Ammonium Thiosulfate and Potassium Thiosulfate as Potential Nitrification Inhibitors in Soils

By

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Abstract

Nitrogen use efficiency (NUE) can vary according to soil properties, precipitation, and soil temperature. Nitrification inhibitors show potential to increase NUE by decreasing the amount of N lost from leaching and denitrification, keeping N in an ammonium form. The objective of this study was to evaluate the effectiveness of ammonium thiosulfate (ATS) and potassium thiosulfate (KTS) products to inhibit nitrification in three soil types: Marvyn loamy sand, Tujunga loamy sand, and Sable silt loam. Two runs of a 10-week incubation experiment were performed, and soil ammonium-N (NH_4^+-N), nitrite-N (NO_2^--N), and nitrate-N (NO_3^--N) concentrations were measured to determine the effectiveness of ATS and KTS to inhibit nitrification in urea-based fertilizers compared to an untreated control, urea, urea + ammonium sulfate, and urea + dicyandiamide (DCD) treatments. Incubation results showed that the higher thiosulfate rate of ATS and KTS reduced nitrification compared to urea alone and urea + DCD in the Tujunga loamy sand from day 21 to day 63 in the first run and from day 28 to day 56 in the second run. Within the Sable silt loam soil, the higher thiosulfate rate in ATS and KTS were similar to urea + DCD in reducing nitrification from day 35 to day 56 in the first run, but no treatment differences were seen in run two. No treatments reduced nitrification compared to the untreated urea treatment in the Marvyn loamy sand soil type. Trends for NO_2^--N concentrations corresponded to trends for NO_3^--N concentrations in the Tujunga soil, showing the treatments inhibiting nitrification are most likely inhibiting the first step in the nitrification process. Greenhouse studies on corn (*Zea mays* L.) were conducted to determine effects on plant growth and soil NH_4^+-N and NO_3^--N. All fertilizer treatments in all soil types resulted in higher NH_4^+-N compared to the control from day 0 to day 7 in run one and from day 0 to day 14 in run two. Nitrate-N concentrations in run one tended to be higher in all fertilizer treatments than the

control in the Marvyn and Tujunga soil from day 0 until day 14, but no differences were seen in the Sable silt loam. However, treatment did not influence NO_3^--N concentrations in the second run. Overall, ATS, KTS and DCD reduced nitrification compared to urea in the Tujunga loamy sand in both incubation runs and Sable silt loam in one out of two runs for the incubation studies. However, similar results were not observed in the greenhouse studies. Both ATS and KTS have potential to inhibit nitrification, but effectiveness of these inhibitors is dependent on soil type and climatic conditions.

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Abbreviations

Nitrogen Use Efficiency (NUE)

Ammonium-N (NH_4^+-N)

Nitrite-N (NO_2^- -N)

Nitrate-N (NO_3^--N)

Dicyandiamide (DCD)

Urea + Dicyandiamide (UDCD)

Urea + Ammonium Sulfate (AMS)

Urea + High S Ammonium Thiosulfate (HSATS)

Urea + Mid S Ammonium Thiosulfate (MSATS)

Urea + High S Potassium Thiosulfate (HSKTS)

Urea + Mid S Potassium Thiosulfate (MSKTS)

Plant Science Research Center (PSRC)

Soil Organic Matter (SOM)

Organic Matter (OM)

I. Literature Review of Nitrification Inhibitor Use in the U.S.

Introduction

Nitrogen (N) is an essential element for all plants. It is a large component of nucleic acids that form DNA and a part of amino acids in the formation of proteins (Canfield et al., 2010). Nitrogen is typically the largest fertilizer input cost, especially for corn (*Zea mays* L.) production, and often has the largest impact on yield and profit from a nutrient deficiency standpoint. Most healthy plants have a dry weight content of 1-6% N (Havlin et al., 2016). Losses of N lead to less nitrogen use efficiency (NUE) in agricultural ecosystems and have negative impacts through greenhouse gas emissions and pollution of ground water systems. Nitrogen use efficiency, defined as the amount of N uptake per amount of N applied, can be different depending on many factors, but is typically below 50% (Chien et al, 2009). The goal of nutrient management and soil fertility planning is to maximize NUE to increase crop productivity while also preventing N input losses. There are many management practices to help prevent N losses including crop rotation, conservation tillage, cover crops, and optimizing fertilizer rates, application timing, placement and sources of fertilizers. Nitrification inhibitors provide mechanisms to improve NUE.

Nitrogen Cycle

The soil N cycle is a complex sequence of biogeochemical processes that are largely controlled by the microbial populations in the soil. Nitrogen can be added to soil through additions of nitrogen fertilizers, symbiotic and asymbiotic biological fixation, lightning and atmospheric deposition, and nitrogen mineralization. Nitrogen can be taken out of the soil solution through plant and microbial uptake, immobilization, volatilization, denitrification, leaching, and runoff. Nitrogen transformations, including biological fixation, mineralization, nitrification, immobilization and denitrification involve biochemical reactions with microorganisms. As a result, N availability to plants is largely controlled by the microbial species.

Most of the N within the soil N cycle is in an organic form. Only 0.03% of the N in the cycle is in a reactive form, including in the atmosphere, soil, and water (Voroney & Derry, 2008). Of the N in the soil, inorganic N is typically never more than 10% (Stevenson, 1982). Total soil N can vary due to factors including soil formation, crop production systems, and susceptibility to erosion. Typically, there will be more N in an organic form within no-till soils, and with increasing temperature, and at lower landscape positions than side slopes (Gregorich & Anderson, 1985).

Additions to the N Cycle

The majority of the inorganic N fertilizers applied today are products of the Haber-Bosch process. Industrial fixation of N has increased from 2.5 Tg of N per year in 1960 to 25.4 Tg of N per year in 2008 (Gu et al., 2013). Along with this, fertilizer use has increased 800% from 1960 to 2000, coinciding with the many advancements in crop production (Canfield et al., 2010). Currently, the Food and Agriculture Organization of the United Nations estimates N fertilizer use at 112 million tons in 2022 (FAO, 2019). Nitrogen fertilizer use was more than 90 million megagrams in 2005 to 2006, but in 1960 was less than 12 million megagrams globally (Emerich et al., 2009). Many common N fertilizers including urea, ammonium sulfate, or urea ammonium nitrate are soluble and become plant available quickly. However, ammonium sulfate becomes less soluble in liquid form and can be a problem in sprayer applicators, but still soluble enough for immediate availability in the soil for plant uptake (Hagstrom, 1986). Not all of the N will be used by the plant, and can be lost through soil runoff, leaching, denitrification, or volatilization, depending on environmental conditions. There are many N fertilizer products that act to slow the release into a mobile form of N to reduce environmental losses.

Nitrogen can be made available to plants through relationships with microorganisms that fix atmospheric N into ammonium for plant uptake, known as biological N fixation (Havlin et al., 2016). They are split into three main groups: symbiotic, associative, and asymbiotic N fixers. Symbiotic N fixation occurs when bacteria form root nodules on specific hosts plants that supply the host with N and receive sugars in return. There are 18 known genera within this group of N fixing bacteria that encompass many different plant species (Lindstrom & Mousavi, 2019). Associative and asymbiotic N-fixing bacteria are less specific or have no specific relationship with higher plants. They generally will not fix a significant amount of N to provide the nutrient needs for crop production.

It is common practice to implement a legume crop into the rotation since the symbiotic relationship with rhizobia bacteria allows for the crop to need little if any N from fertilizers. Cover crops are used to improve soil health and have many benefits including increasing soil organic matter (SOM), soil carbon, improving soil structure and stability of aggregates, decreasing erosion of soil sediment and leaching of nutrients, and overall nutrient use efficiency (Abdalla et al., 2019). Leguminous cover crops also allow some N to be carried over for the next cash crop within the rotation, but the amount that can off-set inputs of fertilizer N is often difficult to be determined and variable between years due to moisture, N applications and source (Rutan et al., 2022). Therefore, many farmers will continue to apply the full recommended rate of N for the succeeding crop.

Organic fertilizer sources have also become a large part of inputs for many farms. However, their nutrient concentration and availability, especially for N, can be variable within the same type of organic source, but can be determined within a range based on the C:N ratio (Lazicki et al., 2020). Estimates can be made on the rate of N mineralization. According to model simulations, typically 16-17% of the N in poultry litter will be plant available at application, and 39.6% is available in a mineral form after 100 days (Geisseler et al., 2021).

More reactive N has been put into the atmosphere due to anthropogenic sources, and lightning strikes can cause the formation of nitrates through the reactions of N_2 and O_2 gases, where both eventually get deposited into the soil (Weil & Brady, 2017). Increases in atmospheric emissions have resulted in more acidic rainfall, and generally forests and aquatic ecosystems are more sensitive to this additional N because of more acidification of soils and bodies of water become prone to eutrophication (Weil & Brady, 2017; Burns et al, 2021).

Nitrogen Transformations within the Soil

Nitrogen mineralization is composed of two major steps, aminization and ammonification. Mineralization involves many microorganisms to break down organic matter (OM) from various sources in almost all soil environments to produce ammonium-N (NH_4^+-N), and through subsequent processes, nitrate (NO_3^- -N), where it can then either be used by plants, accumulate in the soil and immobilized, lost to the atmosphere as nitrogen gases, or leached through the soil (Schepers & Meisinger, 1994). The process is carried out within species of heterotrophic microorganisms that use organic amine groups for energy (Myrold & Bottomley, 2008). The process is often slow and variable. Factors of mineralization include soil temperature, pH, moisture, O_2 and CO_2 concentrations, as well as management practices including tillage, and fertilizer management (Schepers & Meisinger, 1994). Any changes in the soil that affect the microbial community impact the variations in mineralization as well as the whole nitrogen cycle.

Nitrification involves oxidation reactions carried out by microorganisms that convert NH_4^+-N into nitrite (NO_2^- -N) and then into NO_3^- -N. Nitrification of ammonium-based fertilizers can happen quickly with 90% being oxidized into NO_3^- -N within four weeks in some tropical soils with a pH above 6 and with a wide range in organic C and N concentrations (Sahrawat, 1982). Ammonium that is held on the surface of clay minerals and OM is held by the soil's cation exchange capacity (CEC), making it fairly stable in the soil until adsorption and desorption reactions occur (Sahrawat, 2008). However, NO_3^- -N is more mobile in the soil and is easily leached or subject to more conversions leading to denitrification (Subbarao et al., 2007). There are a small number of genera that are involved in nitrification reactions. The most common ammonia oxidizers are *Nitrosomonas* sp. while *Nitrobacter* sp. are the most common nitrite oxidizers. Majority of the nitrifiers are obligate autotrophs.

Multiple factors affect nitrification, including soil moisture, soil pH, texture and temperature. When the soil is saturated with water, there is less oxygen resulting in less nitrification from aerobic bacteria. Denitrification is more likely to occur in saturated soils. With higher amounts of carbon dioxide, less nitrification takes place (Sahrawat, 2008). However, decreases in CO2 from the normal concentration found in the soil will also decrease nitrification since microorganisms are functioning at an elevated concentration than what is found in the atmosphere (Azam et al., 2004).

Soil pH affects nitrification rates by increasing nitrification with increasing pH, up to a pH 8, and essentially stops below a pH of 5 (Sahrawat, 1982; Kyveryga et al., 2004). As nitrification progresses, the soil pH decreases due to the first step of nitrification releasing H^+ protons into the soil solution (Barth et al, 2020). Soils high in sulfate can also accumulate mineralized N in the ammonium form (Sahrawat, 1980). Soil texture influences nitrification and soils higher in sand content generally have fewer nitrifying bacteria (Barth et al, 2020). This could be due to lower organic matter, and because sandy soils typically have a lower water holding capacity. Nitrification typically proceeds at a faster rate with increasing temperature. The maximum temperature that nitrification can occur varies by location but can continue with up to

60° C (Schmidt, 1982). It is one of the main factors when determining the risk of applying anhydrous ammonia in the fall by predicting the rate of nitrification. Generally, cooler temperatures reduce nitrification because of less microbial activity, with nitrification ceasing below 4-5° C (; Schmidt, 1982; Kyveryga et al, 2004).

Ammonium can be fixed to interlayers of some clay minerals including illite, vermiculite, and montmorillonite, reducing its availability to plants. Fixation can occur rapidly after the addition of an ammonium-based fertilizer, with one study showing over 50% is fixed within 6 hours after application (Drury & Beauchamp, 1991). However, fixed NH_4^+-N can be released after exchangeable NH_4^+-N in soil solution is depleted, but usually at a slower rate than fixation to soil colloids.

Losses to the N Cycle

Of all plant essential elements, N is typically considered the one with the most potential to be lost (Kurtz, 1980). Up to 70% of N from fertilizers can be lost (Glass, 2003). Olson (1980), through studies of tagged N-15 fertilizers on a silt loam soil showed that only around one-fourth of the N applied from fertilizers was being removed by the harvested corn grain. Leaching and runoff can lead to high levels of NO_3^- -N being found in surface and groundwaters. Typically, over-fertilization causes more losses, and the extra N applied will not be available for the next crop (Kurtz, 1980).

Plants absorb N in the form of NH_4^+-N and NO_3^- -N (Havlin et al., 2016). Some organic N compounds including urea and amino acids can be taken up by the plant but only in small quantities (Bundy & Meisinger, 1994). A deficiency of N leads to stunted plants and less chlorophyll content resulting in a loss of green pigments, mainly in leaves, and symptoms first appear in older leaves (Fox & Walthall, 2008). Some research states that most plants prefer N in the NH_4^+-N form due to the lower amount of energy required for the plant to assimilate N

(Havlin et al, 2016). Nitrate-N must go through multiple reduction reactions before it can be assimilated into amine groups to form proteins in the plant. Cotton plants in the southeast typically take up 19.9 kg of N per 100 kg of lint, similar to many studies within their review, and an average of 58 kg of N per ha was removed from the soil after harvesting seed cotton (Mullins & Burmester, 1990).

Microbial uptake of N is important due to the small fraction of the N pool being in an inorganic form. Microbes are responsible for converting inorganic N into organic forms through metabolic processes leading to N being immobilized in the soil while others are responsible for implementing the opposite process of mineralization previously discussed. Most immobilization of N occurs biologically. Microorganisms assimilate NH_4^+-N into amino acids to use for metabolic activity. In general, the balance between N mineralization and immobilization is based on the relative availability of C and N in the substrates and the metabolic needs of microorganisms in the soil. Immobilization will occur when the microorganisms cannot meet their N requirements from only metabolizing plant residues, when the ratio of C to N is high. The net process between N mineralization and immobilization determines the N supply that is available to plants as well as other parts of the cycle.

Volatilization is the conversion of N into ammonia gases, which are subsequently lost to the atmosphere. Urea is one of the most common N fertilizers due to being the least expensive granular and has a high concentration of N decreasing transport costs (Freney, 1997). However, it is prone to volatilization loss since it must be hydrolyzed into ammonia before being transformed into NH_4^+-N to be used by plants (Gioacchini et al., 2002). Urease inhibitors are used to prevent losses of N through the ammonia form. It is possible that ammonium sulfate used in a mixture with urea can decrease ammonia volatilization losses compared to urea alone (Watson, 1988; Chien et al., 2009).

Microorganisms convert nitrate into nitric oxide, nitrous oxide and dinitrogen gases through reduction reactions during anaerobic or microaerophilic conditions in the soil through biological denitrification (Kurtz, 1980). Denitrifying microorganisms use N instead of oxygen for an electron acceptor in respiratory metabolism (Coyne, 2008). Nitric and nitrous oxide are both greenhouse gases. Many genera of bacteria have the capacity to carry out denitrification and are present in all agricultural soils. Most denitrifying bacteria are chemoheterotrophs and need an organic C source for energy. However, some are lithotrophic and can use NH_4^+-N as energy substrates (Coyne, 2008). Researchers have shown denitrification increases after irrigation events (Ryden et al., 1979; Mikkelsen et al., 1986). In very acidic soils, denitrification is not a problem since little or no nitrification will take place below a pH of 5 (Sahrawat, 1982). However, non-biological denitrification, happening from chemical reactions, can occur in small amounts in very acidic soils (Kurtz, 1980).

Leaching is the loss of N in soil solution, primarily in the form of NO_3^- -N, as water percolates below the root zone by gravity. It is typically the largest soil N loss pathway in agricultural lands (Weil & Brady, 2017). Leaching causes a major reduction in the efficiency of fertilizers being applied and can cause environmental problems when accumulating in groundwater (Mikkelsen et al., 1986). It is more likely to occur during wet seasons with heavy rainfall (Tao et al., 2018). When excessive amounts of N fertilizer are applied during the growing season, more NO_3^- -N is built up in the soil. However, since it is an anion, NO_3^- -N exists primarily in soil solution, and not readily absorbed to soil minerals. More NO_3^- -N leaching is likely to occur in coarse-textured soils compared to fine-textured soils with a high clay content, due to increased macropore space through these soil types after a rainfall or irrigation event. In addition, fine-textured soils may retain more NH_4^+-N because of a higher CEC (Gioacchini et al., 2002).

Some studies have shown success reducing N leaching potential while maintaining yields when using pre-sidedress nitrate tests (PSNT) to lower recommended N rates. PSNT is a measure of the NO_3^- -N concentration of the top 30 cm of soil, and is typically used to determine the need for a sidedress application on corn based on a yield goal (Durieux et al., 1995) where they have been able to reduce N rates in the Northeast and Midwest because residual NO_3^- -N was able to be observed with a more temperate climate. However, in more humid climates with coarse-textured soils, the ability to detect residual NO_3^- -N can be more challenging (Magdoff et al., 1984), and typically not considered in a routine soil test. The PSNT requires a separate soil test that needs to be analyzed immediately after samples are taken.

Nitrogen can be lost from crop fields through surface runoff, but generally on a smaller scale compared to N losses through leaching (Mulla & Strock, 2008). Nitrogen along with phosphorus, causes a large increase in cyanobacteria growth and eutrophication within bodies of water. This leads to low oxygen levels causing large amounts of aquatic life to die. Soils that do not have a high infiltration rate will be more prone to nutrients being lost through surface runoff, as well as fields under conventional tillage practices (Mulla & Strock, 2008).

Nitrification Inhibitors

Nitrification inhibitors suppress activity of bacteria involved in the processes of nitrification. The inhibitors interfere with the chemosynthesis reactions that are important for the metabolism of the chemoautotrophic microorganisms (Hauck, 1980). The goal of a nitrification inhibitor is to keep N in the more stable NH_4^+-N form (Bronson, 2008). They typically target the ammonia oxidizing bacteria since the NH_4^+-N to NO_2^- -N reduction is the rate limiting step in the nitrification process (Kong et al., 2016). Chemical compounds including nitrapyrin, dicyandiamide (DCD) and thiourea do this by competing for the active site of the ammonia monooxygenase enzyme, a cytochrome enzyme system transferring electrons from one location to another (McCarty, 1999; Hauck, 1980). However, there are also chemicals that inhibit hydroxylamine or nitrite oxidation either directly or indirectly.

Nitrification processes have been studied extensively throughout the last century. Nitrogen fertilizer started being used on a large scale commercially after World War Two with the use of ammonium nitrate (Kurtz, 1980). Fertilizer use became widespread and used excessively once N fertilizer was cheap and affordable. Not long after that, however, it became clear research needed to be done to increase fertilizer use efficiency. Allison (1955) observed the difficulties in accounting for all N inputs through a soil N balance sheet. Research started on the first nitrification inhibitor in the early 1960s (Kurtz, 1980). By this time, it was clear research was needed to reduce losses of N from soil runoff, leaching, denitrification and volatilization (Allison, 1955; Allison, 1965).

Commercial Nitrification Inhibitors and their Mode of Action

Nitrapyrin has been the main nitrification inhibitor product for the last 25 years (Wolt, 2004). It is sold commercially in products including N-Serve^{TM} and Instinct^{TM}. It is often used with applications of anhydrous ammonia (Goos, 2019). However, it can be used effectively when sprayed onto granular urea and broadcasted to the soil surface (Frye et al., 1981). It is considered a bactericide since some studies show suppressed growth of *Nitrosomonas* populations in pure culture with as little as 10 ppm (Zacherl & Amberger, 1990). Nitrapyrin inhibits the enzyme cytochrome oxidase in the ammonia oxidation process (Hauck, 1980). Bundy and Bremner (1973) reported nitrapyrin was more effective in light-textured sandy soils compared to heavier clay soils. The typical amount of nitrapyrin applied for many studies is 0.56 kg ha^{-1} (Touchton & Boswell, 1980). Due to its volatility, it must be band applied (Nelson & Huber, 1980).

Many studies have been done on its effectiveness, and according to a meta-evaluation by Wolt (2004), 75% of experiments show increased N retention, increased yield, and decreased leaching and volatilization. Multiple studies have shown Nitrapyrin increases wheat yields when applied in the fall before seeding (Huber et al., 1980; Liu et al., 1984). Touchton et al. (1979) showed decreases in nitrification and increased corn yield when N was fall or spring applied with Nitrapyrin. However, their studies showed little to no effect on yield. Cerrateo and Blackmer (2013) only observed increased corn yields in two out of 72 site-years. Nitrapyrin decreased potato yields when applied on a sandy soil (Hendrickson et al., 1978). Much of the mixed results may be due to N being applied at higher than optimal rates along with soil and climate conditions not resulting in large N losses. Also, nitrapyrin products are less effective in high-pH soils because nitrifiers can recover at a faster rate (Kyveryga et al., 2004).

Dicyandiamide is a widely known product that can be applied with granular urea (Goos, 2019). It is non-volatile making it more easily applied than nitrapyrin and can be added to different fertilizer sources (Reeves et al., 1988). The mode of action is similar to nitrapyrin with the cytochrome oxidase enzyme of the bacteria being inhibited (Hauck, 1980). Research has shown DCD to reduce leaching of fertilizer N and increase crop yields, but like other inhibitor compounds, there often will be variability (Yang et al, 2016;). When added to urea, DCD reduced ammonia monooxygenase activity and reduced NO_3^--N in a sandy loam soil (Fu et al., 2020) and increased NH_4^+-N retention in a clay loam and sandy loam (Gioacchini et al., 2002). According to Barth et al., (2020), DCD has a higher efficiency in clay and silt loam soils compared to sandy soils. This was due to observing lower amounts of ammonia oxidizing bacteria from most probable number experiments. However, some reports have shown decreases due to phytotoxicity, specifically in cotton, corn and sorghum, but at a relatively high rate. For Reeves & Touchton (1986), a reduction in nitrification did not occur until 5 mg per kg of soil or higher was used. The yield reductions are more likely to occur under drought conditions when water is limited. As DCD rate increased, with optimal moisture, they observed increases in plant dry weight.

Ammonium thiosulfate (ATS) is a liquid N source that is increasingly being used since it supplies sulfur in addition to N (Goos & Johnson, 2001). Once in the soil, thiosulfate decomposes into tetrathionate and elemental S, which then needs to be oxidized to sulfate for plant uptake (Hagstrom, 1986). Oxidation of thiosulfate occurs with increasing temperature, but near 5° C, variations in soil will cause variations in the mineralization process of thiosulfate to sulfate (Goos & Johnson, 2001). Some studies have shown potential to inhibit nitrification, but the studies on its effectiveness have shown mixed results. Thiosulfate can inhibit nitrification similarly to nitripyrin yet a higher concentration was needed, with 100 mg S kg^{-1} of thiosulfate resulting in the same amount of inhibition as 2 mg kg^{-1} of nitrapyrin (Janzen & Bettany, 1986), and in their experiments, the NO_2^- -N to NO_3^- -N step of nitrification was inhibited, along with an accumulation of NO_2^- -N of 42 mg of N kg^{-1} of soil was observed. Goos (1985) found greater inhibition between two to four weeks than one to two weeks, and 32 micrograms of thiosulfate-S per gram of soil resulted in 80% of N being inhibited after 28 days. Some research has shown volatile species of S can inhibit nitrification (Bremner & Bundy, 1974). However, they may not be as effective as nitrapyrin (Maddux, et al., 1985).

Sullivan & Havlin (1992) showed ATS can inhibit urea hydrolysis from 18 to 48% in various Kansas soils, and was more effective in soils low in clay and organic C, along with high temperatures and low soil moisture. They found it is more effective with low organic C and clay content. In their experiments, thiosulfate was oxidized to tetrathionate by reacting with Fe and Mn in the soil, which then may inhibit urease hydrolysis and prevent volatilization. Another study on cotton with subsurface drip irrigation on sandy clay loam soil in Texas found no difference in lint yield due to the application timing of ATS in addition to UAN (Yabaji et al., 2009).

Potassium thiosulfate (KTS) is another liquid fertilizer often used with specialty crops (Goos & Johnson, 2001). Not much research has been conducted with this fertilizer on nitrification. In an incubation study with sandy loam soil in California, KTS applied with UAN reduced N_2O emissions by 48% at a rate of 102 mg S kg^{-1} soil, but no correlation with NH_4^+-N or NO_3^- -N concentrations was seen (Cai et al., 2018). However, they did see a drop in NO_2^- -N accumulation, contrary to reports seeing thiosulfate causing an accumulation of NO_2^- -N (Janzen & Bettany, 1986).

Nitrification Inhibitors in Crop Production

Studies have shown nitrification inhibitors can improve crop yields by more efficiently regulating the transformations of N in the soil (Yang et al., 2016), but all nitrification inhibitors tend to be variable in their effectiveness. Temperature and moisture tend to have a large effect on the efficiencies of the nitrification inhibition products available. Agricultural studies on effects from nitrification inhibitors can be difficult to study due to variables including soil moisture, temperature, pH, organic matter, CEC, minerology and texture. Incubation studies often have clearer results than field trials (Zacher & Amberger, 1990; Vogeler et al., 2007; Barth et al., 2020; Fu et al., 2020). The use of nitrification inhibitors will increase crop yield only when the soil and climate conditions result in large N losses through leaching or denitrification (Touchton & Boswell, 1980; Mikkelsen et al., 1986). When little rainfall occurs, nitrification inhibition may occur and little to no leaching of NO_3^- -N as well, but in turn there may not be an increase in NUE (Rose et al., 2023).

Within the Southeastern United States, majority of soils are Ultisols with a low water holding capacity due to a coarse texture and poor structure, allowing for the rapid percolation of water (Touchton & Boswell, 1980). Leaching of NO_3^- -N is more common during the winter and spring months when majority of the precipitation occurs, especially after heavy rainfall. After 2.5 cm of rainfall, nitrates can leach up to a 30 cm depth. Soil temperatures stay warm enough to allow nitrification to occur during the winter months, preventing applications of N in the fall for succeeding Spring crops.

Some research has shown nitrification inhibition is more difficult in the Southeast, due to the effectiveness of nitrification inhibitors decreasing as temperatures decrease, but inhibitor longevity is limited with higher temperatures (Touchton & Boswell, 1980). In general, nitrification inhibitors can be effectively applied in the Spring to limit nitrification and increase crop yields, but applications of N in the fall are not recommended. Nitrapyrin applied with anhydrous ammonia in December and another study with ammonium sulfate applied in November did not alter nitrification on a Coastal Plain and Piedmont soil in Georgia and did not influence yield (Boswell et al., 1976; Boswell, 1977). However, in another study by Boswell and Anderson (1974), a 4-month reduction in nitrification was seen on a Piedmont soil in Georgia when nitrapyrin was added with ammonium nitrate to polyethylene bags placed in the soil. There are not as many studies that show a yield response in cotton to nitrification inhibitors as in corn (Touchton & Boswell, 1980). However, many studies conducted in the 1970s showed an increase in stand counts when nitrapyrin was applied, due to a decrease in nitrate salt concentration when planting in cooler temperatures. This is more likely to occur in areas like the Mississippi Delta with more alluvial silt and clay that does not allow as much NO_3^- -N leaching.

In the Midwestern Corn Belt, with soil temperatures near 0° C in the winter, more N is typically applied on corn and wheat during the fall months than in other regions of the country, however N losses can often be high (Nelson & Huber, 1980). In general, fall applications are done if the soil temperature is below 10° C at a depth of 10-15 cm (Nelson & Hanse, 1968). To prevent N loss from fall-applications, nitrification inhibitors are often applied with anhydrous ammonia (Kyverga et al., 2004). The most commonly applied chemical is Nitrapyrin since it can be used with fall-applied anhydrous ammonia, and it tends to be more effective in the fall when soil temperatures are lower (Touchton et al., 1978; Nelson & Huber, 1980). Studies in Indiana on different soil textures have shown yield increases in wheat from nitrapyrin added to anhydrous ammonia, ammonium sulfate, calcium nitrate or urea (Huber et al., 1980). Yield increases were more pronounced when conditions were right for high N losses (Nelson & Huber, 1980; Liu et al., 1984). Other areas in the Midwest have had some similar results, while some showed more variability (Hergert & Wiese, 1980).

Research in the Southwest has been conducted mostly on nitrapyrin in coarse and fine-textured soils and arid to semi-arid climates. Research in California on a silty clay loam with nitrapyrin applied up to 2% of N with ammonium sulfate, ammonium nitrate, ammonium phosphate, anhydrous ammonia and aqua ammonia showed recoveries of NH_4^+-N up to 64% at 15 weeks after application (Turner et al., 1962). Soil sampling was conducted between November and April with temperatures between 5° C and 14° C. Another study on irrigated cotton, corn and sugar beets in California applied nitrapyrin with anhydrous ammonia, aqueous ammonia, ammonium sulfate and urea (Swezey & Turner, 1962). With nitrapyrin between 0.5%

to 2% of N added to the fertilizer, a yield increase in cotton was observed compared to a single application of 112 kg ha^{-1} in a sandy loam soil. However, in corn and sugar beet, only with ammonium sulfate was there a yield increase with added nitrapyrin.

Some studies show nitrification inhibitors increase ammonia volatilization losses if applied with a urease inhibitor (Gioacchini et al., 2002). It may be that the decrease in nitrification may increase volatilization due to more NH_4^+-N being available to transform into ammonia or that nitrification inhibitors like DCD affect the properties of urease inhibitors like NBPT (Chien et al., 2009). DCD and other nitrification inhibitors allow more time for volatilization by reducing nitrification of NH_4^+-N (Soares et al., 2012).

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II. Potential of Ammonium Thiosulfate and Potassium Thiosulfate to Inhibit Nitrification in Soils

Introduction

Losses of nitrogen (N) lead to low N use efficiency (NUE) in agricultural ecosystems and can have negative impacts on greenhouse gas emissions and groundwater pollution. Nitrogen use efficiency can vary depending on factors including soil temperature, moisture, pH, cation exchange capacity, organic matter, and crop management practices. However, NUE is generally below 50% globally (Chien et al., 2009). The goal of nutrient management and soil fertility planning is to maximize NUE and increase crop productivity while preventing N input losses. Management practices to help prevent N losses include crop rotation, conservation tillage, and cover crops, as well as optimization of fertilizer rates, timing, placement, and source. Nitrification inhibitors provide one mechanism to improve NUE by slowing down the nitrification process and reducing the amount of N lost through leaching and denitrification. Leaching causes a major reduction in the efficiency of fertilizers and can cause environmental and health problems when accumulating in groundwater (Mikkelsen et al., 1986). When anaerobic or microaerophilic conditions occur in the soil, denitrification results in more greenhouse gases being lost to the atmosphere (Kurtz, 1980).

The soil N cycle is a complex sequence of biogeochemical processes that are largely controlled by soil microbial populations. Inorganic N forms typically make up less than 10% of total soil N (Stevenson, 1982). The process of nitrification involves oxidation reactions that convert ammonium (NH_4^+-N) into nitrite-N (NO_2^- -N) and then into nitrate (NO_3^--N), and is carried out by soil microorganisms. The most common ammonia oxidizers are *Nitrosomonas* sp. while *Nitrobacter* sp. are the most common NO_2^- -N oxidizers (Sahrawat, 2008). The majority of the nitrifiers are obligate autotrophic bacteria. Ammonium that is retained on the surface of clay minerals and organic matter is held by the soil's cation exchange capacity (CEC), making it more stable in the soil until sorption reactions occur (Sahrawat, 2008). However, NO_3^--N is more mobile in the soil, and therefore, more susceptible to leaching loss. Nitrate is also a reactant in the denitrification process, making nitrate more susceptible to gaseous loss (Subbarao et al., 2007).

Multiple factors affect nitrification, including soil moisture, pH, texture, and temperature. When the soil is saturated with water, there is less oxygen for aerobic bacteria to perform nitrification (Sahrawat, 2008). More denitrification tends to occur when soil is saturated. Increases in carbon dioxide (CO_2) result from more microbial activity while also reducing optimal O_2, but eventually reducing the aerobic microbial population. Nitrification typically proceeds at a faster rate with increasing temperature until reaching a maximum anywhere from 20 °C to 60 °C depending on the soil and adaptations made by the microorganisms to their environment (Schmidt, 1982). Generally, cooler temperatures reduce nitrification because of less microbial activity, with nitrification ceasing below 4 to 5 °C (Schmidt, 1982; Kyveryga et al., 2004).

Nitrification, as well as the effectiveness of nitrification inhibitors, is also largely affected by the physical and chemical properties of the soil. Soil texture influences nitrification rates, and soils higher in sand content generally have fewer nitrifying bacteria. This could be due to lower organic matter and water holding capacity of sandy soils (Barth et al, 2020). Soil pH also influences nitrification and occurs at an optimal pH between 6-8. Nitrification essentially stops below a pH of 5 (Sahrawat, 1982; Kyveryga et al, 2004). As nitrification progresses, the soil pH decreases because the first step of nitrification releases H^+ protons into the soil solution (Barth et al., 2020). The mineralogy of a soil also can cause differences in NO_3^--N mobility due to differences caused to cation exchange capacity (CEC) and from pH dependent charge in some soils (Allred et al., 2007).

Nitrification inhibitors suppress activity of bacteria involved in the processes of nitrification to keep N in a more stable NH_4^+-N form (Bronson, 2008). A widely used nitrification inhibitor for many years has been nitrapyrin, and it is sold in various products including N-Serve® and Instinct®(Wolt, 2004). Dicyandiamide (DCD) is also a commonly used nitrification inhibitor that can be applied with granular urea (Goos, 2019). It is non-volatile and non-toxic making it more easily applied than nitrapyrin and can be added to many different fertilizer sources (Reeves et al., 1988). Most nitrification inhibitors target ammonia oxidizing bacteria since the reduction of NH_4^+-N to NO_2^- -N is the rate limiting step (Kong et al., 2016). Chemical compounds including nitrapyrin, DCD, and thiourea target ammonia oxidizing bacteria by competing for the active site of the ammonia monooxygenase enzyme, which is a cytochrome enzyme system transferring electrons from one location to another (Hauck, 1980; McCarty, 1999).

Research has shown that DCD can reduce leaching of fertilizer N and increase crop yields. However, the effectiveness of DCD is variable, and economic benefits are not always seen according to a meta-analysis by Yang et al. (2016). This is most likely due to the multiple factors that can affect nitrification as well as differences in methodology used to assess product efficacy. In an incubation study on a silt loam alfisol, DCD added to urea slowed down the oxidation of ammonium and decreased leaching by 71% (Vogeler et al., 2007). Barth et al. (2020) found the inhibitor had a higher efficiency in clay and silt loam soils compared to sandy soils mainly due to a lower abundance of ammonia oxidizing bacteria through most probable number techniques. DCD slowed down nitrification and reduced leaching in a greenhouse and field experiment on citrus trees in a sandy loam compared to ammonium sulfate and ammonium nitrate blend (Serna et al., 1994).

In some cases, DCD may reduce conversion of NH_4^+-N to NO_3^--N in the soil without increasing plant N uptake or crop yield. This could be due to field conditions in which N losses are minimal when a study is conducted (Reeves et al., 1988; Gioacchini et al., 2002). Some researchers have seen a reduction in NO_3^--N when DCD was added to urea fertilizer on a sandy loam soil, yet a slight reduction in wheat yield occurred as well resulting in no economic benefit (Fu et al., 2020). Some reports have shown decreases in yield due to phytotoxicity, specifically in cotton more than corn and sorghum. For Reeves & Touchton (1986), a reduction in cotton plant growth occured when 5 mg kg $^{-1}$ DCD-N or higher was used. However, additions of DCD may increase photosynthesis due to increased stomatal conductance and result in increased plant dry weight when water is non-limiting (Reeves & Touchton, 1986; Reeves et al., 1988).

Ammonium thiosulfate (ATS) and potassium thiosulfate (KTS) are used as liquid fertilizers in many crops and are often applied as a S source. Ammonium thiosulfate is more commonly applied than KTS, but KTS is used in specialty crop production under drip irrigation (Goos & Johnson, 2001). Some studies have shown thiosulfate has potential to inhibit nitrification (Janzen & Bettany, 1986), but more research has been done with ATS than KTS. Once in the soil, thiosulfate converts into sulfate and elemental S, which then would need to be oxidized to sulfate for plant uptake (Hagstrom, 1986). Tetrathionate is a notable intermediate before sulfate formation (Janzen & Bettany, 1986). Goos (1985) found that 32 micrograms of thiosulfate-S per gram of soil resulted in 80% of N being inhibited after 28 days. Inhibition of nitrification most likely occurs during the oxidation of thiosulfate resulting in an inhibitory effect on ammonia or nitrite oxidizers and their related enzymes.

Some research has shown volatile species of S including hydrogen sulfide and carbon disulfide can inhibit nitrification after conversion from nonvolatile organic compounds by microorganisms (Bremner & Bundy, 1974). While hydrogen sulfide was shown to be a weak nitrification inhibitor, carbon disulfide was more effective than nitrapyrin, in an incubation study. However, they may not be as effective in field studies (Maddux, et al., 1985). It is possible that an intermediate S species forms that can reduce nitrification after thiosulfate is added to the soil (Goos, 1985).

More studies have been done on thiosulfate's effect on volatilization showing ATS can reduce ammonia gas loss from urea-based fertilizers (Goos & Fairlie, 1988). Considering not much research has been conducted on ammonium thiosulfate (ATS) or potassium thiosulfate (KTS) as pertaining to nitrification inhibition, the objective of the incubation study was to evaluate the effectiveness of ATS and KTS on nitrification in soils with different chemical and physical properties while environmental conditions including moisture and temperature were held constant.

Materials and Methods

Incubation Study

An incubation study was conducted to determine the effectiveness of ATS and KTS to prevent nitrification. Soil from three locations with distinct chemical and mineralogical characteristics was used for incubation studies: 1) Marvyn loamy sand (Fine-loamy, kaolinitic, thermic Typic Kanhapludult) from the Auburn University Turf Research Unit in Auburn, AL; 2) Tujunga loamy sand (Mixed, thermic Typic Xeropsamment) from the Tessenderolo Kerley Inc. research farm in Danuba, CA; and 3) Sable silt loam (Fine-silty, mixed, superactive, mesic Typic Endoaquoll) from Bureau County, IL. The study was organized with four replications of each of the following treatments: no N control, urea with ammonium sulfate, urea only, urea with dicyandiamide (DCD), two rates of ammonium thiosulfate with urea and two rates of potassium thiosulfate with urea (Table 2-1). The study was conducted two times (runs) for each of the three soil types. For all fertilizer treatments, a liquid urea solution was made using lab grade granular urea. All fertilizers were applied at a N rate of 135 kg N ha^{-1} based on the surface area of the incubation bin of 49.5 cm^2. Ammonium Thiosulfate and Potassium Thiosulfate were provided to us by Tessenderlo Kerley Inc. (2910 N 44th St, Ste 100, Phoenix, AZ 85018).

Before the start of the experiment, each soil was air-dried and sieved through a 2mm mesh before use. For each incubation bin, 200 grams of soil was used. Water was added to each bin 24 hours before fertilizers were applied to allow for the water to equilibrate within the soil. Water was added and maintained between 22-27% (WHC) for each soil. Temperature in the laboratory was maintained near 20 °C. Fertilizers were applied using a micropipette to the surface of the soil, and then stirred. Soil samples for extractions were taken right after fertilizer application, on days 3, 5, 7 and weekly thereafter for 10 weeks.

Inorganic Nitrogen Analysis

Extractions were done to analyze NH_4^+-N, NO_2^- -N, and NO_3^- -N. A 5-g soil sample was extracted with 25 mL of unbuffered and buffered 2M potassium chloride (KCl). The buffered KCl was only used for extracting NO_2^- -N and buffered to a pH of 8. Extracts were placed on a shaker at low speed for 30 minutes. Samples were then filtered through number 42 fiber glass filter paper and put into freezer storage until time for analysis using a visible light spectrophotometer microplate reader (Biotek® uQuant^{TM}, Agilent, 5301 Stevens Creek Blvd, Santa Clara, CA 95051). Direct determination of NH_4^+-N in 2M KCl extracts was done using a colorimetric procedure by causing an indophenol blue reaction with the concentration of NH_4^+-N, according to the methods of Keeney and Nelson (1982). For determining NO_3^- -N, a colorimetric procedure using cadmium to reduce NO_3^--N to nitrite (Keeney & Nelson, 1982). The microplate procedure for NH_4^+-N and NO_3^- -N was conducted at Auburn University while the procedure for NO_2^- -N was conducted at a TKI laboratory in Arizona following the methods of Griess-Ilosvay (Mulvaney, 1996).

For the NH_4^+-N procedure, 50 microliters (uL) of the sample was placed in a well and then each reagent was added in a specific order: 25 uL citrate reagent, 50 uL salicylate-nitroprusside reagent, 25 uL of hypochlorite reagent and 125 uL of background matrix. After adding each reagent and stirring, the plates would sit for 30 minutes before being placed on a microtiter plate reader using the 695_590 nm method. Microplate standards for 0, 1, 2, 4, 6, 8. 10 and 15 ppm of NH_4-N were used on both sides of each plate.

For the NO_3^--N procedure, each plate was placed in a sonicator with the reductor pins submerged in an HCl and $CuSO_4$ solution to activate the reductor pins before each use. Microplate standards for 0, 0.5, 1, 2, 4, 6, 8 and 10 ppm of NO_3-N were used on both sides of each plate. 20 uL of a sample was placed in a well then 200 uL of EDTA with a pH of 8.5 buffer was added. A reductor plate was then placed on top of the microplate and placed on a shaker for 1 hour. After, 60 uL of the Griess reagent was added and then placed on a shaker for 5 minutes before being placed on a microtiter plate reader at the 542 nm method.

Statistical Analysis

Statistical analysis was completed using SAS Version 9.4 (SAS Institute Inc., Cary, NC) by repeated measures analysis of covariance using PROC GLIMMIX for variables NH_4^+-N, NO_3^- -N and NO_2^- -N. Each run was analyzed separately. The urea with ammonium sulfate treatment was not included in the analysis of the first run due to an incorrect application. The first order heterogeneous autoregressive covariance structure ARH(1) was used to account for repeated measures among days. Treatment, soil, daily trend, and their interactions were used as fixed effects and replication was used as random effects. Ammonium and nitrate differences due to soil and treatment effects were evaluated each day. For all analysis, degrees of freedom were calculated using the Satterthwaite method and the adjust=simulate option was used to adjust mean differences for multiplicity (Littell et al., 2006).

Results and Discussion

Soil Type Effects on Nitrification

Results from two runs of the fertilizer incubation study showed treatment effects on NH_4^+-N, NO_2^- -N and NO_3^--N in some of the soil types while not others (Table 2-3). The interaction between soil types, treatments and time of sampling had a significant effect on NH_4^+-N and NO_3^--N concentrations. However, NO_2^- -N was only significant between sampling day and treatment in the Tujunga loamy sand. In both runs of the experiment, NH_4^+-N increased rapidly during the first week of the experiment (Fig. 2-1 & 2-2), then plateaued and slightly decreased as NO_3^--N increased over the course of the incubation study (Fig. 2-3 & 2-4). This indicated that nitrification was occurring in the Sable silt loam and Tujunga loamy sand. However, no decrease in NH_4^+-N coupled with an increase in NO_3^--N occurred in the Marvyn loamy sand (Fig. 2-3a & 2-4a). The Sable silt loam had the highest concentrations of NH_4^+-N (Fig. 2-1c & 2-2c), while the highest concentrations of NO_3^--N were seen in the Tujunga loamy sand soil towards the end of the incubation in both runs of the experiment (Fig. 2-3b & 2-4b). The Marvyn loamy sand had lower NH_4^+-N (Fig. 2-1a & 2-2a) and NO_3^--N (Fig. 2-3a & 2-4a) compared to the other soil types throughout the duration of the incubation. With a higher CEC, the Sable silt loam had more potential to retain ammonium, and this was evident by the numerically higher NH_4^+-N concentrations towards the end of the incubation. The Marvyn loamy sand had a lower CEC and organic matter that could have resulted in lower NH_4^+-N concentrations being retained throughout the experiment compared to the other soil types. However, it is unclear why there was not an increase in NO_3^--N. There was very little decrease in NH_4^+-N after the initially rapid increase during the first week after application. While in the Tujunga loamy sand and Sable silt loam, a larger decrease in NH_4^+-N resulted in an increase in NO_3^--N concentration. Nitrogen may have been lost through another pathway including volatilization or denitrification. The most likely possibility of lower concentrations is due to a lower nitrifying bacteria population within the soil due to a coarse-texture with lower OM, CEC and water holding capacity than the other soil types (Barth et al., 2020).

Fertilizer Treatment Effects on Nitrification

TUJUNGA LOAMY SAND

Within the Tujunga loamy sand, a reduction in NO_3^--N was seen from day 14 until day 70 for the first run of the incubation study for UDCD, HSATS, MSATS, HSKTS and MSKTS compared to the urea treatment (Fig. 2-3b). Urea with DCD reduced nitrification by 58% and 63% compared to untreated urea on day 14 and day 70, respectively. For the HSATS treatment, a >75% reduction in NO_3^--N concentrations was observed compared to urea from day 14 through 70, resulting in more nitrification inhibition than the UDCD treatment from day 14 through 70. In the second run, nitrification was reduced from day 21 to day 63 by the UDCD, HSATS, MSATS, HSKTS and MSKTS treatments compared to the urea and AMS treatments (Fig. 2-2b & 2-4b). The HSATS treatment resulted in an 86% and 66% reduction in NO_3^--N compared to the urea treatment, on day 21 and day 63, respectively. The HSATS and HSKTS treatments reduced nitrification over a longer period of time compared to the MSATS and MSKTS in the first run (Fig. 2-3b), but there were no significant differences between the treatments in run two (Fig. 2-4b). The concentration of thiosulfate is likely to be a factor in the ability for reducing nitrification, but it is not certain why rates of thiosulfate caused a difference in run one but not in run two. However, Janzen & Bettany (1986) showed that high concentrations are needed to reduce nitrification. Thiosulfate is converted to both sulfate and elemental S once it is in the soil (Hagstrom, 1986), but it is unclear how fast this conversion takes place.

In both runs, the HSATS and HSKTS reduced nitrification compared to the UDCD. The HSATS treatment had lower NO_3^--N than UDCD from day 21 to day 63 in the first run (Fig. 2-3b) and from day 28 to day 56 in the second run (Fig. 2-4b). The HSATS and HSKTS treatments were not significantly different in NO_3^--N concentration compared to the control from day 0 to day 56 in the first run (Fig. 2-3b) and from day 0 to day 49 in the second run (Fig. 2-4b), indicating that more N remained in the NH_4^+-N form. We observed more inhibition earlier in the experiment while Goos (1985) observed a larger inhibitory effect from thiosulfate in the form of sodium thiosulfate later in the experiment; in this study, a higher percent inhibition was seen 28 days than 14 days after application in a four-week incubation study on three fine-textured Mollisols. The inhibition by thiosulfate could be the result of volatile S compounds being formed in the soil after application (Bremner & Bundy, 1974). However, thiosulfate transformations in the soil were not examined in this study.

The Tujunga loamy sand was the only soil to show any significant effects from the fertilizer treatments on NO_2^--N concentration (Fig. 2-5b & 2-6b). The NO_2^- -N tended to follow the same trends according to treatment as the NO_3^--N data (Fig. 2-3b & 2-4b). However, the NO_2^--N concentration from the UDCD treatment decreased sharply between day 42 and 49 in run one (Fig. 2-5b) and day 56 and 63 in run two (Fig. 2-6b) while NO_3^--N concentrations continued to increase. In both runs, the urea had the highest concentration of NO_2^- -N compared to all other treatments (Fig. 2-5b & 2-6b). In run two, the AMS treatment was similar to urea throughout the incubation. The HSATS, MSATS, HSKTS, MSKTS and UDCD seem to be inhibiting the first step of nitrification based on these results. Unlike Janzen & Bettany (1986), we did not see an accumulation of NO_2^--N within the thiosulfate or UDCD treatments (Fig. 2-5 & 2-6). However, NO_2^--N concentrations with the urea treatment reached 15 mg kg^{-1} in the first run, and concentrations reached 83 mg kg^{-1} with the urea and 72 mg kg^{-1} with AMS treatments in run two (Fig. 2-5 & 2-6). Near day 40 in both runs, NO_2^--N started to be observed in the thiosulfate treatments, around the same time NO_3^--N concentrations were increasing, with NO_2^--N reaching 10 mg kg^{-1} in the first run and 40 mg kg^{-1} in the second run. Other studies on DCD seem to show an effect on the first step of the nitrification process by inhibiting ammonia oxidizers (Zacheral & Amberger, 1990; Fu et al., 2020).

SABLE SILT LOAM

In run one for the Sable silt loam, the NH_4^+-N to NO_3^--N conversion was reduced from day 35 until day 56 in the UDCD, HSATS, MSATS, HSKTS, and MSKTS compared to the urea treatment (Fig. 2-1c & 2-3c). The HSATS treatment had 48% and 70% lower NO_3^--N concentrations than urea on day 35 and 56, respectively. The HSATS, HSKTS, and UDCD reduced nitrification compared to the MSATS and MSKTS treatments from day 56 to day 70, showing longer inhibition with the higher rate of thiosulfate. In run two, however, there were no significant differences in NO_3^--N concentrations (Fig. 2-4c). It is not clear why results were different between the two runs. In both experiments, the soil moisture and temperature were maintained similarly. As for NO_2^--N, concentrations were near zero for both runs, and no significant differences were found between treatments (Fig. 2-5c & 2-6c).

One explanation for different results between run one and run two is the possibility of increased denitrification in the second run of the experiment. Research has shown that in more acidic soils, NO_2^--N will convert more rapidly to nitrous acid (HNO_2), followed by conversion to nitric oxide (NO) and nitrous oxide (N_2O) (Venterea & Rolston, 2000). Considering the relatively low NO_3^--N concentrations in the Sable silt loam (Fig. 2-4c) and the low soil pH of 5, there is a possibility that NO_2^- -N accumulated and then transformed rapidly into nitrous gases before measurement.

The Sable silt loam had a numerically higher concentration of NH_4^+-N than the Tujunga loamy sand and Marvyn loamy sand due to a higher CEC, OM and mineralogy able to fix high amounts of NH_4^+-N. A nitrification inhibitor being applied to a soil with a high potential to fix ammonium may increase the rate of fixation. (Drury & Beauchamp, 1991). Higher NH_4^+-N concentrations were seen in the second run which may have resulted in lower NO_3^--N concentrations (Fig. 2-2c & 2-4c).

MARVYN LOAMY SAND

Within the Marvyn soil, HSATS tended to have a higher NH_4^+-N concentration than all other treatments. In run one from day 14 to day 70, HSATS was significantly higher in NH_4^+-N compared to urea and was higher than all treatments from day 21 to 42 (Fig. 2-1a). In run two, HSATS had a higher NH_4^+-N concentration compared to urea, UDCD, MSATS, MSKTS and the control from day 35 to 70, and more than all treatments except for AMS from day 42 to 70 (Fig. 2-2a). However, there were no differences between treatments in nitrification except the control had a significantly higher NO_3^--N concentration throughout the experiment than all the fertilizer treatments (Fig. 3a & 4a). This was seen in both runs of the incubation study. Other research studies have found similar results with coarse-textured soils compared to fine-textured soils. A study on tropical soils with three different soil textures found sandy soils may have limited nitrification due to a lower abundance of ammonium-oxidizing microorganisms, and this was a direct result of lower clay content as well as lower organic matter and water holding capacity

(Barth et al., 2020). Conversely, an incubation experiment by Barth et al. (2001) found more nitrification being inhibited in a sandy soil compared to a loamy soil with the nitrification inhibitor 3,4-dimethylpyrazole-phosphate (DMPP). However, their soil samples were water saturated, and were only incubated for five hours. Water was maintained close to 25% WHC for our incubation study to reduce the possibility of denitrification.

Considering the Marvyn loamy sand had a low OM and coarse texture, there may have been a low microbial population at the beginning of the experiment that increased as N fertilizer was added. One hypothesis for lower NO_3^--N concentrations in the fertilized treatments compared to the control could be due to immobilization of N by soil microorganisms. The addition of nitrification inhibitors like DCD may cause a priming effect for mineralization and immobilization turnover rates due to increased retention of NH_4^+-N from fertilizer-derived N (Gioacchini et al., 2002). Within their study, more leaching occurred when both N-(n-butyl) thiophosphoric triamide (NBPT) and DCD were added to urea, and it was hypothesized to be due to more mineralization of soil organic matter from increased microbial activity. However, within our incubation experiments, we did not observe differences in NO_3^--N in any of the fertilizer treatments, but HSATS did increase NH_4^+-N concentrations compared to all other treatments.

It is possible that some volatilization and denitrification occurred within the incubation bins. We did not measure volatilization or other N fluxes. Studies observing volatilization and denitrification losses with urea-based fertilizers have seen significant losses. In a study within a Mississippi delta cotton production system, researchers observed more N losses from denitrification than volatilization using urea fertilizer (Tian et al., 2015). Overall, more volatilization is likely to occur in course-textured soils like the Marvyn loamy sand compared to fine-textured soils with a high CEC like the Sable silt loam, due to lower NH_4^+-N adsorption capacity. It has also been observed that DCD, when added to urea, can increase volatilization loss (Soares et al., 2012). However, no differences in NH_4^+-N or NO_3^--N were observed between urea and the urea with DCD added for the Marvyn soil.

Some research has shown that ammonium thiosulfate can reduce the rate of urea hydrolysis by 15-35% (Goos, 1985). However, we did not see a reduction in NH_4^+-N concentration for the thiosulfate treatments compared to urea-only in the Marvyn loamy sand. In fact, in run two in the Sable silt loam, urea hydrolysis seemed to be faster within the thiosulfate treatments because urea-only and ammonium sulfate were slower to increase in NH_4^+-N (Fig. 2-2c). In run one, all fertilizer treatments increased in NH_4^+-N at the same rate (Fig. 2-1). Goos & Fairlie (1988) did find that the hydrolysis rate was slower in a more coarse-textured soil.

Conclusion

We conducted an incubation study to determine the effectiveness of thiosulfate products to reduce nitrification of urea-based fertilizers. Studies were conducted for soils with varying chemical and physical characteristics. Thiosulfate products show potential to inhibit nitrification and appear to be more effective in soils with CEC > 5.0. Differences in other soil characteristics including texture, mineralogy, pH and organic matter content may have also influenced the effectiveness of nitrification inhibitors according to soil type. In some cases, the higher rate of ATS and KTS reduced nitrification compared to low rates. However, within the Tujunga loamy, both ATS and KTS reduced nitrification more than DCD. Based on evaluation, ATS and KTS inhibited the first reaction in the nitrification process. This experiment did not evaluate the mechanism by which thiosulfate may inhibit nitrification, and more research may need to be done to determine what mechanism causes nitrification inhibition.

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Tables and Figures

Table 2-1: Description of incubation fertilizer treatment descriptions, fertilizer analysis, percent composition of nitrogen (N) chemical form, and percent composition of sulfur (S) chemical form.

Treatment	Total Analysis	N Forms (%)	S Forms (%)
No N Control	-	-	-
Urea + Ammonium Sulfate (AMS)	9-0-0-1.4(S)	Urea (88%); Ammonium (12%)	Sulfate (100%)
Urea only	20-0-0	Urea (100%)	-
Urea + Mid S Ammonium Thiosulfate (MSATS)	19.2-0-0-2.5(S)	Urea (94%); Ammonium (6%)	Thiosulfate (100%
Urea + High S Ammonium Thiosulfate (HSATS)	18.4-0-0-5.2(S)	Urea (87%); Ammonium (13%)	Thiosulfate (100%)
Urea + Mid S Potassium Thiosulfate (MSKTS)	17-0-3.7-2.5(S)	Urea (100%)	Thiosulfate (100%)
Urea + High S Potassium Thiosulfate (HSKTS)	15-0-6.2-4.2(S)	Urea (100%)	Thiosulfate (100%)
Urea + Dicyandiamide (DCD)	20-0-0	Urea (100%)	-

Table 2-2: Soil series and characterization analysis of particle size fractions, cation exchange capacity (CEC), organic matter (OM) and soil pH for soils used in the incubation study.

Soil Series	**Sand %**	**Silt %**	**Clay %**	**CEC $\mathrm{cmol_c kg^{-1}}$**	**OM %**	**pH**
Marvyn loamy sand	87.5	10	2.5	4.97	0.51	6.5
Tujunga loamy sand	70	25	5	9.66	0.62	7.1
Sable silt loam	22.5	65	12.5	21.55	2.02	5.0

Table 2-3: Summary of ANOVA analysis for ammonium (NH_4^+-N), nitrate (NO_3^--N), and nitrite (NO_2^- -N) in response to soil type, fertilizer treatment, and date of sampling.

Measurement	Source of Variance	ANOVA (P > F)	
		Run One	Run Two
NH_4^+-N	Soil	<0.0001	<0.0001
	Treatment	<0.0001	<0.0001
	Day	<0.0001	<0.0001
	Soil x Treatment	<0.0001	<0.0001
	Soil x Day	<0.0001	<0.0001
	Day x Treatment	<0.0001	<0.0001
	Day x Treatment x Soil	<0.0001	<0.0001
NO_3^--N	Soil	<0.0001	<0.0001
	Treatment	<0.0001	<0.0001
	Day	<0.0001	<0.0001
	Soil x Treatment	<0.0001	<0.0001
	Soil x Day	<0.0001	<0.0001
	Day x Treatment	<0.0001	<0.0001
	Day x Treatment x Soil	<0.0001	<0.0001
NO_2^--N	Treatment	<0.0001	<0.0001
	Day	<0.0001	0.0347
	Day x Treatment	<0.0001	0.1201

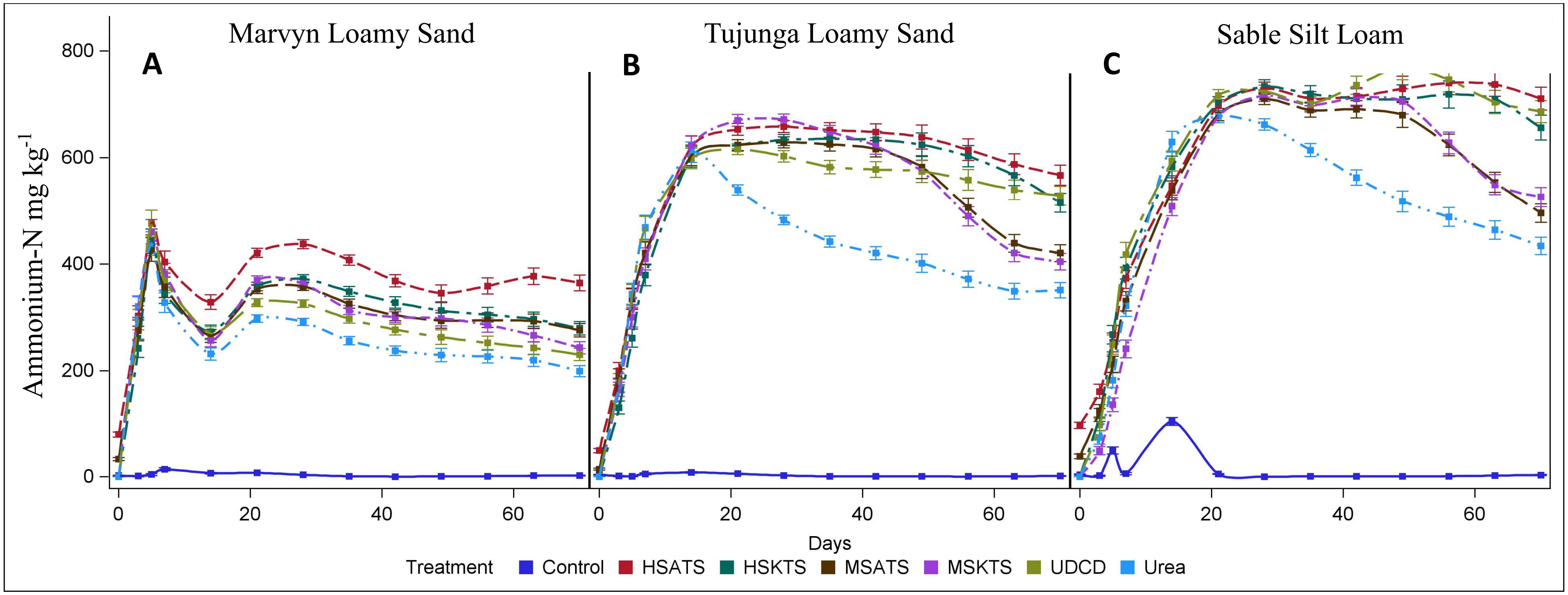

Figure 2-1: Run one of ammonium-N concentrations in mg kg^{-1} over a 70-day incubation for three soil types: (A) Marvyn loamy sand, (B) Tujunga loamy sand, (C) Sable silt loam. Treatments include: control, urea + high S ammonium thiosulfate (HSATS), urea + high S potassium thiosulfate (HSKTS), urea + mid S ammonium thiosulfate (MSATS), urea + mid S potassium thiosulfate (MSKTS), Urea + Dicyandiamide (DCD), and urea only. Error bars represent 95% confidence interval about the mean.

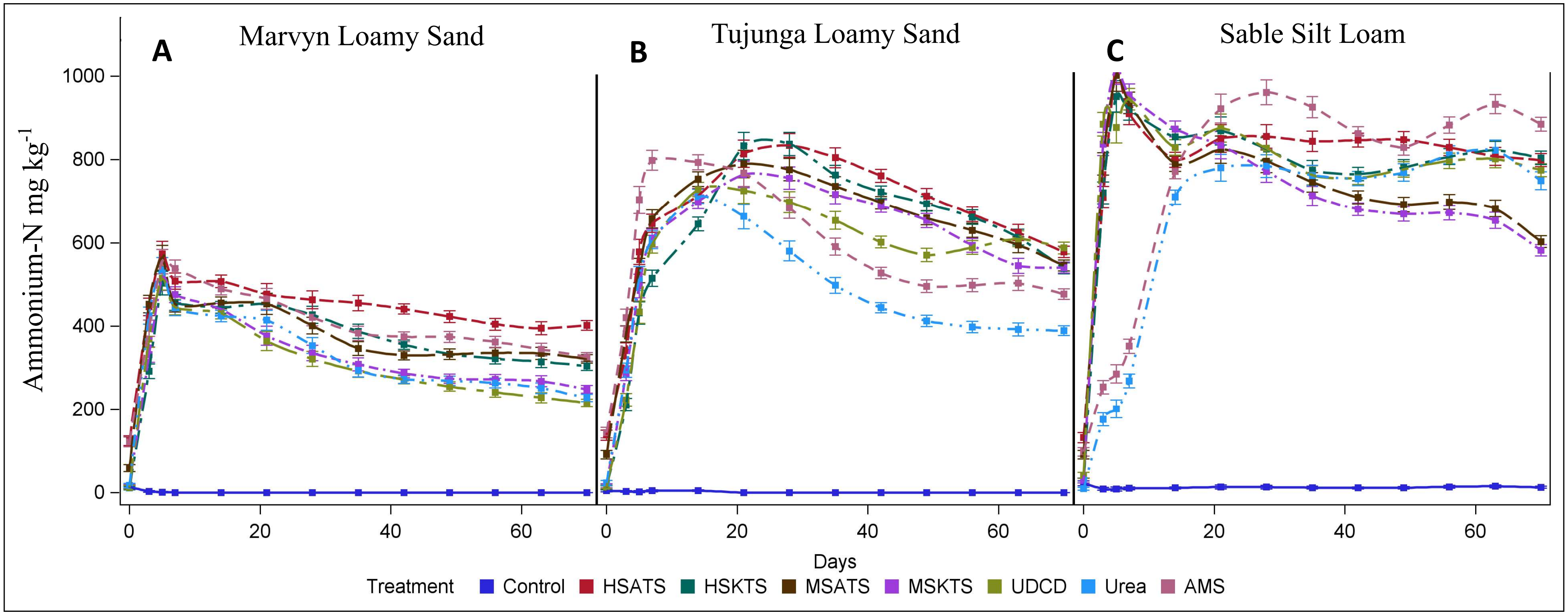

Figure 2-2: Run two of ammonium-N concentrations in mg kg^{-1} over a 70-day incubation for three soil types: (A) Marvyn loamy sand, (B) Tujunga loamy sand, (C) Sable silt loam. Treatments include: control, urea + high S ammonium thiosulfate (HSATS), urea + high S potassium thiosulfate (HSKTS), urea + mid S ammonium thiosulfate (MSATS), urea + mid S potassium thiosulfate (MSKTS), Urea + Dicyandiamide (DCD), and urea only. Error bars represent 95% confidence interval about the mean.

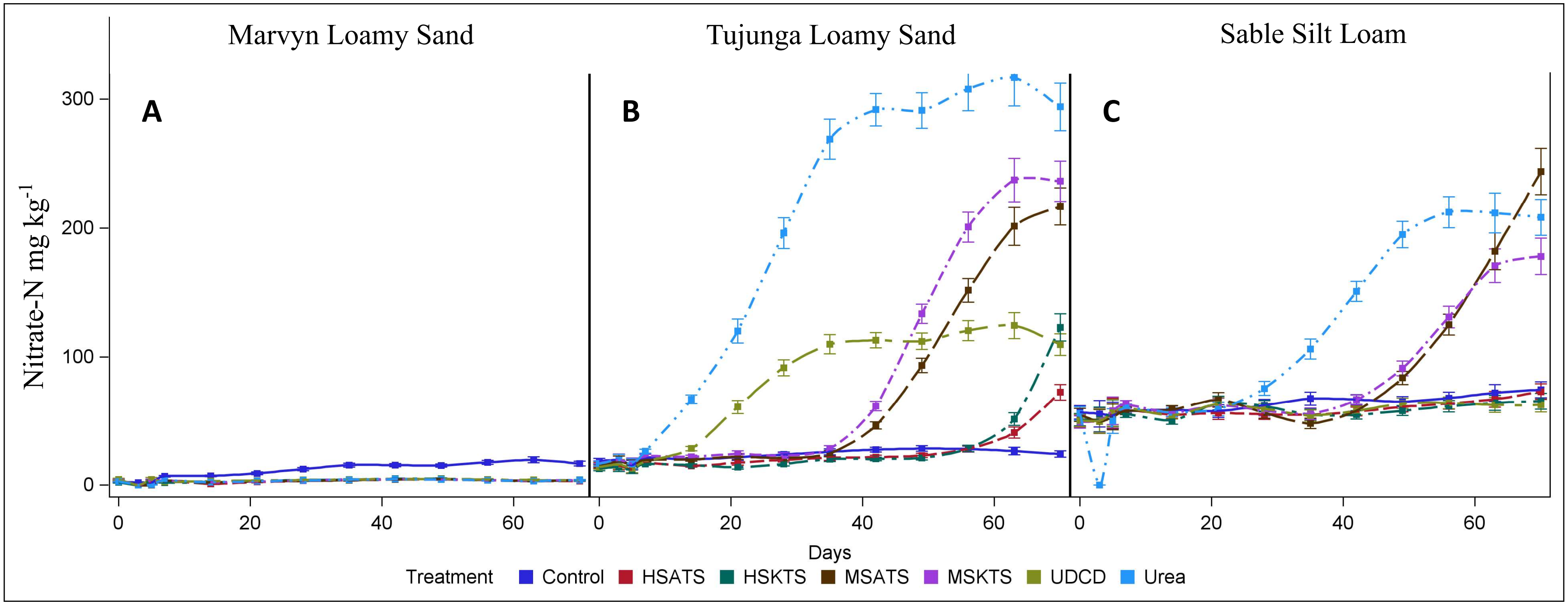

Figure 2-3: Run one of nitrate-N concentrations in mg kg^{-1} over a 70-day incubation for three soil types: (A) Marvyn loamy sand, (B) Tujunga loamy sand, (C) Sable silt loam. Treatments include: control, urea + high S ammonium thiosulfate (HSATS), urea + high S potassium thiosulfate (HSKTS), urea + mid S ammonium thiosulfate (MSATS), urea + mid S potassium thiosulfate (MSKTS), Urea + Dicyandiamide (DCD), and urea only. Error bars represent 95% confidence interval about the mean.

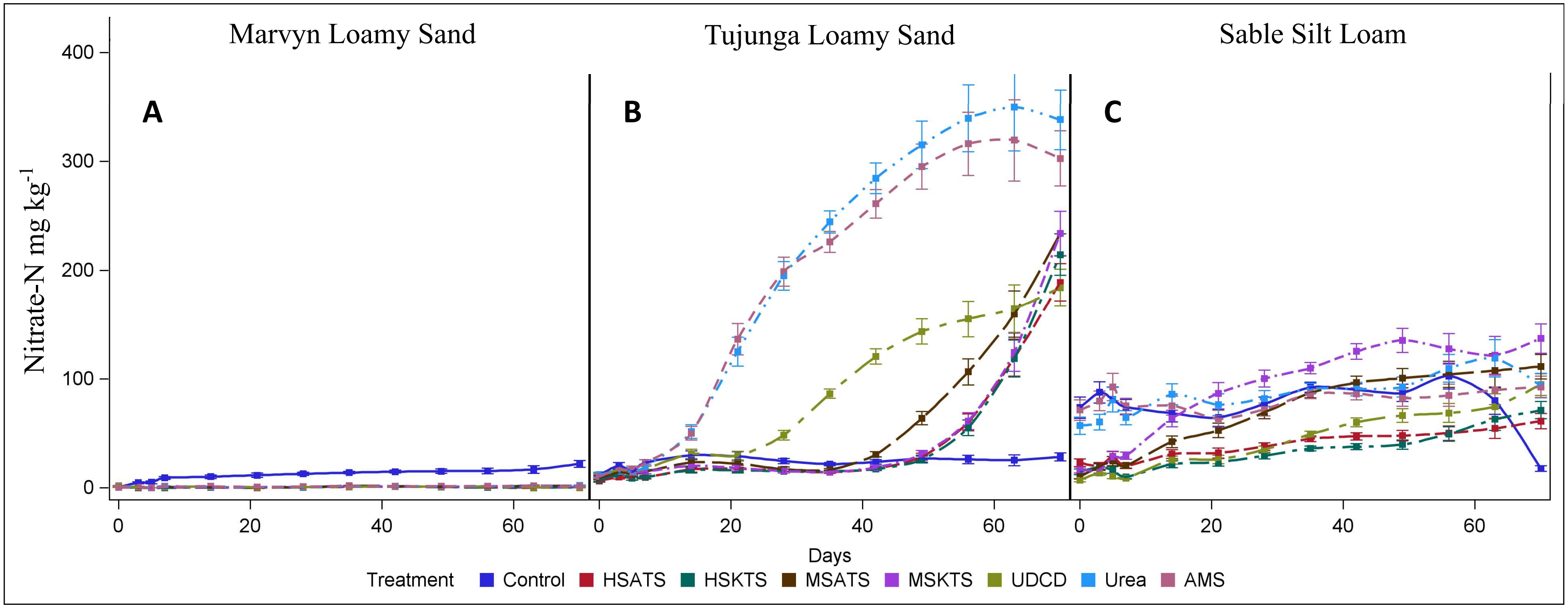

Figure 2-4: Run two of nitrate-N concentrations in mg kg^{-1} over a 70-day incubation for three soil types: (A) Marvyn loamy sand, (B) Tujunga loamy sand, (C) Sable silt loam. Treatments include: control, urea + high S ammonium thiosulfate (HSATS), urea + high S potassium thiosulfate (HSKTS), urea + mid S ammonium thiosulfate (MSATS), urea + mid S potassium thiosulfate (MSKTS), Urea + Dicyandiamide (DCD), and urea only. Error bars represent 95% confidence interval about the mean.

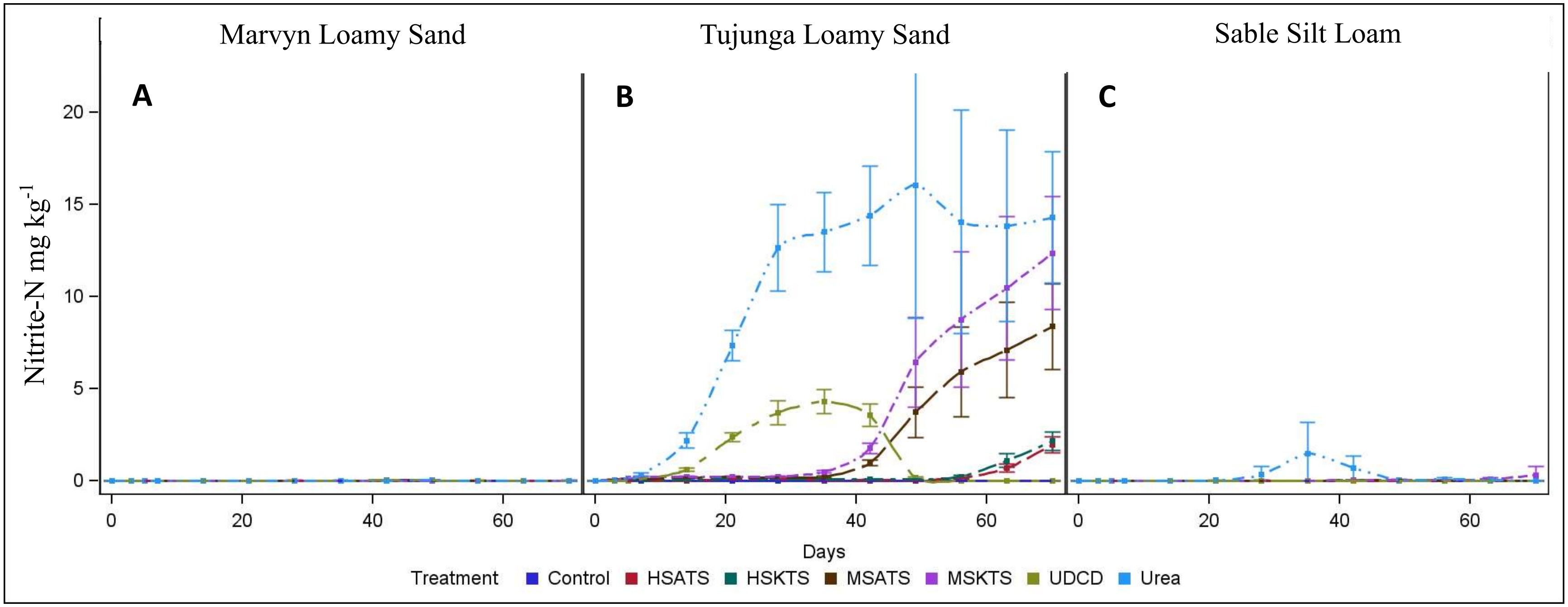

Figure 2-5: Run one of nitrite-N concentrations in mg kg^{-1} over a 70-day incubation for three soil types: (A) Marvyn loamy sand, (B) Tujunga loamy sand, (C) Sable silt loam. Treatments include: control, urea + high S ammonium thiosulfate (HSATS), urea + high S potassium thiosulfate (HSKTS), urea + mid S ammonium thiosulfate (MSATS), urea + mid S potassium thiosulfate (MSKTS), Urea + Dicyandiamide (DCD), and urea only. Error bars represent 95% confidence interval about the mean.

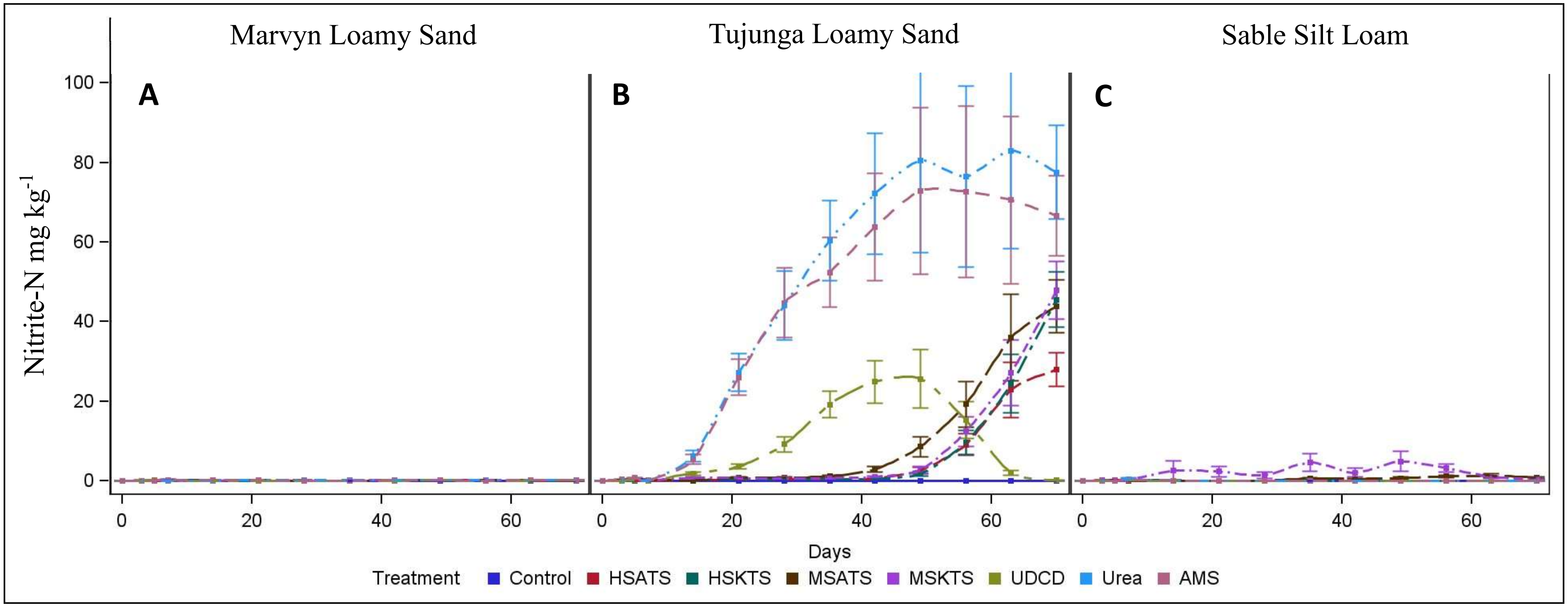

Figure 2-6: Run two of nitrite-N concentrations in mg kg^{-1} over a 70-day incubation for three soil types: (A) Marvyn loamy sand, (B) Tujunga loamy sand, (C) Sable silt loam. Treatments include: control, urea + high S ammonium thiosulfate (HSATS), urea + high S potassium thiosulfate (HSKTS), urea + mid S ammonium thiosulfate (MSATS), urea + mid S potassium thiosulfate (MSKTS), Urea + Dicyandiamide (DCD), and urea only. Error bars represent 95% confidence interval about the mean.

III. Ammonium Thiosulfate and Potassium Thiosulfate Effects on Nitrification in Soils and Vegetative Growth of Corn (*Zea mays* L.)

Introduction

Nitrogen (N) is an essential element for all plants. It is a large component of nucleic acids that form DNA and a part of amino acids that form proteins (Canfield et al., 2010). Nitrogen is typically the largest fertilizer input cost, especially for corn (*Zea mays* L.) production, and often has the largest impact on yield and profit from a nutrient deficiency standpoint. Most healthy plants have a N content of 1-6%, on a dry-weight basis (Havlin et al., 2016). Losses of N lead to a low nitrogen use efficiency (NUE), which can be defined as the amount of N uptake by the crop per the amount of N applied (Alonso-Ayuso et al., 2016), in agricultural ecosystems and have negative impacts from greenhouse gas emissions and pollution of groundwater systems. Nitrogen use efficiency is typically below 50% (Chien et al., 2009). The goal of nutrient management and soil fertility planning is to maximize NUE to increase crop productivity while also preventing N input losses. There are many management practices to help prevent N losses, including crop rotation, conservation tillage, cover crops, and optimizing fertilizer rates, application timing, placement and sources of fertilizers. Nitrification inhibitors provide one mechanism to improve NUE.

The majority of the inorganic N fertilizers applied today are products of the Haber-Bosch process. Industrial fixation of N has increased from 2.5 Tg of N per year in 1960 to 25.4 Tg of N per year in 2008 (Gu et al., 2013). Along with this, fertilizer use has increased 800% from 1960 to 2000, coinciding with many advancements in crop production (Canfield et al., 2010). Nitrogen fertilizer use was more than 90 million megagrams in 2005 to 2006, compared to less than 12 million megagrams globally in 1960 (Emerich et al., 2009). Currently, half the global N fertilizer is applied to rice, wheat, and corn (Yu et al., 2022). However, NUE is still low for the major crops, with average N fertilizer use efficiency being 39%.

One contributing factor to lower NUEs is the use of soluble fertilizers. Nitrogen materials such as urea, ammonium sulfate, or urea ammonium nitrate (UAN) go into soil solution rapidly, where they may be taken up by the plant. However, over half of that soluble N is not used by the plant and can be lost to the environment by various pathways. These pathways can include losses to the air (volatilization, denitrification), groundwater / out of the root zone (leaching) or through surface runoff (Kurtz, 1980). These mechanisms of N loss are both detrimental to the surrounding environment, and to profitable crop production. Various fertilizer application methods and technologies are used to slow N release, metering it out for plant use and an improved NUE. One such technique is the use of nitrification inhibitors, which will be the focus of this introduction.

Most N fertilizers, when applied to the soil undergo nitrification, which is the biologically mediated conversion of ammonium (NH_4^+-N) into nitrite (NO_2^- -N) and then into nitrate (NO_3^--N) (Schepers & Meisinger, 1994). Nitrification of ammonium-based fertilizers can happen quickly, with 90% being oxidized into NO_3^--N within four weeks in some soils (Sahrawat, 1982). Ammonium-N that is held on the surface of clay minerals and organic matter is stable in the soil (Sahrawat, 2008). However, NO_3^--N is more mobile in the soil. Once N is converted from NH_4^+-N into NO_3^--N, it is easily leached or subject to more conversions that lead to another loss pathway known as denitrification (Subbarao et al., 2007). There are a small number of genera that are involved in these reactions. Both leaching and denitrification are two of the main loss pathways resulting in low NUE which occur after N goes through the process of nitrification. It has been studied extensively throughout the last century (Allison, 1955; Allison, 1965).

Nitrification inhibitors prevent the buildup of NO_3^--N within the soil by interfering with the chemosynthesis reactions within the metabolic processes of the nitrifying bacteria (Hauck, 1980). Most nitrification inhibitors target the first step in the nitrification process, activation of the ammonia monooxygenase enzyme. However, there are also chemicals that inhibit the second step of nitrite oxidation directly or indirectly (Hauck, 1980; McCarty, 1999; Kong et al., 2016).

Nitrapyrin has been the main nitrification inhibitor chemical for the last 25 years (Wolt, 2004). It is sold commercially in products including N-Serve^{TM} and Instinct^{TM}. It is often used with applications of anhydrous ammonia due to its volatility (Nelson & Huber, 1980; Goos, 2019). However, it can be used effectively when sprayed onto granular urea and broadcast onto the soil surface (Frye et al., 1981). Dicyandiamide (DCD) is a widely known product that can be applied with many N fertilizers including granular urea (Reeves et al., 1988; Goos, 2019). Much research has shown that DCD can reduce leaching of fertilizer N and increase yields, but economic benefits are not always observed, according to a meta-analysis by Yang et al. (2016).

Typically, laboratory incubation studies conducted with DCD and other nitrification inhibitors tend to have clearer results than field trials (Zacher & Amberger, 1990; Vogeler et al., 2007; Barth et al., 2020; Fu et al., 2020). In a sandy loam soil, Fu and others (2020) observed a reduction in ammonia monooxygenase activity and a reduction in NO_3^--N when DCD was added to urea. However, a slight reduction in wheat (*Triticum aeslivum* L.) yield was also seen with DCD. In another study, on a clay loam and sandy loam, DCD increased the amount of N kept in an NH_4^+-N form but did not increase N recovery by the plant due to low amounts of leaching for the entire study (Gioacchini et al., 2002).

Application of sulfur (S) fertilizer is becoming more common due to cleaner air and fertilizer sources with less impurities (Duke & Reisenauer, 1986). Ammonium thiosulfate (ATS) and potassium thiosulfate (KTS) are liquid fertilizers that are currently being used mostly in conjunction with irrigation (Goos & Johnson, 2001). Limited research has examined the effect of KTS on nitrification, but there are some studies which have examined the effects of ATS or thiosulfate on nitrification (Goos, 1985; Janzen & Bettany, 1986; Goos & Fairlie, 1988).

Thiosulfate is converted to sulfate in the soil before plant uptake (Hagstrom, 1986). Inhibition of nitrification most likely occurs during the oxidation of thiosulfate resulting in an inhibitory effect on ammonia or nitrite oxidizers and their related enzymes. There may be a delayed effect on nitrification with greater inhibition being seen two weeks after application (Goos, 1985). Thiosulfate may have similar inhibition effects to nitrapyrin or DCD, but may be needed in larger concentrations (Goos, 2019). According to Janzen & Bettany, (1986), a 100 mg S kg^{-1} as thiosulfate resulted in the same amount of inhibition as 2 mg kg^{-1} of nitrapyrin in a loamy soil while also causing an accumulation of NO_2^--N of 42 mg N kg^{-1} of soil. In addition to nitrification, some work has observed thiosulfate's effect on volatilization, where application of ATS reduced ammonia gas losses from urea-based fertilizers (Goos & Fairlie, 1988).

The majority of field studies evaluating nitrification inhibitors have been conducted on winter wheat and corn within the midwestern corn belt using nitrapyrin. An average wheat yield increase of 15% was seen when nitrapyrin was added with fall applications of anhydrous ammonia, urea or ammonium sulfate in multiple soil textures (Huber et al., 1980). Another study using anhydrous ammonia on corn in a clay loam soil observed a benefit to using nitrapyrin in a fall N application but no real benefit for spring N applications. However, N recovery values were low for all treatments, ranging from 31% for a fall N application without nitrapyrin to 61% for spring N application with nitrapyrin (Randall et al., 2003; Randall & Vetsch, 2005). Others observed that nitrapyrin added to fall broadcast urea on corn in a poorly drained silt loam resulted in an increase in NH_4^+-N, without NO_3^--N accumulation, an effect not observed when N was Spring applied (Touchton et al., 1979). Nitrapyrin and possibly other nitrification inhibitors may delay leaching losses by 25 to 50 days, but will not necessarily prevent leaching. The delay, however, did allow more time for plant uptake of fertilizer derived N (Walters & Malzer, 1990).

Overall, available nitrification inhibitors widely used in field crop production are largely limited to DCD or nitrapyrin. Both of these chemistries have long been used, with mixed results on both nitrification and crop yield. Thus, new nitrification inhibitor technologies are always of interest, especially those already available, lessening the need for expensive development and licensing. Thiosulfate and the products KTS and ATS have long been explored as potential inhibitors, yet their mode of action, and performance in a range of soils is only lightly studied. So, the objective of this study was to evaluate the application of ATS and KTS in a corn greenhouse experiment, evaluating performance in three distinct soils: an Ultisol from Auburn, AL (Marvyn loamy sand), an Entisol from Danuba, CA (Tujunga loamy sand), and a Mollisol from Bureau County, IL (Sable silt loam).

Along with the incubation study evaluating the effectiveness of ATS and KTS on nitrification in soil types, the objective of this study was to evaluate their effect on nitrification in a greenhouse experiment with corn grown in soils with different chemical and physical properties.

Materials and Methods

Greenhouse Experiment

A greenhouse study was conducted on corn (*Zea mays* L.) with the three soils (Table 3-1 & 3-2) eight fertilizer treatments (Table 3-3) to determine the effects of the different fertilizer sources on crop production in a controlled environment. The soil types consisted of Marvyn loamy sand (Fine-loamy, kaolinitic, thermic Typic Kanhapludult) from the Auburn University Turf Research Unit in Auburn, AL, Tujunga loamy sand (Mixed, thermic Typic Xeropsamment) from the Tessenderlo Kerley Inc. research farm in Danuba, CA, and a Sable silt loam (Fine-silty, mixed, superactive, mesic Typic Endoaquall) from Bureau County, IL.

The greenhouse study was conducted at the Auburn University Plant Science Research Center (PSRC), located in Auburn, AL. The studies were conducted under greenhouses with ambient light. The studies were conducted twice, with the first run from June 13, 2022 to July 18, 2022 and the second run from March 29, 2023 to May 3, 2023. The trial consists of four replications of each treatment in a complete randomized design (Table 3-3), with each soil air dried and sieved through a 2-mm sieve prior to use. Soils were placed in 16.5 cm diameter pots with 2,000 grams of air-dried soil per pot for the Marvyn and Tujunga soil, and 1,600 grams of air-dried soil per pot for the Sable soil type. The corn variety, planted at three seed per pot was P1847VYHR (Pioneer, PO Box 1000, Johnston, IA, 50131). Corn was thinned to one plant per pot at three to five days post emergence.

For the first run, irrigation water was applied uniformly to all soils. However, because of the different water holding capacity (WHC) for each soil, water was not maintained equally. For the second greenhouse experiment, water was initially added to WHC one week prior to fertilizer application and planting, and then maintained up to 60% WHC during the experiment. The goal was to maintain water between 60% and 40% WHC. The study included eight fertilizer treatments: no N control, urea with ammonium sulfate, urea only, urea with dicyandiamide (DCD), and two rates of ammonium thiosulfate with urea and two rates of potassium thiosulfate with urea (Table 3-3). For all fertilizer treatments, a liquid urea solution was made using lab grade granular urea. Fertilizers were applied at planting in a band, roughly 5 cm deep, in the middle of the pot to a rate of 67 kg N ha^{-1} based on the surface area of the pot. Corn was planted 5 cm to the side of the band. Soil samples were taken immediately after fertilizer application and weekly thereafter for 5 weeks, to the point when corn was at the V6 growth stage. Small soil probes (2 cm diameter) were used to take a single soil sample to the depth of the pot for each sample. Each hole was filled with the same soil after taking the sample, and a plastic spatula was used to mark where samples were taken previously to prevent repeated sampling in the same location.

Plant height and SPAD meter readings for relative chlorophyll content were taken periodically throughout each experiment. Five SPAD measurements using a SPAD-502 Plus Chlorophyll Meter (Spectrum Technologies, Inc., 3600 Thayer Court, Aurora, IL, 60504) were taken on the upper most fully developed leaf for each plant, and the average measurement was recorded. At the end of the experiment, above ground plant biomass was collected, placed in a dryer for five days at 60° C, weighed and sent to Water Agricultural Laboratories, Inc. (2101 Calhoun Rd, Owensboro, KY 42301) to be analyzed for total N by combustion using a LECO FP 528 according to Association of Analytical Chemists method 990.03 (AOAC, 2006).

Inorganic Nitrogen Analysis

Soil extractions were performed to determine NH_4^+-N and NO_3^--N on the collected soil samples using the same extraction and microplate procedure as the incubation study. A 5-g soil sample was extracted with 25 mL of unbuffered 2M potassium chloride (KCl). Extracts were placed on a shaker at low speed for 30 minutes. Samples were then filtered through number 42 fiber glass filter paper and put into freezer storage until time for analysis using a visible light spectrophotometer microplate reader (Biotek® uQuant™, Agilent, 5301 Stevens Creek Blvd, Santa Clara, CA 95051). Direct determination of NH_4^+-N in 2M KCl extracts using a colorimetric procedure was done by the indophenol blue reaction to determine the concentration of NH_4^+-N (Keeney & Nelson, 1982). For determining (NO_3^- -N), a colorimetric procedure using cadmium to reduce NO_3^--N to NO_2^- -N (Keeney & Nelson, 1982).

For the NH_4^+-N procedure, 50 microliters (uL) of the sample were placed in a well and then each reagent was added in a specific order: 25 uL citrate reagent, 50 uL salicylate-nitroprusside reagent, 25 uL of hypochlorite reagent and 125 uL of background matrix. After adding each reagent and stirring, the plates would sit for 30 minutes before being placed on a microtiter plate reader using the 695_590 nm method. Microplate standards for 0, 1, 2, 4, 6, 8. 10 and 15 ppm of NH_4^+-N were used on both sides of each plate (Sims et al., 1995).

For the NO_3^- -N procedure, each plate was placed in a sonicator with the reductor pins submerged in an HCl and $CuSO_4$ solution to activate the reductor pins before each use. Microplate standards for 0, 0.5, 1, 2, 4, 6, 8 and 10 ppm of NO_3-N were used on both sides of each plate. 20 uL of a sample was placed in a well then 200 uL of EDTA with a pH of 8.5 buffer was added. A reductor plate was then placed on top of the microplate and placed on a shaker for 1 hour. After, 60 uL of the Griess reagent was added and then placed on a shaker for 5 minutes before being placed on a microtiter plate reader at the 542 nm method (Keeney & Nelson, 1982).

Statistical Analysis

Statistical analysis was completed using SAS Version 9.4 (SAS Institute Inc., Cary, N.C.). NH_4^+-N, and NO_3^- -N variables were subjected to repeated measures analysis of variance using PROC GLIMMIX. All three variables were modeled using splines (piecewise second-degree polynomials). Treatment, soil, sample day, and their interactions were used as fixed effects and replication was used as a random effect. The first order heterogeneous autoregressive covariance structure ARH(1) was used to account for repeated measures among days. Differences due to soil and treatment effects were evaluated at each sampling day. N uptake was analyzed using treatment, soil, and their interaction as fixed effects and replication as random effect using PROC MIXED. Plant height and chlorophyll content were analyzed using a quadratic model and repeated measures analysis of variance. The first order autoregressive covariance structure AR(1) was used to account for repeated measures among days. Splines method was not used, such as for ammonium and nitrate, due to the low number of repeated measures (3-4 data points). For all analyses, degrees of freedom were calculated using the Satterthwaite method and Tukey adjustment was used to adjust for multiple comparisons (Littell et al., 2006).

Results and Discussion

Two runs of the same experiment were conducted in a greenhouse using the same soil types that were used in the incubation experiment (Table 3-1 & 3-2). Measurements were taken on soil NH_4^+-N and NO_3^--N concentrations, plant height, chlorophyll content, total N percent and above ground biomass at the end of the experiment. Both runs had a significant interaction between day, soil and treatment on NH_4^+-N while only run one was significant for differences in extractable NO_3^--N (Table 3-4). A quadratic statistical analysis was used for plant height and chlorophyll content due to the low number of sampling days, and both runs had a significant interaction between soil type, sampling day and treatment. Total N percent at the end of the five weeks was significant between soil and treatment in the first run, but not in the second run. Both soil and treatment effects alone were significant in the second run for total N. Plant dry-weight biomass at the end of the experiment was also significant between soil and treatment in both runs. Total N percent and above ground plant dry-weight biomass were used to determine N uptake at the end of the study (Fig. 3-8 & 3-9). Nitrogen uptake was significant between soil and treatment in both runs of the study.

Soil Type Effects on Nitrification

Slightly higher concentrations in NH_4^+-N were seen in run two for all three soil types compared to the first run (Fig. 3-1 & 3-2). In run one, NH_4^+-N did not increase after day 0, however, in run two, concentrations increased between days 0 and 7, then started to decrease for the rest of the experiment. For NO_3^--N, run two was slightly higher than run one, with a larger difference being seen in the Sable silt loam, but the second run was not significant between soil, treatment, and sampling day. This is possibly due to water being maintained between 40-60% WHC during the second run, but water additions done uniformly in the first run and were not measured. Therefore, a lower amount was maintained consistently throughout the second run. The Sable silt loam tended to have higher concentrations of NH_4^+-N and NO_3^--N in both runs than the Tujunga loamy sand or Marvyn loamy sand. This is not surprising due to a more fine-textured soil, higher OM and higher CEC. Other papers have observed more nitrification proportionally to the amount of clay in the soil (Barth et al., 2020) The Tujunga loamy sand had similar NH_4^+-N levels to the Marvyn loamy sand, but tended to have more NO_3^--N. Overall, in both runs, the Marvyn loamy sand tended to have the lowest NO_3^--N concentrations.

All fertilizer treatments for both runs were significantly higher in NH_4^+-N concentration than the control in all soils from day 0 until day 14 (Fig. 3-1 & 3-2). After that, NH_4^+-N concentrations were near zero by day 14 for all treatments and not significantly different. More variation was seen in the Sable silt loam than the other soil types (Fig. 3-1c & 3-2c). Overall, higher NH_4^+-N concentrations were seen in the Sable silt loam during the first two weeks as well. On day 7 in the first run, the average concentration was 61.7 mg kg^{-1} NH_4^+-N while by day

14, the average concentration was 6.5 mg kg^{-1} NH_4^+-N (Fig. 3-1c). In the second run, the average NH_4^+-N concentration on day 7 was 132.9 mg kg^{-1} and 12 mg kg^{-1} on day 14 (Fig. 3-2c). However, for the Marvyn loamy sand, the average NH_4^+-N concentration was 10 mg kg^{-1} on day 7, and 1.0 mg kg^{-1} NH_4^+-N by day 14 during the first run (Fig. 3-1a). During the second run, the average NH_4^+-N concentration was 94.5 mg kg^{-1} on day 7 and 3.1 mg kg^{-1} on day 14 (Fig. 3-2a). A similar trend was seen in the Tujunga loamy sand with the average concentration being 4.3 mg kg^{-1} NH_4^+-N on day 7, and 1.1 mg kg^{-1} NH_4^+-N on day 14 in the first run then 66 mg kg^{-1} on day 7 and 3.1 mg kg^{-1} on day 14 in the second run (Fig. 3-1b & 3-2b).

Nitrate-N concentrations in run one were near zero by day 14 in the Marvyn loamy sand and Tujunga loamy sand soil types (Fig. 3-3a & 3-3b). For the Sable silt loam, all treatments were near zero by day 14 except for the HSATS and MSKTS treatments (Fig. 3-3c). Nitrate-N tended to not increase after day 0 for all of the treatments except for HSKTS in the Sable silt loam where HSKTS increased in NO_3^--N concentration from day 0 to day 7 and decreased after while urea and UDCD stayed level between day 0 and 7 then decreased until all treatments were near zero by day 21. Within run two, NO_3^--N was measurable through day 21 and near zero for all treatments in all soils by day 28. As with NH_4^+-N, slightly more variation was seen in the Sable silt loam. All treatments, no matter the soil type, increased in NO_3^--N from day 0 through day 7 with some continuing to increase through day 14 in the Sable silt loam and Tujunga loamy sand soil types. However, the interaction between soil, sampling day and treatment was not significant.

Overall, climate conditions in the greenhouse would be considered conducive for N losses through leaching or volatilization due to high moisture and temperature. Even though water was maintained at a lower WHC in the second run, water was added every other day and sometimes daily resulting in the moisture content being high throughout the study. Although volatilization and denitrification were not measured, losses through pathways other than leaching were possible. However, the fertilizers being applied in a band below the surface would reduce potential volatilization losses. Also, research studies have seen a reduction in volatilization with the addition of thiosulfate to UAN (Goos & Fairlie, 1988).

In run one, plant height measurements tended to be lower in the Marvyn loamy sand compared to the Tujunga loamy sand and Sable silt loam (Fig. 3-4). In run two, all three soil types had similar plant height measurements (Fig. 3-5). Also, in both runs, there was no difference in SPAD chlorophyll due to soil type (Fig. 3-6 & 3-7). Corn in run one within the Marvyn loamy sand were significantly lower in N uptake compared to the Tujunga loamy sand and Sable silt loam (Fig. 3-8). In run two, corn grown in the Tujunga loamy sand tended to have lower N uptake in some treatments compared to the Sable silt loam and Marvyn loamy sand, however not significantly different (Fig. 3-9).

Fertilizer Treatment Effects on Nitrification

In both runs, only slight differences in NH_4^+-N and NO_3^--N were seen between treatments, and most were not significantly different (Fig. 3-1, 3-2 & 3-3). However, some trends became apparent. In the first run of the greenhouse trial, all fertilizer treatments were higher in NH_4^+-N than the control at the start of the experiment on day 0 and all treatments were near zero in NH_4^+-N concentration by day 14 (Fig. 3-1). In run two, all fertilizer treatments were higher than the control from days 0 to 14 (Fig. 3-2). In the first run for NO_3^--N, all fertilizer treatments tended to be higher than the control in the Tujunga loamy sand and Marvyn loamy sand from days 0 through 7, but were not different from the control in the Sable silt loam (Fig. 3-3).

In run one, the control in the Marvyn loamy sand was significantly lower in NH_4^+-N on day 7 while no differences were seen between any of the fertilizer treatments (Fig. 3-1a). By day 14, no differences were seen between treatments including the control except on day 28 where UDCD was significantly higher than the control in NH_4^+-N. As for run two, the control was lower in NH_4^+-N than all other treatments from day 0 until day 7 (Fig. 3-2a). On day 14, UDCD was higher in NH_4^+-N than all other treatments except for HSATS. However, UDCD did not appear to hold more NH_4^+-N from day 0 through day 7. The Marvyn tended to have the lowest NO_3^--N concentrations in both runs compared to the other soil types from day 0 until day 21 (Fig. 3-3), but only run one was significant between soil, treatment and day. However, no differences were seen between fertilizer treatments.

Within run one in the Tujunga loamy sand, from day 0 to day 7, all fertilizer treatments were higher in NH_4^+-N concentration than the control (Fig. 3-1b). From day 7 and after, there were no significant differences, including between the control. Yet on day 14, the urea treatment had a similar concentration to the control while all other treatments were higher, suggesting increased nitrification compared to the thiosulfate and UDCD treatments. Then for run two, all fertilizer treatments were higher than the control from day 0 to day 7 (Fig. 3-2b). By day 14 all treatments except UDCD and AMS were similar to the control, but not different from the other fertilizer treatments. On day 21, only UDCD was higher than the control. However, on day 28, only the AMS treatment was higher than the control. Even though there were differences on day 21 and 28, all NH_4^+-N concentrations were near zero by day 21. For NO_3^--N concentrations, all fertilizer treatments were higher than the control from day 0 until day 14 in the first run while fertilizers were higher from day 7 until day 21 in the second run, but not significantly different between soil, day and treatment (Fig. 3-3b). No differences were seen between fertilizer treatments in either run.

Within run one for the Sable silt loam, all fertilizer treatments were higher than the control in NH_4^+-N from day 0 until day 7 (Fig. 3-1c). However, on day 7, MSKTS was similar to the control, but not different from the other fertilizer treatments. By day 14, HSKTS, MSKTS, and MSATS were similar to the control, and not different from the other fertilizer treatments. After day 14, there were no differences between any of the treatments. In run two, all fertilizer treatments were higher than the control from day 0 to day 7 in NH_4^+-N (Fig. 3-2c). On day 7, UDCD was significantly higher in NH_4^+-N concentration than the urea treatment, but not different from MSKTS, HSKTS, HSATS, MSATS or AMS, showing the possibility of retaining more NH_4^+-N. Then, UDCD, HSATS and HSKTS were significantly higher than the control in NH_4^+-N on day 14, but not different from any other treatments. As for NO_3^--N, in run one, no differences were seen between treatments until day 14 where MSKTS was significantly higher than MSATS (Fig. 3-3c). In run two, the interaction between soil, treatment, and sampling day were not significant. Therefore, not many differences were seen as well. On day 7, however, all treatments except HSKTS and UDCD were higher than the control. Therefore, for a short-term after application, nitrification may have been reduced by UDCD, but neither UDCD or HSKTS were significantly different from the other fertilizer treatments.

Overall, DCD did not reduce nitrification or retain more NH_4^+-N than the other fertilizer treatments since it was typically only higher than the control in NH_4^+-N or NO_3^--N concentrations during the first two to three weeks of the experiments. Likewise, either rate of ATS or KTS did not slow nitrification. However, in the Sable silt loam in run one, MSKTS and HSKTS tended to be higher than urea or UDCD in NO_3^--N (Fig. 3-3c), and UDCD tended to be lower in NO_3^--N in run two at the beginning of the experiment. A long-term greenhouse study using DCD with ammonium sulfate plus ammonium nitrate for citrus trees observed increased NH_4^+-N at 100 days and reduced NO_3^--N found in leaching water in the soil at 60 days after application (Serna et al., 1994). While it did reduce the amount of NO_3^--N found in drainage waters than without DCD, there was still more than 300 mg L^{-1} of NO_3^--N found between day 60 and day 100, and concentrations found within the substrate were near 100 mg L^{-1} from day 0 to day 100.

The transformation of thiosulfate and degradation of DCD was not measured within our study. However, with the warm temperatures relative to a humid environment, the compounds may have either decomposed or leached through the soil by week three or four of the experiment. The mineralization rate of DCD increases with temperature regardless of soil type (Guardia et al., 2018).

Fertilizer Treatment Effects on Plant Growth

For plant height, all fertilizer treatments tended to produce corn that was significantly taller than that of the untreated corn in the Tujunga loamy sand and Sable silt loam. There were few consistent differences within the fertilizer treatments. Corn grown in the Marvyn loamy sand did not have any height differences in run one (Fig. 3-4a), while corn grown in run two was taller when fertilized (Fig. 3-5a).

There was a shaper decline in SPAD chlorophyll measurements between fertilized corn and the untreated in run one compared to run two (Fig. 3-6-3-7). Typically, fertilized corn had a higher chlorophyll content than the unfertilized in the Marvyn and Tujunga soils during the first 30 days in run one while the control was not different in the Sable silt loam. In run two, all fertilizer treatments resulted in plants with numerically higher SPAD than the control in all soils, with less variation in the Sable silt loam and Marvyn loamy sand. In run one, all fertilized corn plants achieved higher measurements than the control at 24 days after planting, then no differences were seen between treatments at 31 and 38 days after planting. In run two, no differences were seen between fertilizer treatments. However, HSATS, MSATS and urea were higher than the control at 15 days after planting. Then, HSATS, HSKTS, MSATS and AMS were higher than the control at 22 days after planting. No fertilizer treatments resulted in any differences compared to the control at 30 days after planting. For the Tujunga loamy sand, all fertilizer treatments resulted in similar chlorophyll concentrations for the corn in both runs (Fig. 3-6b & 3-7b). Within the Sable silt loam, no differences in corn chlorophyll content were observed between treatments in either run (Fig. 3-6c & 3-7c). In fact, majority of the time, no differences were measured between the fertilized corn plants and the untreated. Overall, fertilizer source did not result in significant differences in chlorophyll content during the vegetative growth phase of corn.

Nitrogen uptake in above ground biomass at the end of the experiment resulted in few differences between soil and treatment (Fig. 3-8 & 3-9). Within both runs in the Sable silt loam, fertilized corn regardless of treatment resulted in more N uptake compared to the control. In run one, the UDCD treatment was not significantly different than urea, HSATS, HSKTS or MSATS. All thiosulfate treatments were similar to the urea treatment. However in run two, corn fertilized with HSATS were taller than corn fertilized with urea, MSATS or HSKTS. Fertilized corn plants in the Tujunga loamy sand were taller than the control in run one and two, except for HSKTS in run two. However, no differences between fertilizer treatments were seen in either run. For the Marvyn loamy sand, no significant differences were observed in the first run. However, in run two, all fertilized corn plants were taller than the control, and corn fertilized with HSATS were taller than urea, UDCD and HSKTS. Overall, corn fertilized with HSATS or AMS tended to have a larger N uptake. This may be due to a higher initial amount of NH_4^+-N while other treatments had slightly more urea needing to be hydrolyzed. In a field study on a clay loam in Italy, ATS added to 10% of the volume of UAN decreased NO_3^--N found in the top 20 cm (Graziano, 1990). They also found a higher concentration of N in the ear leaf tissue with the addition of ATS, however no significant yield differences in corn were observed. Serna et al., (1994), who observed a reduction in nitrification from DCD, did not see a difference in dry-weight compared to ammonium sulfate + ammonium nitrate alone.

Conclusion

A greenhouse study on corn was conducted to observe the effects of multiple urea-based fertilizers applied with ATS and KTS in three different soil types on nitrification and plant growth. Although temperature and soil moisture were maintained consistently throughout the experiment, the application of HSATS, HSKTS, MSATS, MSKTS and UDCD did not create differences in extractable soil NH_4^+-N or NO_3^--N compared to AMS or urea alone. Dicyandiamide, which is a known nitrification inhibitor that can be applied with urea, did not reduce nitrification in any of the soil types observed. It is unclear why this occurred, but leaching of N may have influenced results. Soil characteristics including texture, OM, CEC and microbial population most likely had an effect on nitrification. The Sable silt loam with a higher CEC and OM resulted in higher NH_4^+-N concentrations but did not necessarily influence plant growth. The Tujunga loamy sand had a lower CEC than the Sable silt loam but was higher than the Marvyn loamy sand and did not result in more NH_4^+-N or plant growth. Nitrogen fertilizer source did not affect plant height, chlorophyll content, total N or dry-weight biomass.

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Tables and Figures

Table 3-1: Run one soil series and characterization analysis of particle size fractions, cation exchange capacity (CEC), organic matter (OM) and soil pH for soils used in the greenhouse study.

Soil Series	**Sand %**	**Silt %**	**Clay %**	**CEC $cmol_c kg^{-1}$**	**OM %**	**pH**
Marvyn loamy sand	87.5	10	2.5	4.97	0.51	6.5
Tujunga loamy sand	70	25	5	9.66	0.62	7.1
Sable silt loam	22.5	65	12.5	21.55	2.02	5.0

Table 3-2: Run two soil series and characterization analysis of particle size fractions, cation exchange capacity (CEC), organic matter (OM) and soil pH for soils used in the greenhouse study.

Soil Series	**Sand %**	**Silt %**	**Clay %**	**CEC cmol$_c$kg $^{-1}$**	**OM %**	**pH**
Marvyn loamy sand	87.5	10	2.5	4.97	1.14	6.2
Tujunga loamy sand	70	25	5	9.66	0.71	7.3
Sable silt loam	22.5	65	12.5	21.55	2.62	6.2

Table 3-3: Description of greenhouse fertilizer treatment descriptions, fertilizer analysis, percent composition of nitrogen (N) chemical form, and percent composition of sulfur (S) chemical form.

Treatment	Total Analysis	N Forms (%)	S Forms (%)
No N Control	-	-	-
Urea + Ammonium Sulfate (AMS)	9-0-0-1.4(S)	Urea (88%); Ammonium (12%)	Sulfate (100%)
Urea only	20-0-0	Urea (100%)	-
Urea + Mid S Ammonium Thiosulfate (MSATS)	19.2-0-0-2.5(S)	Urea (94%); Ammonium (6%)	Thiosulfate (100%
Urea + High S Ammonium Thiosulfate (HSATS)	18.4-0-0-5.2(S)	Urea (87%); Ammonium (13%)	Thiosulfate (100%)
Urea + Mid S Potassium Thiosulfate (MSKTS)	17-0-3.7-2.5(S)	Urea (100%)	Thiosulfate (100%)
Urea + High S Potassium Thiosulfate (HSKTS)	15-0-6.2-4.2(S)	Urea (100%)	Thiosulfate (100%)
Urea + Dicyandiamide (DCD)	20-0-0	Urea (100%)	-

Table 3-4: Summary of ANOVA analysis for ammonium (NH_4^+-N), nitrate (NO_3^--N), plant height, SPAD, total N, above ground dry-weight in response to soil type, fertilizer treatment, and date of sampling.

Measurement	Source of Variance	ANOVA (P > F)	
		Run One	Run Two
Ammonium-N	Soil	<0.0001	<0.0001
	Treatment	<0.0001	<0.0001
	Day	<0.0001	<0.0001
	Soil x Treatment	0.0142	<0.0001
	Soil x Day	<0.0001	<0.0001
	Day x Treatment	<0.0001	<0.0001
	Day x Treatment x Soil	<0.0001	<0.0001
Nitrate-N	Soil	<0.0001	<0.0001
	Treatment	<0.0001	<0.0001
	Day	<0.0001	<0.0001
	Soil x Treatment	0.0008	0.0057
	Soil x Day	<0.0001	<0.0001
	Day x Treatment	<0.0001	<0.0001
	Day x Treatment x Soil	<0.0001	0.0758
Plant Height	Soil	<0.0001	0.0164
	Treatment	0.4285	0.6646
	Day	0.0012	<0.0001
	Soil x Treatment	0.3923	0.4726
	Soil x Day	<0.0001	0.0087
	Day x Treatment	0.2296	0.6838
	Day x Treatment x Soil	0.3999	0.5277
	Day x Day x Soil x Treatment	<0.0001	0.0001
SPAD	Soil	<0.0001	<0.0001
	Treatment	0.3344	0.0024
	Day	0.042	0.1253
	Soil x Treatment	0.3035	0.0286
	Soil x Day	<0.0001	<0.0001
	Day x Treatment	0.2505	0.0008
	Day x Treatment x Soil	0.5852	0.0533
	Day x Day x Soil x Treatment	0.0009	0.0003
Total N	Soil	<0.0001	<0.0001
	Treatment	0.0393	<0.0001
	Soil x Treatment	<0.0001	0.5643
Dry-weight	Soil	<0.0001	<0.0001
	Treatment	<0.0001	<0.0001
	Soil x Treatment	0.0047	0.0031
N Uptake	Soil	<0.0001	<0.0001
	Treatment	<0.0001	<0.0001
	Soil x Treatment	0.0043	0.0060

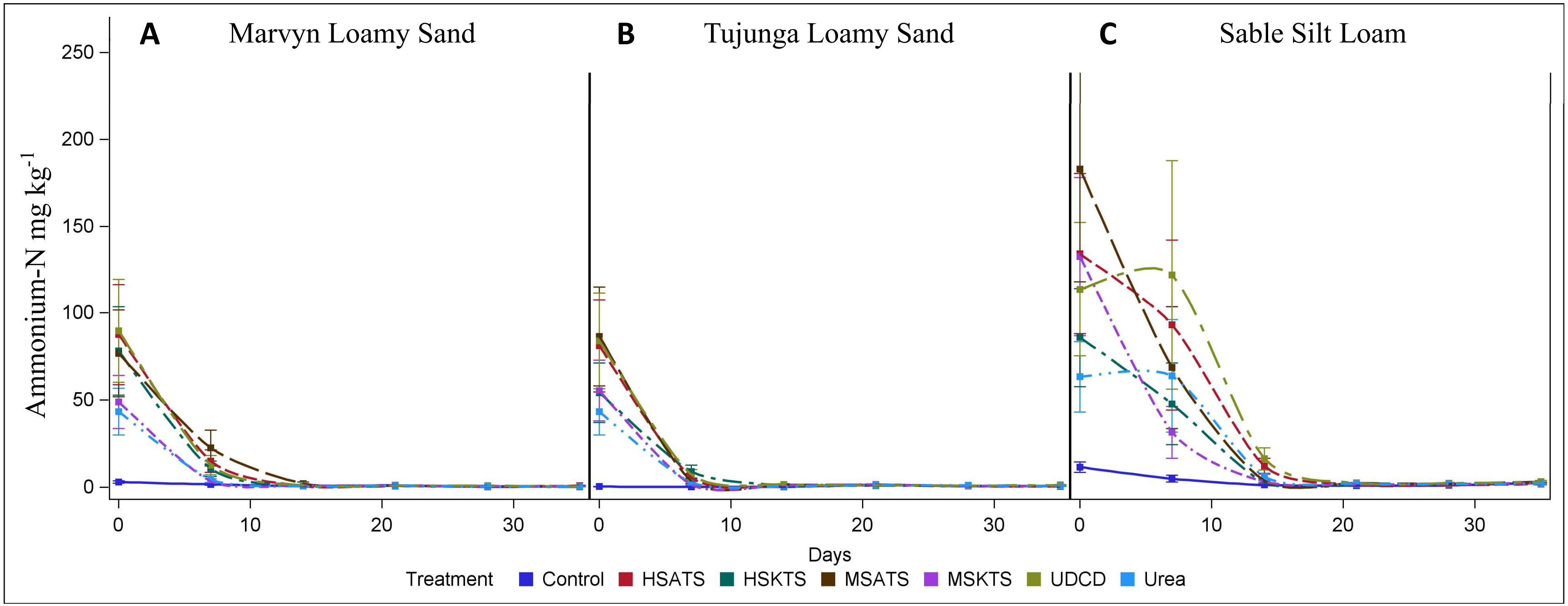

Figure 3-1: Run one ammonium-N concentrations in mg kg^{-1} over a 35-day greenhouse study for three soil types: (A) Marvyn loamy sand, (B) Tujunga loamy sand, (C) Sable silt loam. Treatments include: control, urea + high S ammonium thiosulfate (HSATS), urea + high S potassium thiosulfate (HSKTS), urea + mid S ammonium thiosulfate (MSATS), urea + mid S potassium thiosulfate (MSKTS), Urea + Dicyandiamide (DCD), and urea only. Error bars represent 95% confidence interval about the mean.

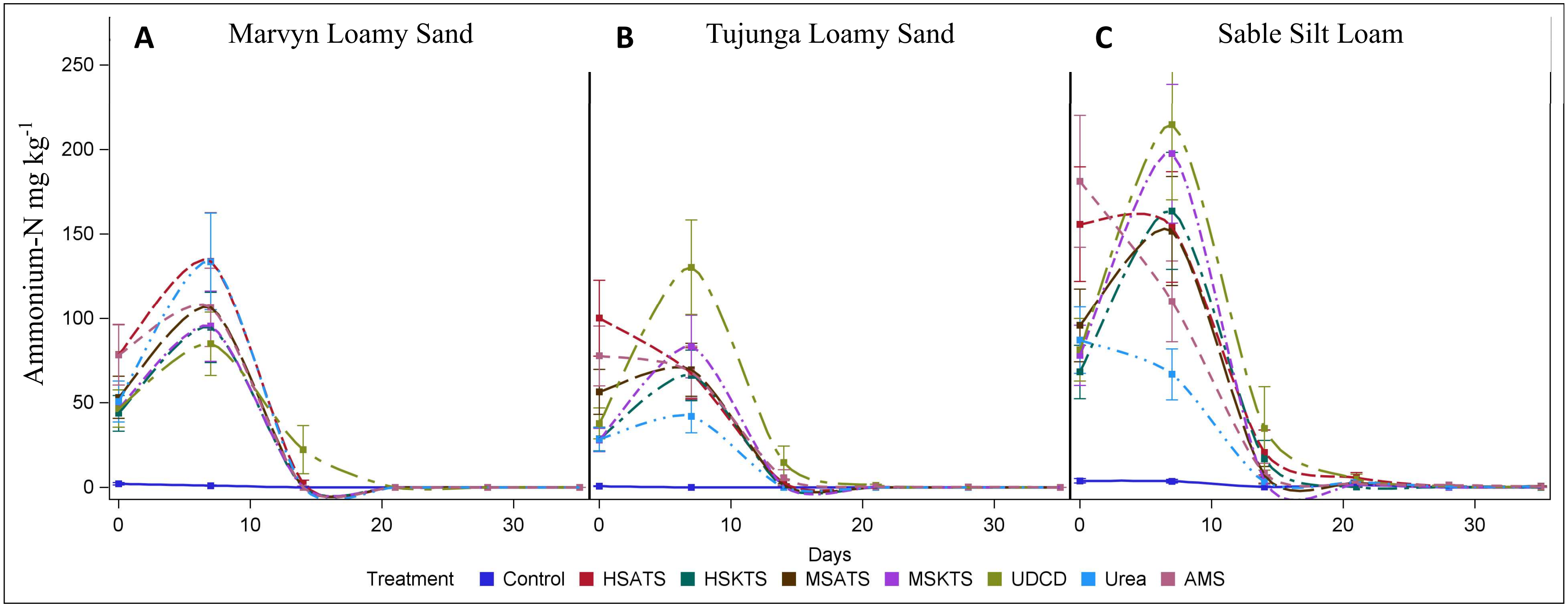

Figure 3-2: Run two ammonium-N concentrations in mg kg^{-1} over a 35-day greenhouse study for three soil types: (A) Marvyn loamy sand, (B) Tujunga loamy sand, (C) Sable silt loam. Treatments include: control, urea + high S ammonium thiosulfate (HSATS), urea + high S potassium thiosulfate (HSKTS), urea + mid S ammonium thiosulfate (MSATS), urea + mid S potassium thiosulfate (MSKTS), Urea + Dicyandiamide (DCD), urea only, and urea + ammonium sulfate (AMS). Error bars represent 95% confidence interval about the mean.

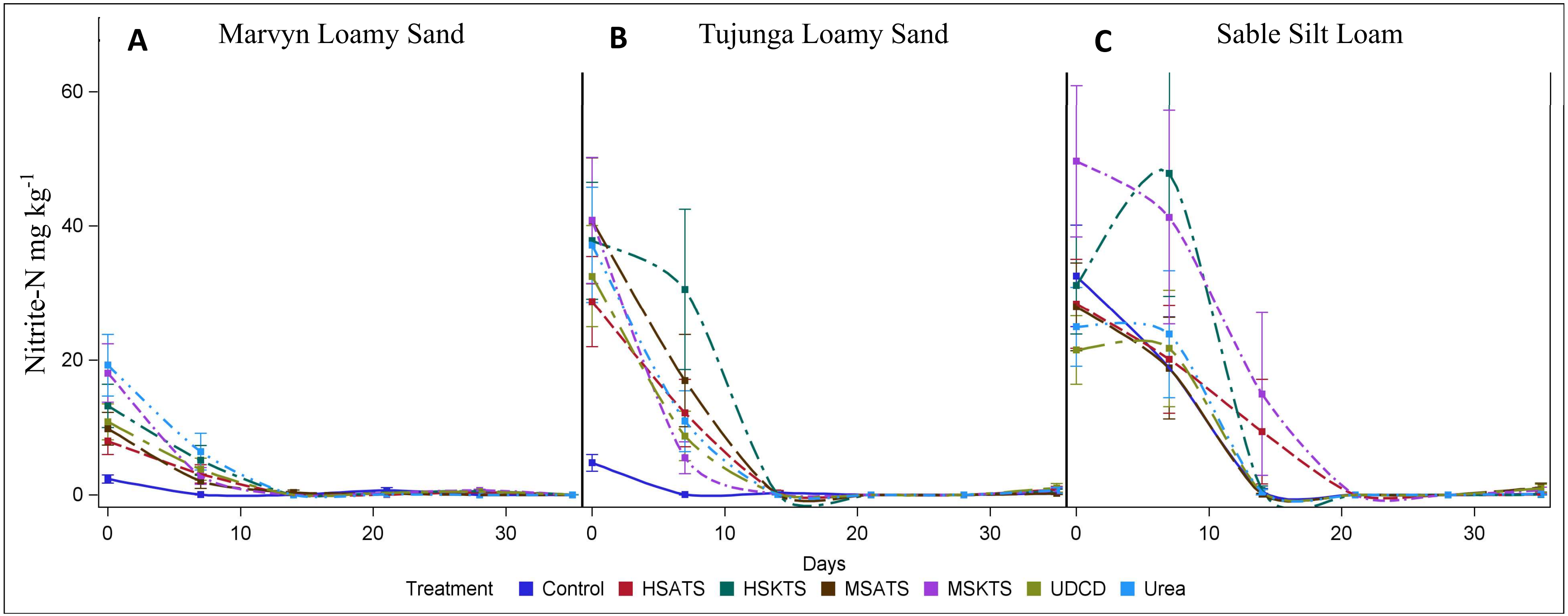

Figure 3-3: Run one nitrate-N concentrations in mg kg^{-1} over a 35-day greenhouse study for three soil types: (A) Marvyn loamy sand, (B) Tujunga loamy sand, (C) Sable silt loam. Treatments include: control, urea + high S ammonium thiosulfate (HSATS), urea + high S potassium thiosulfate (HSKTS), urea + mid S ammonium thiosulfate (MSATS), urea + mid S potassium thiosulfate (MSKTS), Urea + Dicyandiamide (DCD), and urea only. Error bars represent 95% confidence interval about the mean.

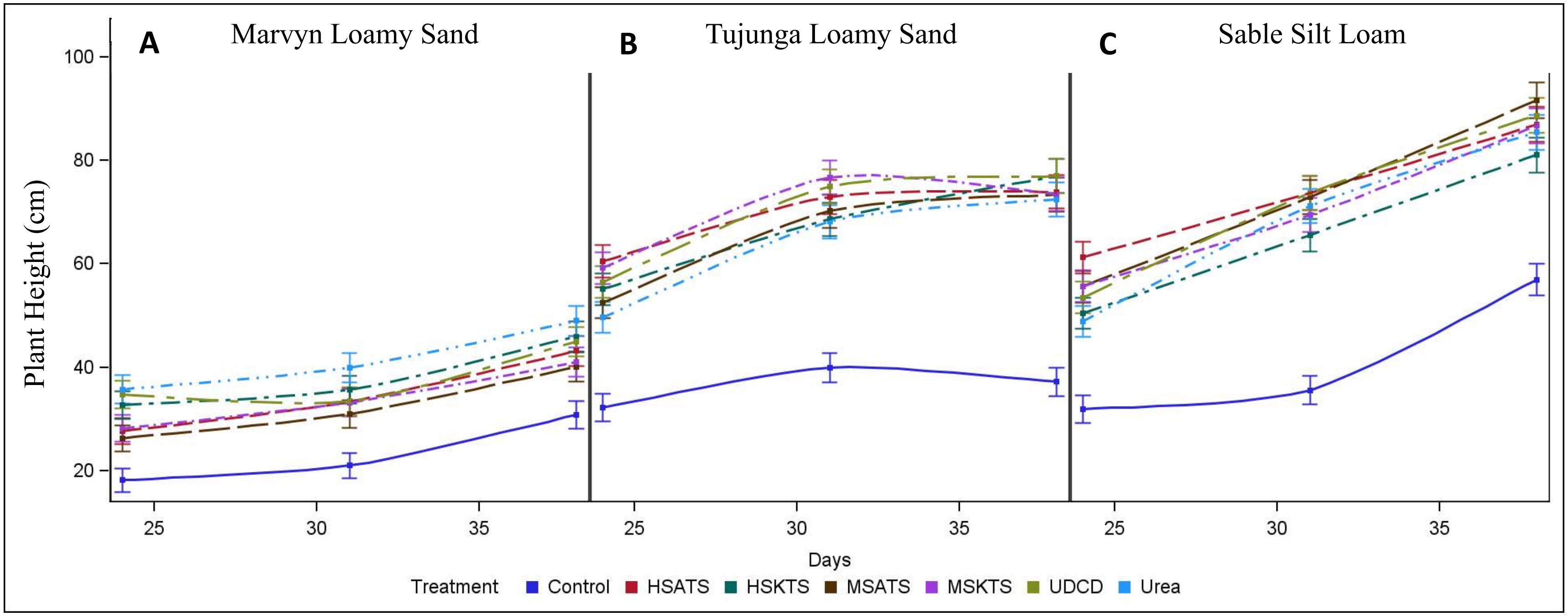

Figure 3-3: Run one plant height measurements in cm for three soil types (A) Marvyn loamy sand, (B) Tujunga loamy sand, (C) Sable silt loam in greenhouse experiment. Treatments include: control, urea + high S ammonium thiosulfate (HSATS), urea + high S potassium thiosulfate (HSKTS), urea + mid S ammonium thiosulfate (MSATS), urea + mid S potassium thiosulfate (MSKTS), Urea + Dicyandiamide (DCD), and urea only. Error bars represent 95% confidence interval about the mean.

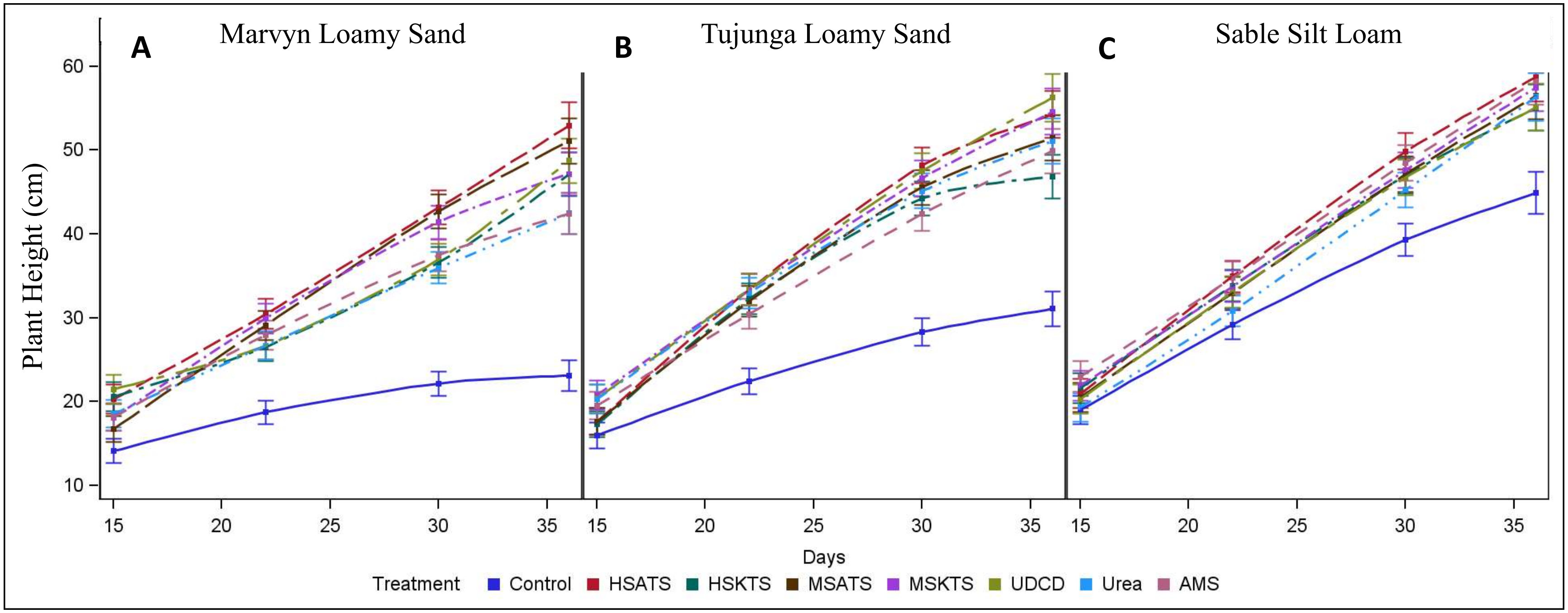

Figure 3-4: Run two plant height measurements in cm for three soil types (A) Marvyn loamy sand, (B) Tujunga loamy sand, (C) Sable silt loam in greenhouse experiment. Treatments include: control, urea + high S ammonium thiosulfate (HSATS), urea + high S potassium thiosulfate (HSKTS), urea + mid S ammonium thiosulfate (MSATS), urea + mid S potassium thiosulfate (MSKTS), Urea + Dicyandiamide (DCD), urea only, and urea + ammonium sulfate (AMS). Error bars represent 95% confidence interval about the mean.

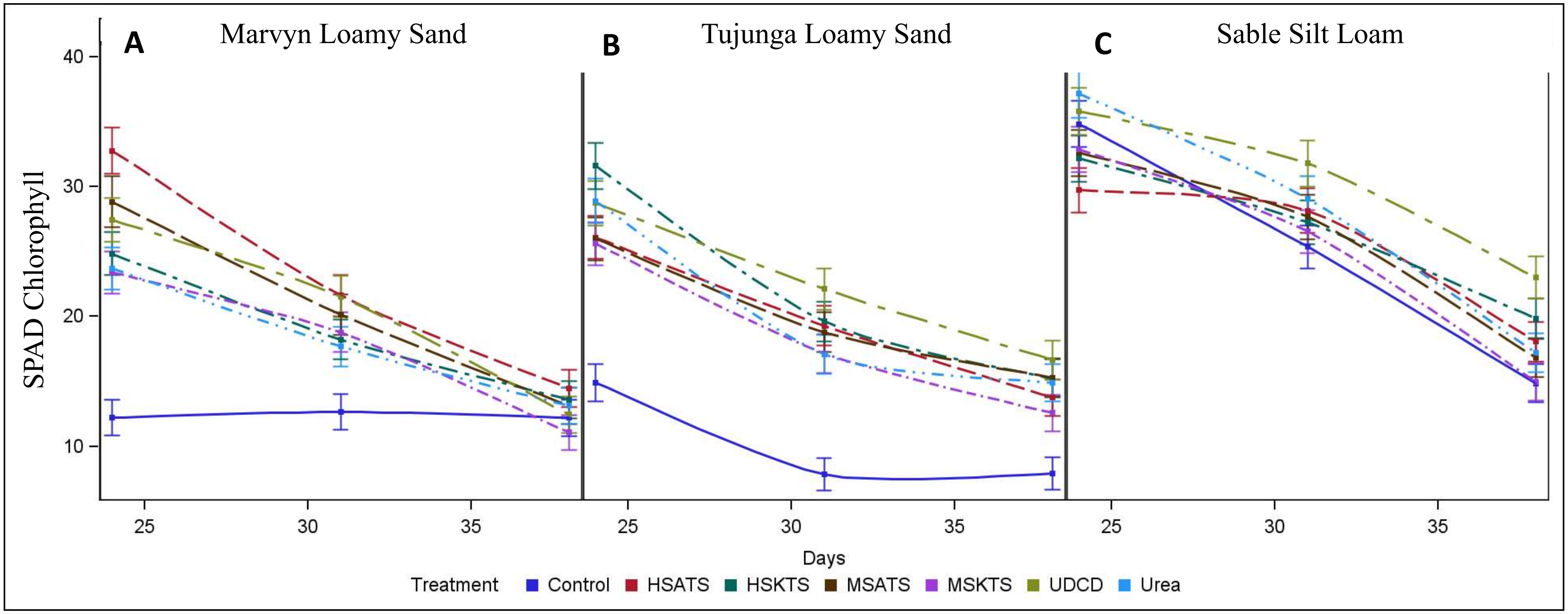

Figure 3-5: Run one chlorophyll concentration in SPAD units for three soil types (A) Marvyn loamy sand, (B) Tujunga loamy sand, (C) Sable silt loam in greenhouse experiment. Treatments include: control, urea + high S ammonium thiosulfate (HSATS), urea + high S potassium thiosulfate (HSKTS), urea + mid S ammonium thiosulfate (MSATS), urea + mid S potassium thiosulfate (MSKTS), Urea + Dicyandiamide (DCD), and urea only. Error bars represent 95% confidence interval about the mean.

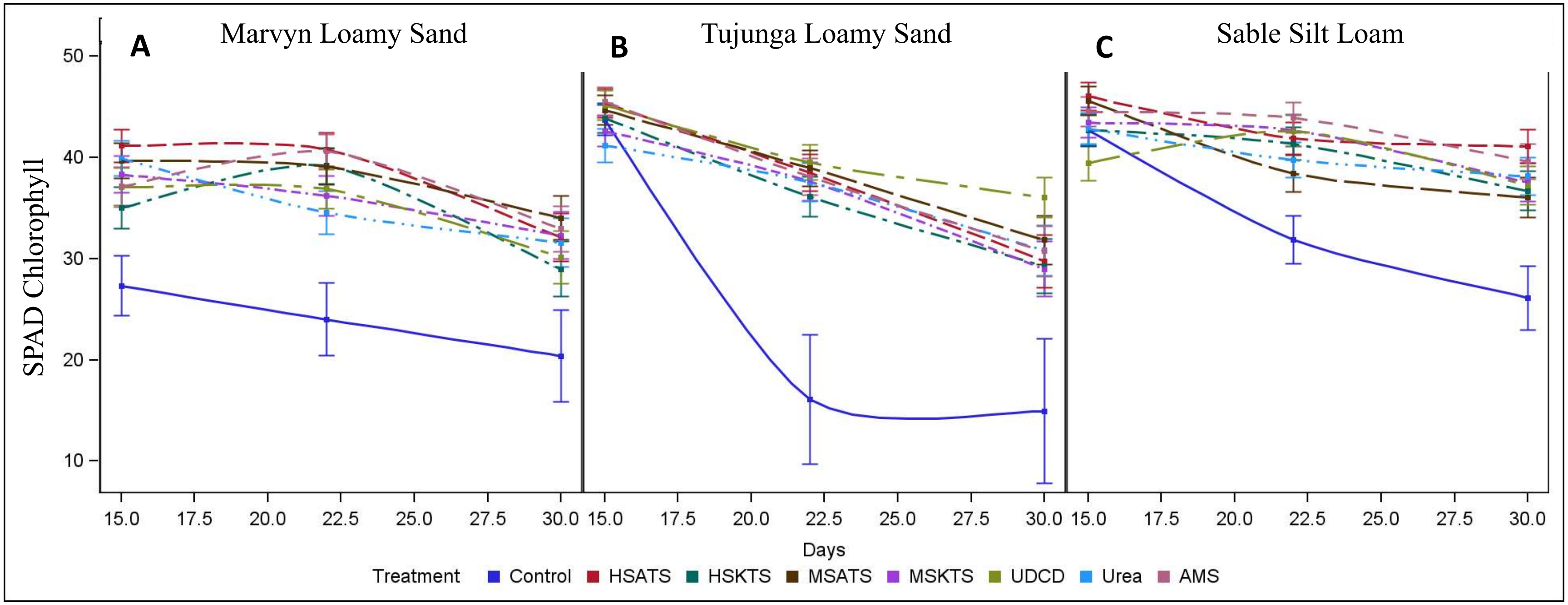

Figure 3-6: Run two chlorophyll concentration in SPAD units for three soil types (A) Marvyn loamy sand, (B) Tujunga loamy sand, (C) Sable silt loam in greenhouse experiment. Treatments include: control, urea + high S ammonium thiosulfate (HSATS), urea + high S potassium thiosulfate (HSKTS), urea + mid S ammonium thiosulfate (MSATS), urea + mid S potassium thiosulfate (MSKTS), Urea + Dicyandiamide (DCD), urea only, and urea + ammonium sulfate (AMS). Error bars represent 95% confidence interval about the mean.

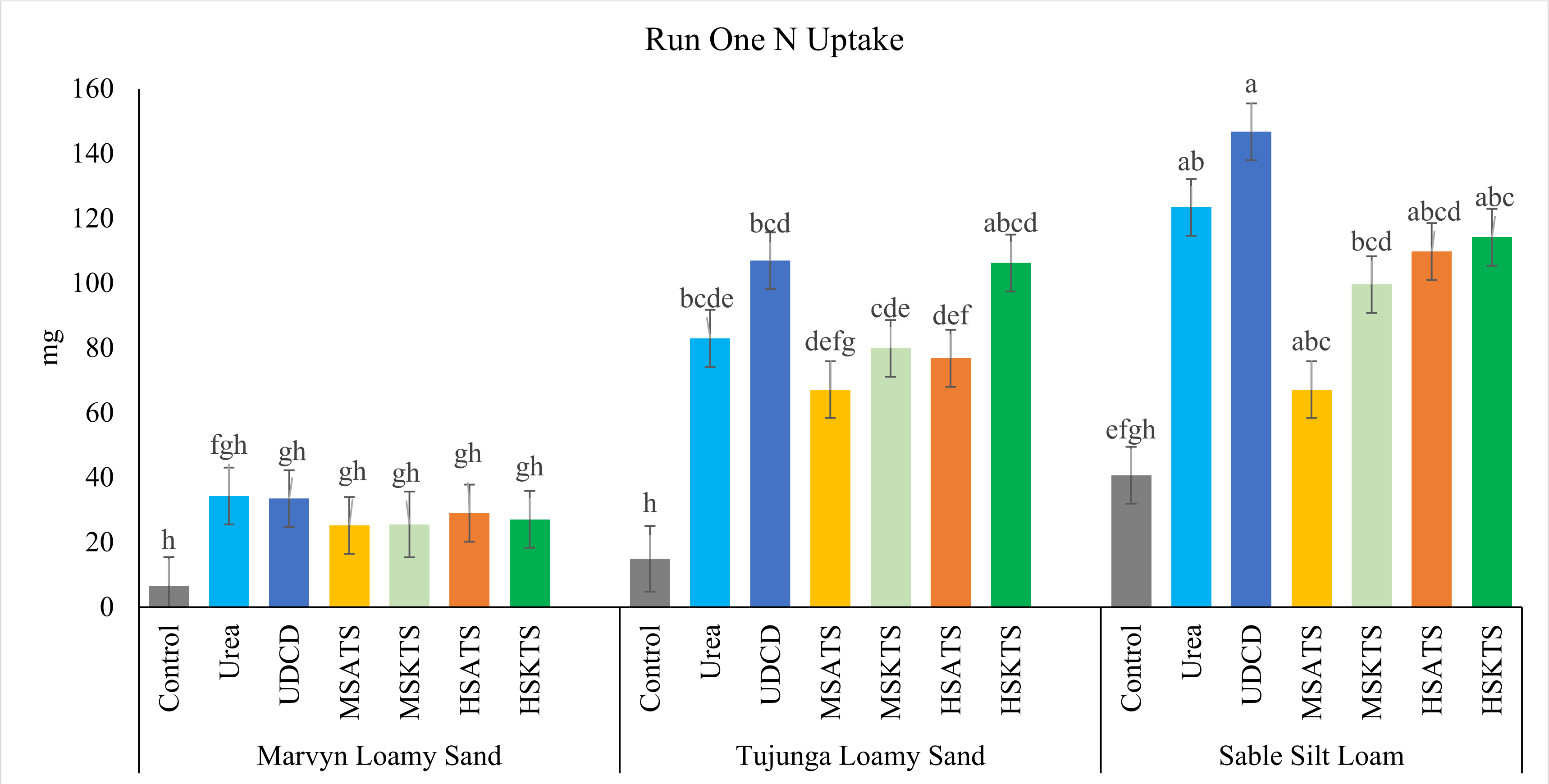

Figure 3-8: Run one above ground biomass nitrogen (N) uptake in milligrams for each soil type: Marvyn loamy sand, Tujunga loamy sand, and Sable silt loam in a greenhouse experiment. Treatments include control, urea, urea + Dicyandiamide (UDCD), urea + mid S ammonium thiosulfate (MSATS), urea + mid S potassium thiosulfate (MSKTS), urea + high S ammonium thiosulfate (HSATS), and urea + high S potassium thiosulfate (HSKTS). Error bars represent 90% confidence interval about the mean.

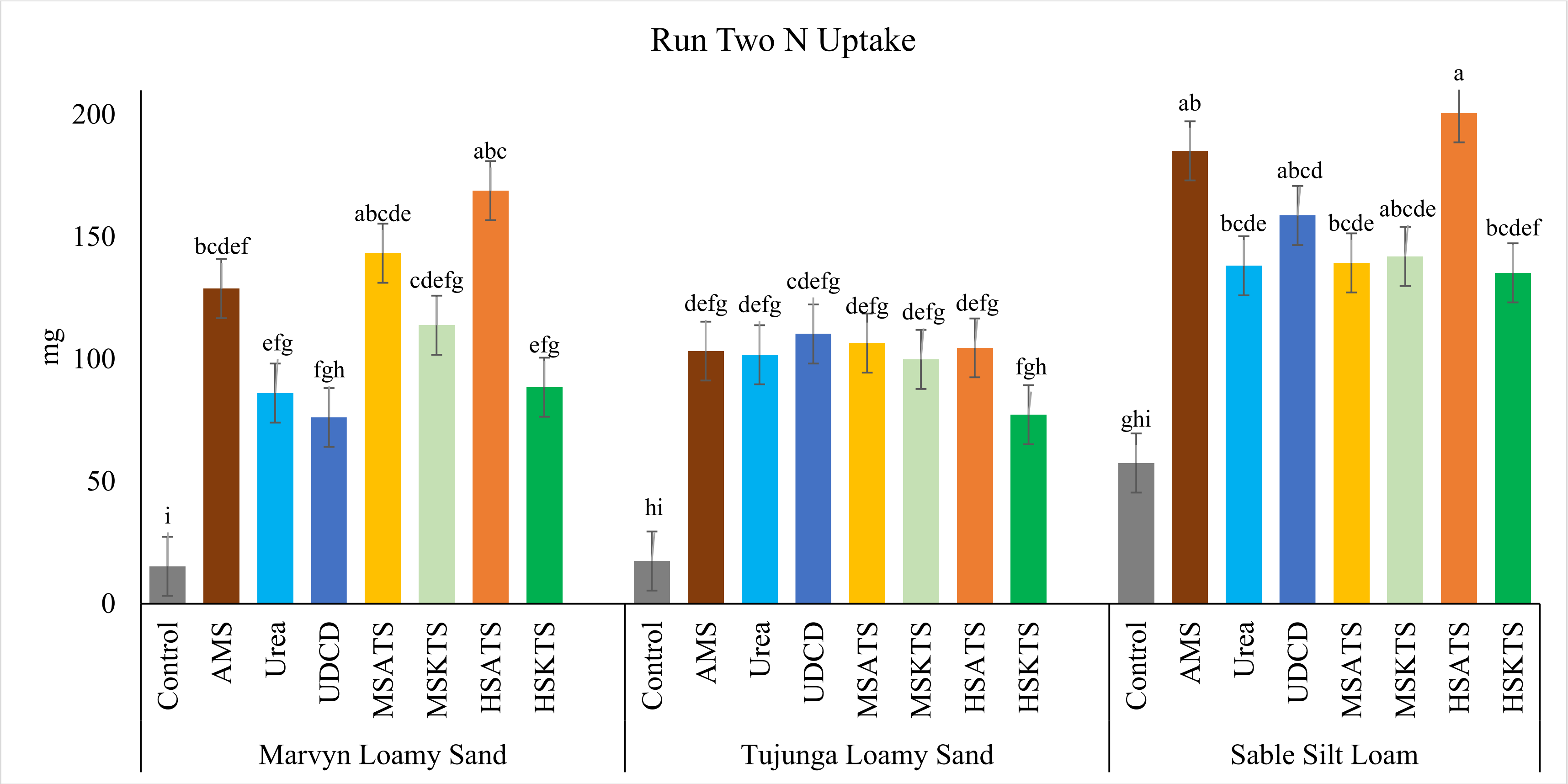

Figure 3-9: Run two above ground biomass nitrogen (N) uptake in milligrams for each soil type: Marvyn loamy sand, Tujunga loamy sand, and Sable silt loam in a greenhouse experiment. Treatments include control, urea + ammonium sulfate (AMS), urea, urea + Dicyandiamide (UDCD), urea + mid S ammonium thiosulfate (MSATS), urea + mid S potassium thiosulfate (MSKTS), urea + high S ammonium thiosulfate (HSATS), and urea + high S potassium thiosulfate (HSKTS). Error bars represent 90% confidence interval about the mean.