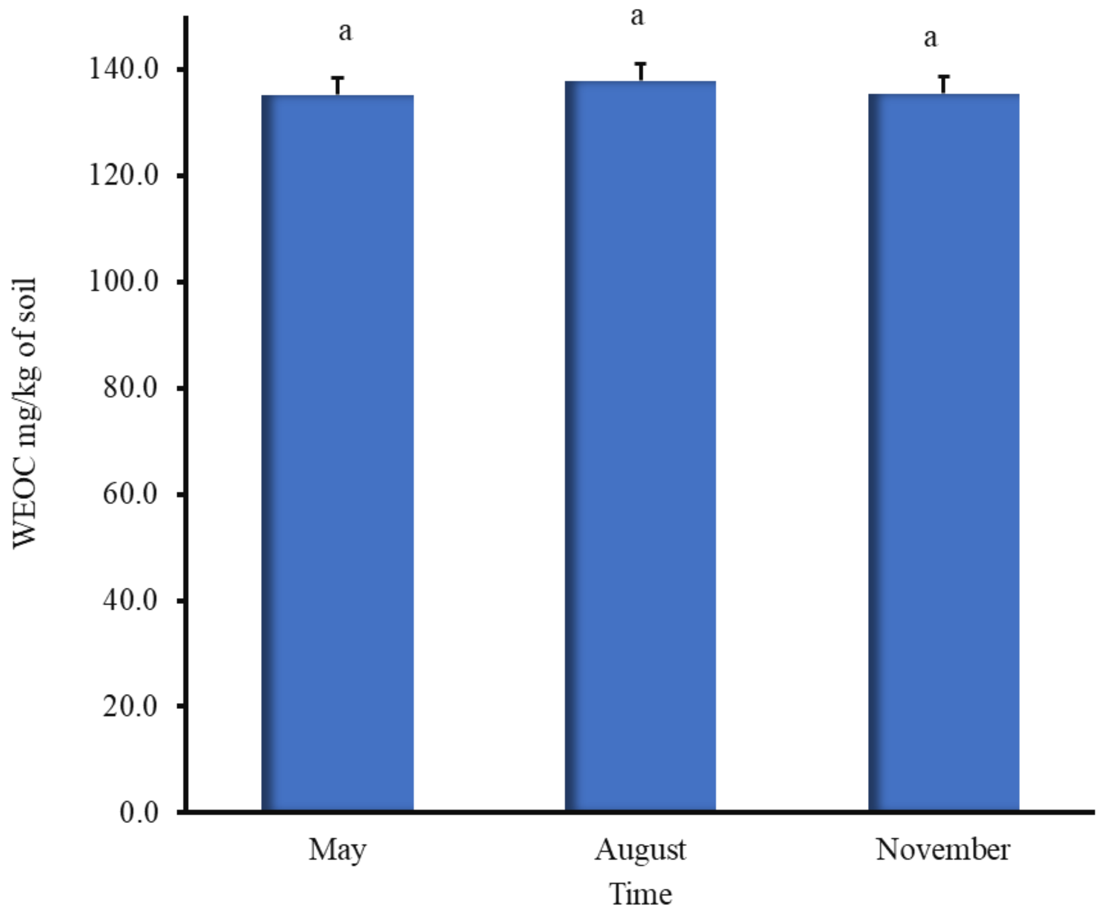
Water extractable organic carbon (WEOC) at different dates of soil sample collection



Note. WEOC analysis was conducted on soil samples collected at a depth of 0-15 cm, from a corn silage field spanning the years 2019 to 2021. Error bars represent standard errors (n = 4). Different lowercases above the bars indicate a significant difference within date of soil sample collection ($p \leq 0.05$).

Figure 4. 10.

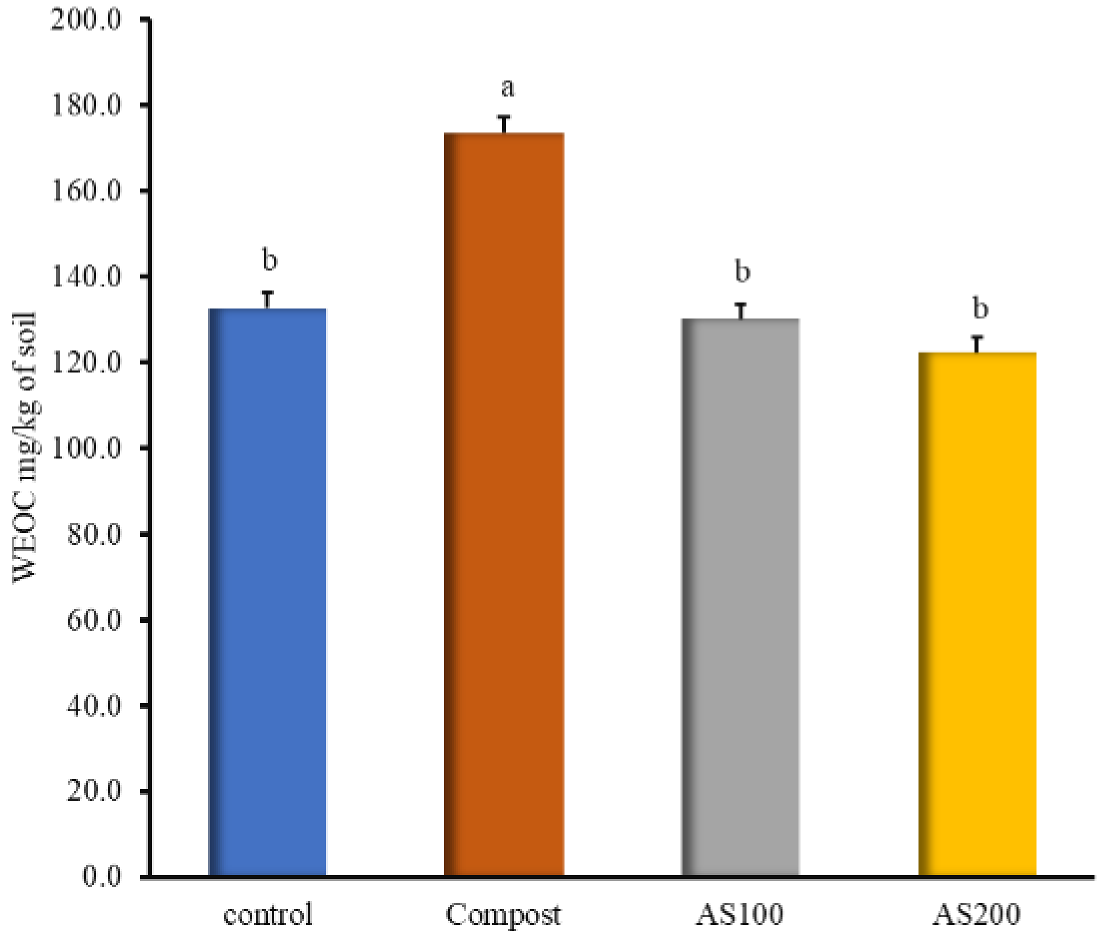
Water extractable organic C (WEOC) at different times of growing season



Note. Repeated measure was employed to analyze *WEOC* at different times of growing season (May, August, and November). Soil samples were collected at a depth 0-15 cm from 2019 to 2021. Error bars represent standard errors (n = 12). Different lowercases above the bars indicate as significant difference among seasons ($p \leq 0.05$).

Figure 4. 11.

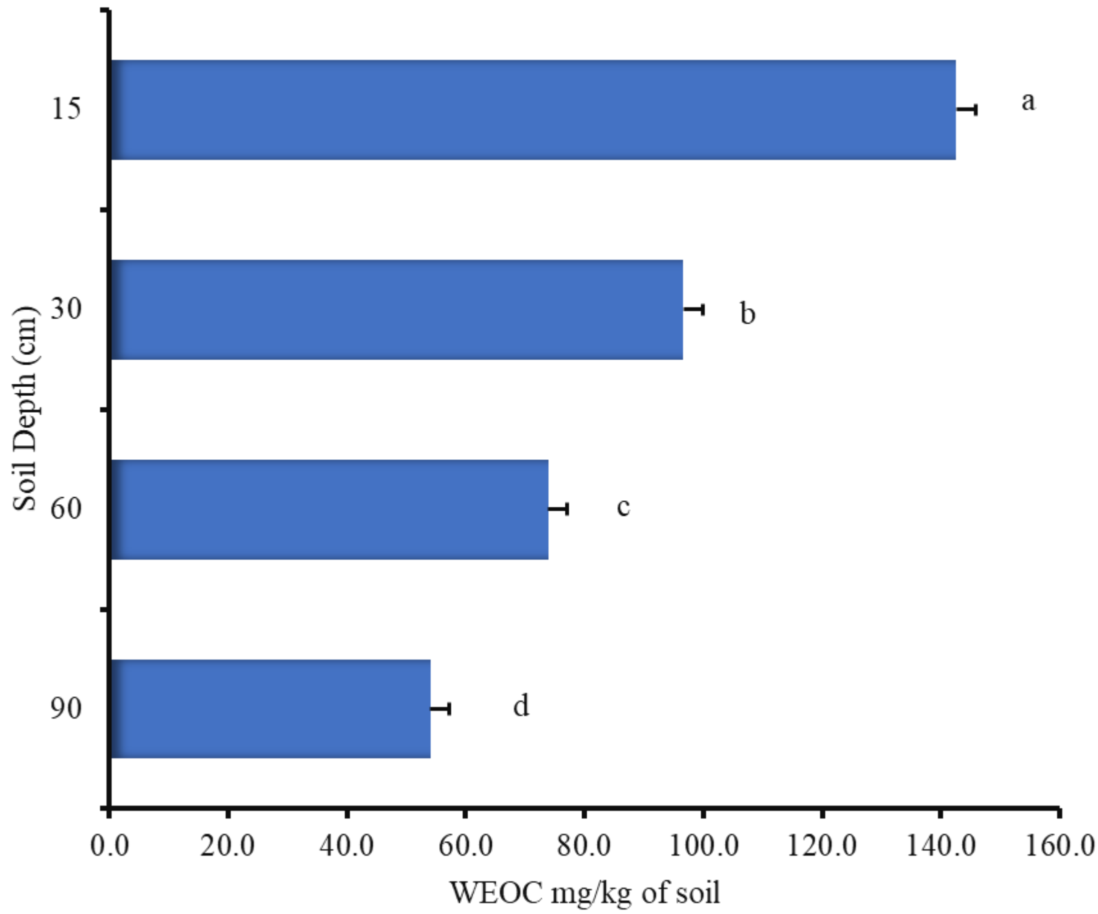
Effects of contrasting N sources on water extractable organic carbon (WEOC)



Note. Repeated measures were employed to analysis the effects of contrasting N sources on WEOC. Soil samples were collected in August at a depth of 0-15 cm spanning the years 2019 to 2021. Error bars represent standard errors (n = 28). Different lowercases above the bars indicate a significant difference among treatments ($p \leq 0.05$).

Figure 4. 12.

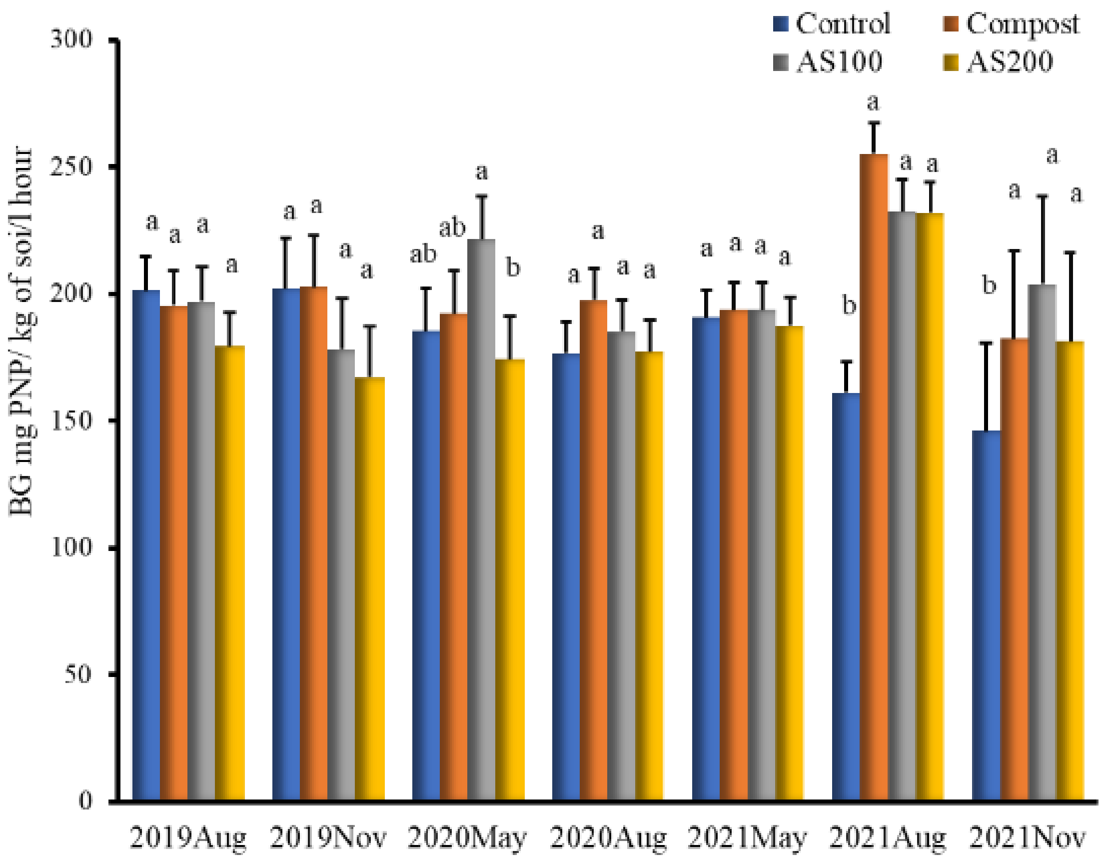
Water extractable organic carbon (WEOC) at different depth of soil



Note. Measurement of WEOC at various soil depths across treatments (0-15 cm, 15-30 cm, 30-60 cm, and 60-90 cm) in November 2019. Bars reflect standard errors (n=4). Lowercase letters above the bars indicate significance at the specified depth ($p \leq 0.05$).

Figure 4. 13.

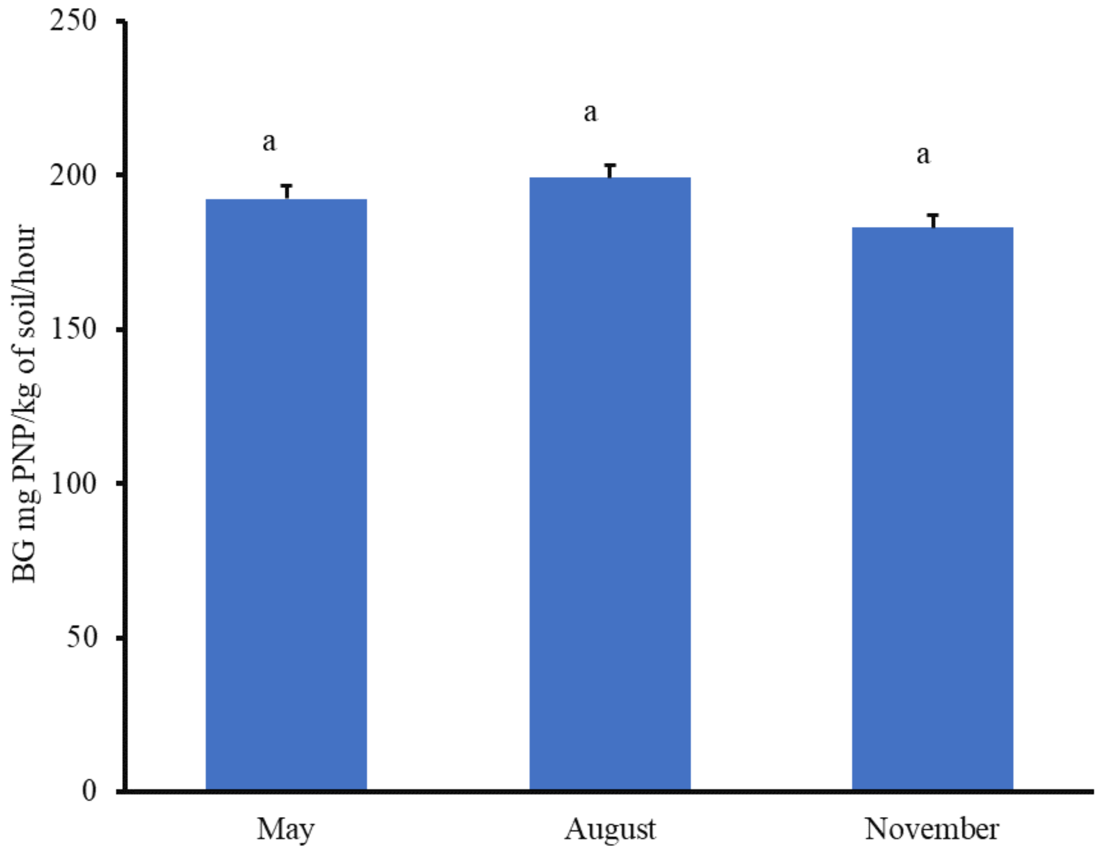
β-Glucosidase (BG) activity in soil at different dates of soil sample collection



Note. BG activity analysis was conducted on soil samples collected at a depth of 0-15 cm, from a corn silage field spanning the years 2019 to 2021. Error bars represent standard errors (n = 4). Different lowercases above the bars indicate a significant difference within date of soil sample collection (p ≤0.05).

Figure 4. 14.

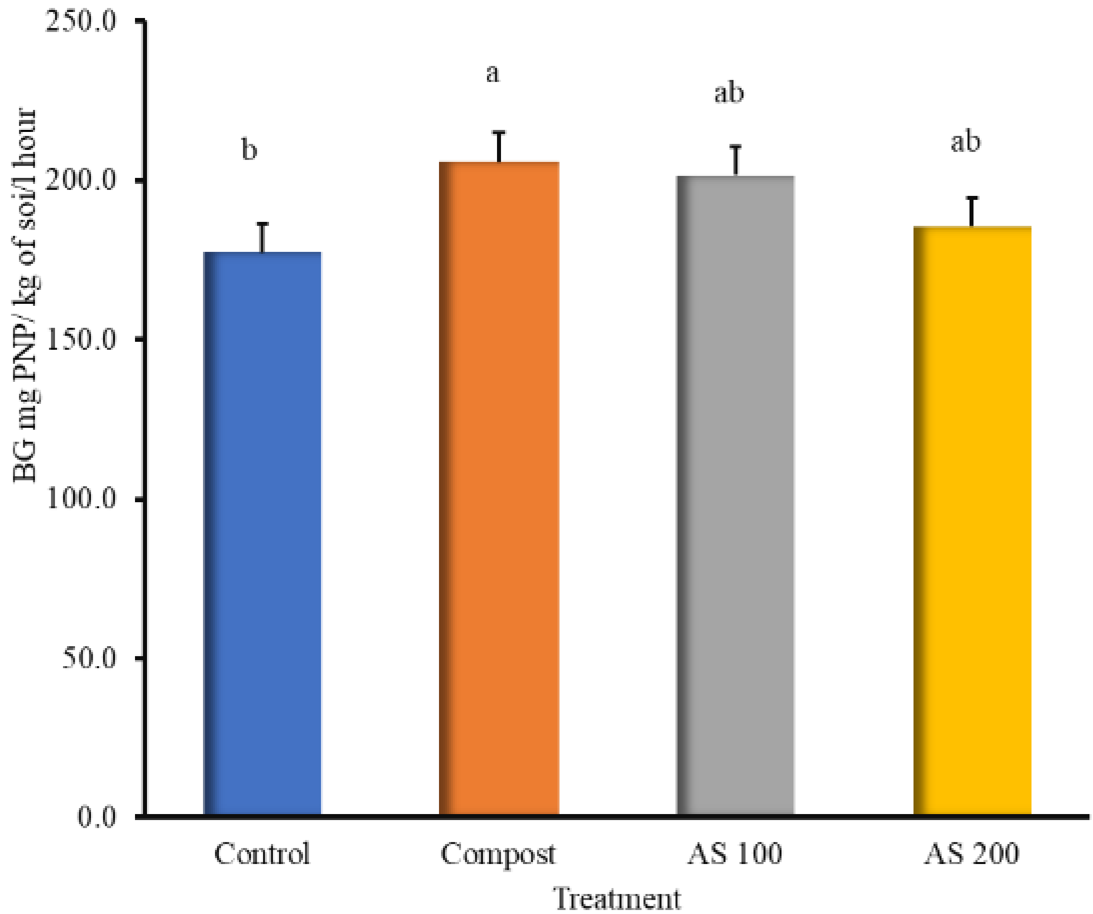
β-Glucosidase (BG) activity at different time of growing seasons



Note. Repeated measurewas employed to analyze BG at different times of growing season (May, August, and November). Soil samples were collected at a depth 0-15 cm from 2019 to 2021. Error bars represent standard errors (n = 12). Different lowercases above the bars indicate as significant difference among seasons (p ≤0.05).

Figure 4. 15.

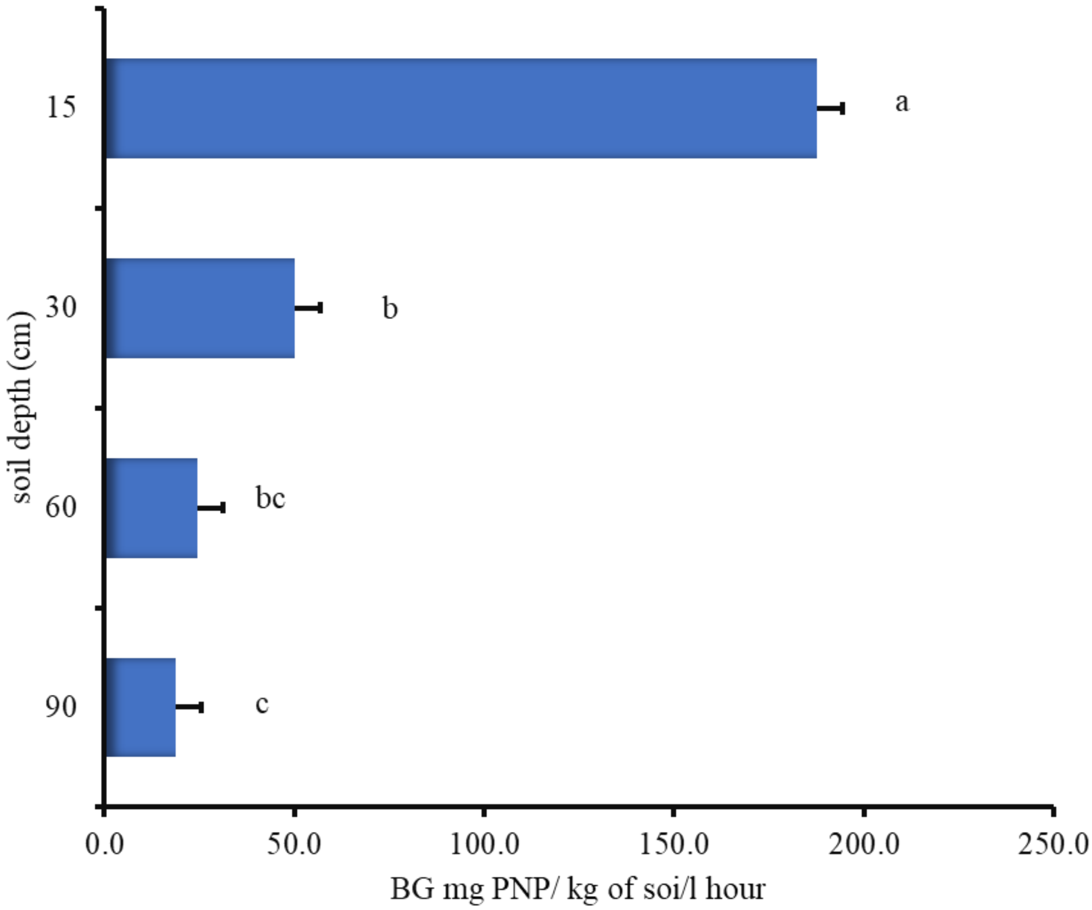
Effects of contrasting N sources on β-Glucosidase (BG)



Note. Repeated measures analysis was employed to assess the effects of contrasting N sources on BG activity. Soil samples were collected at a depth of 0-15 cm spanning the years 2019 to 2021. Error bars represent standard errors (n = 28). Different lowercases above the bars indicate a significant difference among treatments ($p \leq 0.05$).

Figure 4. 16.

β-Glucosidase (BG) activity at different soil depths



Note. Measurement of BG activity at various soil depths across treatments (0-15 cm, 15-30 cm, 30-60 cm, and 60-90 cm) in November 2019. Error bars reflect standard errors (n=4). Lowercase letters above the bars indicate significance at the specified depth (p ≤0.05).

Table 4. 1.

Modelled potentially mineralizable C from 84 days incubation (C_0), potentially mineralizable C at 14 days incubation (C_{014}), the rate constant of carbon mineralization rate over 84 day (k), and the rate constant of carbon mineralization at 14 day (k_{14})

Treatment	C_0	k	R^2	C_{014}	k_{14}	R^2
Control	158.0 b	0.03	0.99	81.1 b	0.10	0.81
Compost	311.7 a	0.03	0.99	205.0 a	0.06	0.75
AS100	161.5 b	0.03	0.97	73.8 b	0.12	0.74
AS200	138.7 b	0.03	0.98	63.4 b	0.10	0.81
P value	***	ns		***	ns	
Standard Error	23.1	0.005		16.6	0.02	

P values indicated as * 0.5, **0.0, *** 0.001
Unit is mg/kg soil, k (per day)

Note. Potential mineralizable C at 84 days incubation (C_0), potential mineralizable C at 14 days incubation (C_{014}), the constant of C mineralization rate at 84 day (k), and the constant of C mineralization rate at 14 day (k_{14}), were estimated with SAS OnDemand using a nonlinear least square curve-fitting technique (Proc NLIN). This regression analysis assumed that C mineralization was a first order reaction $\mathrm{Ct} = \mathrm{C_0}(1 - \mathrm{e}^{-\mathrm{kt}})$. This model was used to estimate the value of C_0 and k of each treatment in each block. Then, the statistical analysis was perform using proc mixed procedure in SAS OnDemand. Two ways ANOVA was employed to determine the significant difference among the treatments. The soil was sampled in August 2019 from 0-15 cm depth.

Table 4. 2.

Pearson correlation matrix for carbon mineralization indicators from 84-day incubation and 14-day incubation

Pearson Correlation Coefficients, N = 16 Prob > |r| under H0: Rho=0				
	C_0	C_{014}	k	k_{14}
C_0	1.00			
C_{014}	0.74 ***	1.00		
k	-0.30	0.09	1.00	
k_{14}	-0.40	-0.64 *	0.36	1.00

*P ≤0.05 , ** p≤0.01, ***0.001

Note. Potentially mineralizable C from 84 days incubation (C_0), potentially mineralizable C from 14 days incubation (C_{014}), the rate constant of carbon mineralization (k) at from 84-day incubation, and the rate constant of C mineralization from 14-day incubation (k_{14}), across different treatments from 84 days incubation experiment.

Table 4. 3.

Pearson correlation matrix for soil C and N indicators

Pearson Correlation Coefficients, N = 48 **Prob > |r| under H0: Rho=0**									
	TC	SOC	POXC	WEOC	BG	STN	ACE	NAGase	WEON
TC	1								
SOC	0.84***	1							
POXC	0.72***	0.7***	1						
WEOC	0.8***	0.7***	0.8***	1					
BG	0.6***	0.5**	0.3	0.5**	1				
STN	0.7***	0.6***	0.5**	0.6***	0.2	1			
ACE	0.8***	0.8***	0.8***	0.9***	0.4*	0.5*	1		
NAGase	0.5**	0.4*	0.6***	0.6***	0.1	0.4*	0.6***	1	
WEON	0.7***	0.7***	0.7***	0.8***	0.4*	0.4*	0.9***	0.4*	1

*Abbreviations: ACE, autoclavable citrate extractable protein; NAGase, N-acetyl β-D-glucosaminidase;STN, total soil nitrogen; WEON, water-extractable organic nitrogen*p≤0.05, **p≤0.01, ***≤0.001*

Note. Pearson correlation is calculated across various treatments and the time of soil sample collection. Soil samples were collected at depths of 0-15 cm from 2019-2021.

CHAPTER V

SUMMARY AND CONCLUSION

Agriculture plays a crucial role in ensuring food security and meeting the demands of a growing global population. However, as we seek to maximize crop production, it is imperative that we do so sustainably, considering the environmental impact and long-term health of our soils. In this context, we conducted a study on contrasting nitrogen management impacts on nitrogen use efficiency (NUE) and soil health under silage corn production in a semi-arid environment. Our study involved a comprehensive examination of the effects of different nitrogen (N) sources on NUE, soil nitrogen and carbon indicators, and the intricate relationship between these soil health indicators.

The dissertation comprises three main data chapters, each scrutinizing the impact of contrasting N fertility management on NUE and soil health in the context of silage corn production within a semi-arid environment. Chapter II delves into the effects of contrasting N fertility sources on the NUE of silage corn. The primary objective is to assess and compare the contrasting effects of contrasting N sources on corn silage yield, plant N uptake, NUE, and soil total N in the semi-arid conditions of northern Utah, USA. Moving to Chapter III, the focus shifts to evaluating soil health N indicators in corn silage production with contrasting nitrogen sources in a semi-arid environment. This chapter aims to comprehensively examine the impact of contrasting N sources on soil N indicators and their dynamic interactions throughout the duration of corn silage production across multiple growing seasons, while simultaneously investigating the intricate relationships between these soil N indicators within a corn silage system under contrasting N sources across different seasons. Lastly, Chapter IV centers on evaluating the effects of contrasting nitrogen sources on soil health carbon indicators and the carbon-nitrogen relationship under corn silage production. The objectives include investigating the impacts of contrasting nitrogen fertilizers on a range of soil carbon indicators and their interrelationships within a corn silage system under contrasting nitrogen sources across multiple growing seasons, with a specific focus on understanding the connections between soil carbon and nitrogen indicators in response to different nitrogen sources.

In Chapter II, the results revealed a substantial 41% improvement in yield under the compost treatment compared to the control treatment. However, it is noteworthy that the yield under the compost treatment was still lower than that achieved with the ammonium sulfate treatments. Additionally, the ammonium sulfate treatments demonstrated increased N uptake and NUE compared to the compost and control treatments. Intriguingly, the AS100 treatment produced yields that were not significantly different from those of the AS200 treatment but outperformed AS200 in terms of NUE. This suggests that increasing N fertilizer rates may not necessarily ensure maximum yield and profit. Despite being less efficient in terms of yield and NUE, the compost treatment significantly contributed by enhancing total soil nitrogen (STN) by 23.1% compared to the other treatments. Therefore, while ammonium sulfate application significantly increased yield and improved NUE, it did not contribute to the enhancement of STN. Conversely, the compost treatment, although yielding lower crop output and NUE due to insufficient N supply, demonstrated a noteworthy advantage by enhancing STN. This improvement is vital for maintaining soil quality, promoting sustainable crop production, and ensuring environmental well-being.

In Chapter III, our study revealed that compost application led to a notable increase in the potential mineralizable nitrogen pool (N_0). This suggests that compost contributes to a reservoir of nitrogen in the soil capable of undergoing mineralization—a microbial decomposition process resulting in the release of mineral nitrogen, thereby augmenting the soil's fertility. Notably, under compost treatment, significant improvements were observed across various N indicators, except for inorganic N. STN levels increased by 23.1%, ACE protein exhibited an approximate 36.7% increase, n-acetyl-glucosamindase enzyme (NAGase) activity saw an increase of about 29.7%, and water extractable organic N (WEON) showed an increase of approximately 29.6%, in comparison to other treatments. Furthermore, the study unveiled moderate to strong linear relationships among these N indicators, highlighting clear interconnections influenced by seasonal variations and specific fertilizer treatments applied.

In Chapter IV, our study demonstrated that compost treatment had a significant impact on soil carbon (TC) and soil organic carbon (SOC) levels, leading to an increase of approximately 4.63 % in TC and a substantial 23.04% increase in SOC. Furthermore, the compost treatment positively influenced other C indicators, including potentially mineralizable C (C_0), permanganate oxidizable C (POXC), water extractable organic C (WEOC), and beta-glucosidase enzyme activity (BG). Specifically, C_0 increased by approximately 103.8%, POXC levels increased by around 20.6%, WEOC showed a substantial boost of roughly 35.2%, and BG activity witnessed an increase of approximately 10.3%, all in comparison to the average values recorded for the other treatments. Additionally, our investigation into the relationship between SOC and other soil N and C indicators revealed strong positive correlations with various C indicators (POXC, WEOC, and BG), emphasizing the intricate relationships among these variables and highlighting the pivotal role of microorganisms in C cycling. Moreover, a strong, positive relationship was found between SOC and soil N indicators such as total N, autoclave citrate extractable (ACE) protein, and WEON. STN also exhibited a moderate correlation with POXC and WEOC, suggesting interconnected dynamics between these variables. These findings underscore the necessity for tailored approaches to address the complex dynamics of soil C and N in agroecosystems.

Additionally, our investigation extended to evaluating the impact of fertilizers on soil health indicators across different soil depths. The distribution of soil health C and N indicators demonstrated higher concentrations in the uppermost soil layer (0-15 cm) and gradually decreased with depth. The topsoil, owing to elevated organic input from plant residues, root activity, and surface organic matter decomposition, displayed a greater accumulation of C and N compounds near the surface. This phenomenon results in higher microbial activity in the topsoil, driven by the availability of organic substrates, which contributes to the decomposition of organic matter and the release of nutrients such as N.

This field experiment was maintained from 2011 through 2021 with the repeated applications of the N source treatments. Treatment impacts on total soil N and soil organic C were not statistically significant in the early years and not in every year throughout the experiment span, highlighting the importance of multiple season studies. The slow response and variability in these measures strengthens the importance of the more responsive soil health C and N indicators determined on surface soil. The indicators of WEON, ACE and NAGase were all correlated positively with total soil N. ACE protein, WEON and NAGase levels were significantly elevated in the surface soils for the compost treatment suggesting that they are more sensitive than STN. Similarly, POXC, WEOC and BG activity were strongly positively correlated with SOC and significantly increased by the repeated compost treatments. POXC and WEOC were also correlated with STN. While STN and SOC are strong baseline measurements for long-term soil health assessments the C and N indicators of the water extractable organic forms and key enzyme activities were confirmed to be useful and more responsive indicators in this long-term experiment.

In summary, our study has unveiled the diverse impacts of different N sources on corn silage yield, NUE, and soil health C and N indicators. The ammonium sulfate treatment resulted in higher yields and NUE, showcasing its effectiveness as a N source. In contrast, the compost treatment, while falling short in meeting crop N demands and yielding lower NUE, exhibited the noteworthy advantage of enhancing soil organic C and N and their associated soil health indicators. This observation highlights the potential benefits of incorporating organic carbon-rich amendments like compost to bolster soil C storage, enhance N dynamics, and boost microbial enzyme activities. The positive shifts in soil indicators underscore the comprehensive advantages of compost application, fostering improved fertility, structure, and sustainable soil management practices. Therefore, for not only maintaining soil health but also optimizing yield and nitrogen use efficiency, farmers may consider adopting a balanced approach that combines compost with commercial fertilizers and incorporates sound soil health practices such as soil testing, crop rotation and cover crops to improve soil health.

APPENDICES

APPENDIX A

SUPPLEMENTARY MATERIAL AND STATISTICAL ANALYSIS FOR CHAPTER II

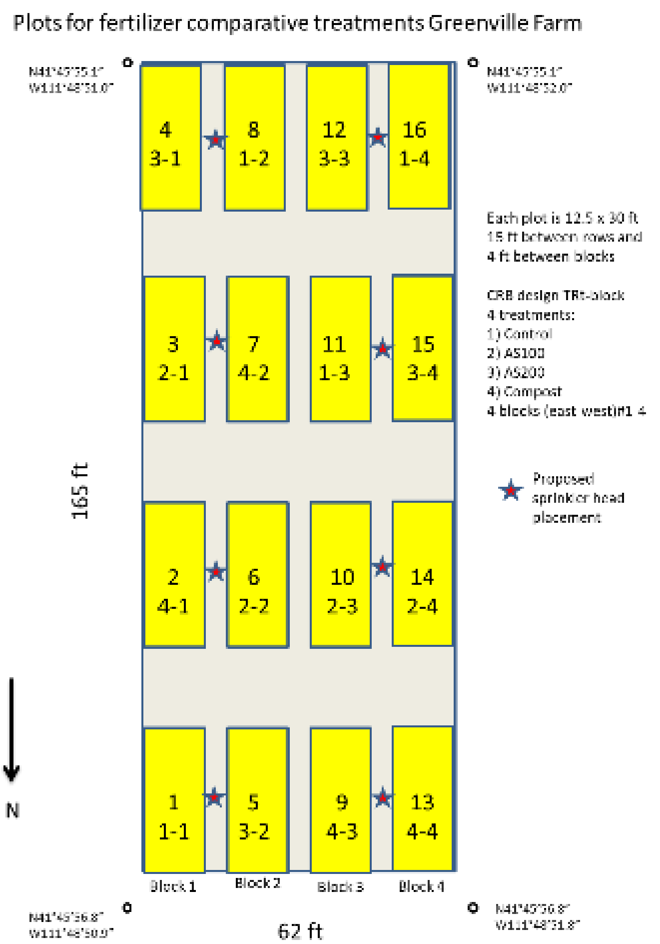
Figure A1.

Plot Layout of Field Study on Contrasting Nitrogen Sources in Corn Silage Experiment

Note. A long-term field study investigating various N fertility sources in corn silage production under semi-arid conditions has been ongoing at the Utah State University Greenville research farm in northern Utah since 2012. This aerial image of the N cycle plots was captured by a drone during the summer of 2020. Each plot measures 3.8 m x 9.1 m. The study comprises four distinct treatments, including: control with no N fertilization (Control), compost with 224 kg total N ha^{-1} (compost), and ammonium sulfate with 112 & 224 kg N ha^{-1} (AS100 and AS200), respectively.

Figure A2.

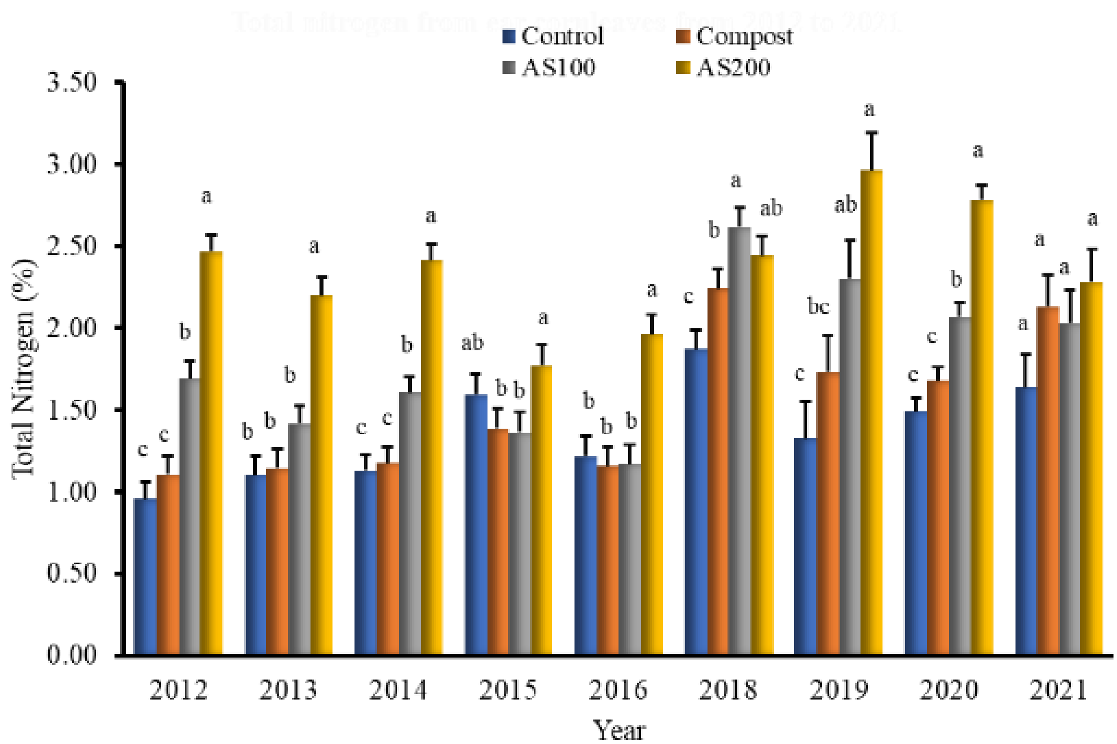
Plot layout diagram



Note. Plot layout for N fertilization studies on contrasting N sources on corn silage production at the Greenville Farm in North Logan, UT. Top number refers to plot number and the bottom number refers to treatment and block. The four treatments: (1) control, (2) low ammonium sulfate (AS100) at a rate of 112 kg N ha^{-1}, (3) high ammonium sulfate (AS200) at a rate of 224 kg N ha^{-1}, and (4) steer manure compost (compost) at 224 kg total N ha^{-1}. Each plot is 3.8 x 9.1 m (4.6 m between rows and 1.2 m between blocks).

Figure A3.

Total nitrogen (TN) concentration from corn ear leaves



Note. TN concentration from ear corn leaves was analyzed from 2012 to 2021. There are four treatments: control with no N fertilization (Control), compost with 224 kg TN ha^{-1} (denoted as Compost), and ammonium sulfate with AS 112 & 224 kg N ha^{-1} (denoted as AS100 and AS200, respectively). Error bars represent standard errors (n = 4). Different lowercases above the bars indicate a significant difference within a year ($p \leq 0.05$).

Table *A1*.

Effects of contrasting N sources nitrogen use efficiency (NUE)

Year	Compost	AS100	AS200	Std error	p value	Corn silage benchmark Efficiency ranges (Augarten et al., 2019)
Partial Factor Productivity (PFP)						
2012	33.11 b	90.2 a	48.47 b	4.3	0.0003	Low 0-80 Medium-Mid 81-95 Mid-High 96-108 High >108
2013	47.48 b	120.3 a	52.32 b	8.0	0.0021	
2014	69.35 c	194.53 a	108.05 b	4.4	≤0.0001	
2015	52.02 b	104.0 a	94.56 ab	13.9	0.0353	
2016	45.69 b	109.35 a	63.46 b	8.4	0.0074	
2018	85.22 b	183.39 a	84.64 b	12.2	≤0.0001	
2019	51.01 c	118.69 a	76.78 ab	11.2	0.0108	
2020	34.75 c	153.72 a	92.47 b	3.4	≤0.0001	
2021	27.94 c	118.90 a	71.35 b	11.3	0.0019	
Partial Nutrient Balance (PNB)						
2012	0.19b	0.53a	0.42a	0.05	0.23	low 0-0.92 Low-Mid 0.92-1.08 Mid-High 01.08-1.29 High >1.29
2013	0.42b	1.30a	0.65b	0.08	0.0011	
2014	0.49b	1.61a	1.36a	0.10	0.0003	
2015	0.34b	0.69a	0.93ab	0.14	0.0549	
2016	0.34b	1.02a	0.80b	0.11	0.011	
2018	0.75b	1.56a	0.86b	0.13	0.0004	
2019	0.27b	0.72a	0.67b	0.08	0.0048	
2020	0.17b	0.77a	0.66b	0.03	<.0001	
2021	0.25a	1.04a	0.77a	0.13	0.0084	
Agronomic Efficiency (AE)						
2012	-10.32a	3.36a	5.04a	5.44	0.1069	Higher AE means more efficient use of nitrogen
2013	20.97b	67.28a	25.81b	8.5	0.0151	
2014	24.53c	104.87a	63.23b	4.0	<.0001	
2015	26.32a	52.60a	68.86a	12.2	0.1074	
2016	19.56a	57.1a	37.34a	8.2	0.0782	
2018	9.69a	32.32a	9.11a	6.1	0.0587	
2019	16.18a	49.04a	41.9a	15.5	0.1984	
2020	7.65c	99.53a	65.37b	3.8	<.0001	
2021	14.71b	92.42a	58.12ab	14.9	0.0079	
Nitrogen uptake efficiency (%)						
2012	-2.3b	9.9ab	21.1a	7.41		UE<50% low UE>50% high UE close to 1 is highest
2013	22.7b	92.3a	45.9b	9.65		
2014	20.9b	105.4a	108.2a	10.6		
2015	16.8a	33.5a	75.2a	13.7		
2016	14.2b	61.4a	59.9a	12.7		
2018	15.0a	36.4a	26.6a	9.25		
2019	10.6b	39.4ab	50.5a	9.71		
2020	7.0b	56.1a	57.9a	3.48		
2021	15.5b	67.3ab	84.3a	15.6		

Note. Effects of contrasting N sources on NUE such as Partial factor productivity (PFP), partial nutrient balance (PNB), and Agronomic Efficiency from 2012 to 2021 Error bars represent standard errors (n = 4). Different lowercases above the bars indicate a significant difference in each year ($p \leq 0.05$)

Results of statistical analysis for chapter II

Repeated measurement of yield of corn silage from 2011 to 2021

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	3	9	33.2	<.0001
year	8	96	12.38	<.0001
year*treatment	24	96	5.47	<.0001

Repeated measurement of nitrogen uptake of silage corn aboveground at harvest from 2019 to 2021

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	3	9	33.44	<.0001
year	8	96	13.37	<.0001
year*treatment	24	96	4.96	<.0001

Repeated measurement of effect of contrasting N sources on nitrogen use efficiency (NUE) indicators_ UE from 2012 to 2021

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	2	6	20.8	0.002
year	8	72	8.34	<.0001
year*treatment	16	72	4.8	<.0001

Repeated measurement of effect of contrasting N sources on nitrogen use efficiency (NUE) indicators_ PFP from 2012 to 2021

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	2	6	84.35	<.0001
year	8	72	11.8	<.0001
year*treatment	16	72	6.59	<.0001

Repeated measurement of effect of contrasting N sources on nitrogen use efficiency (NUE) indicators_ PNB from 2012 to 2021

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	2	6	40.51	0.0003
year	8	72	13.38	<.0001
year*treatment	16	72	6.14	<.0001

Repeated measurement of effect of contrasting N sources on nitrogen use efficiency (NUE) indicators_ AE from 2012 to 2021

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	2	6	28.11	0.0009
year	8	72	9.05	<.0001
year*treatment	16	72	5.13	<.0001

Repeated measurement of effect of contrasting N sources on total N concentration in corn leaves from 2011 to 2021

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	3	9	95.72	<.0001
year	8	96	19.48	<.0001
year*treatment	24	96	6.52	<.0001

Repeated measurement of effect of contrasting N sources on soil total N from 2011 to 2021

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	9	12.49	0.0015
Year	9	108	14.71	<.0001
Year*Treatment	27	108	1.46	0.0879

APPENDIX B

SUPPLEMENTARY MATERIAL AND STATISTICAL ANALYSIS FOR CHAPTER III

Table B1.

Compost analysis

	year	N%	C%	C/N
Ncycle	2011	1.5	37.5	17.9
Ncycle	2012	1.48		
Ncycle	2013	1.5		
Ncycle	2014	1.5		
Ncycle	2015	1.76		
Ncycle	2015	1.85	22.3	12.1
Ncycle	2016	1.68	26.0	15.8
Ncycle	2017	not applied		
Ncycle	2018	1.68		
Ncycle	2019	2.13	22.53	10.6
Ncycle	2020	1.72	25.92	15.1
Ncycle	2021	1.84	28.17	15.3

Table B2.

Means, and standard errors (SE) in inorganic N

	2019		2020		2021		
	August	Nov	May	Aug	May	Aug	Nov
AS 100	1.4	0.7	1.96 a	0.9	0.4	1.7 ab	2.5
AS 200	1.7	1.1	2.28 a	1.0	1.3	2.61 a	2.2
Compost	1.5	0.7	1.35 ab	1.1	1.0	1.73 ab	2.1
Control	0.9	1.0	1.07 b	0.8	1.1	1.13 b	1.6
SE	0.4	0.3	0.4	0.2	0.5	0.2	0.7
p-value	0.3	0.4	0.02	0.5	0.2	0.002	0.8

Unit: $mg\ N\ kg^{-1}\ of\ soil$

Note. Analysis of inorganic N (Ammonium-N+ Nitrate-N) from soil samples collected at a depth of 0-15 cm, spanning the Years 2019 to 2021. Error bars represent standard errors (n = 4). Different lowercases above the bars indicate a significant difference by treatment within date of soil sampling (p≤0.05).

Table B3.

Correlation of N indicators over time (2019-2021)

a) Pearson correlation matrix for N indicators from soil samples taken in August 2019

Pearson Correlation Coefficients, N = 16 Prob > |r| under H0: Rho=0					
	STN	WEON	ACE	NAGase	Inorganic N
STN	1.00				
WEON	0.51*	1.00			
ACE	0.43	0.73**	1.00		
NAGase	0.47	0.47*	0.81***	1.00	
Inorganic N	0.01	-0.27	-0.39	-0.27	1.00

b) Pearson correlation matrix for N indicators from soil samples taken in November 2019

Pearson Correlation Coefficients, N = 16 Prob > |r| under H0: Rho=0					
	STN	WEON	ACE	NAGase	Inorganic N
STN	1.00				
WEON	0.27	1.00			
ACE	0.43	0.33	1.00		
NAGase	0.51*	0.30	0.78**	1.00	
Inorganic N	0.03	0.85	-0.12	-0.001	1.00

c) Pearson correlation matrix for N indicators from soil samples taken in May 2020

Pearson Correlation Coefficients, N = 16 Prob > |r| under H0: Rho=0					
	STN	WEON	ACE	NAGase	Inorganic N
STN	1.00				
WEON	0.49	1.00			
ACE	0.49	0.70**	1.00		
NAGase	0.39	0.49	0.55*	1.00	
Inorganic N	0.03	0.04	0.03	0.44	1.00

d) Pearson correlation matrix for N indicators from soil samples taken in August 2020

Pearson Correlation Coefficients, N = 16 Prob > |r| under H0: Rho=0					
	STN	WEON	ACE	NAGase	Inorganic N
STN	1.00				
WEON	0.43	1.00			
ACE	0.68**	0.87***	1.00		
NAGase	0.14	0.32	0.41	1.00	

Inorganic N	0.20	-0.04	-0.05	-0.42	1.00

e) Pearson correlation matrix for N indicators from soil samples taken in May 2021

Pearson Correlation Coefficients, N = 16 Prob > |r| under H0: Rho=0					
	STN	WEON	ACE	NAGase	Inorganic N
STN	1.00				
WEON	0.21	1.00			
ACE	0.19	0.70**	1.00		
NAGase	0.43	0.48	0.65**	1.00	
inorganic N	-0.40	-0.09	0.01	-0.38	1.00

f) Pearson correlation matrix for N indicators from soil samples taken in August 2021

Pearson Correlation Coefficients, N = 16 Prob > |r| under H0: Rho=0					
	STN	WEON	ACE	NAGase	Inorganic N
STN	1.00				
WEON	0.48	1.00			
ACE	0.66**	0.77***	1.00		
NAGase	0.49	0.74**	0.66**	1.00	
inorganic N	0.24	-0.06	0.15	-0.09	1.00

g) Pearson correlation matrix for N indicators from soil samples taken in November 2021

Pearson Correlation Coefficients, N = 16 Prob > |r| under H0: Rho=0					
	STN	WEON	ACE	NAGase	inorganicN
STN	1.00				
WEON	0.11	1.00			
ACE	0.75**	0.38	1.00		
NAGase	0.09	0.61**	0.32	1.00	
inorganicN	-0.21	0.77**	-0.09	0.56*	1.00

Note. Appendix table 3.1 (a-g) showed the correlation values between the N indicators for each time of taking soil sample at depth 0-15 cm. The specific month and year for each set of correlation values are indicated in the corresponding titles, such as “Aug2019” representing samples taken in August 2019.

Table B4.

Effects of STN, treatments and years on ACE protein

a) Estimated Effects of STN, Treatment, and Year on ACE

Solution for Fixed Effects							
Effect Intercept	Treatment	Year	Estimate	Standard Error	DF	t Value	Pr > |t|
STN			3933.17	218.12	3	18.03	0.0004
STN*Treatment			0.03318	0.1362	38	0.24	0.8089
STN*Treatment	AS 100		-0.1118	0.06507	38	-1.72	0.094
STN*Treatment	AS 200		-0.103	0.06385	38	-1.61	0.115
STN*Treatment	Compost		0.7729	0.06164	38	12.54	<.0001
STN*Year	Control		0	.	.	.	.
STN*Year		2019	0.228	0.08562	38	2.66	0.0113
STN*Year		2020	6.33E-16	0.02287	38	0	1
Effect		2021	0	.	.	.	.

b) Type 3 Tests of Fixed Effects for STN, Treatment, and Year on ACE

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
STN	1	38	3.33	0.0759
STN*Treatment	3	38	94.75	<.0001
STN*Year	2	38	3.61	0.0367

Note. Appendix 6 (a and b) presents the estimated effects of “STN,” “Treatment,” and “Year” on the response variable “ACE.” The table displays the estimates, standard errors, degrees of freedom, t-values, and p-values for each effect. Notably, it reveals the strength and significance of the relationships between the predictor variables and “ACE

Table B5.

Effects of STN, treatments and years on WEON

a) Estimated Effects of STN, Treatment, and Year on WEON

Solution for Fixed Effects							
Effect	Treatment	Year	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept			12.7286	1.6269	3	7.82	0.0043
STN			0.000059	0.001123	38	0.05	0.9584
STN*Treatment	AS 100		-0.00014	0.000701	38	-0.21	0.8375
STN*Treatment	AS 200		-0.00103	0.000703	38	-1.47	0.151
STN*Treatment	Compost		0.002474	0.000642	38	3.85	0.0004
STN*Treatment	Control		0	.	.	.	.
STN*Year		2019	0.001184	0.000416	38	2.85	0.0071
STN*Year		2020	-2.54E-18	0.000319	38	0	1
STN*Year		2021	0	.	.	.	.

b) *Type 3 Tests of Fixed Effects for STN, Treatment, and Year on WEON*

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
STN	1	38	0.51	0.4788
STN*Treatment	3	38	10.17	<.0001
STN*Year	2	38	4.75	0.0144

Note. Appendix Correlation 3.5 a and b presents the estimated effects of “STN,” “Treatment,” and “Year” on the response variable “WEON.” The table displays the estimates, standard errors, degrees of freedom, t-values, and p-values for each effect. Notably, it reveals the strength and significance of the relationships between the predictor variables and “WEON.

Table B6.

Effects of STN, treatments and years on NAGase

a) *Estimated Effects of STN, Treatment, and Year on NAGase*

Solution for Fixed Effects							
Effect	Treatment	Year	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept			44.2907	8.8939	3	4.98	0.0156
STN			0.005721	0.00602	38	0.95	0.348
STN*Treatment	AS 100		-0.0039	0.002851	38	-1.37	0.1797
STN*Treatment	AS 200		-0.00534	0.002859	38	-1.87	0.0696
STN*Treatment	Compost		0.006098	0.002679	38	2.28	0.0285
STN*Treatment	Control		0	.	.	.	.
STN*Year		2019	1.86E-06	0.002555	38	0	0.9994
STN*Year		2020	2.17E-17	0.002016	38	0	1
STN*Year		2021	0	.	.	.	.

b) *Type 3 Tests of Fixed Effects for STN, Treatment, and Year on NAGase*

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
STN	1	38	0.67	0.42
STN*Treatment	3	38	5.62	0.0027
STN*Year	2	38	0.00	1

Note. Appendix Correlation 3.6 (a and b) presents the estimated effects of “STN,” “Treatment,” and “Year” on the response variable “*NAGase”.* The table displays the estimates, standard errors, degrees of freedom, t-values, and p-values for each effect. Notably, it reveals the strength and significance of the relationships between the predictor variables and “*NAGase”.*

Results of statistical analysis for chapter III

Repeated measurement of effect of contrasting N sources on soil total N (STN) from 2011 to 2021

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	9	12.49	0.0015
Year	9	108	14.71	<.0001
Year*Treatment	27	108	1.46	0.0879

Repeated measurement of effect of contrasting N sources Seasonal Variation in Soil Total Nitrogen (STN) at 0-15 cm Depth (2019-2021

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	27	12.85	<.0001
month	2	9	5.08	0.0334
month*Treatment	6	27	0.82	0.5670

Soil total nitrogen (STN) by soil depths

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	45	1.47	0.2345
depths	3	45	28.79	<.0001
Treatment*depths	9	45	1.01	0.4451

Net N mineralization (NetMin) 84-day incubation (NetMin84)

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	25	2.06	0.1311

Net N mineralization (NetMin) at 14-day incubation

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	3	25	15.51	<.0001

Repeated measurement of effects of contrasting nitrogen sources on autoclaved citrate-extractable protein (ACE) from 2019 to 2021 for the 0-15 cm soil depth

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	3	9	89	<.0001
Date	6	152	134.56	<.0001
Date*treatment	18	152	4.98	<.0001

Repeated measurement of ACE under different times of growing season

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	3	27	17.56	<.0001
Month	2	9	5.42	0.0285
Month*treatment	6	27	0.78	0.5893

Repeated measurement of effects of contrasting N sources on ACE protein at different soil depths

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	3	109	5.39	0.0017
depth	3	109	506.98	<.0001
treatment*depth	9	109	3.8	0.0003

Repeated measurement of effects of contrasting N sources on water extractable organic nitrogen (WEON) from 2019 to 2021 for the 0-15 cm soil depth

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	9	14.75	0.0008
Date	6	119	23.92	<.0001
Date*Treatment	18	119	2.16	0.0074

Repeated measurement of WEON under different times of growing season

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	9	6.72	0.0113
Month	2	135	15.37	<.0001
Month*Treatment	6	135	2.26	0.041

Water extractable organic nitrogen (WEON) at different depth of soil

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	3	45	6.65	0.0008
depth	3	45	171.82	<.0001
treatment*depth	9	45	1.56	0.1555

Repeated measurement of effects of contrasting N sources on inorganic N from 2019 to 2021 for the 0-15 cm soil depth

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	9	4.85	0.0283
Date	6	119	3.33	0.0046
Date*Treatment	18	119	1.27	0.2225

Repeated measurement of inorganic N under different times of growing season

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	9	3.96	0.0471
Month	2	135	0.95	0.3879
Month*Treatment	6	135	0.49	0.815

Inorganic N at different soil depths

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	41	2.16	0.1075
Depth	3	41	6.64	0.0009
Treatment*Depth	9	41	0.53	0.8471

Repeated measurement of effects of contrasting N source treatment on N-acetyl-β-D-glucosaminidase activity (NAGase) 2019 to 2021 for the 0-15 cm soil depth

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	3	9	12.13	0.0016
Date	6	184	0.89	0.5008
Date*treatment	18	184	2.34	0.0024

Repeated measurement of N-acetyl-β-D-glucosaminidase activity (NAGase) under different times of growing season

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	3	9	10.4	0.0028
time	2	200	0.11	0.8981
time*treatment	6	200	0.95	0.4593

NAGase at different soil depths

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	45	1.33	0.2757
Depth	3	45	83.04	<.0001
Treatment*Depth	9	45	0.91	0.5235

APPENDIX C

SUPPLEMENTARY MATERIAL AND STATISTICAL ANALYSIS FOR CHAPTER IV

Table C1.

Means, and standard errors (SE) of soil bulk density at different soil depths

Depth (cm)	Bulk Density $\mathbf{g\ cm^{-3}}$
0-15	1.33 a
15-30	1.20 b
30-60	1.14 b
60-90	1.15 b
SE	0.03
p-value	0.001

Unit: g cm^{-3}

Note. Analysis of bulk density from soil samples collected in November 2021 at a depth of 0-15, 15-30, 30-60, and 60-90 cm. Different lowercases letters indicate a significant difference by depth ($p \leq 0.05$).

Table C2.

The Pearson correlation between the carbon mineralization from 14-day incubation and 84 days incubation

	C084	K84	c014	k14	RateC14	CumC14	Rate84	CumC84
C084	1							
K84	-0.3	1						
c014	0.73*	0.17	1					
k14	-0.4	0.25	-0.64*	1				
RateC14	0.83***	-0.048	0.94***	-0.61*	1			
CumC14	0.8**	0.30	0.85***	-0.27	0.91***	1		
Rate84	0.8***	-0.39	0.51	-0.39	0.58*	0.5	1	
CumC84	0.95***	-0.09	0.81**	-0.39	0.9***	0.89***	0.78**	1

$p \leq 0.05$ *, $p \leq 0.001$**, $p \leq 0.0001$***

Potential carbon mineralizable C from 84 days incubation, C084; Potential carbon mineralizable C from 14 days incubation, C014; constant of organic carbon mineralization rate from 84 days incubation, k84; constant of organic carbon mineralization rate from 14 days incubation, k14; Rate of C mineralization from 84 day incubation, Rate84; Rate of C mineralization from 14 day incubation, Rate14; Cumulative C mineralization from 84 day incubation, CumC84; Cumulative C mineralization from 14 day incubation, CumC14.

Note. The soil analysis for this correlation was from August soil 2019 at depth 0-15 cm.

Table C3.

The Pearson correlation between the potential carbon mineralization from 14 days incubation and other soil health indicators

	CO14	ACE	STN	WEON	NAGase	WEOC	POXC	TC	BG	SOC
CO14	1	0.69*	0.72*	0.63*	0.62*	0.90***	0.41	0.64*	0.26	0.34

$p \leq 0.05$ *, $p \leq 0.001$**, $p \leq 0.0001$***

Note. The soil analysis for this correlation was from August soil 2019 at depth 0-15 cm. Potential carbon mineralizable C from 14 days incubation, Co14, Autoclaved citrate-extractable protein, ACE, Soil Total nitrogen, STN, Water extractable organic nitrogen, WEON, N-acetyl-β-D-glucosaminidase, NAGase, Water extractable organic carbon, WEOC, Permanganate-oxidizable carbon, POXC, Soil Total carbon, TC and β-glucosidase, BG, soil organic C, SOC

Table C4.

Correlation of carbon indicators over time (2019-2021)

h) Pearson correlation matrix for carbon indicators from soil samples taken in August 2019

Pearson Correlation Coefficients, N = 16 Prob > |r| under H0: Rho=0					
	TC	SOC	POXC	WEOC	BG
TC	1				
SOC	0.79**	1			
POXC	0.51*	0.34	1		
WEOC	0.75**	0.37	0.52*	1	
BG	0.03	-0.17	0.29	0.21	1

b)Pearson correlation matrix for carbon indicators from soil samples taken in November 2019

Pearson Correlation Coefficients, N = 16 Prob > |r| under H0: Rho=0					
	TC	SOC	POXC	WEOC	BG
TC	1				
SOC	0.81**	1			
POXC	-0.29	-0.39	1		
WEOC	0.65*	0.44	-0.17	1	
BG	0.73*	0.66*	-0.17	0.56*	1

C) Pearson correlation matrix for carbon indicators from soil samples taken in May 2020

Pearson Correlation Coefficients, N = 16 Prob > |r| under H0: Rho=0					
	TC	SOC	POXC	WEOC	BG
TC	1				
SOC	0.84***	1			
POXC	0.77**	0.75**	1		
WEOC	0.69*	0.57*	0.64*	1	
BG	0.47	0.39	0.2	0.2	1

i) Pearson correlation matrix for carbon indicators from soil samples taken in August 2020

Pearson Correlation Coefficients, N = 16 Prob > |r| under H0: Rho=0					
	TC	SOC	POXC	WEOC	BG
TC	1				
SOC	0.88***	1			
POXC	0.19	0.12	1		
WEOC	0.88***	0.81**	0.43174	1	
BG	0.49	0.44	0.33	0.4	1

e) Pearson correlation matrix for carbon indicators from soil samples taken in May 2021

Pearson Correlation Coefficients, N = 16 Prob > |r| under H0: Rho=0					
	TC	SOC	POXC	WEOC	BG
TC	1				
SOC	0.61*	1			
POXC	0.06	0.058	1		
WEOC	0.27	0.51*	0.36	1	
BG	0.27	0.16	0.42	0.31	1

f) Pearson correlation matrix for carbon indicators from soil samples taken in August 2021

Pearson Correlation Coefficients, N = 16 Prob > |r| under H0: Rho=0					
	TC	SOC	POXC	WEOC	BG
TC	1				
SOC	0.75**	1			
POXC	0.78**	0.81**	1		
WEOC	0.83***	0.79**	0.95***	1	
BG	0.56*	0.41	0.43	0.54*	1

j) Pearson correlation matrix for carbon indicators from soil samples taken in November 2021

Pearson Correlation Coefficients, N = 16 Prob > |r| under H0: Rho=0					
	TC	SOC	POXC	WEOC	BG
TC	1				
SOC	0.81**	1			
POXC	0.74*	0.63*	1		
WEOC	0.67*	0.71*	0.76**	1	
BG	0.41	0.45	0.2	0.19	1

p≤0.05 *, p≤0.001**, p≤0.0001***

Note: Appendix table 3.1 (a-g) showed the correlation values between the nitrogen indicators for each time of taking soil sample at depth 0-15 cm . The specific month and year for each set of correlation values are indicated in the corresponding titles, such as “Aug2019” representing samples taken in August 2019.

Table C5.

Effects of SOC, treatments and years on POXC

a)Estimated Effects of SOC, Treatment, and Year on POXC

Solution for Fixed Effects							
Effect	**Treatment**	**Year**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Intercept			604.63	50.9933	3	11.86	0.0013
SOC			0.003866	0.002966	38	1.3	0.2003
SOC*Treatment	AS 100		-9.51E-06	0.000874	38	-0.01	0.9914
SOC*Treatment	AS 200		-0.00031	0.000866	38	-0.35	0.7255
SOC*Treatment	Compost		0.005302	0.000985	38	5.38	<.0001
SOC*Treatment	Control		0	.	.	.	.
SOC*Year		2019	-0.00122	0.000691	38	-1.76	0.0861
SOC*Year		2020	0.000667	0.000554	38	1.2	0.2363
SOC*Year		2021	0	.	.	.	.

b)Type 3 Tests of Fixed Effects for SOC, Treatment, and Year on POXC

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
SOC	1	38	3.19	0.0821
SOC*Treatment	3	38	13.7	<.0001
SOC*Year	2	38	3.04	0.0594

Note: Appendix Correlation 3.4 a and b presents the estimated effects of “SOC,” “Treatment,” and “Year” on the response variable “POXC”. The table displays the estimates, standard errors, degrees of freedom, t-values, and p-values for each effect. Notably, it reveals the strength and significance of the relationships between the predictor variables and “POXC”.

Table C6.

Effects of SOC, treatments and years on WEOC

a) Estimated Effects of SOC, Treatment, and Year on WEOC

Solution for Fixed Effects							
Effect	Treatment	Year	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept			160.57	14.169	3	11.33	0.0015
SOC			-0.00054	0.000823	38	-0.65	0.5188
SOC*Treatment	AS 100		-0.00051	0.000418	38	-1.23	0.2272
SOC*Treatment	AS 200		-0.00094	0.000413	38	-2.28	0.0283
SOC*Treatment	Compost		0.001994	0.000403	38	4.95	<.0001
SOC*Treatment	Control		0	.	.	.	.
SOC*Year		2019	-0.00045	0.000136	38	-3.34	0.0019
SOC*Year		2020	5.50E-19	0.000135	38	0	1
SOC*Year		2021	0	.	.	.	.

b) Type 3 Tests of Fixed Effects for SOC, Treatment, and Year on WEOC

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
SOC	1	38	0.56	0.4598
SOC*Treatment	3	38	21.73	<.0001
SOC*Year	2	38	7.41	0.0019

Note. Appendix Correlation 3.5 (a and b) presents the estimated effects of “SOC,” “Treatment,” and “Year” on the response variable “WEOC.” The table displays the estimates, standard errors, degrees of freedom, t-values, and p-values for each effect. Notably, it reveals the strength and significance of the relationships between the predictor variables and “WEOC.

Table C7.

Effects of SOC, treatments and years on BG

a) Estimated Effects of SOC, Treatment, and Year on BG

Solution for Fixed Effects							
Effect	Treatment	Year	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept			117.92	34.1824	3	3.45	0.0409
SOC			0.003847	0.00198	38	1.94	0.0594
SOC*Treatment	AS 100		0.000755	0.000749	38	1.01	0.32
SOC*Treatment	AS 200		-0.00073	0.00074	38	-0.99	0.3299
SOC*Treatment	Compost		-0.00013	0.000758	38	-0.17	0.8694
SOC*Treatment	Control		0	.	.	.	.
SOC*Year		2019	0.000136	0.000396	38	0.34	0.7333
SOC*Year		2020	-3.57E-06	0.000324	38	-0.01	0.9913
SOC*Year		2021	0	.	.	.	.

b) Type 3 Tests of Fixed Effects for SOC, Treatment, and Year on BG

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
SOC	1	38	4.49	0.0408
SOC*Treatment	3	38	1.32	0.2837
SOC*Year	2	38	0.1	0.9072

Note. Appendix Correlation 3.6 (a and b) presents the estimated effects of “SOC” “Treatment,” and “Year” on the response variable ***“ BG.”*** The table displays the estimates, standard errors, degrees of freedom, t-values, and p-values for each effect.

Results of statistical analysis for chapter IV

Repeated measurement of effect of contrasting nitrogen sources on TC of 0-15 cm soil

Tests of Fixed Effects				
Num DF	Num DF	Den DF	F Value	Pr > F
treatment	3	9	11.16	0.0022
Date	6	152	6.73	<.0001
Date*treatment	18	152	1.70	0.0443

Repeated measurement of effect of contrasting nitrogen sources on IC of 0-15 cm soil

Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	3	63	36.81	<.0001
Date	6	21	3.18	0.0223
Date*treatment	18	63	0.86	0.6264

Repeated measurement of effect of contrasting nitrogen sources on OC of 0-15 cm soil

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	3	9	10.67	0.0025
Date	6	152	4.36	0.0004
Date*treatment	18	152	1.07	0.3850

Soil TC at different soil depths

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	45	7.97	0.0002
Depth	3	45	65.05	<.0001
Treatment*Depth	9	45	2.44	0.0237

Soil IC at different soil depths

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	45	0.39	0.7593
Depth	3	45	103.38	<.0001
Treatment*Depth	9	45	0.45	0.902

Soil OC at different soil depths

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	45	2.13	0.1091
Depth	3	45	43.30	<.0001
Treatment*Depth	9	45	0.29	0.9738

Effects of N sources on Co at 84 days of incubation

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	9	16.4	0.0005

Effects of N sources on k at 84 days of incubation

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	9	0.11	0.9538

Effects of N sources on Co at 14 days of incubation

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	9	15.23	0.0007

Effects of N sources on k at 14 days of incubation

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	9	2.27	0.1489

Rate of carbon mineralization at different dates of incubation

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	9	12.3	0.0016
day	7	84	53.94	<.0001
day*Treatment	21	84	2.16	0.0073

Effect of N sources on carbon mineralization indicators from 84-day incubation

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	9	16.40	0.0005

Effect of N sources on carbon mineralization indicators at14 days incubation

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	9	15.23	0.0007

Repeated measurement of effects of contrasting nitrogen sources on soil permanganate-oxidizable carbon (POXC) from 2019 to 2021

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	9	31.76	<.0001
date	6	72	1.57	0.1679
date*Treatment	18	72	1.73	0.0534

Repeated measurement of effects of contrasting nitrogen sources on soil permanganate-oxidizable carbon (POXC) under different times of growing season

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	9	19.08	0.0003
Month	2	88	2.44	0.0934
Month*Treatment	6	88	0.85	0.5351

Permanganate-oxidizable carbon (POXC) at different soil depth

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	45	0.76	0.5217
Depth	3	45	47.72	<.0001
Treatment*Depth	9	45	1.75	0.1053

Repeated measurement of effects of contrasting nitrogen sources on water extractable organic carbon (WEOC) from 2019 to 2021

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	3	9	62.96	<.0001
date	6	120	22.13	<.0001
date*treatment	18	120	1.74	0.0416

Repeated measurement of effects of contrasting nitrogen sources on water extractable organic carbon (WEOC) under different growing season

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	3	9	30.87	<.0001
Month	2	136	0.33	0.7218
Month*treatment	6	136	0.81	0.5644

Water extractable organic carbon (WEOC)at different depth of soil

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	3	45	7.42	0.0004
depth	3	45	142.1	<.0001
treatment*depth	9	45	2.23	0.0377

Repeated measurement of effects of contrasting nitrogen sources on β-Glucosidase activity from 2019 to 2021 for the 0-15 cm soil depth

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	3	9	5.59	0.0193
date	6	184	3.58	0.0022
date*treatment	18	184	2.98	0.0001

Repeated measurement of β-Glucosidase activity under different times of growing season

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	3	27	6.94	0.0013

time	2	9	1.12	0.3693
time*treatment	6	27	0.98	0.4555

β-Glucosidase (BG) activity at different depths of soil

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	45	0.52	0.6699
Depth	3	45	133.41	<.0001
Treatment*Depth	9	45	0.41	0.924

APPENDIX D

Curriculum Vitae For Phearen Miller

Phearen Miller/Graduate Student
Contact: 435-294-5915
E-mail: kitphearen@gmail.com

EDUCATION

Doctor of Philosophy (Ph.D.) in Plant Science — Jan 2019 – May 2024 (Expected)
Plants, Soils and Climate Department, Utah State University, Utah

- Conducted extensive research on soil health, nitrogen cycling, and cropping systems.
- Proficient in data management, statistical analysis, and laboratory work.
- Guest Lecturer on soil health, carbon and nitrogen cycles, and soil enzyme activity.
- Collaborated with partners for impactful research outcomes.

Dissertation:

Contrasting Nitrogen Management Impacts on Nitrogen Use Efficiency and Soil Health under Silage Corn Production in a Semi-Arid Environment

Master of Science (M.S.) in Plant Science — Aug 2015–Nov 2018
Plants, Soils and Climate Department, Utah State University, Utah

- Conducted research on interactions between biochar and compost soil amendments for organic winter wheat production and soil quality.
- Skilled in fieldwork, laboratory processing, and data analysis.

Thesis:

Interaction between Biochar and Compost Soil Amendments on Organic Winter Wheat and Soil Quality in Dryland Production — Dec 2018

Bachelor's degree in Agricultural Technology and Management — 2009-2013
Royal University of Agriculture, Cambodia

Undergraduate project:

Effect of Drip Irrigation System and Different Rates of Fertilizer on Growth and Yield of Eggplant (Solanum melongena) — Mar 7- Aug 31, 2013

WORK EXPERIENCES

Graduate Research Assistant and Teaching Assistant, PhD Program, Utah State University — Logan, Utah, USA — Jan 2019- Dec 2023

- Conducted fieldwork involving corn and hairy vetch, including experiment setup, irrigation, weed control, soil sampling, harvesting, and data collection.
- Performed laboratory tasks, focusing on soil sample preparation and analysis, particularly nitrogen and carbon analysis, and lab organization.
- Performed data analysis and interpretation

- Managed data collection and recording, as well as statistical analysis.
- Served as a Teaching Assistant for PSC 5560, 6560 "Analytical Techniques for the Soil Environment" and PSC 5530, 6530 "Soil Health and Fertility."
- Developed protocols for analyzing soil organic carbon for the Lab and PSC 6530 class.
- Delivered guest lectures on topics such as the nitrogen cycle, soil salinity, soil acidification, and soil enzymes.
- Instructed students in performing laboratory analyses.

Graduate Research Assistant, Master's Program, Utah State University

Logan, Utah, USA

Aug 2015-Nov2018

- Conducted fieldwork on wheat and barley, including seed packing, Nested Association Mapping panel nursery planting, irrigation, phenotypic data monitoring, and harvesting.
- Performed laboratory tasks such as post-harvest storage, weighing, test weight measurements, and wheat quality testing using NIR and Mixograph.
- Planned and executed research on "Interactions between Biochar and Compost Soil Amendments on Organic Winter Wheat and Soil Quality in Dryland Production
- Performed data analysis and interpretation

Royal University of Agriculture, Cambodia, and Michigan State University:

Kompong Speur, Cambodia

June 2014-March 2015

Project Coordinator for “Pass Swine on Project for rural farmers” which is a rural development initiative to supported small swine farms in Cambodia

- Orchestrated workshops, training, and awareness programs for local farmers to initiate sustainable sow farming practices
- Oversaw operations, monitoring, and follow-ups with sow farmers
- Prepared comprehensive reports to encapsulate project outcomes

World Vegetable Center: Survey Researcher – Post-harvest production and marketing chain of vegetable production - Designed and conducted questionnaire surveys to analyze the value chain of vegetable production around the Tonle Sap Lake - Collected and analyzed survey data Provided translation support	Siem Reap, and Battambang, Cambodia	Nov 2014-Jan, 2015
United States Agency for International Development (USAID): Survey Researcher – Post-harvest production of vegetable production - Designed and conducted questionnaire surveys to analyze the value chain of vegetable production and assess the microfinance system's impact - Collected and analyzed survey data. - Provided translation support.	Kandal Province, Cambodia	Jun-Dec, 2013
Royal University of Agriculture, Cambodia and University of Kuala Lumpur, Malaysia: Project Assistant – Promoting Agri-Cambodian portal system for safe vegetables - Conducted survey research, designed questionnaires, and collected data to assess the effectiveness of promoting Agri-tech adoption in Cambodia - Provided translation services	Kandal Province, Cambodia	Mar 2014-Mar, 2015
Royal University of Agriculture, Cambodia, and Nagoya University: Program Facilitator and Research Surveyor-Woman Leaders Program to Promote Well-being in Asia - Designed questionnaires, facilitated workshops, and conducted research surveys gain insights into gender roles and barriers within Cambodia's farming system - Provided translation and facilitation services.	Kompong Cham province, Cambodia	Mar 3-8, 2014
Royal University of Agriculture, Cambodia - Contractual staff- Planning and International Cooperation Office - Managed administrative tasks, coordinated workshops, and screened scholarship candidates. - Assisted students with scholarship applications. - Conducted tours and facilitated events	Phnom Penh, Cambodia	Jan 2011-Jan, 2015
Royal University of Agriculture Part-time English teacher at Language Center - Conducted English language classes.	Phnom Penh, Cambodia	2014-2015

INTERNATIONAL EXCHANGE AND TRAINING PROGRAMS

Joint Oversea Training Program Only 8 students from Cambodia were selected (Fully funded by Japanese Government)	Nagoya University, Nagoya,Japan	Oct 17-25, 2013
International Student Summit Forum Only 1 student from Cambodia was selected (Fully funded by Japanese Government)	Tokyo University of Agriculture, Tokyo, Japan	Sept 29-Oct 5, 2013
World Congress of Global Partnership for Young Woman Only 3 students from Royal University of Agriculture were selected (Fully funded by United Nation)	Duksunk Woman's University, Seoul, South Korea	Aug 10-13, 2012
Undergraduate Intensive English Language Study Program Only 8 students from Cambodia were selected (Fully funded by US Department of State)	Utah State University, Utah, USA	Jun 13- Aug 5, 2011

ACADEMIC ACHIEVEMENT AND AWARDS

Robert N Love Scholarship	Utah State University, Utah, USA	2023
Academic Opportunity Fund Application	Utah State University, Utah, USA	2023
School of Graduate Studies Graduate Student Travel Award	Utah State University, Utah, USA	2023
Utah State University College of Agriculture and Applied Sciences Graduate Student Travel Grant	Utah State University, Utah, USA	2023
Southard Soil Science Graduate Fellowship.	Utah State University, Utah, USA	2022-2023
DeVere McAllister Scholarship	Utah State University, Utah, USA	2022-2023
Ronald L. Moshier Scholarship	Utah State University, Utah, USA	2022-2023
Ambassador Ardeshir Zahedi International Endowment Scholarship	Utah State University, Utah, USA	2021-2022
DeVere McAllister Scholarship	Utah State University, Utah, USA	2020-2021

Outstanding presentation award at the 7th AG-BIO/ PERDO Graduate Conference on Agricultural Biotechnology & KU-UT Joint Seminar IV	Kasetsart University, Thailand	2016
Frank O. & Ina Seeley Morgan Scholarship	Utah State University, Utah, USA	2016 and 2017
Level II Schoenl Family Undergraduate Grant for Dire Needs Overseas Michigan State University	Phnom Penh, Cambodia	2014-2015

VOLUNTEERING ACTIVITY

Fulbright and Undergraduate State Alumni Association of Cambodia (FUSAAC) - Project Coordinator – Enhanced outreach programs in Kompong Cham province. - Facilitated diverse outreach initiatives, including education, leadership, and career fairs	US embassy, Phnom Penh, Cambodia	2011-2015
Youth Volunteer for Environment - Vice president – Organized and led environmental activities and initiatives	Phnom Penh Cambodia	Nov 2014-Jan, 2015
Cambodian Red Cross - Engaged in fundraising, charity, and environmental cleanup events. - Contributed to blood donation campaigns.	Royal University of Agriculture, Phnom Penh Cambodia	2009-2015
Royal University of Agriculture, Cambodia, and Nagoya University - Identified suitable training locations and communicated with local authorities and farmers. - Trained students in developing questionnaires and conducting surveys. - Assisted students in teamwork and survey activities with farmers	Royal University of Agriculture, Phnom Penh Cambodia	Mar 2013-Mar 2015
TEDx USU - Participated in organizing TEDx event at Utah State University.	Utah State University, Utah, USA	Oct, 2016

Fundraiser for the hospital - Led efforts to raise funds for the labor ward construction at Y Tounseang health center.	Kampot Cambodia	Oct 2020-2021
Student research symposium (SRS) - Participated in organizing TEDx event at Utah State University	Utah State University, Utah, USA	Apr 11-12, 2023

CONFERENCES/WORKSHOP/TRAINING FOR PROFESSIONAL DEVELOPMENT

ASA-CSSA-SSSA International Annual Meeting	St. Louis, Missouri, USA	Oct 29-Nov 1, 2023
The 14th International Conference on Environmental and Rural Development	Siem Reap, Cambodia	Mar 3-5, 2023
ASA-CSSA-SSSA International Annual Meeting	Salt Lake City, Utah, USA	Nov 7-10, 2021
ASA-CSSA-SSSA International Annual Meeting	San Antonio, Texas, USA	Nov 10-13, 2019
Our Farm, Our Future Conference	St. Louis, Missouri, USA	Apr 3-5, 2018
OREI wheat meeting	Washington State University, Washington, USA	July 17-19, 2017
The Synergy of Science and Industry: Biochar's Connection to Ecology, Soil, Food, and Energy	Oregon State University, Oregon, USA	Aug 22-25, 2016
Wheat Project Meeting	Wyoming Organic Wheat Research Site, Wyoming, USA	May 8-10, 2016
University of Idaho-Limagrain Cereal seeds field day	Aberdeen, Idaho, USA	Jul 15, 2015
Blue Creek Research Farm Field Day	USU Blue Creek Research Farm, Utah, 2016	Jun 22, 2016
33rd International Vegetable Training Course “Vegetables: From Seed to Table and Beyond”	Kasetsart University, Kamphaengsaen Campus, Thailand	Sept 15-Dec 5, 2014
Joint Oversea Training Program	Nagoya University, Nagoya,Japan	Oct 17-25, 2013

International Student Summit Forum	Tokyo University of Agriculture, Tokyo, Japan	Sept 29-Oct 5, 2013
World Congress of Global Partnership for Young Woman	Duksunk Woman's University, Seoul, South Korea	Aug 10-13, 2012
Undergraduate Intensive English Language Study Program	Utah State University, Utah, USA	Jun 13- Aug 5, 2011

PUBLICATION

Miller, P. (2018). Interactions Between Biochar and Compost in Organic Winter Wheat Production and Soil Quality Under Dryland Conditions. All Grad. Theses Diss. https://digitalcommons.usu.edu/etd/7359

Kit, P. (2013). Analyzing adoptions and problems in sow raising of Osaray farmers after establishing pig raising farmer groups in Cambodia. The Thirteen International Students Summit (ISS) on Food, Agriculture and Environment in the New Century (pp107-115). Tokyo: Tokyo University of Agriculture Press.

Kit. P. (2013). Effect of drip irrigation system and different rates of fertilizer on growth and yield of eggplant (Solanum Melongena). Graduation thesis of Royal University of Agriculture.

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ORAL PRESENTATION

Miller, P., & Norton, J.M. (2023). Nitrogen Use Efficiency and Soil Health Indicators in Corn Silage Production in a Semi-Arid Environment, ASA-CSSA-SSSA International Annual Meeting at St. Louis, Missouri (October 29-Novemner 1,2023).

Miller, P., &Norton J.M. (2023). Nitrogen Use Efficiency and Soil Health Indicators in Corn Silage Production in a Semi-Arid Environment, Student Research Symposium (April 11-12,2023).

Miller, P., Norton J.M., Cardon, G., Jones, SB., and MacAdam, J. (2023). Nitrogen Use Efficiency and Soil Health Indicators in Corn Silage Production in a Semi-Arid Environment, The 14th International Conference on Environmental and Rural Development at Angkor Paradise Hotel, Siem Reap, Cambodia (March 3-5,2023).

Miller, P. &Norton, J.M. (2022). Nitrogen Use Efficiency and Soil Bioavailable Nitrogen in Corn Silage Production in a Semi-Arid Environment. Online International Webinar on Agricultural Sciences (Virtual), Kasetsart University, Kamphaengsaen, Thailand. (Apr 27-28, 2022).

Miller, P. (2021). Carbon Mineralization and Relationships to Nitrogen in Soils from Corn Silage Production in A Semi-Arid Environment (virtual) at PSC 7890 Graduate Seminar. Utah State University, Logan, Utah, USA. (Apr 9, 2021).

Miller, P. (2020). Nitrogen Use Efficiency and Soil Bioavailable Nitrogen in Corn Silage Production in a Semi-Arid Environment at PSC 7890 Graduate Seminar. Utah State University, Logan, Utah, USA. (Dec 7, 2020).

Miller, P. (2020). Hairy Vetch Response to different sources of Nitrogen at PSC 6430 Plant Nutrition. Utah State University, Logan, Utah, USA. (Dec 10, 2020).

Miller, P. (2019). Nitrogen Use Efficiency and Soil Bioavailable Nitrogen in Corn Silage Production Under Semi-Arid Conditions: Poster and 5 Minute Rapid--Soil Biology and Biochemistry Oral (includes Society-wide student competition selection) at ASA-CSSA-SSSA International Annual Meeting. San Antonio, Texas, USA. (Nov 10-13, 2019).

Miller, P., & Hole, D. (2019). Interaction between Biochar and Compost on Organic Winter Wheat Production and Soil Quality in Dryland Conditions, Utah. Invited speaker for sharing experience workshop. University of Battambong, Battambong, Cambodia. (May 19, 2019).

Kit, P., & Hole, D. (2016). Interaction between biochar and compost on organic winter wheat production and soil quality in dryland conditions, Utah at the 7th AG-BIO/ PERDO Graduate Conference on Agricultural Biotechnology & KU-UT Joint Seminar IV. Kasetsart University, Kamphaengsaen, Thailand. (Dec 8-9, 2016).

Kit, P. & Hole, D. (2016). Interaction between biochar and compost on organic winter wheat production and soil quality in dryland conditions, Utah at the 3rd National Conference on Agricultural and Rural Development: Enhance the Rural Economy through Sustainable Development in Agriculture. Svay Reang University, Sv 252
Reang, Cambodia. (Nov 26-27, 2016).

Kit, P. (2014). Enhancing women participation in capacity building program at Kompong Thom Province, Cambodia at 33rd International Vegetable Training Course "Vegetables: From Seed to Table and Beyond". Kasetsart University, Thailand. (Dec 4, 2014).

Kit, P. (2014). Value chain of rice production in Kompong Cham Province, Cambodia at Woman Leaders Program to Promote Well-being in Asia, at 33rd International Vegetable Training Course on Sustainable Vegetable Development. Royal University of Agriculture, Phnom Penh, Cambodia. (Mar 8, 2014).

Kit, P. (2014). Irrigation management in Chita prefectures, Japan at Sharing Experience Workshop. Royal University of Agriculture, Phnom Penh, Cambodia. (Feb 6, 2014).

Kit, P. (2014). The effect of Aichi Canal on resident's lives in Chita prefectures, Japan at Join Oversea Training Program. Nagoya University, Nagoya, Japan. (Oct 24, 2013).

Kit, P. (2013). Analyzing adoptions and problems in sow raising of Osaray farmers after establishing pig raising farmer groups in Cambodia at 13th International Student Summit. Tokyo University of Agriculture, Tokyo, Japan. (Oct 3, 2013).

Kit, P. (2013). Effect of drip irrigation system and different rates of fertilizer on growth and yield of eggplant (Solanum Melongena) at Research Workshop of Agricultural Development Research Interest Group, Development Research Forum Phase II. Royal University of Agriculture, Phnom Penh, Cambodia. (Sept 9, 2013).

Kit, P. (2012). Water quality and waste management at waste division and water quality campaign Sangkat Chrang Chamres. Youth volunteer for environment. Phnom Penh, Cambodia. (May 13, 2012).

Kit, P. (2012). Water quality at student presentation of Undergraduate Intensive English Language Study Program. Utah State University, Utah, USA. (Aug 4, 2011).

Poster Presentations

Miller, P., &Norton, J.M. (2021). Carbon and Nitrogen Mineralization in Soils from Corn Silage Production in a Semi-Arid Environment at ASA-CSSA-SSSA International Annual Meeting. Salt Lake City Utah. (Nov 7-10, 2021)

Cunningham, K., Hendon, M., Miller, P. and Norton J.M. (2021). Biological Nitrogen Fixation, Nodule Organisms and Nitrogen Dynamics in Silage Corn Following a Vetch Cover Crop. ASA-CSSA-SSSA International Annual Meeting. Salt Lake City Utah. (Nov 7-10, 2021).

Miller, P., Kakkar, A. and Norton, J.M. (2021). Nitrogen Use Efficiency and Soil Bioavailable Nitrogen in Corn Silage Production Under Semi-Arid Conditions at Student Research Symposium. Utah State University, Logan, Utah, USA. (Ap 12-13, 2021).

Miller, P., & Norton, J.M (2020). Nitrogen Use Efficiency and Soil Bioavailable Nitrogen in Corn Silage Production Under Semi-Arid Conditions at Student Research Symposium. Utah State University, Logan, Utah, USA. (April 8-9, 2020).

Miller, P., Kakkar, A., Ouyang, Y., and Norton, J.M. (2020). Nitrogen Use Efficiency and Soil Bioavailable Nitrogen in Corn Silage Production Under Semi-Arid Conditions at ASA-CSSA-SSSA International Annual Meeting. San Antonio, Texas, USA. (Nov 10-13, 2019).

Kit, P., Stukenholtz, P., Koenig. R., Reeve, J., Hole, D., and Miller, B. (2018). Long term effects of compost addition in an arid organic dryland wheat system for the past 22 years at Our Farm Our Future Conference. St. Louis, Missouri, USA. (April 3-5 2018).

Kit, P., Dara, C and Hoeung, S (2012). Promoting gender equality in agriculture sector, Cambodia at global congress of global partnership for young woman. (Aug 12, 2012).

Kit, P. (2012). Effect of drip irrigation system and different rates of fertilizer on growth and yield of eggplant (Solanum Melongena) at Research Workshop of Agricultural Development Research Interest Group, Development Research Forum Phase II. (Oct 11, 2013).

Kit, P. (2011). Water quality project at student presentation of Intensive English Language Study Program summer program. Utah State University, Logan, Utah, USA. (Aug 5, 2011).

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