

SPRING WHEAT (TRITICUM AESTIVUM L.) RESPONSE TO NITROGEN (N) LOSS
MANAGEMENT AND SULFATE-BASED SOIL SALINITY

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ABSTRACT

The first study was conducted during 2014 growing season at Glyndon, MN to evaluate the effectiveness of nitrification inhibitor or both urease and nitrification i.e. double inhibitors on reducing N losses in a rainfed spring-wheat (*Triticum aestivum* L.) system. Our findings suggested that amending urea with double inhibitors might be an effective strategy to reduce all possible N losses without compromising crop yields from urea-fertilized soils.

The second study was conducted to understand the responses of spring-wheat to sulfate-based salinity stress under greenhouse and field conditions. Results from the greenhouse study indicated that the threshold soil EC_e (EC using saturated-paste-extract method) affecting grain and straw yields were 8.2 and 2.9 dS m⁻¹, respectively. However in fields, crop roots were subjected to heterogeneous salinity and the preferential root-growth in the least saline surface 0-60 cm soil layers resulted in greater salinity-tolerance to crops than that observed in a greenhouse study.

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DEDICATION

This work is dedicated to my parents Mr. Hira Bahadur Thapa and Mrs. Thum Kumari Thapa.

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INTRODUCTION

The world human population recently crossed the 7 billion mark (Tollefson, 2011) and is expected to reach 9.6 billion by 2050 (United Nations, Department of Economic and Social Affairs, Population Division, 2013). In the past, expansion of agricultural lands and crop intensification played a crucial role to meet the food and nutritional demands of rapidly increasing human population (Cassman and Wood, 2005). Even with recent gains in crop productivity, there are still 1 billion undernourished people in the world (Davidson et al., 2015; Foley et al., 2011). Agricultural expansion occurs when croplands and pastures extend into new areas that were not previously used for production. About 38% of the Earth's terrestrial surface is currently used for agriculture at the expense of forests, savannas, and grasslands (Foley et al., 2011). Agricultural expansion is no longer a viable option to meet global food demands because of its adverse effects on biodiversity, carbon storage, ecosystem and environmental services (Cassman et al., 2003; Foley et al., 2011; Linqvist et al., 2012; Tilman et al., 2001).

Agricultural intensification, on the other hand, refers to increasing productivity of existing agricultural lands through the use of irrigation, fertilizers, herbicides, fungicides, pesticides, and mechanization. The production of synthetic nitrogen (N) fertilizers through Haber-Bosch process in the early 20th century and its subsequent use in agriculture was mainly responsible for increasing the productivity of existing agricultural lands (Erisman et al., 2008). The global use of N fertilizers in 2010 was estimated to be 104.3 million Mega grams (Heffer, 2013). Wheat (*Triticum aestivum* L.), corn (*Zea mays* L.) and rice (*Oryza sativa* L.) are the main crops that consume nearly 50% of all N applied to crops globally in 2010 (Heffer, 2013). With increasing food demands from expanding population, the future demand for N fertilizer is expected to increase in similar magnitude to that of food (Wood et al., 2004). The future N

demands could be negotiated to a certain extent through the efficient use of N fertilizers and implementation of management practices such as improved water and fertilizer technologies. Many studies (Cassman et al., 2003; Raun and Johnson, 1999; Snyder et al., 2014) reported that less than 50% of the total N applied in fields is typically recovered with the crops and the other half either resides in soils or escape into the environment as nitrate (NO_3^-), dissolved organic nitrogen (DON), ammonia (NH_3), nitric oxide (NO), nitrous oxide (N_2O), and dinitrogen (N_2) (Galloway et al., 2004).

Among the various unintended pathways of N losses, the return of N fertilizer as N_2 is environmentally safe. In contrast, NH_3 upon deposition to recipient ecosystems can cause N enrichment, soil acidification, eutrophication of surface water bodies, and can become a secondary source of N_2O emissions (Aneja et al., 2009; de Klein et al., 2006; Erisman et al., 2007; Sutton et al., 2008). Nitrous oxide is a primary greenhouse gas and a notable stratospheric ozone-depleting substance (Ravishankara et al., 2009). Similarly, NO is a precursor to tropospheric ozone pollution, NO_3^- and DON can cause eutrophication and algal blooms in downstream aquatic ecosystems (Davidson et al., 2015; Di and Cameron, 2002; Howarth, 1988). Therefore, efficient use of N fertilizers is crucial in food production systems to meet future food demands and sustainability needs, as improperly managed N would be environmentally and economically detrimental.

One way of improving the crop N use efficiency (NUE) is to tailor soil N release from applied fertilizers with crop N demands. Conventional N-fertilizers such as urea (U), anhydrous ammonia, urea ammonium nitrate (UAN), and ammonium nitrate are readily soluble and release N quickly when applied to the soils. If greater amount of N fertilizer is released into the soil too early and the crop is not able to fully uptake it, the excess N in soil will likely be lost through

various pathways. Such asynchrony between N supply (fertilizers) and N demand (crops) not only decrease crop NUE, yield potential and economic returns, but also pose a threat to the environment. In this context, various enhanced efficiency fertilizer products (EEF) are developed to synchronize soil N release from applied fertilizers with the crop N demands and minimize the environmental degradation associated with N fertilizer application (Halvorson et al., 2014; Trenkel, 2010).

Enhanced efficiency N-fertilizers can be categorized into four broad categories depending upon their mode of action: urease inhibitors (UI), nitrification inhibitors (NI), combination of both urease and nitrification inhibitors i.e. double inhibitors (DI), and controlled release N-fertilizers (CRF). The UI delays the hydrolysis of urea by temporarily blocking the urease enzyme binding site (Trenkel, 2010). The most commonly used UI is N-(n-butyl) thiophosphoric triamide (NBPT, trade name Agrotain®). The NI blocks the first step of nitrification (i.e. the conversion of NH_4^+ to NO_2^-) by inhibiting the activity of nitrifiers in soil (Trenkel, 2010). The most commonly used NI is nitrapyrin (2-chloro-6-trichloromethyl-pyridine, trade name N-Serve® or Instinct®), dicyandiamide (DCD), and DMPP (3, 4-dimethylpyrazole phosphate). The DI (trade name SuperU®, AgrotainPlus®) release N in a more conserved manner by slowing down urea hydrolysis as well as inhibiting nitrification in soils. The CRF release N by diffusion through semi-permeable coating membrane in a controlled manner such that the release of nutrient is more synchronized with crop demands (Blaylock et al., 2004). The most commonly used CRF is polymer coated urea (PCU, trade name ESN®), sulfur-coated urea (SCU), resin-coated urea (RCU), and polymer-sulfur-coated urea (PSCU).

With increase in climate change concerns, several studies were conducted in the past to evaluate the effectiveness of various EEF products in reducing N losses and improving NUE.

However, the results obtained from them were highly inconsistent. For example, Halvorson et al. (2014) showed ESN reduced N₂O emissions by 42% compared with U and 14% compared with UAN, SuperU reduced N₂O emissions by 46% compared with U and by 21% compared with UAN, and AgrotainPlus reduced N₂O emissions by 61% compared with U and 41% compared with UAN. Maharjan et al. (2014) observed SuperU significantly reduced N₂O emissions compared with ESN. Similarly, SuperU significantly reduced NH₃ volatilization (Jantalia et al., 2012; Zaman et al., 2009) and NO₃⁻ leaching losses (Sanz-cobena et al., 2012) compared with U. Abalos et al. (2012) observed NBPT decreased NH₃ volatilization, N₂O and NO emissions by 58%, 86% and 88%, respectively, and increased grain yield and crop N uptake by 5% and 6%, respectively, compared with U. While others (Dell et al., 2014; Nash et al., 2012; Parkin and Hatfield, 2014; Sistani et al., 2011; Venterea et al., 2011) found no effect or slightly higher N losses (N₂O emissions) compared with U or UAN. Such variability in the response of EEF across studies reinforced the fact that effectiveness of EEF is highly soil-, crop-, climate-, and management-specific; more research should be conducted at the local scale in order to identify the optimum conditions under which their usage would be economically viable.

First half of this thesis deals with evaluating the effectiveness of various EEF such as UI, NI, DI, and CRF on reducing N losses and increasing crop yields under different soil and management conditions. In the first chapter, we collected the data from all published literatures and conducted a meta-analysis to evaluate the effectiveness of different EEF category (UI, NI, DI, and CRF) under three major cereal crops (rice, corn, and wheat) production systems, and to identify the soil and management conditions under which they are more efficient. In the second chapter, we compared the effectiveness of U with and without inhibitors (NI and DI) on reducing N losses (NH₃ volatilization, N₂O emissions and NO₃⁻ leaching) and increasing grain and protein

yields in a spring wheat rainfed production systems. The effects of soil water-filled pore space (WFPS), soil temperature, and soil inorganic nitrate (NO_3^-) contents on nitrous oxide (N_2O) flux were also investigated.

Second half of this thesis deals with understanding the response of spring wheat to sulfate-based soil salinity under controlled greenhouse and naturally saline field conditions. About 10% of the Earth's terrestrial surface is affected by salinity (Pessarakli and Szabolcs, 1999). The most predominant salts are chlorides, sulfates, carbonates, and bicarbonates of sodium, calcium, and magnesium. Crop growth and productivity are adversely affected in salt-affected soils primarily due to osmotic stress and specific ion toxicity (Munns and Tester, 2008). Osmotic stress is due to high salt concentrations outside plant roots disrupting water uptake, while the ion toxicity effect is due to excessive accumulation of salts in the plant tissues and their inability to tolerate the salts (Munns and Tester, 2008).

Wheat is considered to be more salt-tolerant crop compared to corn and soybean (*Glycine max* L.). Maas and Hoffman (1977) concluded that the maximum salinity (ECe: electrical conductivity determined following standard saturated paste extract method) tolerance level of wheat without any decline in grain yields is 6.0 dS m^{-1} , while that for corn and soybean is 1.7 and 5.0 dS m^{-1} , respectively. Above the threshold value, wheat grain yields declined by 7.1% per unit increase in soil ECe. The threshold salinity value reported by Mass and Hoffman (1977) was based on the studies where salinity gradients were artificially created using chloride salts (NaCl , CaCl_2). There are many more studies conducted across the globe to understand the responses, tolerance mechanisms, and adaptive strategies of wheat to chloride-based salinity stress (Francois et al., 1986; Julkowska et al., 2014; Rahnema et al., 2011; Stepphun and Wall, 1997; Wilson et al., 2002; Yousfi et al., 2009, 2010; Zolla et al., 2010). Results from such studies

might be of limited use to farmers in eastern North Dakota, USA where the predominant salts is sulfate-based (Na_2SO_4 , CaSO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) (Keller et al., 1986). When plants are subjected to chloride salts, they suffer from both Na^+ and Cl^- ion toxicities (Hasegawa et al., 2000; Munns and Tester, 2008; Tavakkoli et al., 2010). Dang et al. (2006) found that the decrease in relative grain yields of wheat was associated with increased concentrations of Cl^- rather than Na^+ in the young mature leaves. On the other hand, plants exposed to sulfate salts do not encounter Cl^- ion toxicity. Thus, sulfate salts may not be as toxic as chloride salts. In the third chapter of this thesis, we determined the impact of sulfate-based soil salinity on above-ground (plant height, chlorophyll content, number of tillers per plant), below-ground (root length, root surface area, root volume, root biomass), absolute and relative yields of spring wheat under both artificially-salinized-controlled greenhouse and naturally-saline field conditions.

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**CHAPTER 1 - EFFECT OF ENHANCED EFFICIENCY FERTILIZERS ON NITROUS
OXIDE EMISSIONS AND CROP YIELDS IN MAJOR CEREAL SYSTEMS - A
GLOBAL META-ANALYSIS**

Abstract

Enhanced efficiency fertilizers (EEF) have the potential to reduce nitrous oxide (N₂O) emissions and improve crop productivities. However, the impact of soil and management conditions on their effectiveness is less clear. Here we conducted a meta-analysis to evaluate the effectiveness of different EEF category under three major cereal crops [rice (*Oryza sativa*), corn (*Zea mays*), and wheat (*Triticum aestivum*)] production systems, and to identify the soil and management conditions under which they are more efficient. Our results showed the effect of EEF on N₂O emissions and crop yields greatly varied with their mode of action, soil, and management conditions. Nitrification inhibitors (NI), double inhibitors (DI: urease and nitrification inhibitors), and controlled release N-fertilizers (CRF) consistently reduced N₂O emissions compared to conventional N-fertilizers across wide range of soil and management conditions (grand mean decrease of 38%, 30%, and 19%, respectively). DI was more effective in reducing N₂O emissions in alkaline soils compared to NI, but the trend was opposite in case of acidic soils. Urease inhibitors (UI) also significantly reduced N₂O emissions compared to conventional N-fertilizers in coarse-textured soils and irrigated systems. Overall crop yields were significantly increased by 7% with the addition of NI alone. Compared to conventional N-fertilizers, DI also significantly increased crop yields in alkaline soils, coarse-textured soils, and irrigated systems. CRF had no effect on crop yields. Our findings showed that NI and DI applications would not only mitigate N₂O emissions, but also provide monetary benefits among all other currently available EEF categories.

Abbreviations: EEF, enhanced efficiency fertilizers; UI, urease inhibitors; NI, nitrification inhibitors; DI, both urease and nitrification inhibitors; CRF, controlled release N-fertilizers; PCU, polymer coated urea; N₂O, nitrous oxide; NBPT, (n-butyl)-thiophosphoric triamide; DCD, dicyandiamide; DMPP, 3,4-dimethylpyrazol phosphate; CI, confidence interval

Key words: enhanced efficiency fertilizers, urease inhibitor, nitrification inhibitor, controlled release N-fertilizers, N₂O, yields

Introduction

Nitrous oxide (N₂O) is a potent greenhouse gas and also the single most ozone-layer depleting substance (Ravishankara et al., 2009). Atmospheric N₂O concentrations have risen from about 270 parts per billion (ppb) during the pre-industrial era to around 327 ppb today (Blasing, 2015). Nitrous oxide is mainly emitted as an intermediate by-product during the oxidation of NH₄⁺ to NO₃⁻ by nitrifiers (nitrification) and also during the reduction of NO₃⁻ to N₂ by denitrifiers (denitrification). Agricultural activities, mainly the production and consumption of synthetic nitrogen (N) fertilizers, are responsible for the substantial buildup of N₂O in the atmosphere (Denman et al., 2007; Snyder et al., 2009). Globally, 50% of the synthetic N fertilizers were applied to three major cereal crops (rice; *Oryza sativa*, corn; *Zea mays*, and wheat; *Triticum aestivum* L.) which supplies bulk of the human food calories and proteins either directly as grains or indirectly through livestock products (Ladha et al., 2015). Even with recent gains in crop productivity, more than 1 billion people in the world lack access to food (Foley et al., 2011). To meet the future food demands of additional 2 to 3 billion people by 2050, the cereal crop production need to be increased dramatically. This requires additional production and consumption of synthetic N fertilizers in rice, corn, and wheat production systems as 48% of the N harvested by these major cereal crops was supplied through synthetic N-fertilizers (Ladha et

al., 2015). Therefore, judicious management of N fertilizers in rice, corn and wheat production systems can potentially play a crucial role in N₂O mitigation and future sustainability of cropping systems.

Use of enhanced efficiency fertilizers (EEF) instead of conventionally used N-fertilizers (for example, urea) are often claimed to be an effective means to mitigate N₂O emissions and improve N use efficiency of crops. As per the Association of American Plant Food Control Officials (AAPFCO, 2013), EEF are defined as “fertilizer products with characteristics that allow increased plant uptake and reduce the potential of nutrient losses to the environment (e.g., gaseous losses, leaching, or runoff) when compared to an appropriate reference product.” Different EEF products have different modes of action that control the rate of nutrient release and improve synchronization of soil N availability with crop N demands (Halvorson et al., 2014; Shaviv, 2001; Trenkel, 2010). For example, urease inhibitors (UI) delay the hydrolysis of urea, nitrification inhibitors (NI) inhibit the nitrification process by suppressing the activity of nitrifiers in soil, and controlled release N-fertilizers (CRF) slow the release of nutrient through coatings (Trenkel, 2010). Combined application of both urease and nitrification inhibitors (DI) have the potential to delay urea hydrolysis as well as inhibit nitrification in soils. All these EEF products have been proposed to have agronomic, economic, and environmental advantages over conventional N-fertilizers (conventional N-fertilizers hereafter refers to those N fertilizers without any inhibitors and coatings) (Trenkel, 2010; Shaviv, 2001; Venterea et al., 2012; Snyder et al., 2009).

Previous studies that investigated the effectiveness of EEF in reducing N₂O emissions reported mixed results depending upon their mode of action, soil and environmental conditions, and management factors. For example, some studies reported decrease in N₂O emissions by 14-

61% with EEF as compared to conventional N-fertilizers (Halvorson et al., 2014), while others showed no effect or slightly higher N₂O emissions (Dell et al., 2014; Parkin and Hatfield, 2014; Sistani et al., 2011; Venterea et al., 2011). Such variability in the response of EEF indicated that the effectiveness of EEF is highly soil-, crop-, climate-, and management-specific. Therefore, a meta-analysis, which combines the results from a number of independent studies, is needed to derive broad conclusions (Rosenberg et al., 2000). A meta-analysis by Akiyama et al. (2010) found that NI as well as PCU (polymer coated urea; the most commonly used CRF), but not UI, were effective in reducing N₂O emissions from wide range of agricultural soils. With increase in climate change concerns, more studies were conducted and published since 2008 in various peer-reviewed journals across the globe that might have changed the conclusions drawn by Akiyama et al. (2010). Furthermore, increase in global cropland area under major cereal systems (namely, rice, corn, and wheat) which shares more than 50% of the global N consumption necessitate specific meta-analysis for major cereal systems alone. The effect of EEF on crop yields is also required to evaluate the economic viability of the system. Given that EEF, in particular CRF, have high prices, it is also necessary to identify the optimum combination of soil and management factors in which EEF are most effective. Therefore, the main objectives of this meta-analysis were (a) to evaluate the effects of individual EEF (UI, NI, DI, and CRF) on soil N₂O emissions and crop yields, and (b) to identify the soil and management factors in which EEF are most effective.

Materials and Methods

Data collection

An extensive search of literature, using Web of Science and Google Scholar (Google Inc., Mountain View, CA, USA) databases, was conducted in May 2015 for articles that reported N₂O

emissions with and without EEF in major cereal production systems. The following key words and their combinations were used for searching literatures: enhanced efficiency fertilizers, urease inhibitor, nitrification inhibitor, polymer coated urea, N source, nitrous oxide emissions, rice or paddy, wheat, corn or maize, and cereals. The search was supplemented by searching through the reference lists of the articles found and the literatures used in the previous meta-analysis by Akiyama et al. (2010) and Decock (2014). Literatures were scrutinized and included only if they met the following criteria: 1) only field studies conducted in rice, corn and wheat systems were included and the laboratory incubation and greenhouse experiments were excluded; 2) studies should measure nitrous oxide emissions for at least one complete growing season without missing N₂O fluxes during the days following fertilization, tillage, rainfall or irrigation events; 3) means and number of replicates for each treatment comparisons had to be reported. We found a total of 43 studies that fulfilled these selection criteria (See Appendix B).

From each selected articles, data pertaining to study site location (longitude and latitude), soil characteristics (pH, texture), management factors (fertilizer N types, application rates, timing and mode of fertilizer application, tillage, and irrigation), crop types (rice, corn or wheat), number of replicates, and the response variables (cumulative N₂O emissions and crop yields) were recorded. Data provided in the graphical format were extracted using Webplotdigitizer version 3.8. Each treatment comparison between EEF and conventional N-fertilizers served as observation in our meta-analysis. Conventional N fertilizers include both organic (pig slurry, poultry manure) and inorganic (urea, urea ammonium nitrate (UAN), ammonium sulphate nitrate (ASN), potassium nitrate (KNO₃), anhydrous ammonia) forms of N fertilizers. The EEF were grouped based on their mode of action: urease inhibitors (UI), nitrification inhibitors (NI), or combination of both urease and nitrification inhibitors (DI), and controlled release N-fertilizers

(CRF). In addition, data were also categorized on the basis of soil characteristics (soil texture, soil pH) and management practices (time of fertilizer application, mode of fertilizer application, tillage, and irrigation) for each study. Soil texture was sub-divided into three categories: fine (>30% clay), medium (<30% clay and <45% sand), and coarse (>45% sand). Whenever the particle size distribution data were not available, we classified the soil texture based on the textural class: fine (clay, silty clay, sandy clay), medium (clay loam, loam, silty clay loam, silt, silt loam), and coarse (sandy loam, sandy clay loam, loamy sand) (USDA, 1999). Studies were also grouped into three categories based on soil pH: alkaline (>7.5), neutral (6.5 to 7.5) and acidic (<6.5). Similarly, with respect to management practices, we broadly classified the studies based on the time of N application (single vs split), mode of fertilizer application (broadcast vs banded), tillage (no-tilled vs tilled) and irrigation (irrigated vs rainfed).

Meta-analysis

For side-by-side comparisons of EEF with conventional N-fertilizers, we used natural log-transformed response ratio ($\ln R$) as a measure of effect size (Hedges et al., 1999).

$$\ln R = \ln \left(\frac{X_t}{X_c} \right) = \ln(X_t) - \ln(X_c) \quad (1.1)$$

where X_t and X_c are the mean values of cumulative N₂O emissions or crop yields for the EEF (treatment group) and conventional N-fertilizer (control group), respectively. The variance (v) of $\ln R$ was estimated using the following equation:

$$v = \left(\frac{s_t}{X_t} \right)^2 + \left(\frac{s_c}{X_c} \right)^2 \quad (1.2)$$

where s_t and s_c represent the standard errors of the EEF treatment and conventional N-fertilizer control groups, respectively. If the standard error was not reported in the studies, we computed the average coefficient of variation (CV), and then estimated the missing standard error by multiplying the reported mean by 150% of the average CV (Decock, 2014).

Using response-ratio ($\ln R$) and variance (v) from individual study, MetaWin version 2.1 statistical software was used to calculate weighted mean effect sizes and generate bias-corrected 95% confidence intervals (CIs) using a bootstrapping procedure (4999 iterations) for each category (Rosenberg et al., 2000). This software allowed us to perform categorical random- or fixed-effects meta-analytic models for the calculation of group effect sizes and/or compute the random-effects variance component (pooled study variance or between-study variance). At first, categorical random-effects meta-analytic model was selected. A fixed-effects meta-analytic model was used in place of random-effects model only when the estimated pooled variance was ≤ 0 (Rosenberg et al., 2000). For both random- and fixed-effects models, the weighted mean effect sizes for each category were computed using the following equation:

$$\overline{\ln R} = \frac{\sum_i (\ln R_i \times w_i)}{\sum_i w_i} \quad (1.3)$$

where $\ln R_i$ and w_i are the $\ln R$ and weighting factor of the i^{th} observation, respectively. The weighting factor (w_i) vary between the models used.

In case of categorical random-effects model:

$$w_i = \frac{1}{(v_i + \sigma^2_{pooled})} \quad (1.4)$$

In case of categorical fixed-effects model:

$$w_i = \frac{1}{v_i} \quad (1.5)$$

where v_i and σ^2_{pooled} are the individual study variance (variance (v) of the i^{th} observation) and pooled study variance (between-study variance), respectively. To facilitate the interpretation, the results of the meta-analyses were exponentially transformed and depicted in graphs as percentage change under EEF relative to conventional N-fertilizer applications ($[e^{\overline{\ln R}} - 1] \times 100$). The mean effect sizes of EEF applications on N_2O emissions and crop yields were considered significantly

different relative to the conventional N-fertilizers only when the 95% CI did not overlap with zero. The mean effect sizes for different subgroups are considered significantly different from one another only if their 95% CIs do not overlap each other.

Results and Discussion

Overview of the dataset

We found 43 studies (See Appendix B) with a total of 246 observations that were conducted to evaluate the effect of EEF on soil N₂O emissions in rice, corn and wheat cropping systems. Out of them, 31 studies (172 observations) also documented crop yields. The studies used here represent the global dataset and were from South America (Brazil), North America (USA, Canada), Asia (China, India, Japan and Indonesia), and Europe (Germany, Spain) (Fig. 1.1). The studies conducted in rice systems were located in china (n=1) and India (n=4), respectively. The study sites related to corn systems were mainly distributed around the world, of which 21 sites located in North America, 5 located in Asia, 2 in Europe, 1 in South America, respectively. Similarly, the study sites related to wheat systems were mainly distributed in Asia (n=7), North America (n=4), and Europe (n=2), respectively.

Effect of enhanced efficiency fertilizers (EEF)

Urease inhibitors (UI)

Urease inhibitors (UI) delay the hydrolysis of urea into NH₄⁺ by blocking the urease enzyme binding sites (Trenkel, 2010). The most commonly used UI is NBPT ((n-butyl)-thiophosphoric triamide). Manunuza et al. (1999) reported that NBPT in soil gets quickly converted to N-(n-butyl)-phosphoric triamide (NBPTO), which in turn forms tridentate ligand with urease enzyme to suppress its activity. By slowing down urea hydrolysis, UI reduces NH₃ volatilization and improves synchronization between soil N availability and crop N demand

(Trenkel, 2010). Moreover, UI may reduce N₂O emissions by decreasing availability of NH₄⁺ substrate for nitrification. Consistent with Akiyama et al. (2010), the overall effect of UI on N₂O emissions in the present meta-analysis did not differ from zero.

When data were separated based on individual crop species, soil texture, and management practices, significant reduction in N₂O emissions with UI was observed in few cases (Fig. 1.2). For example, UI significantly reduced N₂O emissions in corn systems by 36% (CI: -55 to -17%) compared to conventional fertilizers. Similarly, N₂O emissions were reduced with UI applications in coarse-textured soils (mean: -28%, CI: -55 to -4%) and when fertilizers were applied in multiple split doses (mean: -19%, CI: -37 to -5%) and under irrigated field conditions (mean: -32%, CI: -40 to -23%). It should be noted that the results presented in this analysis were based on relatively fewer number of studies; more field studies are needed to validate these findings.

Nitrification inhibitors (NI)

Nitrification inhibitors (NI) are compounds that delay the microbial oxidation of NH₄⁺ to NO₂⁻ by inhibiting the activity of nitrifiers in soil (Subbarao et al., 2006; Weiske et al., 2001). By slowing down the first step of nitrification, NI retain NH₄⁺ for extended time periods and decrease the NO₃⁻ contents in the soil. Thus, NI have the potential to reduce N₂O emissions by suppressing both nitrification and denitrification pathways. Akiyama et al. (2010) estimated that NI reduced N₂O emissions by 38% (CI: -44 to -31%) compared with those of conventional fertilizers. In this meta-analysis, we acquired similar result of 38% N₂O (CI: -44 to -33%) emissions reduced by NI compared to conventional fertilizers (Fig. 1.3a).

Application of NI might also increase N use efficiency and crop yields by facilitating the uptake of N in NH₄⁺ form which can be assimilated with less energy as compared to NO₃⁻

(Subbarao et al., 2006; Zaman et al., 2009). In this meta-analysis, we observed that NI significantly increased cereal yields by 7.1% (CI: 4.7 to 9.5%) compared to conventional N fertilizers (Fig. 1.3a, b). Similar benefit of NI applications over conventional fertilizers was found in a recent meta-analysis conducted by Qiao et al. (2015).

The effectiveness of NI varied for different cereal types (Fig. 1.3). Among the three major cereal crops, NI were more effective in reducing N₂O emissions in corn (mean: -51%, CI: -61 to -42%) compared to wheat (mean: -30%, CI: -36% to -24%) and rice (mean: -27%, CI: -37 to -18%) systems. This indicated that NI were more effective in reducing N₂O emissions in those cropping systems which demand higher N inputs and have relatively high mean N₂O emissions for conventional fertilizers. In the studies included in this meta-analysis, higher amount of N were applied in corn systems (mean N application rate of 184 kg N ha⁻¹) which ultimately resulted in relatively high mean N₂O emissions for conventional fertilizers (3.05 kg N₂O-N ha⁻¹). In contrast, the mean application rates and mean N₂O emissions for conventional fertilizers were relatively low for rice (146 kg N ha⁻¹ and 0.57 kg N₂O-N ha⁻¹) and wheat (135 kg N ha⁻¹ and 1.18 kg N₂O-N ha⁻¹) systems. Although NI greatly reduced N₂O emissions in corn systems, there was no significant effect of NI on corn yields. NI significantly increased rice and wheat yields by 5.5% (CI: 0.1 to 12%) and 7.2% (CI: 4.6 to 9.6%), respectively compared to conventional fertilizers.

Our results also showed that the efficacy of NI varied for different NI forms (Fig. 1.4). Among the most commonly used NI, dicyandiamide (DCD) and 3, 4-dimethylpyrazol phosphate (DMPP) significantly reduced N₂O emissions and also increased crop yields compared to conventional fertilizers (Fig. 1.4a,b). We expected greater effectiveness of DMPP compared to DCD because the relative mobility of DMPP in soil was same as that of NH₄⁺ (Pasda et al.,

2001). Due to the same mobility of DMPP and NH_4^+ in soil, DMPP stays close to where NH_4^+ is adsorbed, and thus is supposed to inhibit nitrification more effectively (Subbarao et al., 2006). However, our analysis showed that DMPP and DCD reduced N_2O emissions by similar amount (Fig. 1.4a). Nitrapyrin, on the other hand, also reduced N_2O emissions by 41% (CI: -54 to -32%) but had no effect on crop yields (mean: 3.3%, CI: -10 to 11%). Despite these benefits, large-scale application of DCD should be viewed with caution because low levels of DCD residues were detected in milk products and the use of DCD was suspended in New Zealand in 2012 (MPI, 2013). Thus, a complete life-cycle assessment of these NI products in addition to their toxicity effects on plant growth and human health need to be conducted in future studies.

Double (urease and nitrification) inhibitors (DI)

The combined application of both UI and NI not only increased NH_4^+ availability by delaying urea hydrolysis, but also prolong NH_4^+ retention by inhibiting nitrification in soil. A more conserved release of N by DI has the potential to reduce all possible N losses (NH_3 and N_2O emissions and NO_3^- leaching) and improve N use efficiency of crops. Therefore, DI may be more effective compared to UI or NI alone. Results indicated that DI and NI were equally effective in reducing N_2O emissions compared to conventional fertilizers for their confidence intervals overlapping each other (Fig. 1.3a, 5a). This suggests that supplemental addition of UI to NI did not necessarily mitigate direct- N_2O emissions more effectively. However, the presence of UI in DI may enhance their efficacy in reducing indirect- N_2O emissions which occur via NH_3 volatilization (Kim et al., 2012). Application of NI alone, on the other hand, significantly increased NH_3 volatilization by prolonging NH_4^+ retention in soil, which in turn lead to greater indirect- N_2O emissions (Kim et al., 2012; Thapa et al., 2015).

We also expected significantly higher crop yields with DI compared to conventional fertilizers. But the overall effect of DI on crop yields was non-significant (Fig. 1.5b). For DI, significant yield benefits were found only in alkaline soils (mean: 2.0%, CI: 0.5 to 3.7%), medium (mean: 2.3%, CI: 0.3 to 4.7%) to coarse-textured (mean: 5.7%, CI: 1.4 to 9.9%) soils, and under irrigated (mean: 2.0%, CI: 0.5 to 1.9%) field conditions. Such an inconsistent response of crop yields to DI suggests that the current combination of UI (NBPT) and NI (DCD) might be unable to synchronize soil N release to crop N demands. More research is needed to determine the optimum combination of NBPT and DCD within DI to optimize both economic and environmental benefits.

Controlled release N-fertilizers (CRF)

Controlled release N-fertilizers (CRF) include coated or encapsulated fertilizers with inorganic or organic materials that control the rate, pattern, and duration of nutrient release (Shaviv, 2001; Chien et al., 2009). These products are designed to release nutrients by diffusion through semi-permeable polymer coating membrane (e.g. polymer coated urea) in a controlled manner such that the release of nutrient is more synchronized with crop demands (Blaylock et al., 2004). Thus, CRF limited the availability of N substrates to nitrifiers and denitrifiers and potentially reduce N₂O emissions. Our analysis indicated that CRF reduced N₂O emissions by 19% from cereal systems relative to conventional N fertilizers (Fig. 1.6a), which is smaller than the 35% reduction reported by Akiyama et al. (2010). Variation in N₂O reduction potential of CRF observed between these studies could be due to the differences in the size of datasets (89 comparisons in this study vs. 20 comparisons in Akiyama et al. (2010)). Also, Akiyama et al. (2010) included studies conducted in grasslands where the mean N₂O emissions were relatively high (5.63 kg N₂O-N ha⁻¹ for conventional fertilizers). With greater N₂O emissions from

grasslands, the effectiveness of CRF in reducing N₂O emissions might also be relatively higher in grasslands than in croplands where the N₂O emissions were relatively low even with conventional fertilizers (3.56 kg N₂O-N ha⁻¹ for conventional fertilizers in this study).

The major bottleneck in the widespread adoption of CRF over conventional fertilizers is their cost. To be economically feasible, the use of CRF should increase crop yields such that the added costs are compensated. In this meta-analysis, CRF consistently showed no or negative effect on crop yields (Fig. 1.6b). Quemada et al. (2013) also reported that CRF had negative effect on crop yields, although the NO₃⁻ leaching losses were significantly reduced compared to conventional fertilizers. This demands the need of invention of new generation of CRF products that can effectively reduce N losses in an economically sustainable manner.

Factors affecting the effectiveness of enhanced efficiency fertilizers (EEF)

Soil factors

Soil pH

Soil pH greatly influences the efficacy of EEF products by regulating the N loss mechanisms. In general, the rate of NH₃ volatilization (Francis et al., 2008) as well as that of nitrification (Norton, 2008; Simek and Cooper, 2002) following urea fertilization increases with increasing soil pH. Thus, the benefit of using EEF might be higher in soil with higher pH values. Linquist et al. (2013) observed EEF increased crop yields and N uptake in rice systems only in neutral to alkaline soils, but not in acidic soils. In sharp contrast, Abalos et al. (2014) observed the overall effect of EEF (urease and nitrification inhibitors) on crop yields and N uptake decreased in neutral to alkaline soils as compared in acidic soils due to increase in N losses via NH₃ volatilization in case of alkaline soils.

Given that different EEF products differ in their mode of action, the effectiveness of these products might vary in soil depending on pH values. In acidic soils, only NI significantly reduced N₂O emissions (mean: -55%, CI: -72 to -40%) compared to conventional fertilizers (Fig. 1.3a). Both DI and CRF did not reduce N₂O emissions to that of conventional fertilizers for their CI did not differ from zero (Fig. 1.5a, 1.6a). In alkaline soils, DI (mean: -43%, CI: -47 to -38%) were more effective in reducing N₂O emissions compared to NI (mean: -23%, CI: -29 to -17%) (Fig. 1.3a, 1.5a). This could be attributed to rapid hydrolysis of NI at high soil pH, which in turn leads to reduced efficacy of NI in inhibiting nitrification in alkaline soils (Briggs, 1975). In alkaline soils, the effectiveness of NI in enhancing crop yield and N uptake may be further reduced due to their tendency to increase NH₃ volatilization by prolonging NH₄⁺ retention in soil for longer duration (Kim et al., 2012; Thapa et al., 2015; Qiao et al., 2015). It is because higher soil pH lead to overall increase in NH₃ loss by favoring the conversion of NH₄⁺ to NH₃ due to decrease in H⁺ activity. Therefore, DI which has the ability to inhibit both N loss mechanisms might be the most effective form of EEF in alkaline soils. However, results from this analysis indicated that both NI and DI were equally effective in increasing crop yields compared to conventional fertilizers for their CI overlapping each other (Fig. 1.3b, 1.5b).

Soil texture

Soil texture affect gas diffusivity, control soil moisture loss, and influence N₂O production (Del Grosso et al., 2008; Skiba and Ball, 2002; Rochette et al., 2004). Fine-textured, poorly-drained soils tend to remain wetter and anaerobic for longer duration following rainfall or irrigation, thereby making conditions conducive for denitrification (Del Grosso et al., 2008). In contrast, proper aeration status in coarse-textured, well-drained soils facilitates nitrification. Therefore, applied fertilizers are more susceptible to N₂O emissions in fine and medium-

textured, poorly-drained soils; applications of EEF might be more beneficial on reducing N₂O emissions in such soils. Unfortunately, Bundy and Bremer (1973) observed that NI was considerably more effective in inhibiting nitrification in coarse-textured soils than in fine-textured soils. Slangen and Kerkhoff (1984) also concluded that the mobility, bioactivity, and effectiveness of inhibitors are reduced in fine-textured soils than in coarse-textured soils due to greater adsorption of inhibitors in fine-textured soils. In this meta-analysis, NI, DI, and CRF significantly reduced N₂O emissions compared to conventional fertilizers in all soil types, but the response did not vary with soil texture (Fig. 1.3a, 1.5a, 1.6a).

The effect of NI and DI on crop yields varied for different soil types. In coarse-textured soils, both NI and DI significantly increased crop yields by 5.5% (CI: 2.1 to 8.6%) and 5.7% (CI: 1.4 to 9.9%), respectively compared to conventional fertilizers (Fig. 1.3b, 1.5b). In medium-textured soils, only DI significantly increased crop yields by 2.3% (CI: 0.3 to 4.7%). While in fine-textured soils, only NI significantly increased crop yields by 8.8% (CI: 4.9 to 11.5%) compared to conventional fertilizers. On the other hand, CRF showed no or negative effect on crop yields in medium to coarse-textured and fine-textured soils, respectively (Fig. 1.6b). Based on these results, the positive response of crop yields to EEF application seems to be more consistent in coarse-textured soils.

Management factors

Timing of fertilizer application

Timing of fertilizer application to synchronize soil N release with crop N demands is essential for improving the yield and quality of crops. This could be achieved through split application of N fertilizers in multiple doses throughout the growing season. The enhanced efficiency of crops to recover fertilizer N during split N applications might help to reduce

unwanted N losses, including N₂O emissions, and lessen the environmental impact of fertilization (Velasco et al., 2012). On the other hand, single applications of N fertilizers at or before planting are more prone to early season N losses when the crop N uptake is very low. Various EEF products are designed to reduce this early season N losses. Our meta-analysis suggested that NI were more effective in reducing N₂O emissions from conventional fertilizers when applied in single (mean: -51%, CI: -59 to -44%) dose compared at multiple split (mean: -25%, CI: -30 to -19%) doses (Fig. 1.3a). This reduction in N₂O emissions with NI during single N applications was not sufficient enough to significantly increase crop yields (Fig. 1.3b). The positive response of crop yields to NI was only observed during split N applications (mean: 7.3%, CI: 4.9 to 9.5%).

Mode of fertilizer application

Sub-surface placement of fertilizers in bands is often promoted to enhance agronomic efficiency or N fertilizer recovery efficiency of crops compared to broadcast applications (Malhi et al., 2001; Yadvinder-Singh et al., 1994; Zhu and Chen, 2002). There is a discrepancy among existing literatures on the effect of fertilizer placement on soil N₂O emissions. When the fertilizers are applied in bands, the contact between fertilizers and soil microbes may be greatly reduced, which in turn slows N transformation and results in less accumulation of NO₃⁻ substrate for leaching and denitrification processes. Supporting this hypothesis, Drury et al. (2006), Nash et al. (2012), and Pfab et al. (2012) observed reduced N₂O emissions in banded above broadcast fertilizer applications. While others (Engel et al., 2010; Fujinuma et al., 2011) reported banding of N fertilizers increases soil pH and NH₄⁺ levels which favor NO₂⁻ production in soils, and ultimately increases N₂O emissions above broadcasted N fertilizers.

The effectiveness of EEF is also impacted by its mode of application. Subbarao et al. (2006) suggested greater effectiveness of EEF (nitrification inhibitors) when applied on banded than on broadcasted fertilizers. In this meta-analysis, we observed greater effectiveness of DI in reducing N₂O emissions when applied on banded (mean: -45%, CI: -53 to -36%) fertilizers than on broadcasted (mean: -14%, CI: -22 to -5%) fertilizers (Fig. 1.5a). However, the overall effect of NI and CRF on N₂O emissions did not vary between broadcasted vs banded applications (Fig. 1.3a, 1.6a). Slangen and Kerkhoff (1984) further reported NI, in particular nitrapyrin, is not effective as coatings on broadcasted fertilizers. It is because nitrapyrin has a relatively high vapor pressure and is therefore incorporated or injected into the soil to enhance its effectiveness. We thus separately evaluated the efficacy of nitrapyrin, but found similar response under both broadcasted (mean: -40%, CI: -50 to -19%) and banded (mean: -42%, CI: -56 to -18%) fertilizer applications.

Tillage

No-tillage or minimal tillage management practices are promoted to reduce soil erosion, enhance agricultural sustainability, build soil health, and reduce greenhouse gas emissions through carbon sequestration (Cole et al., 1997; Six et al., 2004). However, the effect of no-tillage on N₂O emissions is highly variable. No-tillage can enhance N₂O emissions by increasing soil moisture content and bulk density (Liu et al., 2007; Rochette et al., 2008) or decrease N₂O emissions by lowering soil temperature and improving soil structure (Six et al., 2002; Venterea et al., 2011). Also by regulating soil moisture and soil temperature, tillage practices affects the mobility, persistence and effectiveness of inhibitors in soil. The relative effectiveness of most NI decreased with increasing soil temperature (Bundy and Bremer, 1973) due to decreased persistence of inhibitors in the soil and increased nitrifiers activity at higher soil temperatures

(Slangen and Kerckhoff, 1984). In this context, NI should be more effective in reducing N₂O emissions and enhancing crop yields under no-tilled compared to tilled soil conditions.

Results from this meta-analysis, however, indicated that the overall effect of NI on N₂O emissions and crop yields did not vary between no-tilled (mean: -46%, CI: -58 to -34%) and tilled (mean: -42%, CI: -55 to -30%) conditions (Fig. 1.3a, 1.3b). Similarly, DI also showed similar reduction in N₂O emissions under both no-tilled (mean: -26%, CI: -37 to -14%) and tilled (mean: -34%, CI: -44 to -23%) conditions (Fig. 1.5a). On the other hand, CRF significantly reduced N₂O emissions compared to conventional fertilizers only under tilled soil conditions (mean: -28%, CI: -36 to -19%), but the effect was non-significant under no-tilled soil conditions (Fig. 1.6a).

Irrigation

Irrigating the fields soon after fertilizer application facilitate incorporation of broadcasted fertilizers into the soil which lead to reduction in N losses through NH₃ volatilization (Holcomb et al., 2011). However, irrigated systems are more prone to NO₃⁻ leaching losses due to frequently occurring drainage events than the rainfed systems. Moreover, irrigated systems are vulnerable to denitrification-induced N₂O emissions due to the fact that irrigated systems tend to have higher soil water-filled-pore-space for most of the growing season. By reducing the availability of NO₃⁻ substrate for denitrification and leaching losses, the positive benefits of EEF applications might be more prominent in irrigated systems than in rainfed systems.

In this meta-analysis, we also observed that the benefits of different EEF products were more pronounced in irrigated than in rainfed systems. UI significantly reduced N₂O emissions in irrigated (mean: -30%, CI: -45 to -11%) systems, but the effect was non-significant in rainfed systems (Fig. 1.2a). NI also significantly reduced N₂O emissions compared to conventional

fertilizers, but the effect size did not vary between irrigated and rainfed systems (Fig. 1.3a). However, combined application of both urease and nitrification inhibitors (DI) reduced N₂O emissions more effectively in irrigated (mean: -45%, CI: -51 to -39%) compared to rainfed (mean: -17%, CI: -29 to -5%) systems (Fig. 1.5a). Besides N₂O emissions, the use of EEF significantly reduced NO₃⁻ leaching losses compared to conventional fertilizers in irrigated systems (Quemada et al., 2013), which were generally the most dominant N loss processes. Thus, application of EEF products may consistently increase crop yields in irrigated systems. Supporting this hypothesis, we found that NI and DI significantly increased crop yields by 5.2% (CI: 2.9 to 7.8%) and 2.0% (CI: 0.5 to 3.8%), respectively compared to conventional fertilizers (Fig. 1.3b, 1.5b). In rainfed systems, however, the effect of NI and DI on crop yields was non-significant.

Knowledge Gaps and Future Considerations

Soil N₂O emissions have a high degree of spatial (hotspots) and temporal (hot moments) variability (Groffman et al., 2009). The most commonly accepted snapshot measurements of N₂O emissions using closed chambers at weekly intervals (as used in most of the studies included in this meta-analysis except Liu et al. (2013)) might have missed out short-termed emissions peaks (hot moments). Moreover, these studies deployed only one chamber within a plot which might have missed out N₂O fluxes from potential hot spots. Missing hotspots and hot moments of N₂O fluxes will probably mask the true treatment effects. Moreover, capturing such hotspots and hot moments of N₂O fluxes will further help to improve our understanding on biogeochemical processes responsible for N₂O emissions. Therefore, it is necessary to capture spatial, temporal and diurnal variability in N₂O emissions. This could be achieved by facilitating continuous N₂O measurements through the deployment of multiple automated chambers over

small area or by using micrometeorological methods coupled with optical analytical techniques over wide area (Rapson and Dacres, 2014).

Until now, vast majority of studies have focused on determining the impact of EEF at the field scale over the crop growing season. However, the positive benefits associated with EEF application may remain even after the growing season for extended time periods. Therefore, future research endeavors should consider taking year-round measurements of N₂O emissions at landscape level even during the crop non-growing season. In periods of year when it is very difficult to manually take gas samples such as snow cover periods, N₂O measurements could be facilitated through the use of automated chambers. Similarly, the economic and environmental benefits associated with fall applications of various EEF products need to be evaluated. It is because fall applications of fertilizers will help to minimize work load of farmers during planting season in early spring. Few researchers in the past have evaluated the potential benefits of fall-applied nitrapyrin (commonly used NI in North America) over fall-applied conventional N-fertilizers in croplands. For example, Goos and Johnson (1999) and Parkin and Hatfield (2010) found fall-applied nitrapyrin significantly increase crop yields and N uptake above conventional fertilizers. However, Parkin and Hatfield (2010) found no effect of fall-applied nitrapyrin on cumulative year-round N₂O emissions.

Future studies should also clearly report sample sizes and some measure of variability while reporting mean cumulative N₂O emissions as well as information pertaining to the production and quality of the crops in their future studies. This will facilitate comparative data analysis and helps in formulating the most effective and economically feasible management decisions. It is because nearly 63% of the studies included in the present analysis did not report any measures of variance such as standard deviation, standard error, coefficient of variation, etc.

Similarly, crop yields were not reported in 28% of the studies included in this meta-analysis. Future studies should also report information on other factors that could affect the effectiveness of EEF such as soil temperature, soil organic carbon, cation exchange capacity and other environmental variables.

To estimate the overall effect of EEF on total N₂O emissions, results obtained in our meta-analysis must be accompanied with information on indirect N₂O emissions which occur via NH₃ volatilization, NO₃⁻ leaching, runoff and erosion losses. The short and long-term effects of the continuous use of EEF on targeted and non-targeted soil microorganisms and biogeochemical processes, plant growth and metabolism, human and animal health, and biodiversity should be evaluated in upcoming studies. Future studies should also consider developing new generation of EEF which could effectively reduce N losses in most vulnerable environmental conditions (high temperature, alkaline pH) in a cost-effective manner. A complete life-cycle assessment and cost-benefit analysis is needed to assess the net benefits of these new products before implementation for widespread adoption.

Conclusions

Ensuring global food security while reducing environmental costs associated with N fertilizer application has become a great challenge in the 21st century. Among the 4R (right source, right amount, right time, right placement) nutrient stewardship to achieve sustainable intensification is the selection of right N source such as EEF (UI/NI/DI/CRF) over conventional N-fertilizers. As anticipated, EEF showed variable response depending upon the soil (soil pH, texture) and management (timing and mode of fertilizer application, tillage, irrigation) factors. Urease inhibitors (UI) significantly reduced N₂O emissions from conventional fertilizers only in coarse-textured soils and under irrigated conditions. Nitrification inhibitors (NI) consistently

reduced N₂O emissions, but their effectiveness was more pronounced in neutral soils, coarse-textured soils, and under irrigated conditions. Combined application of both UI and NI (DI) were more effective in alkaline soils, medium to coarse-textured soils, irrigated field conditions, and when the fertilizers were applied in bands. Controlled release N-fertilizers (CRF) significantly reduced N₂O emissions across wide range of soil and management conditions, but had no or negative effect on crop yields. Based on our findings, the use of NI can be recommended as a potential option for N₂O mitigation while enhancing the economic viability of the cropping systems. Alternatively, applications of DI in alkaline soils, coarse-textured soils, and irrigated systems would provide an additional advantage over NI in terms of reduced direct as well as indirect N₂O emissions. Future work should be directed towards developing new generation of EEF products that works effectively under wide range of soils, crops, climate, environments, and management conditions to ensure its widespread application for future sustainability of the cropping systems.

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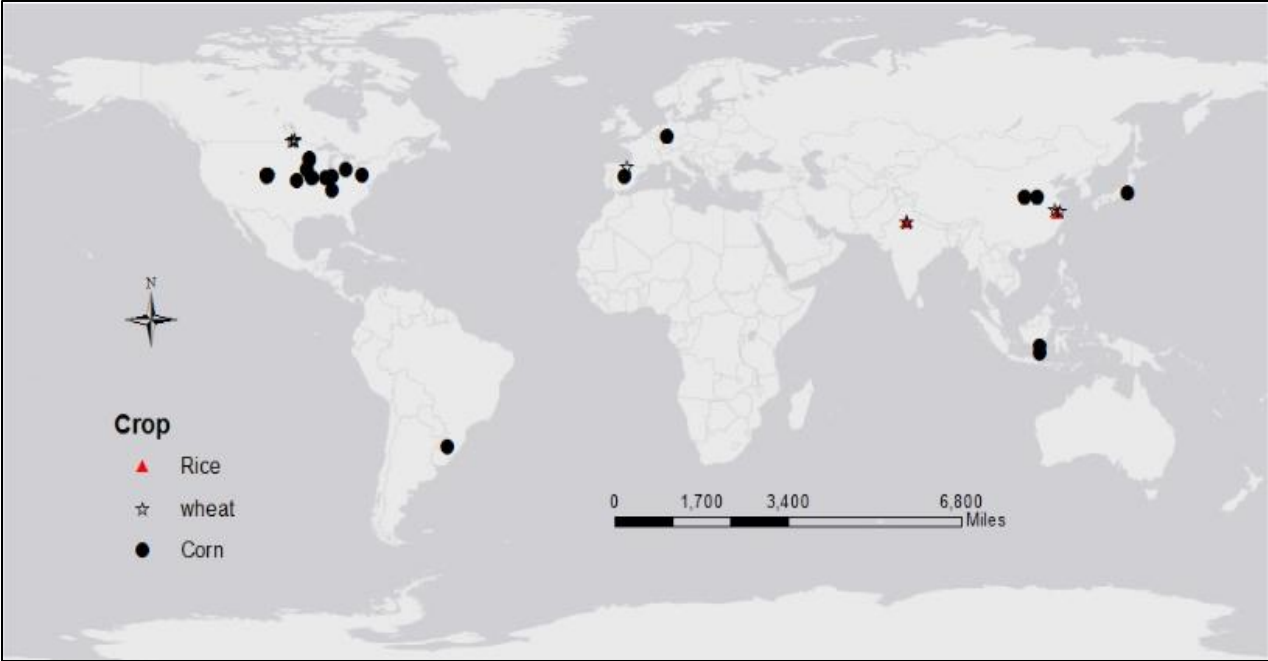


Figure 1.1. The global distribution of study sites included in this meta-analysis.

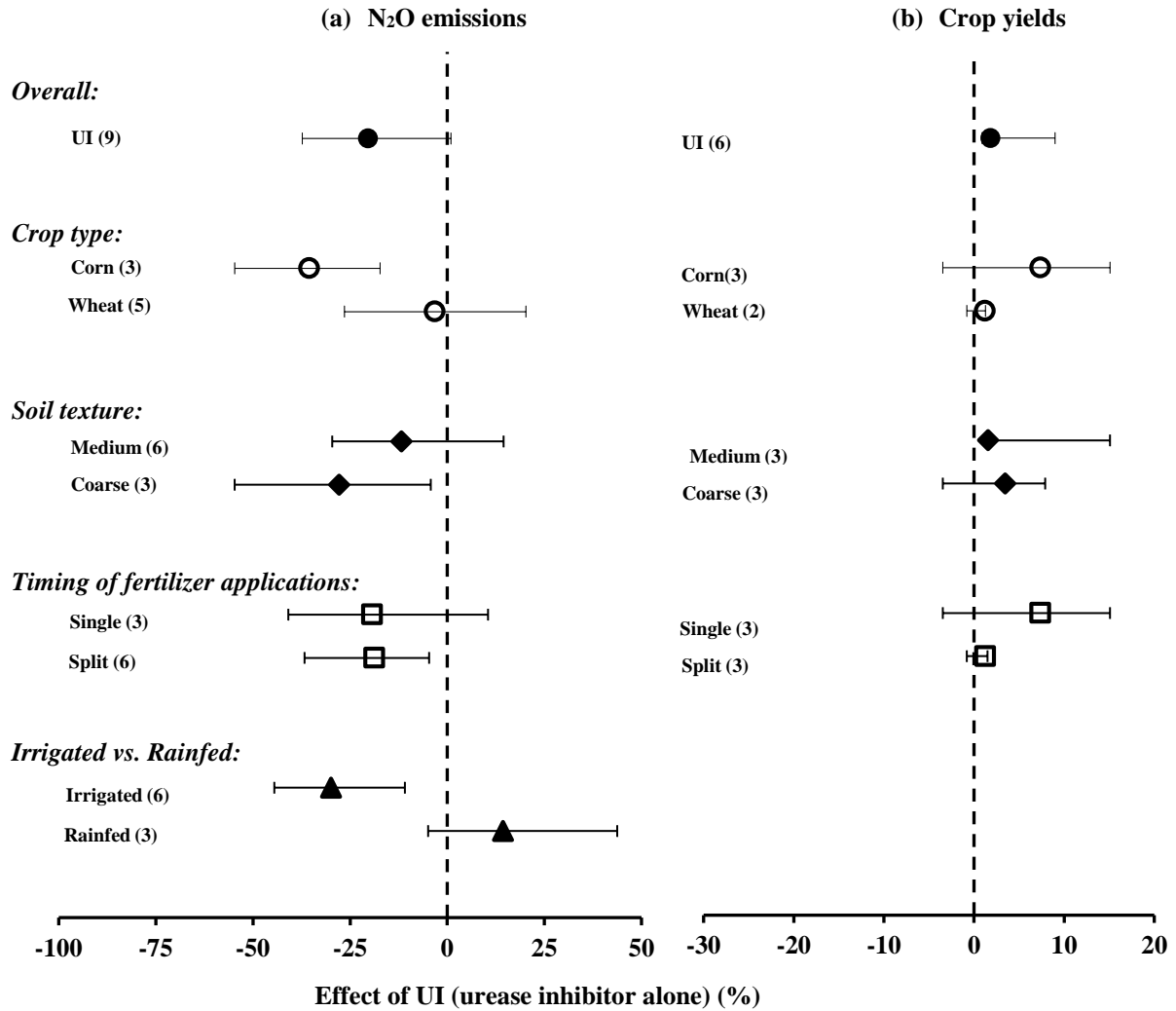


Figure 1.2. The effect of urease inhibitors (UI) on: (a) nitrous oxide (N₂O) emissions and (b) crop yields relative to conventional N-fertilizers for different cereal types, soil types, and management conditions. Mean effect sizes and 95% confidence intervals (CIs) are shown. Numbers in parentheses indicate the sample sizes (the number of pair-wise comparisons). The mean effect sizes were considered significantly different only when the 95% CIs did not overlap with zero. The mean effect sizes for different subgroups are considered significantly different from one another only if their 95% CIs do not overlap.

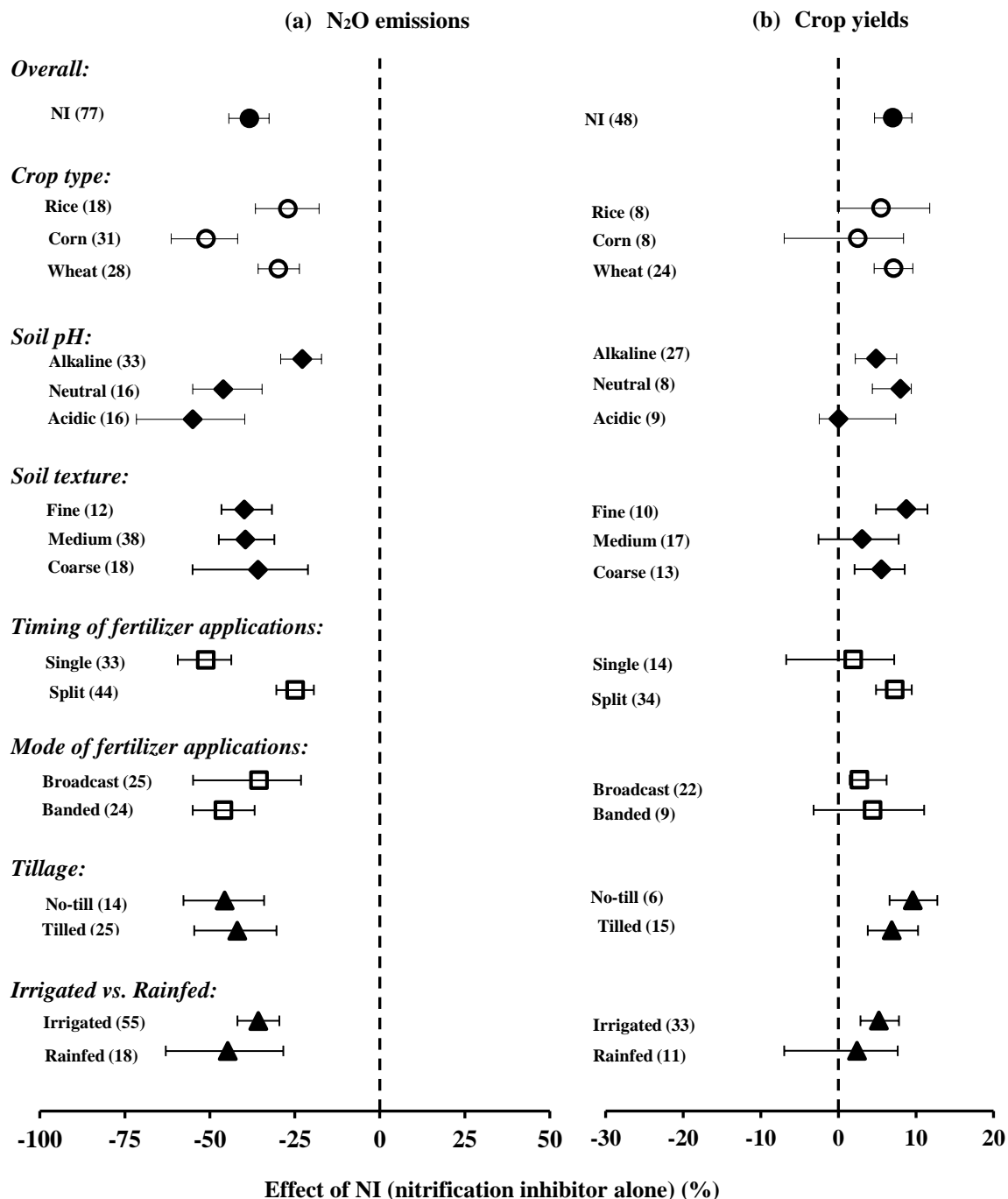


Figure 1.3. The effect of nitrification inhibitors (NI) on: (a) nitrous oxide (N₂O) emissions and (b) crop yields relative to conventional N-fertilizers for different cereal types, soil types, and management conditions. Mean effect sizes and 95% confidence intervals (CIs) are shown. Numbers in parentheses indicate the sample sizes (the number of pair-wise comparisons). The mean effect sizes were considered significantly different only when the 95% CIs did not overlap with zero. The mean effect sizes for different subgroups are considered significantly different from one another only if their 95% CIs do not overlap.

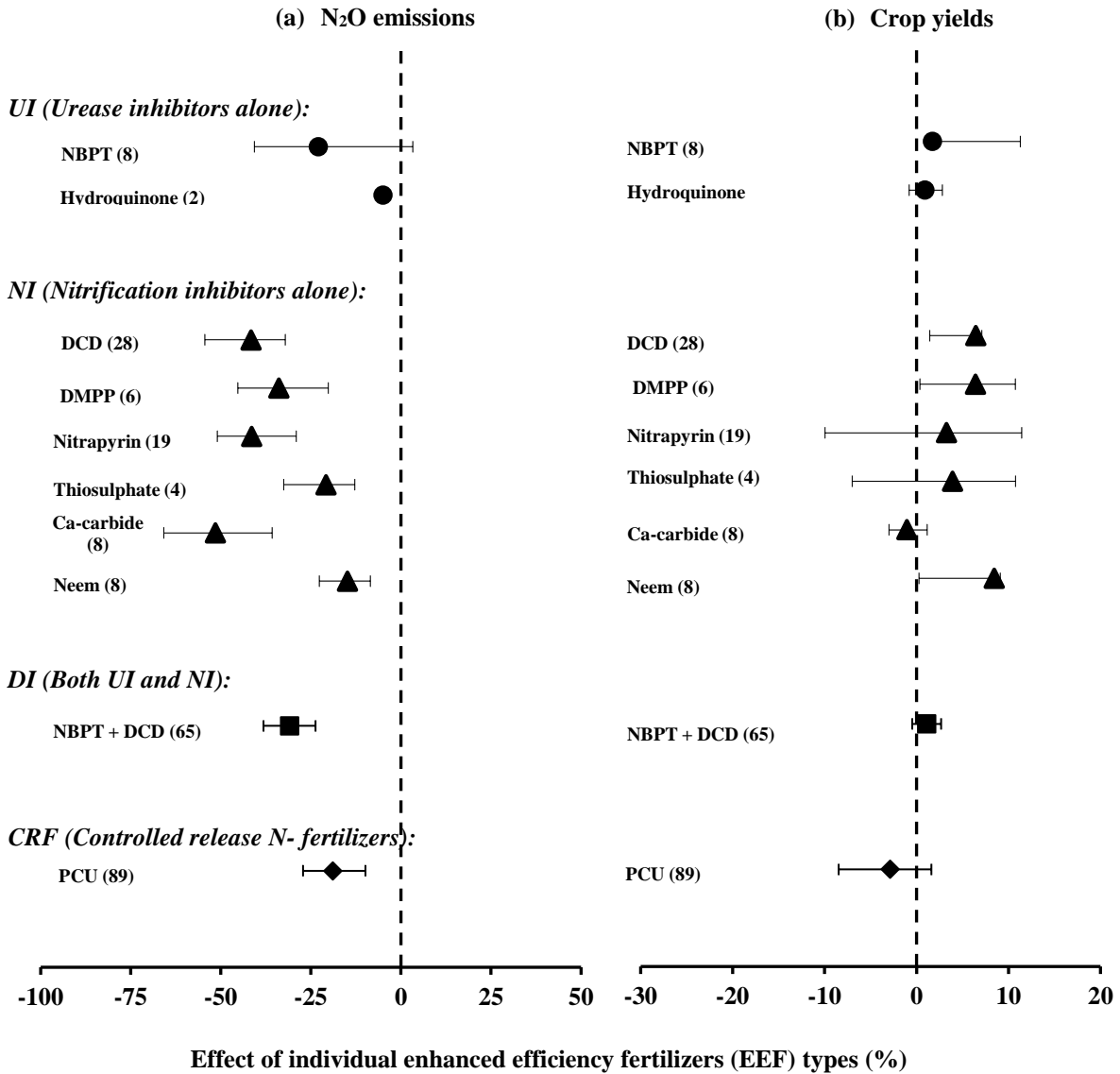


Figure 1.4. The effect of individual enhanced efficiency fertilizers (EEF) on: (a) nitrous oxide (N₂O) emissions and (b) crop yields relative to conventional N-fertilizers for different cereal types, soil types, and management conditions. Mean effect sizes and 95% confidence intervals (CIs) are shown. Numbers in parentheses indicate the sample sizes (the number of pair-wise comparisons). The mean effect sizes were considered significantly different only when the 95% CIs did not overlap with zero. The mean effect sizes for different subgroups are considered significantly different from one another only if their 95% CIs do not overlap.

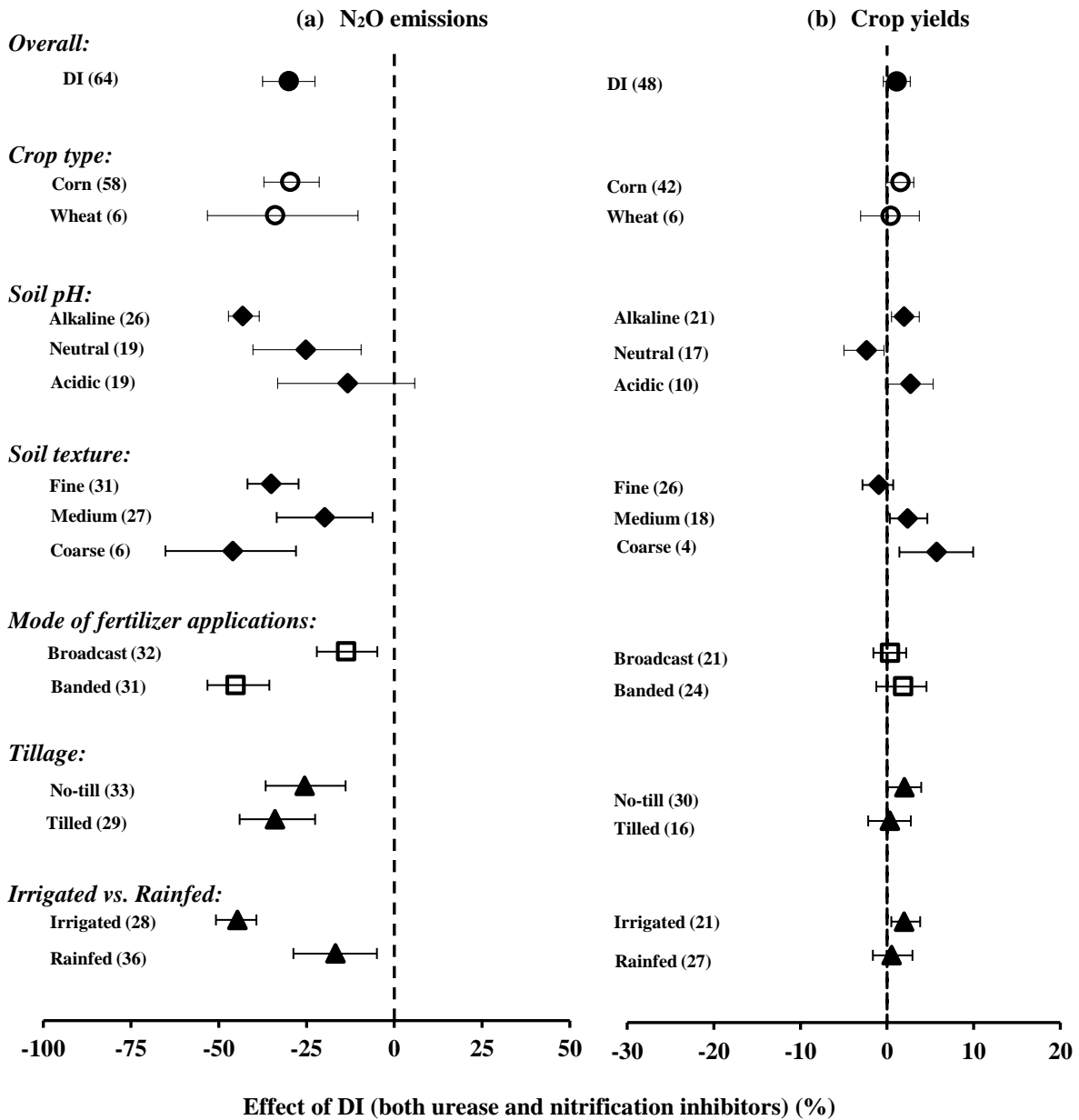


Figure 1.5. The effect of double inhibitors (DI) on: (a) nitrous oxide (N₂O) emissions and (b) crop yields relative to conventional N-fertilizers for different cereal types, soil types, and management conditions. Mean effect sizes and 95% confidence intervals (CIs) are shown. Numbers in parentheses indicate the sample sizes (the number of pair-wise comparisons). The mean effect sizes were considered significantly different only when the 95% CIs did not overlap with zero. The mean effect sizes for different subgroups are considered significantly different from one another only if their 95% CIs do not overlap.

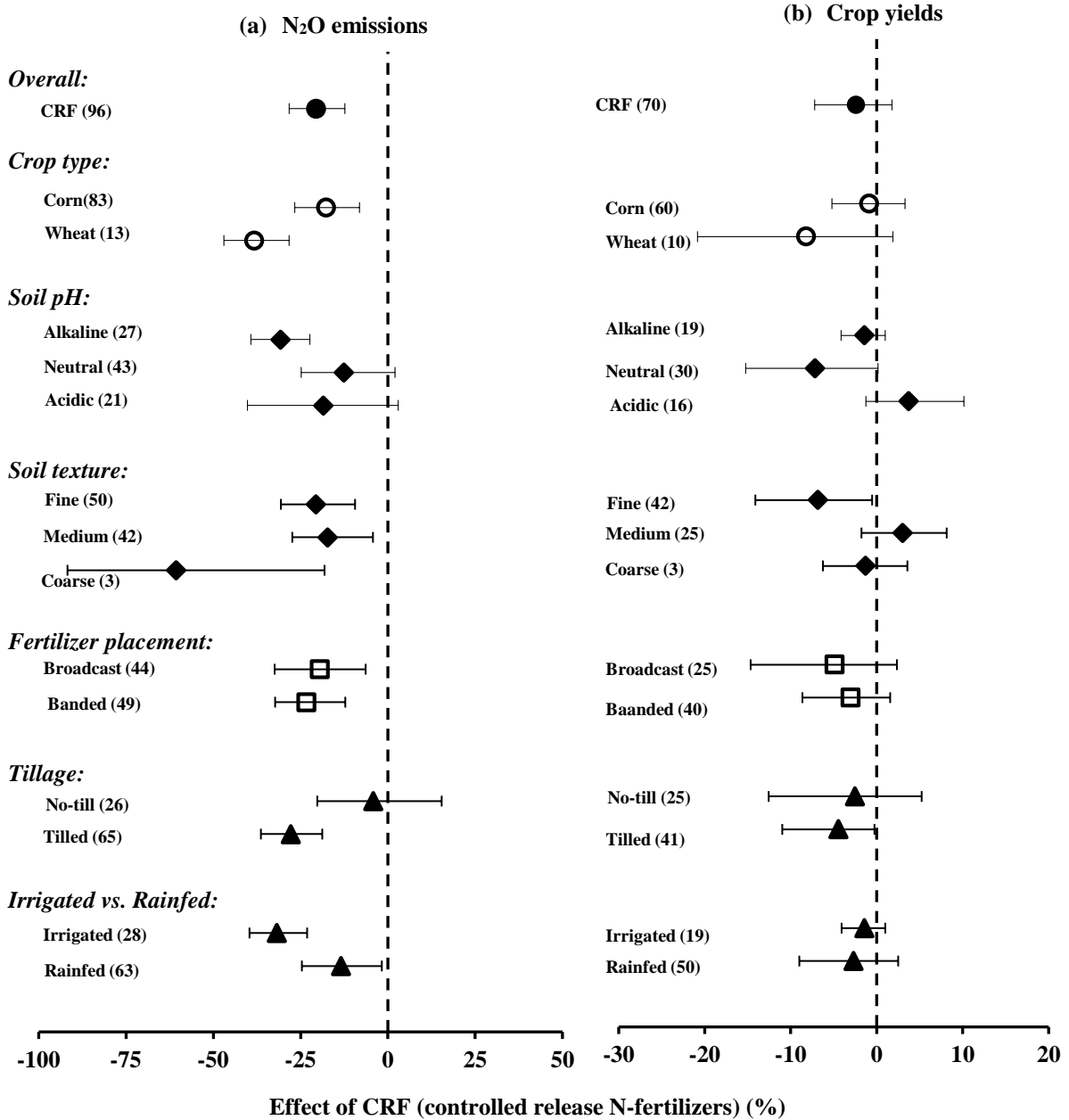


Figure 1.6. The effect of controlled release N-fertilizers (CRF) on: (a) nitrous oxide (N₂O) emissions and (b) crop yields relative to conventional N-fertilizers for different cereal types, soil types, and management conditions. Mean effect sizes and 95% confidence intervals (CIs) are shown. Numbers in parentheses indicate the sample sizes (the number of pairwise comparisons). The mean effect sizes were considered significantly different only when the 95% CIs did not overlap with zero. The mean effect sizes for different subgroups are considered significantly different from one another only if their 95% CIs do not overlap.

CHAPTER 2 - STABILIZED NITROGEN FERTILIZERS AND APPLICATION RATE INFLUENCE NITROGEN LOSSES UNDER RAINFED SPRING WHEAT¹

Abstract

Nitrogen (N) losses associated with fertilizer application have negative economic and environmental consequences, but urease and nitrification inhibitors have potential to reduce N losses. The effectiveness of these inhibitors has been studied extensively in irrigated but not in rainfed systems. This study was conducted at Glyndon, MN, under rainfed conditions to assess the impact of urease and nitrification inhibitors on NH₃ volatilization, N₂O emissions, and NO₃⁻ concentrations below the spring wheat (*Triticum aestivum* L.) rooting zone. Urea (U), urea with urease and nitrification inhibitors (SU), and urea with nitrification inhibitor only (UI) were applied at 146 and 168 kg N ha⁻¹ along with the control treatments. Cumulative NH₃ volatilization was reduced by 26%, N₂O emissions measured 18 d after planting were reduced by 50% with SU, but no significant reduction was observed with UI compared to U. We did not observe a significant effect of higher N rate on N₂O emissions, but lower N application rate (146 kg N ha⁻¹) significantly reduced NH₃ volatilization by 26% compared to 168 kg N ha⁻¹. Nitrate concentration below the rooting zone was reduced by applying N at lower rate and also through the use of SU and UI instead of U. Soil inorganic N intensity was significantly related with cumulative N₂O emissions. Nitrogen source and rate did not influence grain yield and protein content. This single-growing season study under rainfed conditions suggests that fertilizer N-

¹ The material in this chapter was co-authored by Resham Thapa, Amitava Chatterjee, Jane M F Johnson, and Rakesh Awale. Resham Thapa had primary responsibility for collecting samples in the field and lab analysis. Resham Thapa drafted and revised all versions of this chapter. Amitava Chatterjee, Jane M F Johnson, and Rakesh Awale served as proofreader and checked the math in the statistical analysis conducted by Resham Thapa.

stabilizers can be successfully used to minimize N losses without compromising grain yield and protein content.

Abbreviations: DCD, dicyandiamide; WFPS, water-filled pore space; SU, stabilized urea containing both urease inhibitor and nitrification inhibitor; U, Urea; NBPT, N-(n-butyl)-thiophosphoric triamide; UI, stabilized urea containing nitrification inhibitor.

Introduction

Spring wheat, an important cereal crop, typically receives N-based fertilizer. In 2010, about 18% of global N fertilizer was used for wheat production (Heffer, 2013). Nitrogen losses associated with fertilizer application have negative economic and environmental consequences. Therefore, reducing N losses through denitrification (N_2O), volatilization (NH_3) and leaching (NO_3^-) from wheat production systems has global environmental implications with regard to greenhouse gas emissions, air and water quality.

Environmental impact of N-loss depends on the form N is lost. Nitrous oxide is a very potent greenhouse gas and the most dominant ozone-layer depleting substance (Ravishankara et al., 2009). Agriculture is the primary anthropogenic source of N_2O , accounting 74.8% of the U.S. N_2O emissions (USEPA, 2014). Furthermore, agricultural activities have been reported as the major contributor toward NH_3 volatilization (Aneja et al., 2009) and NO_3^- leaching. Ammonia emissions, through interactions with other compounds in the atmosphere, contribute to soil acidification, eutrophication, and can also pose a threat to human health through particulate matter formation (Aneja et al., 2009). Further, all these forms of N losses decrease N use efficiency of a crop.

Excess amount of inorganic N after plant uptake is prone to N losses through N_2O emissions (McSwiney and Robertson, 2005; Van Groenigen et al., 2010), NH_3 volatilization

(Rochette et al., 2013) and NO_3^- leaching (Liang et al., 2011). Mineralization of inorganic N from organic matter contributes to significant portion of crop-N demand. But, it is hard to accurately assess the inorganic N supply from soil organic matter mineralization; as it depends on precipitation and temperature during the growing season (Dinnes et al., 2002). Variability in mineralization patterns lead to low N supply or immobilization of fertilizer N and plant response to additional fertilizer N without significant N losses. Zebarth et al. (2008b) reported no significant increase in N_2O emissions with increasing N application rate. Tian et al. (1998) also reported that the fraction of applied N lost as NH_3 was not affected by N application rate. This variability in response with N rate brought about the need of conducting further studies to assess the effect of N rates on N losses.

Urease and/or nitrification inhibitors provide another way to reduce N losses and increase N use efficiency from urea fertilized fields. Such products have the potential to increase crop yields by delaying N transformation processes, thereby synchronizing N availability with the peak crop N demand (Franzen, 2011). Urease inhibitors prevent or delay the rate of urea hydrolysis for 7 to 14 d by blocking the urease enzyme-binding sites (Trenkel, 2010). Whereas, nitrification inhibitors inhibit the biological oxidation of NH_4^+ into NO_3^- for 4 to 10 week by inhibiting the activity of nitrosomonas and nitrobacter bacteria (Trenkel, 2010; Franzen, 2011). Furthermore, these products facilitates single pre-plant application, avoiding the economic and time constraints associated with multiple applications of urea fertilizers throughout the crop growth period. This characteristic is advantageous in North-Central plains of the United States where an application of N fertilizer before planting is the common practice (Franzen, 2011). Many studies conducted in irrigated corn (*Zea mays* L.) cropping system reported decreased N_2O emissions with the use of urease inhibitor, nitrification inhibitor or both (Bronson et al., 1992;

Halvorson et al., 2014). A recent meta-analysis by Decock, (2014) using 20 observations from three independent studies from corn production belts in mid-western United States hypothesized that the combined use of both urease inhibitor N-(n-butyl)-thiophosphoric triamide (NBPT) and the nitrification inhibitor dicyandiamide (DCD) could significantly reduce N₂O emissions. Consistent with these findings, Dawar et al. (2011) also observed that urea + NBPT could reduce N₂O emissions by 7 to 12%, NH₃ volatilization by 65 to 69%, and NO₃⁻ leaching by 36 to 55% in a silt loam soil under irrigated grasslands. Furthermore, Di and Cameron, (2002b) also reported decrease in NO₃⁻ leaching and N₂O emissions with DCD as compared to urine-N from irrigated grasslands.

Despite intensive works in irrigated conditions, only few studies were conducted in rainfed production systems. In rainfed systems, many researchers (Venterea et al., 2011; Sistani et al., 2011; Parkin and Hatfield, 2013; Dell et al., 2014) reported limited or no reductions in N₂O emissions with NBPT+DCD, whereas Abalos et al. (2012) reported reduction in N₂O emissions by 86% with NBPT as compared to urea. Ammonia volatilization loss was significantly reduced by NBPT (Clay et al., 1990; Abalos et al., 2012) and NBPT+DCD (Zaman et al., 2009; Jantalia et al., 2012) as compared to urea. However, NH₃ loss may increase, decrease, or remains constant with the use of nitrification inhibitor alone (Kim et al., 2012). No studies evaluating the efficacy of inhibitors in reducing soil water NO₃⁻ concentrations below the rooting zone were found under rainfed systems. There is an even greater paucity of data that assessed N₂O emissions, NH₃ volatilization, and soil water NO₃⁻ concentrations below the rooting zone, as a function of inhibitors within the same study in either irrigated/rainfed production systems.

The primary objective of this study was to assess the impact of urease and nitrification inhibitor, and N rate on (i) cumulative NH_3 volatilization, N_2O emissions, and soil water NO_3^- concentration below the rooting zone, and (ii) grain yield and protein content under rainfed spring wheat production system. The secondary objective was to correlate cumulative NH_3 volatilization and N_2O emissions with soil inorganic N intensity. We hypothesized that urease and nitrification inhibitor would significantly reduce all forms of N losses and increase crop yield and protein content from urea-fertilized soils.

Materials and Methods

Site description and experimental design

A field trial was conducted during 2014 growing season at Glyndon, south central MN (282 m above sea level; 46°54 '45" N, 96°36'35" W) on a Bearden silt loam soil (a fine-silty, mixed, superactive, frigid Aeric Calciaquolls) (Soil Survey Staff, 2013). The field was chisel plowed and soybean (*Glycine max* L.) was grown in the 2013 growing season. Basic physical and chemical properties of the soil are reported in Table 2.1. The pre-plant soil NO_3^- level (0-60 cm) was 45 kg N ha⁻¹.

Field experiment was laid out in a randomized complete block design with four replicates. Seven treatments comprised of: (i) Control (0 kg N ha⁻¹), (ii) SU at 146 kg N ha⁻¹ (urea stabilized with both urease inhibitor N-(n-butyl)-thiophosphoric triamide and the nitrification inhibitor dicyandiamide (DCD) (SuperU, Koch Agronomic services)), (iii) SU at 168 kg N ha⁻¹, (iv) U at 146 kg N ha⁻¹ (conventional urea), (v) U at 168 kg N ha⁻¹, (vi) UI at 146 kg N ha⁻¹ (urea stabilized with the nitrification inhibitor nitrapyrin (2-chloro-6-(trichloromethyl) pyridine) only (Instinct, Dow Agrosiences)), and (vii) UI at 168 kg N ha⁻¹. Individual plot dimension was 9 by 3 m and any two adjacent plots were separated by 1 m buffer zone to

segregate the potential treatment effects. Fertilizer treatments were uniformly broadcasted on 16 May (day of year: 136) on the same day of planting. The field was then chisel plowed using field cultivator to the depth of 7.5 cm before planting.

Spring wheat variety Glenn was planted at the seeding rate of 135 kg ha⁻¹ seeds using a 20 cm wide, small plot sized grain drill. At physiological maturity, the middle five rows of each plot were harvested using the small plot combine harvester on 25 August (day of year: 237). Wheat grains were dried at 60°C for 3 d; grain yield was adjusted and reported at 14% moisture content. Grain protein content was analyzed following near-infrared reflectance method at 12% grain moisture using Infratec 1241 Grain analyzer (FOSS analytical AB, Hoganas, Sweden).

Sampling procedures

Ammonia volatilization measurements

Ammonia (NH₃) volatilization loss was quantified using open chamber ammonia traps as described by Jantalia et al. (2012). This trap uses a 2-L polyethylene terephthalate bottle (covering 79 cm² surface area of soil) and polyfoam strips (25 cm long × 3.5 cm wide × 0.5 cm thick) as NH₃ traps. Polyfoam strips were rinsed thoroughly twice with deionized water; excess water removed, and then rinsed with 0.5 M H₃PO₄ solution, finally the excess solution was removed. A single strip was then hung from the bottle lid inside each chamber using a wire hook. The lower end of the polyfoam strip was dipped into 30 mL H₃PO₄ solution which was inside a 60 mL plastic cup suspended from the wire hook. Chambers were installed toward the center of the plot within a week of N fertilization.

Ammonia volatilization was measured from 7 to 14, 14 to 21, 21 to 28, 28 to 33, 33 to 40, 40 to 55, and 55 to 70 d after N fertilization. At the end of each sampling period, the ammonia traps and the acid solution in plastic cup, if any, from each chamber were collected in 125 mL of

2 M KCl. Fresh polyfoam strips and H₃PO₄ solution were placed inside the chambers as explained above to facilitate NH₃ trapping till next sampling. The solution containing NH₃ traps were transferred to the laboratory, and maintained at 5°C until analysis within 2 d. In the laboratory, the solution was brought to 250 mL by further rinsing the strips with KCl solution. Fifty milliliters of this solution was then sealed and frozen at -18°C in polypropylene vials, until analysis within 2 d using Automated Timberline TL2800 Ammonia Analyzer (Timberline Instruments, Colorado). Ammonia loss during consecutive sampling dates (kg NH₃ ha⁻¹) is obtained by multiplying NH₃ concentration (µg mL⁻¹) by the total volume of solution (250 mL), divided by the surface area of the soil covered by the respective chamber (79 cm²).

Field nitrous oxide flux measurements

The N₂O fluxes were measured by static chamber methods as recommended by Parkin and Venterea, (2010). Headspace air sampling was done during 0900 to 1200 local hours because during this time, surface soil temperature was near to its daily average (Maharjan et al., 2014). After planting, polyvinyl chloride (PVC) rings (25.4 cm i.d. by 8-cm deep) were inserted 5-cm deep into the soil in the middle of each plot. At each gas sampling day, insulated, vented, and reflective PVC chamber tops were placed above the rings (anchors). Headspace air samples were collected at 0, 0.5, and 1 h ± 1 min following chamber deployment using 30 mL polypropylene syringe and transferred to 12 mL pre-evacuated glass vials sealed with butyl rubber septa. Gas sampling for N₂O flux determination was conducted at 18, 26, 32, 40, 45, 55, 62, 69, 75, 81, 89, and 96 d after N fertilization. Air samples were analyzed for N₂O concentration using DGA-42 Master Gas Chromatograph (Dani Instruments, Milan, Italy) fitted with a ⁶³Ni electron capture detector (ECD) and a master SHS headspace autosampler. The Ar/CH₄ (95:5) mixture was used as carrier gas, and the ECD was operated at an oven temperature of 300°C. Analytical gas

standards (0.1, 0.5, 2, 5, 10, 100 mg kg⁻¹; Scott Specialty Gases) were included after every nine samples on each sample analyzing day to construct standard curves.

The N₂O fluxes (μL N L⁻¹ h⁻¹) were determined from N₂O concentrations vs. time graph using the linear regression or quadratic regression (QR) (Wagner et al., 1997) and using correction factors to account for theoretical flux underestimation generated as a result of chamber deployment (Venterea, 2010). Linear regression was used with linear or convex-upward curves (i.e., when second derivative of QR ≥ 0), while QR was used with convex-downward curves (Venterea et al., 2012). The N₂O fluxes were then converted into μg N₂O-N m⁻² h⁻¹ using ideal gas law equation. Minimum detectable flux of gas chromatograph was estimated by sampling ambient air samples from the experimental site (Parkin et al., 2012) and ranged from 5.7 to 17.5 μg N₂O-N m⁻² h⁻¹. Even if the N₂O flux lies below the detection limit, actually measured N₂O flux data have been reported and used for estimating cumulative N₂O emissions. Soil temperature and volumetric water content at a depth of 5 cm were measured on every N₂O gas sampling day using GS3 soil moisture temperature sensor (Decagon Devices, Inc., Pullman, WA) by inserting its probe within 10 cm from the PVC rings. Water-filled pore space (WFPS) was calculated for each flux measurement day using the equation:

$$\text{WFPS} = \frac{q_v}{\left(1 - \frac{r_b}{r_s}\right)} \times 100\% \quad (2.1)$$

where, q_v is the current volumetric water content on each N₂O flux measurement day, r_b is the bulk density of the soil (1.28 g cm⁻³) and r_s is the particle density of the soil (assumed to be 2.65 g cm⁻³).

Soil water nitrate concentrations below the rooting zone

Ceramic suction cup lysimeters (130 cm long by 1.60 cm i.d.) were installed to a depth of 0.9 m within a week of N fertilization. Before the installation, the ceramic end of the lysimeters was soaked in deionized water for 24 h at a constant vacuum of 40 kPa. For lysimeter installation, 1-m deep soil hole was bored using a probe (3.6 cm inner diameter) at the center of each plot, a lysimeter was inserted into the hole, and the gap around the lysimeter was re-filled with silica slurry along with excavated soil. A continuous vacuum of 40kPa was created inside the lysimeters using hand pump and rubber septum throughout the sampling period. Soil water were collected for a total of seven times during the growing season in 50 mL polypropylene tubes and then frozen at -18°C until analysis using Automated Timberline TL2800 Ammonia Analyzer (Timberline Instruments, CO, USA).

Temporal inorganic N dynamics

Intact soil cores (2 cm diam.) were collected to a depth of 15 cm from each plot at 15 d interval to determine the soil inorganic N levels throughout the growing season. Three soil cores from each plot were composited, transferred to laboratory at 5°C , and stored at -18°C until analysis within a week. After thawing and homogenizing frozen soil, 6.5 g of moist soil were extracted with 25 mL of 2 M KCl (1:5 soil/extractant ratio) after shaking for 30 min. The KCl extracts were analyzed using Timberline TL2800 Ammonia Analyzer (Timberline Instruments, CO, USA). Soil moisture content was determined by soil weight loss method at 105°C using separately weighed subsamples of soil. Additional soil cores were taken randomly from the field sites to determine the bulk density (1.28 g cm^{-3}) following core method and soil texture following hydrometer method.

Data Analysis

Cumulative N₂O emissions (direct soil-to-atmosphere) from each plot were calculated using trapezoidal integration of daily measured N₂O fluxes using the following equation:

$$\text{Cumulative N}_2\text{O emission } (t) = \sum_i^n \frac{X_i + X_{i+1}}{2} (t_{i+1} - t_i) \quad (2.2)$$

where X_i is the N₂O-N flux measurement on day t , X_{i+1} is the succeeding N₂O-N flux measurement on day t_{i+1} and n is the final date of N₂O-N flux measurement. Cumulative NH₃ volatilization loss (kg N ha⁻¹) was determined by summing the amount of NH₃ volatilized during each sampling period throughout the growing season. Soil inorganic N intensity is an index that represents the extent and duration of exposure of soil inorganic N (NH₄⁺ + NO₃⁻) accumulation for microbial action. Soil inorganic N intensity (g N d kg⁻¹ soil) was calculated in similar manner to that of cumulative N₂O emissions using trapezoidal integration of daily soil inorganic N concentrations over the growing season (Burton et al., 2008; Zebarth et al., 2008a; Maharjan and Venterea, 2013).

Statistical analysis

Statistical analyses were performed using PROC GLM procedure for RCBD in SAS 9.3 (SAS Institute, 2010). Comparison of the means was conducted using single degree of freedom contrasts for N source and rate. Linear regression analysis was conducted with PROC REG in SAS 9.3 and the significant correlation coefficients (R²) were reported.

Results and Discussion

Environmental conditions and drainage

The mean daily air temperature and wind speed over the growing season (16 May 16–20 August) were 20°C and 3.5 m s⁻¹, respectively (Fig. 2.1A), being identical to the long-term (1990-2013) normal air temperature and wind speed. Soil temperature, and WFPS measured at

each N₂O gas flux measurement day averaged $25.9 \pm 1^\circ\text{C}$, and $60.1 \pm 2.6\%$, respectively (Fig. 2.1B). Cumulative precipitation during the 2014 growing season was 212 mm, which was lower than the total rainfall (259 mm) of the long-term (1990–2013) normal. The first rainfall event that occurred 4 d after fertilizer application was 11.9 mm. Rainfall patterns mimic the past years with most of the rainfall occurring May through June. Approximately 85% of the total rainfall during the growing period was measured within 60 d following N fertilization, which resulted in total of six drainage events.

Grain yield and protein content

All of the N fertilized treatments had significantly greater grain yield and protein content compared to control (Table 2.2). Among the N sources used in this study, grain yield and protein content were similar. This is in line with numerous studies conducted in both irrigated conditions (Halvorson et al., 2010, 2011) as well as in rainfed conditions (Sistani et al., 2011). McKenzie et al. (2010) also reported that urease inhibitor-NBPT had no influence in grain yield and grain protein content of winter wheat in southern Alberta. Similarly, Abalos et al. (2012) also observed no significant difference in grain yield, biomass yield, grain N uptake and biomass N uptake of rainfed barley with urea+ NBPT as compared to urea alone in New Zealand. Conversely, a recent meta-analysis by Abalos et al. (2014) reported that use of urease and nitrification inhibitors could increase the grain yield by 7.5%, and also concluded that the response would be substantial in coarse-textured soil, under irrigated conditions and with crops having higher N demands. Therefore, fine-textured soil, low N application, and limited water availability during this study might be the reason of having no variation in grain yield among N sources.

Nitrogen application rate had no effect on grain yield (Table 2). Averaged across N sources, grain protein content was significantly increased by 4.3% when N was applied at higher

rate of 168 kg N ha⁻¹ as compared to 146 kg N ha⁻¹. Franzen et al. (2011) also reported little or no difference in grain yield and protein content of wheat to urea at the rate of 101kg N ha⁻¹ with and without nitrapyrin, in North Dakota.

Ammonia volatilization

Between 70 and 86% of the cumulative NH₃ volatilization loss occurred during the first 30 d for all treatments (Fig. 2.2). Daily NH₃ loss was significantly lower with SU as compared to UI and U during the initial sampling days. The cumulative NH₃ volatilization loss ranged from 2.5 to 7.0 kg N ha⁻¹ (Table 2.2). Lower NH₃ loss in this experimental period might be due to the occurrence of 11.9 mm of rainfall following N fertilization. Jantalia et al. (2012) also reported that irrigating the fields the day following fertilization could significantly limit NH₃ loss from urea-based fertilizers to <4%. Furthermore, Holcomb et al. (2011) reported that irrigation rate of 14.6 mm would be sufficient to substantially incorporate the broadcasted urea into the soil. Sanz-Cobena et al. (2011) further stated that supplemental water inputs of 7 to 14 mm following urea application demonstrated similar NH₃ loss reduction efficiency as obtained through the use of NBPT. Another possibility would be the use of urea and fine-textured soil in our study. Other investigators (Rawluk et al., 2001; Singurindy et al., 2006) also observed lower cumulative growing season NH₃ loss with fine-textured soil as compared to coarse-textured soil. Chantigny et al. (2004) concluded that significant portion of NH₄⁺ released from urea hydrolysis get fixed in the clay lattices of fine-textured soil, making NH₄⁺ unavailable for microbial processes. Further, incorporating broadcasted fertilizer into soil by tillage has been reported to reduce NH₃ loss by 50% as compared to broadcasting without incorporation (Bouwman et al., 2002).

Cumulative NH₃ volatilization loss was significantly increased by N fertilization as compared to control (Table 2.2). Considering all N rates, SU (SuperU) statistically reduced

cumulative NH_3 volatilization loss by 26% as compared to U (Urea) (Table 2.2). But, UI (Urea amended with Instinct) did not show any reduction in cumulative NH_3 volatilization loss compared to U. This finding is consistent with Clay et al. (1990) who reported that nitrification inhibitor (DCD) does not have any role in abating NH_3 loss, whereas treating urea with urease inhibitor (NBPT) could significantly reduce NH_3 loss by 100-folds in Minnesota. In contrast, Zaman et al. (2009) observed that NH_3 loss will increase with nitrification inhibitor (DCD), decrease with urease inhibitor (NBPT) and decrease with both DCD and NBPT as compared to urine alone over all the seasons in grazed pasture system in New Zealand. Jantalia et al. (2012) also reported reduction in NH_3 volatilization loss with SU as compared to U, when both applied at 200 kg N ha^{-1} under corn cropping system in Colorado. A meta-analysis by Kim et al. (2012) further highlighted that the application of U with NBPT and DCD would significantly reduce NH_3 loss, but the use of nitrification inhibitor alone would either increase, decrease, or have no effect in NH_3 loss. Our result, consistent with these studies, suggests that the inhibitory effect of SU on NH_3 volatilization loss was associated with the presence of urease inhibitor, NBPT, which slowed down urea hydrolysis during the initial days following fertilization. But, the presence of nitrification inhibitor, nitrapyrin with UI resulted in NH_4^+ retention for longer duration, providing more opportunity for NH_3 volatilization loss.

Consistent with Tian et al. (1998), N application rate significantly increased NH_3 volatilization loss but had no significant effect on fertilizer-induced volatilization factor. Averaged across N sources, higher N application rate of 168 kg N ha^{-1} increased cumulative NH_3 volatilization loss by 26% as compared to 146 kg N ha^{-1} (Table 2.2). This result suggests that application of N fertilizer at the optimum recommended rate would also aid in reducing NH_3 volatilization loss without compromising crop yield.

Nitrous oxide emissions

Over the measurement dates, mean daily N₂O fluxes ranged from 2.30 μg m⁻² h⁻¹ (control) to 239 μg m⁻² h⁻¹ (U at 168 kg N ha⁻¹) with the highest fluxes on day of year 154 (Fig. 2.3). On this sampling date, averaged N₂O emissions were greater by 14, 44, and 82% respectively with SU, UI, and U than the control. No significant difference was found on other sampling dates. Nitrous oxide fluxes during the time period (154–205 day of year), accounted for 72 to 86% of the cumulative N₂O emissions, and corresponded to WFPS > 60%. When WFPS > 60%, denitrification is anticipated to be the major pathway toward N₂O emission (Linn and Doran, 1984; Bateman and Baggs, 2005).

Cumulative N₂O emissions over the measurement period was not significantly affected by N fertilization, but there was a trend (P = 0.06) of higher N₂O emissions with N fertilization (0.65 kg N₂O ha⁻¹) as compared to the control (0.25 kg N₂O ha⁻¹) (Table 2.2). Among the N fertilizer sources, SU statistically reduced cumulative N₂O emissions by 50% as compared to U, but application of UI did not reduce N₂O emissions as compared to U. Abalos et al. (2012) also reported significant reduction in N₂O emissions by 86% with U treated with NBPT as compared to U in rainfed Mediterranean barley (*Hordeum vulgare* L.). In contrast, other studies conducted in rainfed corn cropping system (Venterea et al., 2011; Parkin and Hatfield, 2013; Dell et al., 2014) reported that SU did not significantly reduce N₂O emissions as compared to U. This difference in response within rainfed systems could be associated with relatively wet soil conditions (WFPS > 60%) due to the frequent rainfall events during this study as well as during Abalos et al. (2012), which facilitated denitrification processes. However, the rainfall patterns were erratic during the experimental period of studies by other investigators (Venterea et al., 2011; Parkin and Hatfield, 2013; Dell et al., 2014), which resulted in low WFPS. This rationale

can be further supported by the numerous studies conducted in irrigated corn cropping system that reported significantly greater N₂O emissions with U with respect to SU (Halvorson et al., 2014). Frequent irrigation and rainfall events throughout the growing period resulted in consistently higher soil WFPS, improved synchronization between N availability and N uptake with SU, thereby reducing N₂O emissions with SU (Dell et al., 2014). Thus, the effectiveness of fertilizer containing inhibitors in reducing N₂O emissions might be greatly influenced by the rainfall patterns and soil moisture conditions within rainfed systems.

Many previous studies concluded that increasing rate of N application increased N₂O emissions (Zebarth et al., 2008a; Millar et al., 2010; Gao et al., 2013). This was based on the premise that N substrate for N₂O production pathways (i.e., nitrification and denitrification) increased with more application of N. Although our study, consistent with Zebarth et al. (2008b), showed that cumulative N₂O emissions were not significantly affected by N rates (Table 2.2), increase in N application from 146 to 168 kg N ha⁻¹ resulted in 1.73-folds greater cumulative area-based N₂O emission with U, 1.5-folds with SU and 1.05-folds with UI.

Soil water nitrate concentrations

During this field experiment, soil water NO₃⁻ concentrations below the rooting zone ranged from 0.07 to 46 mg L⁻¹ (Fig. 2.4). Six drainage events that occurred between day of year 139 and 170 following heavy rainfall (Fig. 2.1B) were responsible for higher below root zone NO₃⁻ concentration. Consistent with Liang et al. (2011), our results suggest that rainfall is the primary driving force responsible for downward movement of NO₃⁻ in dryland cropping systems. Di and Cameron (2002a) further noted that greater NO₃⁻ concentrations in the soil during or before heavy rainfall or irrigation events were susceptible to leaching losses. Different N fertilizer sources nitrified at different speed (as expected), thus creating soil water

NO_3^- concentration peaks at different times (Fig. 2.4). Presence of urease and nitrification inhibitors with SU and UI slowed the nitrification rate, whereas U with no inhibitor nitrified at greater rate, giving greater soil water NO_3^- concentration in the initial sampling days.

Soil inorganic n concentration, nitrogen intensity, and its relation to gaseous form of nitrogen losses

Soil inorganic N concentrations in 0 to 15 cm soil depth varied from $>2 \text{ mg kg}^{-1}$ to as high as 175 mg kg^{-1} over the entire growing season (Fig. 2.5). Soil inorganic N concentrations were found to be highest (day of year: 142, 154) following N fertilizer application, and then decreased. Soil inorganic N intensity was not significantly affected by N sources used in this study (Table 2.2). But, soil inorganic N intensity was significantly increased at higher N application rate of 168 kg N ha^{-1} as compared to 146 kg N ha^{-1} .

Studies reported a linear relationship between cumulative area-based N_2O emissions and soil NO_3^- intensity (Burton et al., 2008; Zebarth et al., 2008a; Gagnon et al., 2011); cumulative area-based N_2O emissions and soil NO_2^- intensity (Maharjan and Venterea, 2013); cumulative area-based N_2O emissions and soil NO_3^- plus NO_2^- intensity (Engel et al., 2010); as well as cumulative area-based N_2O emissions and soil NH_4^+ intensity (Gagnon et al., 2011). Likewise, our results revealed that cumulative area-based N_2O emissions were significantly correlated (Fig. 2.6A; $R^2 = 0.71$, $P < 0.05$) with the soil inorganic N intensity. This was in contrast with the finding of Venterea et al. (2011) who reported that the soil inorganic N intensity was not significantly correlated with cumulative N_2O emissions for a rainfed corn cropping system grown in silt loam soil in Minnesota. This difference in response could be ascribed to the difference in the timing of the N- fertilizer application. Venterea et al. (2011) applied N-fertilizers when the corn was at V4 to V6 stage as single sidedress application. This might have

provided less chance for microbial N transformation before significant plant N uptake, thereby reducing N₂O emissions despite having high soil inorganic N intensity. However, in this current study, we applied all of the N- fertilizer as single time pre-plant application, which resulted in greater soil inorganic N intensity and N₂O emissions during the initial days after N application; corresponding to a linear relationship.

Cumulative NH₃ volatilization loss was not significantly related with soil inorganic N intensity (Fig. 2.6B; R² = 0.42, P > 0.05). This was not surprising as soil inorganic N content might be dominated by NO₃⁻ forms rather than NH₄⁺ forms. Thus, our result suggests that higher N loss with higher N application rates as well as with U was most probably due to the greater soil inorganic N intensity available for microbial processes.

Conclusions

Our single-growing-season study revealed that urease and nitrification inhibitors can be successfully used to minimize N losses without compromising spring wheat grain yield and protein content, under silt-loam soil conditions. Controlled release of inorganic N from SU containing both urease- and nitrification-inhibitors might reduce volatilization and denitrification losses; whereas, UI containing nitrification inhibitor only can prolong NH₄⁺-N retention in soil, making it prone for NH₃ volatilization loss. Besides this, urease and nitrification inhibitors might have the potential to reduce soil water nitrate concentrations below the rooting zone. Moreover, current spring wheat fertilizer N recommendation rate of 146 kg N ha⁻¹ was sufficient to meet crop N demand, and increasing N rate at 168 kg ha⁻¹ significantly increased N losses. Significant linear relationship was observed between cumulative N₂O emissions and soil inorganic N intensity. These findings should be verified for multiple growing seasons to develop a sound nutrient management stewardship program.

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Table 2.1. Basic soil physical and chemical properties at the study site.

Depth	pH [†]	EC [†]	CEC	Macronutrients						Micronutrients			OM [§]	Particle size distribution		
				NO ₃ -N [‡]	S	P	K	Ca	Mg	Zn	Fe	Cu		Sand	Silt	Clay
cm		dS m ⁻¹	cmol kg ⁻¹	— kg ha ⁻¹ —			mg kg ⁻¹			g kg ⁻¹						
0-15	8.4	0.21	26.1	15	18	19	240	660	140	1.44	11.9	1.37	47	88	587	325

[†] pH and EC determined in 1:1 soil:water extractant.

[‡] Nitrate-N determined using 2M KCl (1:5 soil:KCl extraction).

[§] Organic matter (OM) determined using loss-on-ignition method.

Table 2.2. Grain yield (Mg ha⁻¹), grain protein content (g kg⁻¹ grain), cumulative growing season N₂O emissions (kg N ha⁻¹), NH₃ volatilization (kg N ha⁻¹) and soil inorganic N (NH₄⁺ + NO₃⁻) intensity (g N d kg⁻¹ soil) under rainfed spring wheat cropping system.

N source	N Rate	Grain yield	Grain protein content	Cumulative growing season		Soil inorganic N intensity
				N ₂ O emissions	NH ₃ volatilization	
	kg N ha ⁻¹	Mg ha ⁻¹	g kg ⁻¹ grain	kg N ha ⁻¹		g N d kg ⁻¹
Control	0	2.52 (0.13)	121 (2.70)	0.25 (0.03)	2.50 (0.14)	0.81 (0.12)
SU	146	3.26 (0.08)	132 (2.46)	0.36 (0.07)	4.36 (0.81)	0.95 (0.08)
	168	3.25 (0.06)	137 (3.22)	0.55 (0.09)	4.08 (0.75)	1.65 (0.50)
U	146	3.17 (0.14)	127 (3.28)	0.67 (0.18)	4.41 (0.79)	0.82 (0.07)
	168	3.24 (0.06)	135 (0.82)	1.16 (0.36)	7.01 (0.99)	2.65 (0.56)
UI	146	3.18 (0.20)	128 (4.43)	0.57 (0.10)	3.62 (0.28)	1.45 (0.43)
	168	3.35 (0.12)	132 (2.40)	0.60 (0.16)	6.36 (0.83)	1.15 (0.19)
Single df contrasts			Significance probabilities for F-statistic			
Response to fertilization		***	**	NS	**	NS
146 vs. 168		NS	*	NS	**	*
SU vs. U		NS	NS	*	*	NS
UI vs. U		NS	NS	NS	NS	NS
SU vs. UI		NS	NS	NS	NS	NS

* Significant at $\alpha = 0.05$; NS, not significant.

** Significant at $\alpha = 0.01$.

*** Significant at $\alpha = 0.001$.

† SU, stabilized urea with urease inhibitor NBPT and nitrification inhibitor DCD; U, urea; UI, stabilized urea with nitrification inhibitor nitrapyrin.

‡ Values in parenthesis represents standard error (n=4).

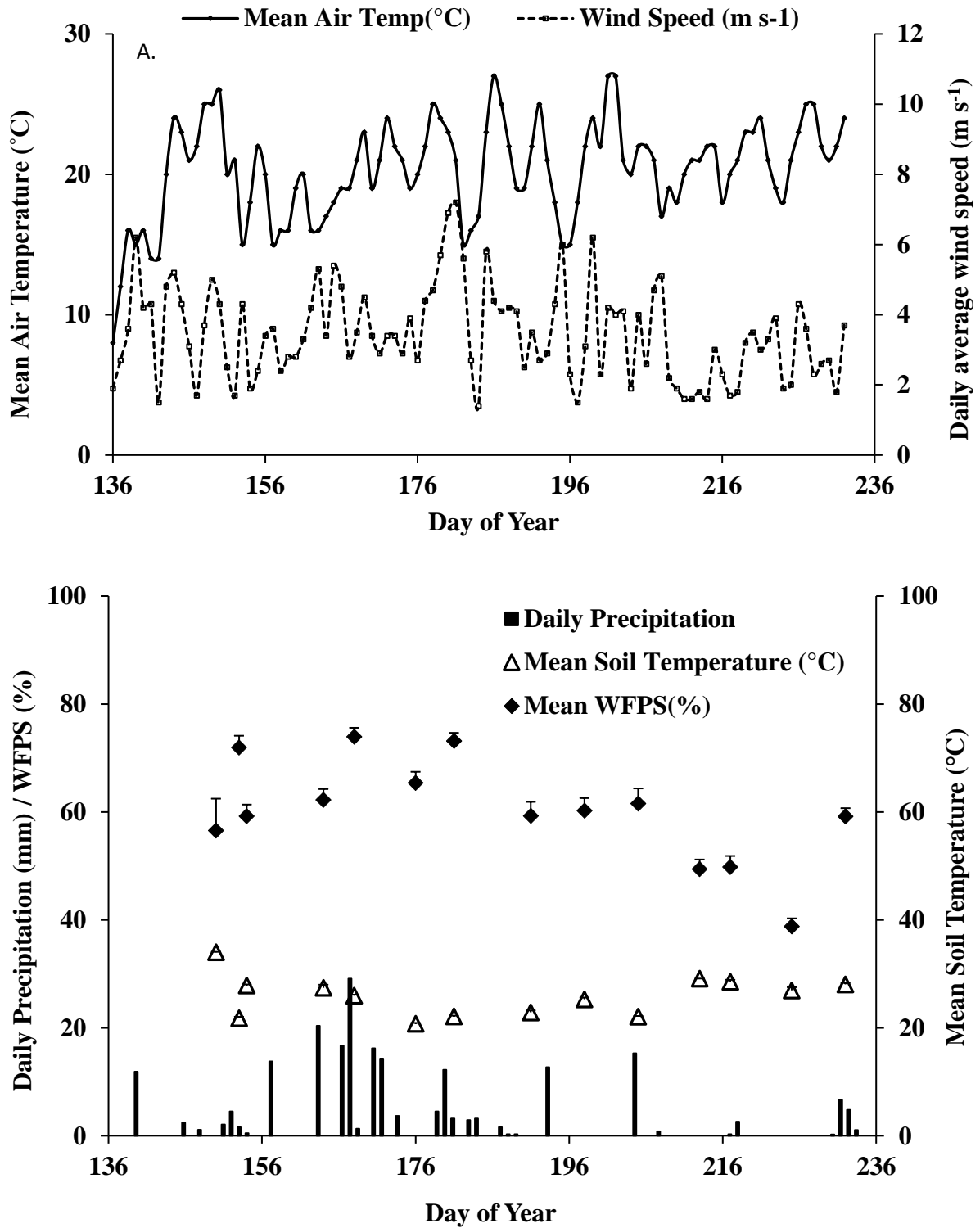


Figure 2.1. (A) Daily mean air temperature and daily mean wind speed, and (B) daily precipitation (mm); mean soil temperature (°C) and mean water-filled pore space (WFPS) at the 0.05 m soil depth at the time of N₂O sampling across all the treatments. Vertical bars represents the standard errors (n=28).

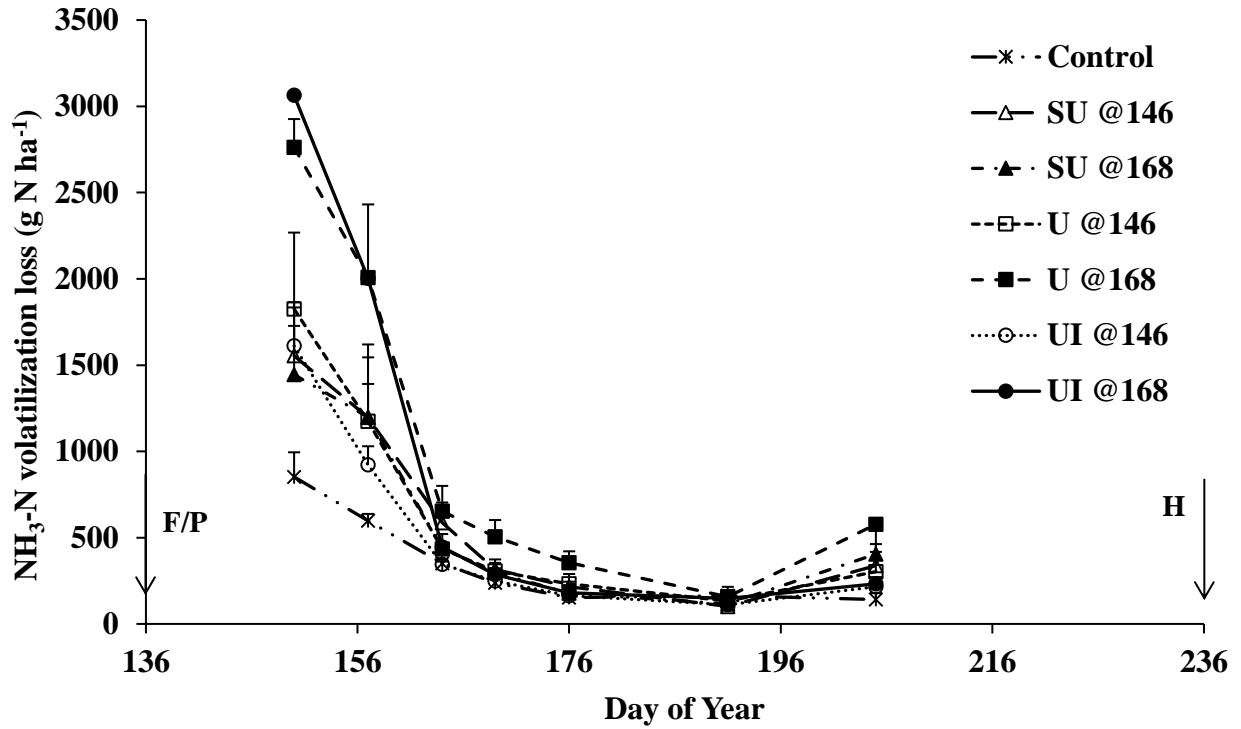


Figure 2.2. Ammonia (NH₃) volatilization loss measured on each sampling date under different N sources and rates: no N addition (Control); urea stabilized with NBPT and DCD (SU) at 146, 168 kg N ha⁻¹; urea (U) at 146, 168 kg N ha⁻¹; and urea stabilized with nitrapyrin (UI) at 146, 168 kg N ha⁻¹. Ammonia volatilization measurement on day of year 150 represents the total ammonia that was volatilized from day of year 143 to 150. Downward pointing arrows indicate the date of planting (P), fertilizer application (F), and harvesting (H). Vertical bars represents the standard errors (n=4).

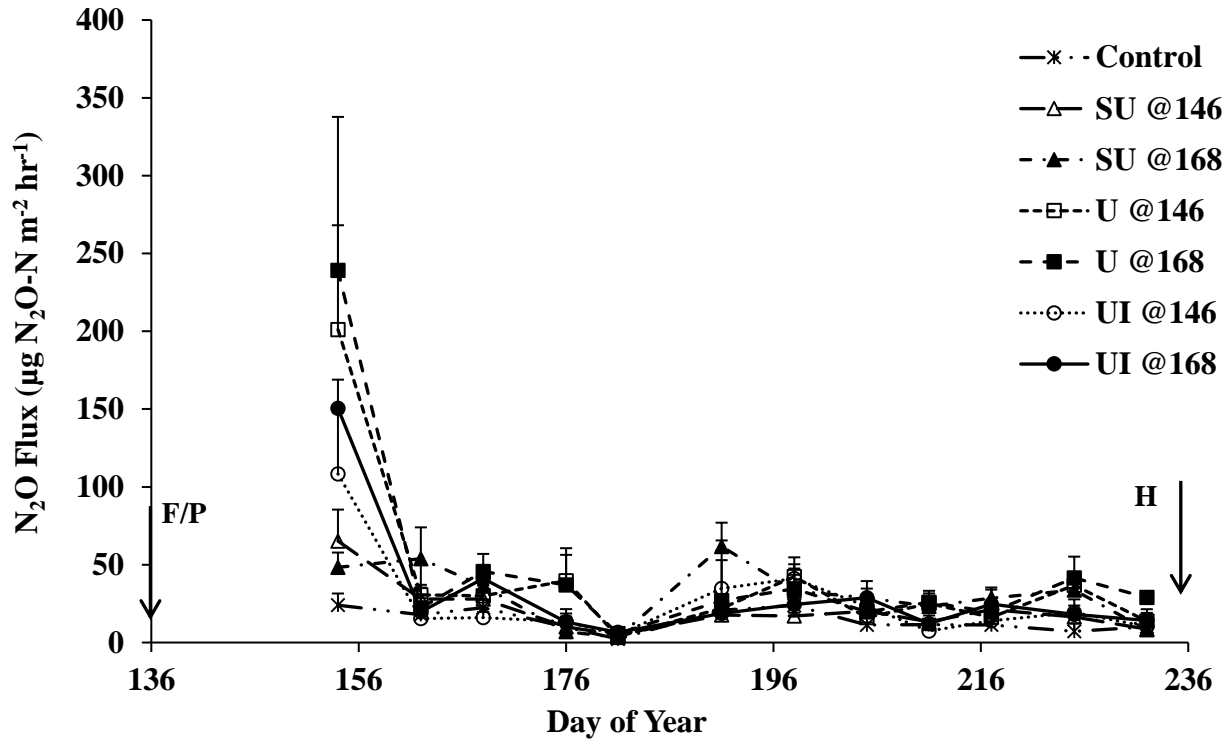


Figure 2.3. Nitrous oxide (N₂O) fluxes measured on each sampling date under different N sources and rates: no N addition (Control); urea stabilized with NBPT and DCD (SU) at 146, 168 kg N ha⁻¹; urea (U) at 146, 168 kg N ha⁻¹; and urea stabilized with nitrapyrin (UI) at 146, 168 kg N ha⁻¹. Downward pointing arrows indicate the date of planting (P), fertilizer application (F), and harvesting (H). Vertical bars represents the standard errors (n=4).

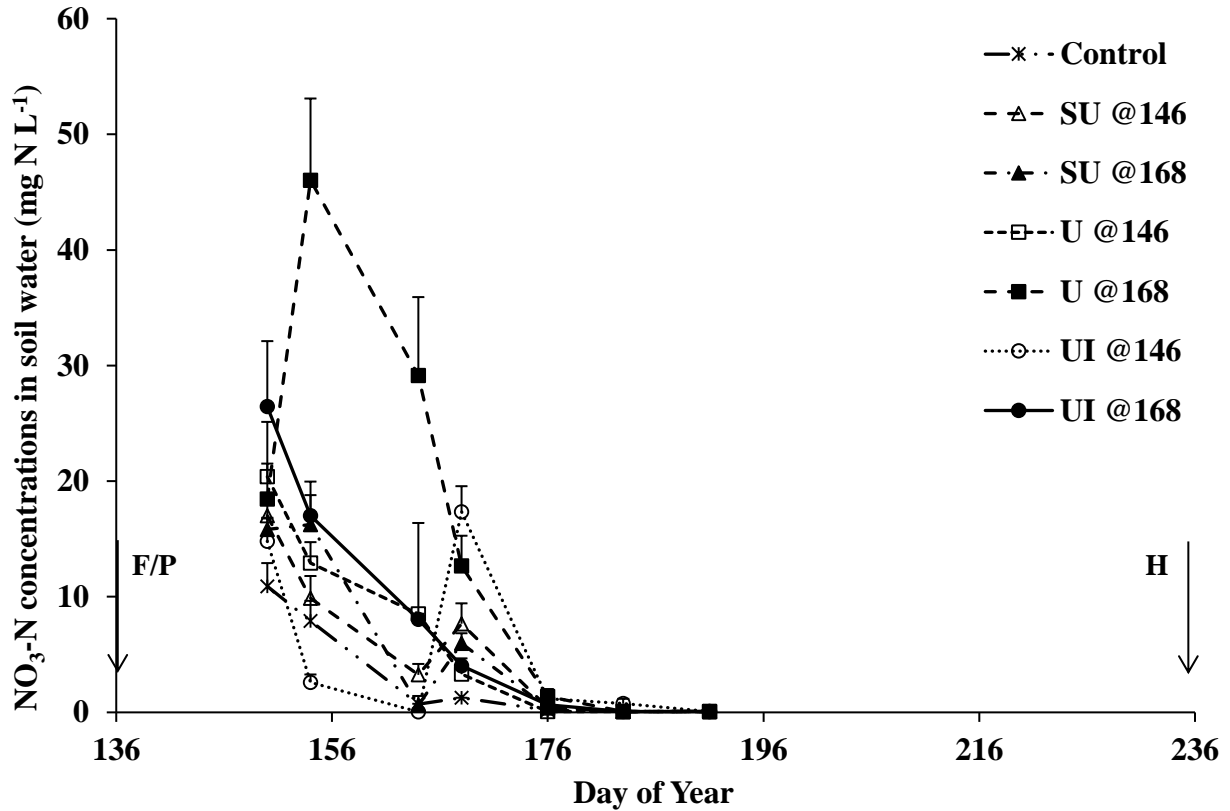


Figure 2.4. Soil water nitrate (NO_3^-) concentrations below the spring wheat rooting zone (0.9 m depth) on each sampling date under different N sources and rates: no N addition (Control); urea stabilized with NBPT and DCD (SU) at 146, 168 kg N ha^{-1} ; urea (U) at 146, 168 kg N ha^{-1} ; and urea stabilized with nitrapyrin (UI) at 146, 168 kg N ha^{-1} . Downward pointing arrows indicate the date of planting (P), fertilizer application (F), and harvesting (H). Standard errors are represented by vertical bars (n=4).

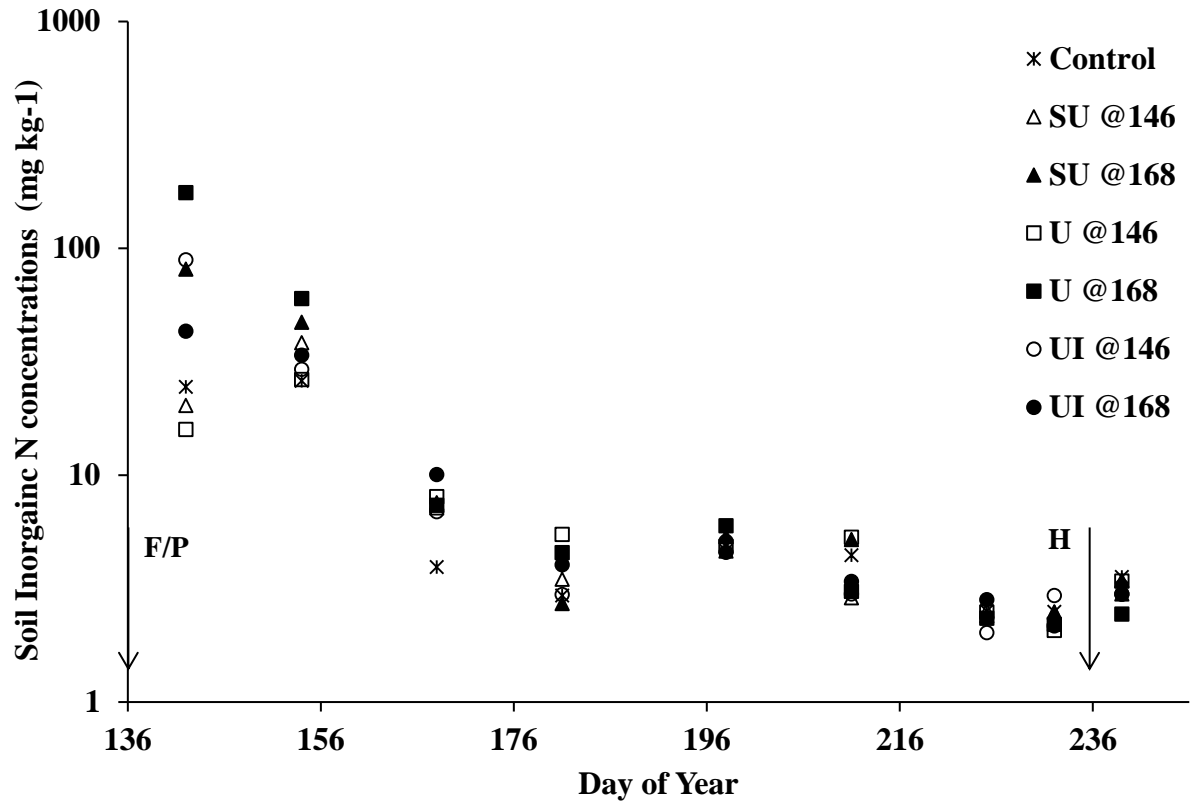


Figure 2.5. Mean Soil Inorganic N ($\text{NH}_4^+ + \text{NO}_3^-$) concentrations collected on each sampling date under different N sources and rates: no N addition (Control); urea stabilized with NBPT and DCD (SU) at 146, 168 kg N ha⁻¹; urea (U) at 146, 168 kg N ha⁻¹; and urea stabilized with nitrapyrin (UI) at 146, 168 kg N ha⁻¹. Downward pointing arrows indicate the date of planting (P), fertilizer application (F), and harvesting (H).

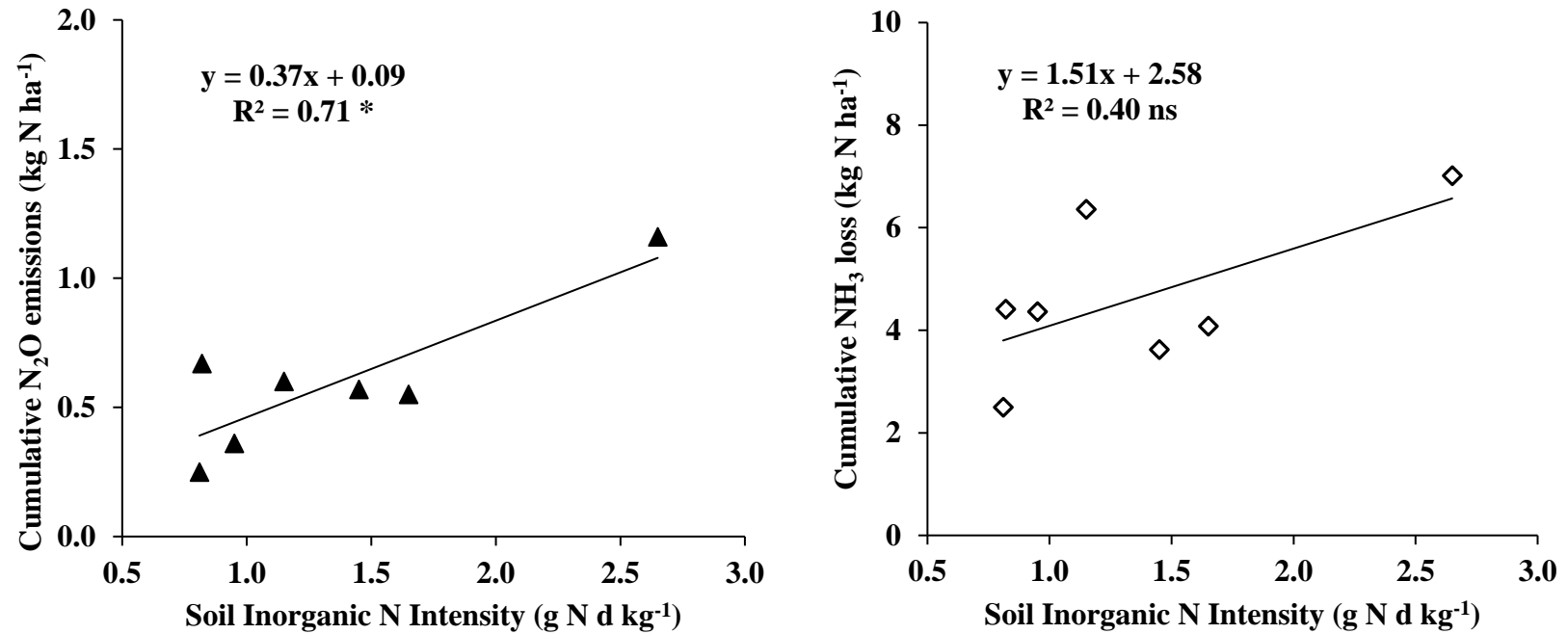


Figure 2.6. Relationship among soil inorganic N (NH₄⁺ + NO₃⁻) intensity with A) cumulative area-based N₂O-N emissions, and B) cumulative NH₃-N volatilization loss under rainfed spring wheat production system. Soil inorganic N intensity is calculated as the summation of daily inorganic N concentrations in the surface soil (0-15 cm deep) over the growing season.

Suction cup lysimeters (installed to 0.9 m depths)



Open chamber ammonia traps

anchors for nitrous oxide chambers

Image 2.1. Field plots at Glyndon, MN showing the anchors (nitrous oxide sampling), open chambers (ammonia measurements), and suction cup lysimeters (soil water nitrate sampling at 0.9 m soil depths).

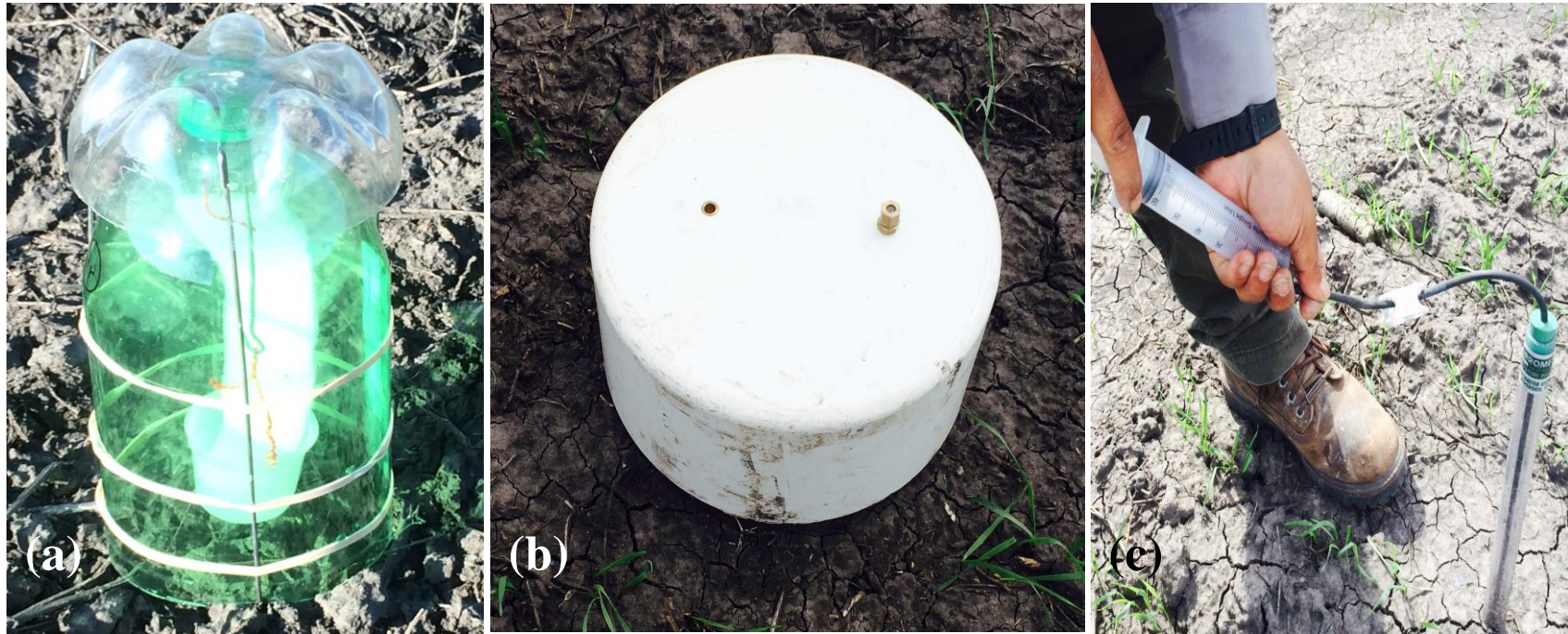


Image 2.2. (a) Open chamber ammonia traps (polyfoam strips act as ammonia traps), (b) Polyvinyl chloride chamber tops with vent and port for sampling nitrous oxide, and (c) Sampling of soil water at 0.9 m soil depths using suction cup lysimeters.



Image 2.3. Harvesting of spring wheat with small plot combine harvester at Glyndon, MN.

CHAPTER 3 - RESPONSE OF SPRING WHEAT TO SULFATE-BASED SALINITY STRESS DIFFERS BETWEEN GREENHOUSE AND FIELD CONDITIONS

Abstract

Spring wheat (*Triticum aestivum* L.), a moderately salt-tolerant crop, is often grown on saline areas worldwide. This study was conducted to compare the response of spring wheat to sulfate-based salinity stress between greenhouse and field conditions. In a greenhouse experiment, salinity treatments (control, 3.0, 5.0, 9.0, and 15.0 dS m⁻¹) were established by adding Na₂SO₄ and MgSO₄·7H₂O salts in soil-silica mixes. Similarly, field studies were conducted in four different naturally occurring sulfate-based saline fields at Richland County, North Dakota, USA during 2014-2015. In fields, soil was sampled up to 120 cm soil depths and the depth-weighted mean root-zone salinity was calculated. Results indicated variable response of spring wheat to salinity between greenhouse and field conditions. Under greenhouse conditions, shoot growth (plant height, number of tillers per plant) decreased significantly at soil ECe of 5 dS m⁻¹ and above. Similarly, root growth decreased significantly at soil ECe of 9.0 dS m⁻¹ and above. Relative kernel and straw yields were unaffected by sulfate salts up to 8.2 and 2.9 dS m⁻¹, respectively. Above the threshold value, the kernel and straw yields were declined by 12.0 and 4.9% per unit increase in soil ECe, respectively. Under field conditions, soil salinity was highly heterogeneous and the spring wheat responded by decreasing plant heights. However, the root growth and relative crop yields were maintained. The preferential root growth and water uptake from the least saline surface soil layers may result in greater salinity tolerance to crops in naturally saline fields than in uniform salinity conditions.

Abbreviations: RY, relative yields; ECe, electrical conductivity of soil determined using saturated paste extract method.

Key words: sulfate salts, shoot and root growth, absolute and relative yields, root-weighted mean salinity, spring-wheat.

Introduction

Soil salinity is one of the major environmental constraints limiting agricultural production (Rengaswamy, 2006). Salinity affects 955 million ha of land worldwide, which amounts to 10% of the total land area (Pessarakli and Szabolcs, 1999). In the Red River Valley (RRV) of North Dakota, approximately 0.60 million ha of land has been classified as either slightly or moderately saline (Hadrich, 2011). The extent of salinization in RRV is continuously increasing due to the close proximity of saline groundwater tables to the crop root zone in response to frequent wet periods (Franzen, 2007; Hadrich, 2011). Crop growth and productivity are adversely affected in salt-affected soils primarily due to osmotic stress and specific ion toxicity (Munns and Tester, 2008). Osmotic stress is due to high salt concentrations outside plant roots disrupting water uptake, while the ion toxicity effect is due to excessive accumulation of salts in the plant tissues and their inability to tolerate the salts (Munns and Tester, 2008). The level of salinity stress that a crop can withstand varies among crops species.

Wheat (*Triticum aestivum* L.) is considered to be a moderately salt-tolerant crop. Maas and Hoffman (1977) concluded that the maximum salinity (ECe: electrical conductivity determined following standard saturated paste extract method) tolerance level of wheat without any decline in grain yields is 6.0 dS m^{-1} , with an average decline of 7.1% per unit increase in soil ECe above the threshold. A study by Francois et al. (1986) also reported that the semi-dwarf bread (*Triticum aestivum* L.) as well as durum wheat (*Triticum durum* L., Durum Group) cultivars were both tolerant to soil salinity with the thresholds of 8.6 and 5.9 dS m^{-1} , respectively. In all these studies, the salinity treatments were established using chloride salts (NaCl , CaCl_2).

Many more studies were conducted using chloride salts to determine the response and tolerance mechanisms of wheat to salinity stress (Ashraf et al., 2002; Julkowska et al., 2014; Rahnama et al., 2011; Stepphun and Wall, 1997; Wilson et al., 2002; Yousfi et al., 2009, 2010; Zang et al., 2009; Zolla et al., 2010). However, wheat may respond differently to sulfate-based (Na_2SO_4 , CaSO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) salinity stress conditions predominant in RRV. When plants are subjected to chloride salts, they suffer from both Na^+ and Cl^- ion toxicities (Hasegawa et al., 2000; Munns and Tester, 2008; Tavakkoli et al., 2010). Dang et al. (2006) found that the decrease in relative grain yields of wheat was associated with increased concentrations of Cl^- rather than Na^+ in the young mature leaves. On the other hand, plants exposed to sulfate salts do not encounter Cl^- ion toxicity. Thus, sulfate salts may not be as toxic as chloride salts; but more research is needed to confirm this.

Another major drawback associated with the past studies was that most of them were conducted under controlled experimental conditions (hydroponics, sand tank cultures, and greenhouse environments) or in artificially salinized fields (Munns et al., 2002; Stepphun and Wall, 1997). However, crop responses to salinity stress under controlled environments may not correspond to those observed under real field conditions for two reasons. First, the adverse effects of salinity stress on plants grown in salt-affected fields may be exacerbated by number of other stress factors such as high diurnal temperatures, low humidity, and drought, which act simultaneously in fields (Jafari-Shabestari et al., 1993; Munns and James, 2003). Second, the soil salinity in fields is spatially and temporally heterogeneous (Lam et al., 2014); the plants are exposed to non-uniform salinity gradient and the plants suffer from varying degree of salinity stress at different growth stages. In sharp contrast, plants under controlled greenhouse experiments are exposed to uniform salinity gradient throughout their growth stages. Despite the

possibility of variable responses of crop to salinity stress between greenhouse and field experiments, very little effort has been made to compare crop responses under these experimental conditions. Therefore, the main objective of this study was to compare the responses of spring wheat to sulfate-based salinity stress under greenhouse and field conditions. We hypothesized greater tolerance of crops to salinity stress in non-uniform salinity conditions (field studies) than in uniform salinity conditions (greenhouse study).

Materials and Methods

Greenhouse study

The controlled greenhouse study was conducted in a completely randomized design at the Agricultural Experiment Station Greenhouse Facility at North Dakota State University. The soil for this experiment was collected from 0-15 cm depths from an agricultural field near Wyndmere, North Dakota, USA. The soil was classified as very deep, somewhat poorly drained Glyndon series (Coarse-silty, mixed, superactive, frigid Aeric Calciaquolls) (Soil Survey Staff, 2014). The soil was air-dried, and ground to pass through 2 mm sieve before conducting any analysis. Basic soil properties were: nitrate-N, 19 kg ha⁻¹; Olsen-P, 23 mg kg⁻¹; 1 N ammonium acetate-K, 340 mg kg⁻¹; pH, 5.6; EC, 0.31 dS m⁻¹; and sodium absorption ratio (SAR), 0.17.

A 50:50 mixture of the non-saline, non-sodic Glyndon series soil and 2040 grade silica sand (TCC materials, Mendota Heights, MN) was used as growth medium. In a plastic bag, 500 g each of soil and silica sand were separately weighed and moistened silica was mixed with soil. The soil-silica mixtures were divided into two equal halves. A known quantity of soluble sulfate salts (Na₂SO₄, MgSO₄·7H₂O) was added to the soil-silica mixes to create artificial salinity gradient ranging from 0.3 to 15.3 (ECe) dS m⁻¹ (Table 3.1). For simplicity, these salinity treatments were hereafter referred as control, 3.0, 5.0, 9.0, and 15.0 dS m⁻¹. All of the soluble

salts were added to lower half of soil-silica mixes and the other salt-free half of soil-silica mixture was added from the top to ensure good seed germination. The soil-silica mixture was established in a plastic bag kept inside the pot to prevent leaching of salts. Each salinity treatment was replicated ten times.

Spring wheat variety 'Faller' was planted at the rate of 8 seeds per pot on 23 January 2015. After planting, the nutrient solution (160 mg of Urea dissolved in 125 ml water; per soil testing recommendation) was uniformly added from the top to achieve 12% gravimetric water content. The pots were watered frequently (every 3 days in the beginning and every 1 day later in the experiment as the wheat matured) gravimetrically to prevent the plants from experiencing drought stress. Plants were thinned to 4 seedlings per pot 8 days after planting (DAP). The day and night temperature in the greenhouse were maintained at 18.3-21.1°C (16 hours) and 15.5-18.3°C (8 hours), respectively.

Plant measurements

Number of tillers per plant and chlorophyll content (using a SPAD 502 plus Chlorophyll meter, Spectrum Technologies, Inc.) were recorded for individual plants at 42 DAP. Plant height was measured at harvest. At harvest, the kernel and straw of all the four plants from each pot were bagged separately. The kernel and straw were dried at 70 °C for 48 hours before recording oven-dried biomass.

For root analysis, soil along with root tissues were gently washed with tap water using nested 4 mm mesh sieves. Root materials were collected from both sieves using tweezers. The cleaned individual roots were transferred in a 20 × 30 cm tray and spread across the tray such that the overlapping between the root tissues was minimized. The tray was then placed on a dual-scan optical scanner (Regent Instruments, Inc.) and the gray-scale root images were obtained at

800 × 800 dpi resolution. Analysis of the root images was conducted using WinRHIZO Pro software (Regent Instruments, 2009). The cumulative root length, root surface area and root volume were used for further analysis. After scanning, the root tissues were collected in plastic pans and dried for 24 hours at 55°C for recording root dry biomass.

Data analysis and Statistics

The effects of soil salinity on shoot growth, root growth and yield parameters were determined by running anova test in R statistical environment (R Core Team, 2014). When the main effect was significant, multiple comparisons of means among salinity treatments was conducted by performing post-hoc Tukey's test using multcomp package (Hothorn et al. 2008). Significant differences were mentioned at $P < 0.05$, unless otherwise stated.

To determine the salinity response curve for wheat, the relative yields (Y_r) were calculated by dividing the absolute yields (Y) obtained at respective ECe levels by the average yields (Y_m) obtained at control (normal) treatments. The relative yields normalize the dataset. The relative yields were then regressed with soil ECe using the sigmoidal response model in SigmaPlot version 13.0. The sigmoidal response model was first proposed by van Genuchten (1983) and is given as (Equation 3.1):

$$Y_r = 1 / [1 + (C/C_{50})^p] \quad (3.1)$$

Where Y_r is the relative grain or straw yields; C is the soil ECe level; C_{50} is the ECe level at which the grain or straw yield is reduced by 50%; and p is the shape parameter. The 'p' can be substituted by $[\exp (s.C_{50})]$, where 's' is a steepness parameter.

Field study

Site location and characteristics

Four studies were conducted on farmers' fields in Richland county, eastern ND, USA during 2014 and 2015 growing seasons (Fig. 3.1). Each of these four fields has been historically identified as saline. The dominant soil in the study region was a poorly drained, slowly permeable Fargo silty clay loam (a fine, smectitic, frigid Typic Epiaquerts) formed in calcareous, clayey lacustrine sediments (Soil Survey Staff, 2014). In 2014, the field experiment was conducted on Soil Health and Agriculture Research Extension (SHARE) Farm. The SHARE Farm is a long-term ongoing research farm established to answer the fundamental soil health and management issues faced by the ND producers. In 2015, three more research trials were conducted in the nearby wheat fields. Spring wheat was planted and raised by growers following conventional practices (Table 3.2).

Unlike in many past research studies, where salinity gradient was created by irrigating with poor quality water, these fields are naturally saline. Frequent wet periods since 1993 raised saline groundwater tables, resulting in the accumulation of salts in crop root zone. The predominant salts were hydrated form of Na and Mg sulfates (Keller et al., 1982). In-depth analysis of major cations and anions in the soil samples collected from SHARE farm further suggested that the primary salts in eastern ND region were sulfates (SO_4^{2-}) of sodium (Na^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) (Derby et al., 2014).

Transect and sampling points establishments

The salinity map for each site was initially developed in ArcGIS 10.2 (ESRI) through apparent electrical conductivity (ECa) mapping (Fig. 3.2). The ECa measurements were taken during relatively moist field conditions using EM38 electromagnetic induction meter (Geonics

Limited, Ontario, Canada) vertical readings on 5 m spacing. The ECa maps indicated that the soil salinity in the selected fields were spatially heterogeneous. This provided us the unique opportunity to determine the impact of natural sulfate-salts based salinity gradient on spring wheat growth and yield parameters. Using the spatial patterns of the soil ECa maps, transects (each 100 m in length) were randomly delineated in each field along the salinity gradient. At each transect, four sampling points were marked at 33 m intervals to represent varying levels of root-zone salinity.

Plant measurements

At each sampling point on established transects, wheat response to root zone salinity was determined by measuring the above-ground parameters (plant height, chlorophyll content), below-ground parameters (root length, root surface area, root volume, root dry biomass), and yield parameters (above-ground biomass, grain yield, protein content). For above-ground parameters, the plant height and the chlorophyll content was recorded from the five randomly selected plants at each point. All measurements taken at each sampling point were averaged before relating to root-zone salinity.

For root-growth parameters, a soil core (3.6 cm inner diameter) was collected to 120 cm depth after wheat harvest from the center of each sampling point with a truck-mounted Giddings hydraulic probe (Image 3.1). The soil core was divided and bagged separately at incremental depth intervals: 0-15, 15-30, 30-60, 60-90 and 90-120 cm. Soil from each depth intervals was soaked in water plus 5% sodium hexametaphosphate solution for 24 hours to disperse soil particles and facilitate root washing. The extraction of root tissues from the soil and its further analysis was conducted by following the procedure as discussed earlier.

For yield parameters, wheat was hand-harvested at maturity from the plots at each sampling point (1.5 m by 1.5 m) and dried at 70 °C for 48 hours. The above-ground biomass was recorded. The wheat was then threshed by passing it through the combine harvester; the wheat grains were collected, cleaned, and weighed. The grain samples were then analyzed for protein content using Infratec 1241 Grain analyzer (FOSS analytical AB, Hoganas, Sweden). Both the grain yield and protein content were adjusted and reported at 12% moisture level.

Soil measurements

Three additional soil cores (3.6 cm inner diameter) were sampled to 120 cm with a truck-mounted Giddings hydraulic probe at each point (Giddings machine company, CO, USA; Image 3.1). The soil cores were divided at varying depth intervals as mentioned earlier, composited, and then transported back to the laboratory. The composited soil cores were homogenized, air-dried, and ground to pass through 2 mm sieve. Electrical conductivity (ECe) and pH of the soils was determined following saturated paste extract method (Whitney, 1998).

Data analysis and statistics

To determine the response of wheat to salinity under field conditions, the plant parameters were averaged for each sampling point and then regressed against the root-zone salinity. The root-zone salinity was determined as depth-weighted mean salinity, calculated by using the following equation 3.2:

$$\text{Depth – weighted mean salinity} = \frac{\sum_{i=1}^n (\text{ECe} \times D)_n}{\sum_{i=1}^n (D)_n} \quad (3.2)$$

where n is the number of soil layers, ECe and D represent the soil salinity and soil depth in nth soil layer, respectively. Relative yields were calculated by dividing the absolute yields by the maximum yield obtained in each year. The relative yields standardize the data and facilitate data comparison across sites and years. The effect of depth-weighted ECe on shoot, root and harvest

parameters of spring wheat was determined by performing linear mixed effects analysis using lme4 (Bates et al., 2015) package in R statistical environment (R Core Team, 2014). Depth-weighted ECe was treated as fixed effect. As random effects, we had intercepts for transects nested within fields. P-values were obtained by likelihood ratio tests of the full model (including both fixed and random effects) against the intercept-only null model (only random effects without fixed effect component in the model). To determine the proportion of variance explained by the fixed effect, marginal R-squared values were calculated with a script based on sem.model.fits from R package piecewiseSEM (Lefcheck, 2015).

Results

Greenhouse study

Shoot growth parameters

The effect of salinity on plant height, chlorophyll content, and number of tillers per plant are presented in Table 3.3. The plant height measured at maturity was significantly reduced by soil ECe above 3.0 dS m⁻¹. Plant height decreased by 4.4% and 5.5% at 5.0 and 9.0 dS m⁻¹, respectively, compared to the control treatment. Chlorophyll content increased linearly with increasing salinity. Compared to the salt-free control treatment, soil ECe at 3.0, 5.0, and 9.0 dS m⁻¹ significantly increased chlorophyll content by 9.1, 13.3, and 20.2%, respectively. The number of tillers per plant decreased significantly at higher levels of soil salinity. At 5.0 and 9.0 dS m⁻¹, the number of tillers per plant decreased by 14.6 and 29.3%, respectively, compared to the control treatment.

Root growth parameters

Soil salinity significantly reduced root growth at higher ECe levels (Table 3.4). At lower soil ECe levels less than 5.0 dS m⁻¹, the cumulative root length, root surface area, and root

volume were similar. At 9.0 dS m⁻¹, the cumulative root length, root surface area, and root volume were decreased by 19, 26, and 33 %, respectively, compared to the control treatment.

Absolute and relative yields

Root, straw, above-ground, and total biomass were statistically similar up to 3.0 dS m⁻¹, but were reduced at higher ECe levels (Fig. 3.3). At 5.0 and 9.0 dS m⁻¹, root biomass was significantly reduced by 22 and 29%, respectively, compared to the control treatment. Similarly, straw yield was significantly decreased by 12 and 30% at 5.0 and 9.0 dS m⁻¹, respectively. The above-ground and total biomass were also reduced by 8 and 10%, respectively, at 5.0 dS m⁻¹ and by 22 and 23% at 9.0 dS m⁻¹, respectively, compared to the control treatment. Kernel yield showed no significant reduction up to 5.0 dS m⁻¹. But at 9.0 dS m⁻¹, kernel yield was reduced by 15% relative to the salt-free control treatment.

The relative kernel and straw yields decreased non-linearly with increasing soil ECe (Fig. 3.4). The relative yields were calculated by dividing the absolute yields by the mean kernel yield (4.7 g pot⁻¹) and mean straw yield (6.4 g pot⁻¹) for the salt-free control treatment. The sigmoidal-shaped response function proposed by van Genuchten (1983) showed good fits of relative yields with increasing root-zone salinity. Based on the fitted curve, the threshold soil ECe at which the kernel yield started to decline was 8.2 dS m⁻¹ (Fig. 3.4a). The kernel yield decreased sharply at root-zone salinity above the threshold value. The kernel yield was reduced by 20% and 50%, respectively, at 10 and 12 dS m⁻¹, respectively. Straw yield was more sensitive to ECe than kernel yield, with the threshold ECe at 2.9 dS m⁻¹ (Fig. 3.4b). Above the threshold ECe, the straw yield showed a general decline. As per the fitted function, the straw yield was decreased by 50% at 12.7 dS m⁻¹.

Field Study

Soil Salinity

Apparent electrical conductivity (ECa) was highly variable and ranged from 0.3 to 12.8 dS m⁻¹ across four fields. Variability in soil salinity was also observed with depths as evident from differences in ECe values (minimum and maximum) (Table 3.5). The high coefficient of variation (CV) of 60 to 118% further confirmed the spatial heterogeneity in ECe at various soil depths over the study sites. Averaged across four fields, the mean ECe increased from 0.6 dS m⁻¹ at surface soils to 4.8 dS m⁻¹ at deeper soils.

Descriptive statistics of ECe within the same field indicated that the soil salinity changes dramatically over a short distance, even below 100 meters (Fig. 3.5). For example in Field 1, the ECe ranged from 0.4 to 3.5, 0.5 to 5.8, 0.6 to 7.9, 1.7 to 9.1, and 0.5 to 8.6 dS m⁻¹ within 0-15, 15-30, 30-60, 60-90, and 90-120 cm depths, respectively. The depth-weighted ECe ranged from 0.8 to 6.6 dS m⁻¹ for Field 1, 0.4 to 5.4 dS m⁻¹ for Field 2, 0.6 to 4.1 for Field 3, and 1.1 to 6.6 dS m⁻¹ for Field 4, respectively.

Shoot growth parameters

The plant height ranged from 75 to 88 cm in Field 2, 86 to 105 cm in Field 3, and 74 to 85 cm in Field 4, respectively. When the datasets were classified based on the commonly accepted threshold ECe (6.0 dS m⁻¹), the average plant height was 85 (74 to 105) and 80 (77 to 83) cm below and above 6.0 dS m⁻¹, respectively. Our analysis showed that the depth-weighted ECe affected plant height ($\chi^2=12.9$, $p<0.001$), lowering it by 1.20 ± 0.29 cm per unit increase in depth-weighted ECe (Fig. 3.6).

There was no significant effect of depth-weighted ECe on leaf chlorophyll contents ($\chi^2=0.95$, $p=0.33$). Numerically, the mean chlorophyll content at depth-weighted ECe below and

above 6.0 dS m^{-1} were 47 and 43, respectively and ranged from 42 to 52 and 42 to 44, respectively.

Root growth parameters

Root growth of spring wheat was mainly concentrated in the upper 0 to 60 cm soil depths. Approximately 80 to 90% of the total root surface area was observed in 0 to 60 cm soil depths. There was a high degree of spatial variability in root-growth within and across fields. The root growth parameters decreased with depth. The cumulative root surface area ranged from 6 to 56 cm^2 for 0-15 cm, 4 to 31 cm^2 for 15-30 cm, 4 to 45 cm^2 for 30-60 cm, 3 to 19 cm^2 for 60-90 cm, and 1 to 4 cm^2 for 90-120 cm soil depths, respectively. Similar trends were found with respect to root length, root volume, and root biomass (data not shown).

The growth of root at each soil depths appeared to be affected by soil ECe levels. For example, the cumulative root length, root surface area, root volume, and root biomass were comparatively less at all soil depths for Field 1 which also showed relatively higher ECe levels compared to other fields (data not shown). There was no significant effect of depth-weighted ECe on any of the root growth parameters. Numerically, the mean root surface area across all fields decreased from 64 to 49 cm^2 at depth-weighted ECe below and above 6.0 dS m^{-1} . Similarly, the mean root volume and root biomass decreased from 0.50 to 0.30 cm^3 and 0.15 to 0.07 g, respectively. However, the mean root length increased from 736 to 750 cm when the ECe was increased above 6.0 dS m^{-1} .

Absolute and relative yields

In 2014, the average grain and protein yields were 3.9 and 0.5 Mg ha^{-1} , respectively and ranged from 2.2 to 5.0 and 0.4 to 0.6 Mg ha^{-1} , respectively. The protein content ranged from 406 to 553 g kg^{-1} and averaged to 144 g kg^{-1} . Similarly in 2015, the average grain and protein yields

were 3.3 and 0.5 Mg ha⁻¹, respectively, and ranged from 2.00 to 4.3 and 0.3 to 0.6 Mg ha⁻¹, respectively. The straw and above-ground biomass averaged to 6.7 and 10.0 Mg ha⁻¹, respectively and ranged from 4.1 to 9.7 and 6.1 to 13.5 Mg ha⁻¹, respectively.

When data were pooled across both years, the average grain yields at depth-weighted ECe below and above 6.0 dS m⁻¹ was 3.40 and 4.08 Mg ha⁻¹, respectively. However, the average protein yields were similar at depth-weighted ECe below (0.48 Mg ha⁻¹) and above (0.47 Mg ha⁻¹) 6.0 dS m⁻¹. When the relative grain and straw yields were plotted against the depth-weighted ECe, the response was best captured by the quadratic regression curve but the relation was not significant (Fig. 3.7a, b).

Discussion

Soil salinity in greenhouse and field studies

The nature of soil salinity in greenhouse and field studies was quite different. In the greenhouse study, in which the artificial salts were added in 1 kg of soil-silica, salts were accumulated within the top 0 to 10 cm soil depths. As a result, the roots of spring wheat were subjected to uniform salinity gradient and the plants suffered from salinity stress earlier and for a longer duration. In sharp contrast, soil salinity was greatly heterogeneous in field conditions. The soil ECe was variable both horizontally and vertically. The roots of spring wheat were, therefore, exposed to non-uniform salinity gradient in the fields.

The salinization process occurring in the study region was distinct from most of the previous field studies in which salinization were of secondary origin (Francois et al., 1986). The secondary salinization was due to the use of poor quality irrigation water and the soil ECe decreased with soil depths. In sharp contrast, soil ECe increased with soil depth across study sites in both years. Soil ECe values were lowest on the surface 0-15 cm soil layer and were

highest at the deeper 60-90 and 90-120 cm soil layers (Fig. 3.5). This trend in soil EC_e suggested that the source of soluble salts in the studied fields was a shallow and saline groundwater table coming in contact with soluble salts within the soil profile at depth. Frequent wet periods in this region raised the ground water table containing soluble salts. Soluble salts from such shallow groundwater were carried at or near the soil surface through capillary rise (Franzen, 2013). Salts subsequently accumulate within the crop root-zone when the soil water evaporates (Bakker et al., 2010; Franzen, 2013). Other research (Choudary et al., 2008; Devkota et al., 2015) indicates surface salt accumulation in bare soils over shallow and saline groundwater table is directly proportional to the rate of evaporation. Thus, the extent of salinization in the study region could be minimized by reducing the rate of evaporation or by preventing the rise of saline groundwater tables. Evaporation rates can be reduced by raising cover crops during the fallow period or through crop residue retention (Devkota et al., 2015; Forkutsa et al., 2009). Similarly, raising deep-rooted crops and installing sub-surface tile drainage helps to prevent the upward movement of salts and also the rise of saline groundwater tables.

Impact of soil salinity on shoot growth

Soil salinity inhibits plant growth and yield by two mechanisms: a rapid osmotic phase followed by a slower ion toxicity phase (Munns and Tester, 2008). In this study, the effect of salinity was observed in terms of decrease in plant height under both greenhouse and field experiments. Based on the results from greenhouse study, threshold soil EC_e at which the reduction in plant height occurred is between 3.0 and 5.0 dS m⁻¹. There are numerous reports of decreased plant height with increasing salinity. For example, Stepphun and Wall (1997) conducted an experiment in water tanks using hydroponic solution and found that plant height of different spring wheat cultivars decreased linearly with increasing salinity in similar EC_e ranges

to this study. The only difference was the use of chloride salts (NaCl and CaCl₂) to establish different salinity treatments, while this study used sulfate salts (Na₂SO₄, MgSO₄.7H₂O). The decrease in plant height at higher salinity levels was also reported in more recent studies (Ashraf et al., 2002; Wilson et al., 2002; Yousfi et al., 2009, 2010). Under field studies where the different salinity plots were established by adding NaCl and CaCl₂ in irrigation water, Francois et al. (1986) observed that plant height of both bread and durum wheat species were reduced with increasing soil salinity. Fowler and Hamm, (1980) conducted an experiment on naturally saline fields dominated by sulfate salts (CaSO₄, Na₂SO₄ and MgSO₄) and found that plant height of spring wheat started to decline at soil ECe of 4.1 dS m⁻¹. Their findings matched the trend observed in this study.

The chlorophyll content has been considered as the most simple, non-destructive and practical way of screening large number of genotypes for salinity tolerance (El-Hendawy et al., 2007; Munns and James, 2003). Generally, salinity-induced stress decreased chlorophyll content (Parida et al., 2004; Yousfi et al., 2010). In the current study, the effects of soil salinity on chlorophyll contents greatly vary between greenhouse and field conditions. In greenhouse, soil ECe significantly increased chlorophyll contents. In fields, we found no effect of depth-weighted ECe on chlorophyll contents. This variation in the response of chlorophyll content might be associated with the differences in experimental conditions. Under greenhouse conditions, plants experienced salinity stress much earlier due to the close proximity of salts to roots as compared to field conditions where salinity was at lower depth and highly variable. Leaf growth rate was greatly inhibited and thus, any uptake of N from soil might have been concentrated to smaller leaf area. Higher leaf N concentrations at higher soil ECe were also observed in corn and soybeans in a similar pot experiment (Heglund et al., 2013). Greater leaf N concentrations in

wheat leaves grown in pots ultimately resulted in darker and succulent leaves with higher chlorophyll content. Furthermore, increase in chlorophyll content at higher salinity levels might be attributed to reduction in relative leaf tissue water contents (Wang and Nil, 2000).

Root-zone salinity reduces the number of tillers per plant by hindering the development of primordia (Grieve et al., 1993), leading to reduction in absolute yields (Mass and Grieve, 1990; Mass et al., 1996). Pearson correlation coefficients in this study also support these hypotheses. Tillers per plant were negatively correlated with soil ECe ($r=-0.60$). There was a positive correlation between tillers per plant and kernel yield ($r=0.37$) as well as between tillers per plant and straw yield ($r=0.61$).

Impact of soil salinity on root growth

The response of plants to salinity stress greatly depends upon its ability to adjust root morphology under stress conditions (Julkowska et al., 2014). Therefore, characterizing the response of root growth parameters under stress conditions would help predict the overall performance of crops. Rahnama et al. (2011) observed that the increase in root-zone salinity to 150 mM NaCl significantly decreased the total root length of three out of four wheat cultivars. Results from the greenhouse experiment conducted in this study also suggested that the root growth parameters (total root length, root surface area, and root volume) were significantly reduced at higher levels of sulfate-based salinity (9.0 dS m^{-1}). The decrease in total root length at higher salt concentrations may be attributed to the decrease in the length of primary rather than lateral roots. There are multiple reports which reported that under high salt stress, the growth of primary roots was severely affected, whereas the growth of lateral root was stimulated (Julkowska et al., 2014; Rahnama et al., 2011; Zang et al., 2009; Zolla et al., 2010). Salt stress suppressed the growth of primary roots by reducing the activity of apical meristem cells (West et

al., 2004). Whereas the growth and proliferation of lateral roots was triggered due to the salt-mediated transport of auxin and phloem water from shoot to lateral root tips (Boyer et al., 2010; Zolla et al., 2010). Under moderate stress (25 mM NaCl \approx 2.5 dS m⁻¹), Zolla et al., (2010) observed that the moderate level of soil salinity may stimulate primary root growth. Although not significant, numerically higher root growth at moderate level of soil salinity (3 dS m⁻¹) was observed in this study. The stimulatory effect of low salinity on root growth may be due to increase in osmotic potential of apical meristem cells, enhancing cell elongation and cell division (Zolla et al., 2010). Results for root biomass in this study also supported the hypothesis that primary but not lateral roots are affected under salt stress. Despite having similar total root length, the root biomass decreased significantly at 5.0 dS m⁻¹ compared to the control treatment. This was possible only if the total root system was dominated by lateral roots at 5.0 dS m⁻¹ which are generally thinner and lighter in weight compared to primary roots.

Under field conditions, the depth-weighted root-zone salinity did not showed any significant relation with root growth. First, this may be a result of low soil ECe levels in the top 0-60 cm soil profile where more than 80% of root growth occurred. Second, roots at low salinity, surface soil layers may supply sufficient water and nutrients to roots at high salinity, depth layers to enable their growth even under high external salt concentrations.

Impact of soil salinity on yields

Impacts of soil salinity on yields varied under greenhouse and field conditions. Under greenhouse conditions, the above-ground and total biomass started to decline earlier than the grain yield, presumably due to greater sensitivity of straw yield to soil salinity. The threshold soil ECe for straw yield was 2.9 dS m⁻¹, which was much lower than that for kernel yield (8.2 dS m⁻¹) (Table 3.5). Average decline in kernel and straw yields per unit increase in soil ECe beyond

respective thresholds were 12.0 and 4.9%, respectively. Our values were slightly higher than the globally accepted threshold ECe value of 6.0 dS m⁻¹ and -7.1% for wheat grain yield (Maas and Hoffman, 1977). Maas and Hoffman (1977) noted that the latter values can be applied only when wheat is salinized to uniform salinity of chloride salts throughout the crop growth stages. Also, the threshold ECe obtained in this greenhouse study was higher than those obtained in other studies where chloride salts was used (Francois et al., 1986; Stepphun and Wall, 1997) (Table 3.5). These observations suggest greater tolerance of spring wheat to sulfate salts compared to chloride salts. Under sulfate salts (Na₂SO₄, MgSO₄·7H₂O), plants may be subjected to Na⁺ toxicity alone. However, plants stressed with chloride salts (NaCl, CaCl₂) may be subjected to both Na⁺ and Cl⁻ ion toxicities (Munns and Tester, 2008).

Furthermore, variation may have been resulted from differences in experimental conditions and the varietal differences among studies (Table 3.6). Spring wheat may have greater salt tolerance under soil systems, resulting in higher threshold soil ECe than in a study by Stepphun and Wall (1977). Stepphun and Wall (1997) conducted a greenhouse experiment in hydroponics using chloride salts and obtained much smaller threshold ECe value (<3.0 dS m⁻¹) for grain yield. Tavakkoli et al. (2010) also reported growth reductions and uptake of inhibitory ions such as Na⁺ and Cl⁻ in barley were more severe under hydroponics than in soil systems. They concluded that the cation exchange capacity of soil reduces salt stress by facilitating the adsorption of Na⁺ in the surfaces of soil colloids, while the soil buffering capacity provide enough time for the plants to acclimate salt stress.

Under field conditions, no significant reduction in relative grain, protein, and straw yields was obtained up to depth-weighted ECe of 7.5 dS m⁻¹. This correlated with the findings from the greenhouse study where 8.2 dS m⁻¹ was obtained as the maximum tolerance ECe level without

any reduction in grain yields. Even at depth-weighted ECe above 8.2 dS m⁻¹, the relative crop yields might not be reduced in naturally saline fields. This is based on our observation that soil ECe increased gradually with depths in naturally saline fields and the bulk of plant root systems encountered lower levels of soil salinity on the surface soil layers. Similar observation was made by Rahnama et al. (2011) in an artificially created NaCl gradient using germination paper in plastic tubes. This nature of salinity not only avoid crops from facing salinity stress during the early growth stages, but also minimizes its negative effects by facilitating compensatory water and nutrient uptake from the low salinity side. Bazihizina et al. (2009) observed that exposing one-half of halophyte (*Atriplex nummularia*) roots in 10 Mm NaCl maintained shoot growth, shoot water potential and net photosynthesis even if the other root-half from the same plant was exposed to 670 mM NaCl due to preferential water uptake from the low salinity side. Thus in the current field studies, the bulk of the plant water may be taken up from the less saline, shallow soil layers alluding greater tolerance of plants to salinity stress in the field than in uniform salinity conditions as that in greenhouse pot experiment. More research in fields with much higher levels of salinity or in greenhouse with non-uniform salinity gradients is needed to confirm this. However, this hypothesis may not be true in all cases as salinity stress is accompanied by numbers of other biotic and abiotic stresses such as drought and heat stresses in fields.

Conclusions

Results from this study demonstrated that spring wheat responded differently to salinity stress under greenhouse and field conditions. Under greenhouse conditions, spring wheat responded to salt stress by decreasing shoot growth (plant heights, number of tillers per plant), root growth (root length, root surface area, root volume, root biomass), absolute and relative

yields (straw, kernel). The threshold sulfate-based salinity level obtained under greenhouse conditions (8.2 dS m^{-1}) was higher than the globally accepted chloride-based threshold (6.0 dS m^{-1}), suggesting the greater tolerance of wheat to sulfate salts than chloride salts.

Under field conditions, plants were exposed to both horizontally and vertically non-uniform salinity gradients. The response to salt stress was seen in terms of decrease in plant heights but the root growth and relative yields were maintained. Given that majority of the plant roots are concentrated in surface soil layers where soil salinity is relatively low compared at deeper depths, depth-weighted mean root-zone salinity may possibly overestimate the actual salinity stress experienced by plant roots. Furthermore, the preferential uptake of water from shallow and least saline soil layers act as osmotic adjustment mechanism for crops. Thus, crops can have greater salinity tolerance and can withstand higher soil salinities in fields than the threshold values determined under uniform salinity conditions in a greenhouse experiments. Future controlled greenhouse experiments should be conducted by developing non-uniform salinity gradients to evaluate the performance of crops under heterogeneous salinity conditions.

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Table 3.1. Salinity levels achieved after the addition of soluble sulfate salts in a soil-silica mix for a greenhouse experiment.

Target EC _{1:1} dS m ⁻¹	Amount of salts added per kg of soil-silica mix		Achieved EC _{1:1} dS m ⁻¹	Estimated EC _e † dS m ⁻¹
	Na ₂ SO ₄	MgSO ₄ ·7H ₂ O g		
0.3	0.0	0.0	0.38 ± 0.13	0.33 ± 0.14
2.0	1.3	1.2	1.76 ± 0.21	2.86 ± 0.40
3.0	3.1	2.7	3.09 ± 0.59	5.45 ± 1.15
4.0	4.7	4.1	4.93 ± 0.54	9.05 ± 1.05
8.0	9.4	8.2	8.14 ± 0.47	15.3 ± 0.92

† The EC_e was estimated by using the linear equation: $EC_e = 1.98 \times EC_{1:1} - 0.78$ obtained for fine textured soil (Fig. A1). The ± represents the standard deviation (n=10).

Table 3.2. Experimental conditions and management practices at four field site-years.

Variables	Field 1	Field 2	Field 3	Field 4
Year	2014	2015	2015	2015
Latitude	46°13'35.04" N	46°14'48.49" N	46°14'48.49" N	46°12'59.23" N
Longitude	96°53'25.08" W	96°50'21.55" W	96°50'21.55" W	96°51'09.03" W
Dominant soil series	Fargo	Fargo	Fargo	Fargo
Soil type	Silty clay loam	Silty clay loam	Silty clay loam	Silty clay loam
Spring wheat variety	Prosper	Forefront	Prosper	Forefront
Planting date	4/21/2014	4/2/2015	4/1/2015	4/2/2015
Seeding rate (kg ha ⁻¹)	135	118	118	118
N-P-K (kg ha ⁻¹)	135-56-0	150-0-0	140-34-0	140-22-0
Harvest date	7/30/2014	8/5/2015	8/5/2015	8/5/2015
Total rainfall (mm) †	294	273	273	273

† **Source: North Dakota Agricultural Weather Station network (<https://ndawn.ndsu.nodak.edu>).**

Table 3.3. Impact of salinity gradient on: (a) plant height, (b) chlorophyll content on the third leaves from the top, and (c) number of tillers per plant, under greenhouse conditions.

Salinity gradient (ECe)	Plant height	Chlorophyll content	Number of tillers per plant
<u>dS m⁻¹</u>	<u>cm</u>		
Control	58.5 ± 2.12 A	45.4 ± 1.80 D	4.10 ± 0.44 A
3.0	59.0 ± 2.75 A	49.6 ± 2.50 C	3.85 ± 0.49 AB
5.0	55.9 ± 1.91 B	51.5 ± 2.15 B	3.50 ± 0.55 B
9.0	55.3 ± 1.64 B	54.6 ± 1.22 A	2.90 ± 0.60 C

The ± sign represents the standard deviation (n=10). Columns with different uppercase letter were significantly different at p≤0.05.

Table 3.4. Impact of salinity gradient on root parameters of wheat under greenhouse conditions.

Salinity gradient (ECe)	Root length	Root surface area	Root volume
<u>dS m⁻¹</u>	<u>m</u>	<u>cm²</u>	<u>cm³</u>
Control	46.8 ± 7.25 A	743 ± 133 A	9.57 ± 2.67 A
3.0	49.4 ± 2.96 A	778 ± 88.5 A	9.89 ± 2.14 A
5.0	44.3 ± 7.18 A	714 ± 122 A	9.32 ± 2.44 A
9.0	37.8 ± 5.59 B	549 ± 111 B	6.41 ± 1.74 B

The ± sign represents the standard deviation (n=10). Columns with different uppercase letter were significantly different at p≤0.05.

Table 3.5. Descriptive statistics of electrical conductivity (ECe) across four field sites.

Soil depths	Mean	Min.	Max.	SD †	CV ‡
Cm	dS m⁻¹				%
0-15	0.55	0.15	3.46	0.51	92.9
15-30	0.92	0.09	5.84	1.09	118
30-60	2.25	0.10	7.90	2.26	101
60-90	3.99	0.34	9.12	2.83	70.8
90-120	4.82	0.17	10.4	2.88	59.7

† SD represents the standard deviation.

‡ CV represents the coefficient of variation.

Table 3.6. Comparison of salinity tolerance of wheat under different experimental conditions and salt types.

Experimental setup	Wheat type	Common salts	Three-piece linear parameters †		Sigmoidal response parameters ‡				References
			C _t dS m ⁻¹	b slope % (dS m ⁻¹) ⁻¹	C ₅₀ dS m ⁻¹	p shape	s steepness	ST- Index	
Grain yield									
A. Greenhouse									
Hydroponics	Spring wheat	NaCl, CaCl ₂	<3	12.2-17.9	2.8-6.1	1.7-3.7	0.186- 0.273	3.3- 7.4	Stepphun and Wall, 1997
Soil systems	Spring wheat	Na ₂ SO ₄ , MgSO ₄ .7H ₂ O	8.2	12.0	11.9	8.9	0.183	14.1	This study
B. Fields									
Artificially salinized	Wheat	NaCl, CaCl ₂	6.0	7.1	12.6	3.9	0.108	14.0	Maas and Hoffman, 1977
	Durum wheat	NaCl, CaCl ₂	5.9	3.8	18.6	2.9	0.058	19.7	Francois et al., 1986
	Semi-dwarf wheat	NaCl, CaCl ₂	8.6	3.0	24.7	3.1	0.046	25.8	
Naturally saline	Spring wheat	Na ₂ SO ₄ , MgSO ₄	4.1	10.7	-	-	-	-	Fowler and Hamm, 1980
	Winter wheat	Na ₂ SO ₄ , MgSO ₄	3.4	11.5	-	-	-	-	
Straw yield									
A. Greenhouse									
Soil systems	Spring wheat	Na ₂ SO ₄ , MgSO ₄ .7H ₂ O	2.9	4.9	12.7	2.6	0.075	13.6	This study
B. Fields									
Artificially salinized	Durum wheat	NaCl, CaCl ₂	3.2	2.5	22.7	2.4	0.038	23.5	Francois et al., 1986
	Semi-dwarf wheat	NaCl, CaCl ₂	4.5	2.6	23.2	2.5	0.040	24.1	
Naturally saline	Spring wheat	Na ₂ SO ₄ , MgSO ₄	4.2	11.0	-	-	-	-	Fowler and Hamm, 1980
	Winter wheat	Na ₂ SO ₄ , MgSO ₄	3.4	11.0	-	-	-	-	

† The three-piece linear function (Maas and Hoffman, 1977) is the widely used response function to explain the crop response to salinity and is given as: $Y_r = 1 - b(C - C_t)$. Where Y_r is the relative yield; b is the absolute value of declining slope in Y_r with C ; C is the level of soil salinity; C_t is the maximum value of salinity without a yield reduction.

‡ The sigmoidal response model (van Genuchten, 1983) is given as: $Y_r = 1 / [1 + (C/C_{50})^p]$. Where Y_r is the relative yield; C is the level of soil salinity; C_{50} is the level of salinity at $Y_r = 0.5$; p is shape parameter and can be estimated as $[\exp(s.C_{50})]$. 's' is the curve steepness parameter. The ST-index is the salinity tolerance index and is defined as: $ST\text{-index} = C_{50} + s.C_{50}$.

The parameter estimates for each of these response model, if not provided, was conducted by following Stepphun et al. (2005).

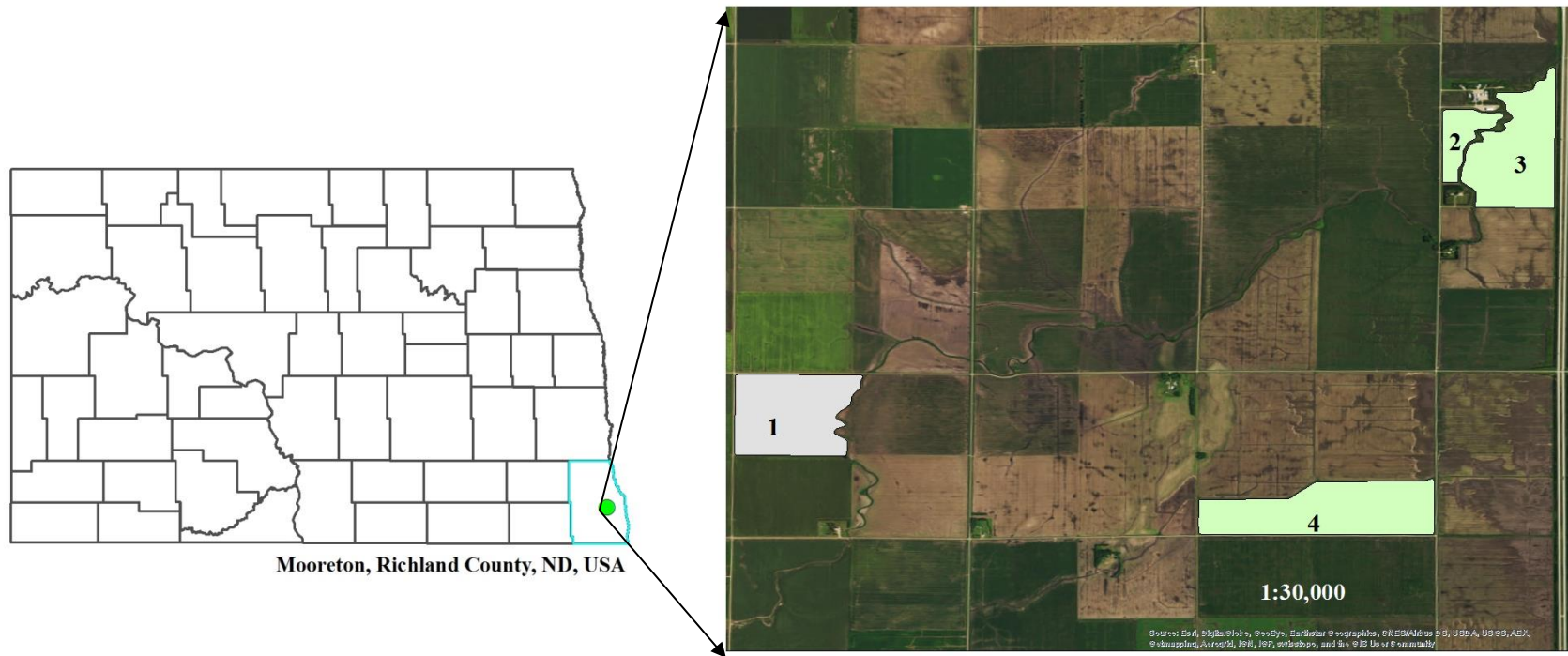


Figure 3.1. Location of four field sites in the Richland County, North Dakota, USA. Research was conducted in 2014 growing season in field 1. In field 2, 3, and 4, research was conducted in 2015 growing season.

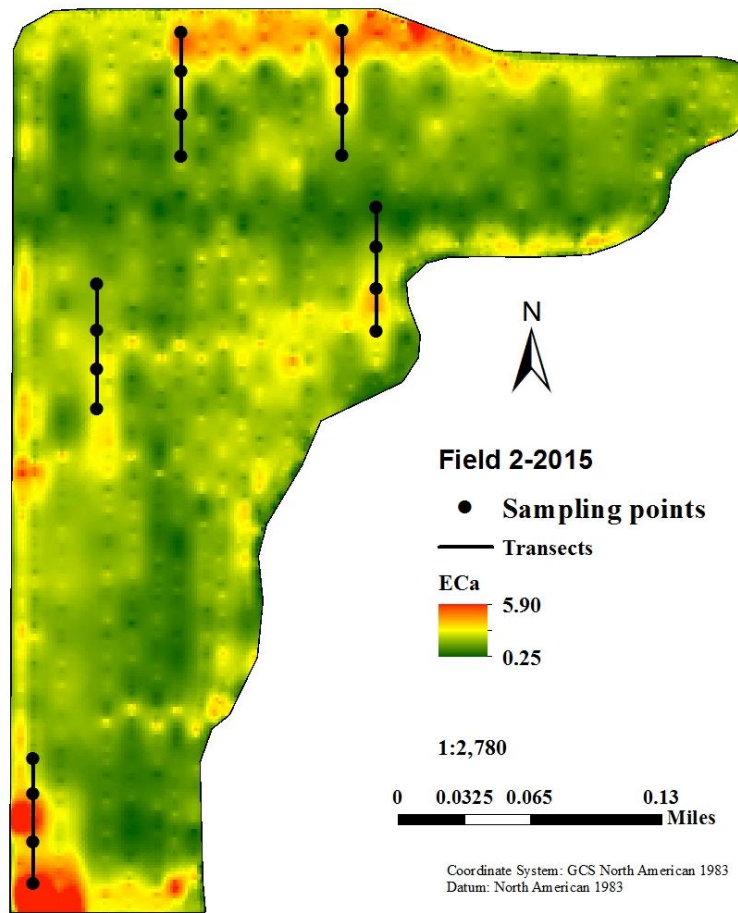


Figure 3.2. Variation in apparent electrical conductivity (ECa) across field 2. The line represents transects established along a salinity gradient and the points represents the sampling positions. [Note: *Similar salinity maps were also constructed for other fields*].

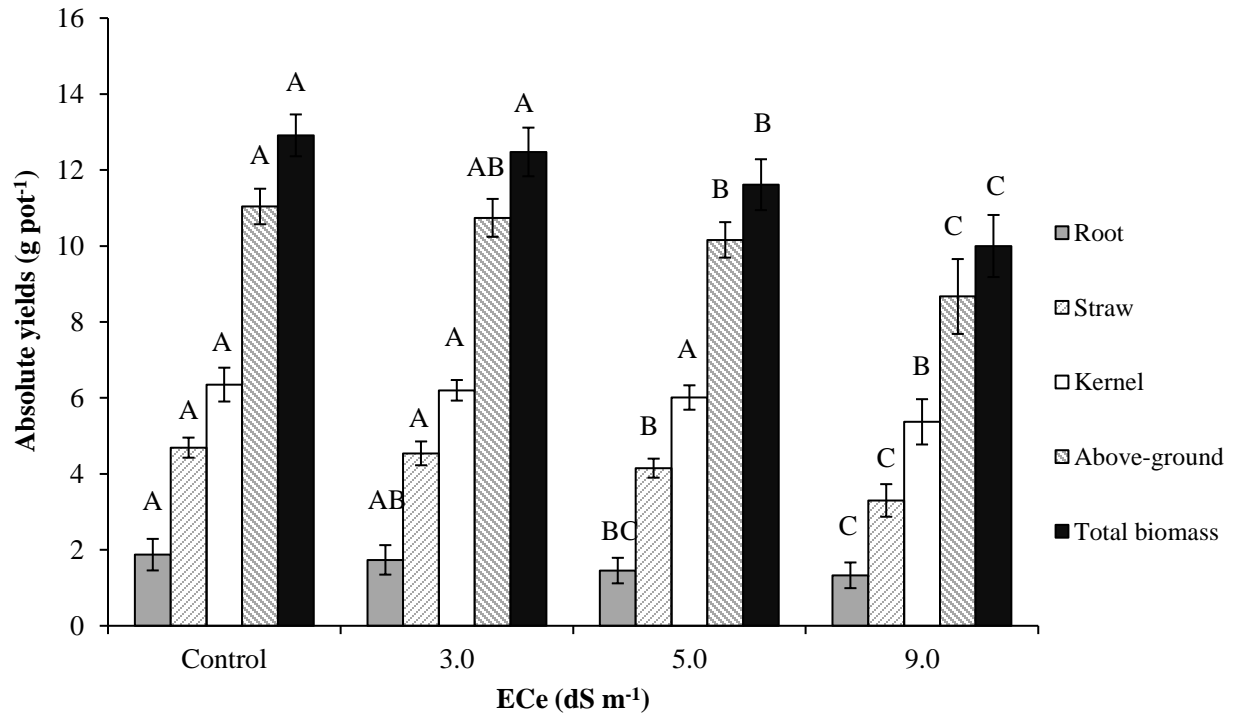


Figure 3.3. Impact of salinity gradient on absolute yields (g pot⁻¹) of different plant fractions under greenhouse conditions. Different uppercase letter indicate significant effect of salinity for particular plant fractions at $p \leq 0.05$. Vertical bars represent standard deviation (n=10).

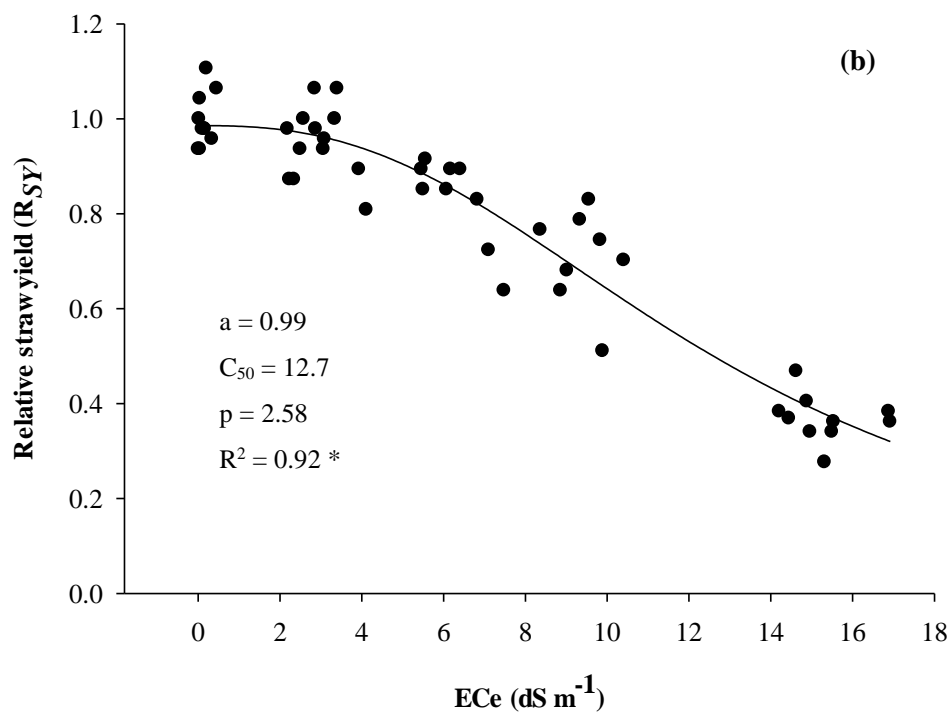
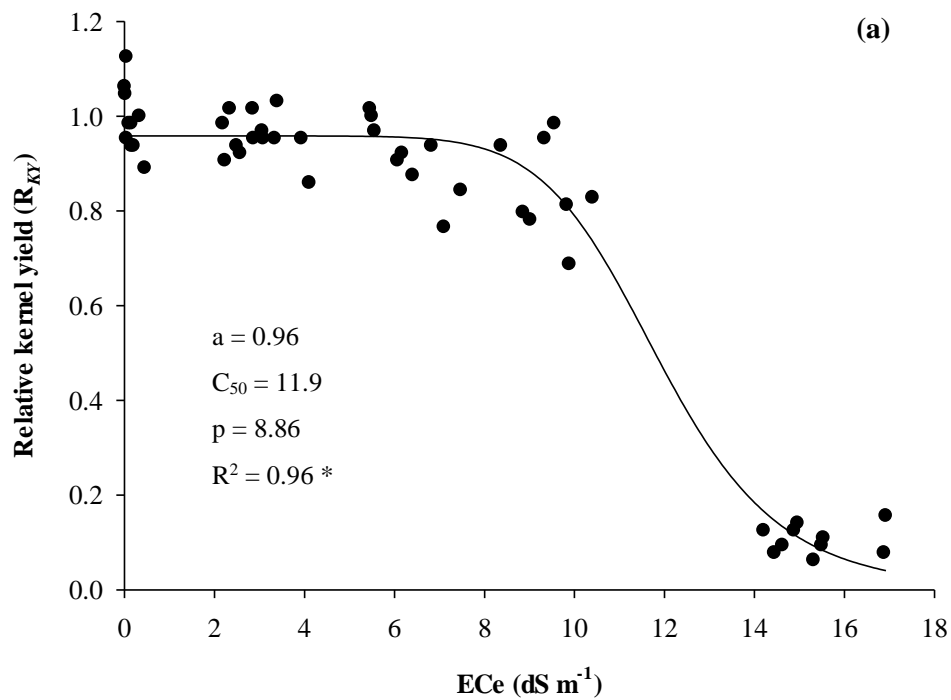


Figure 3.4. Impact of salinity gradient on (a) relative kernel yield (R_{KY}) and (b) relative straw yield (R_{SY}) under greenhouse conditions.

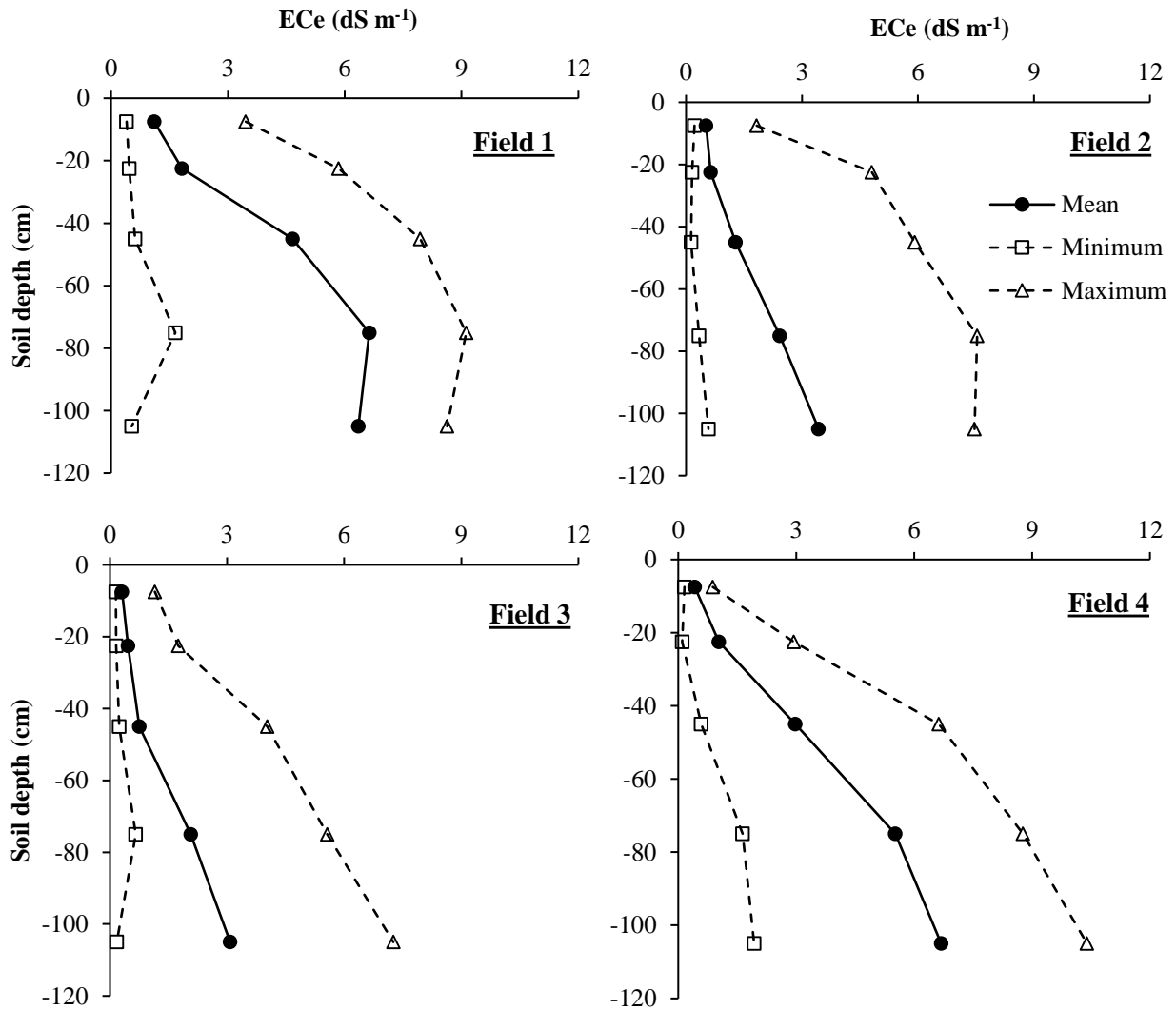


Figure 3.5. Mean, minimum and maximum soil ECe levels at various soil depths in each of the four field sites.

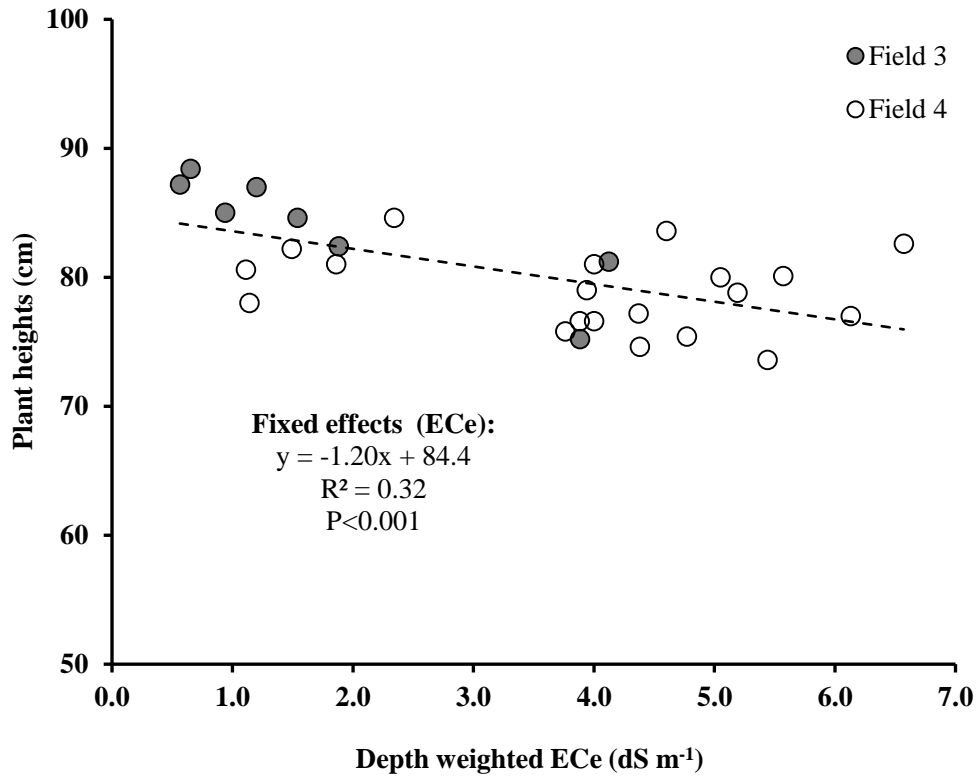


Figure 3.6. Effect of depth-weighted mean root-zone salinity (ECe) on plant heights of spring wheat under field conditions. The regression line was tested by linear mixed effect regression and the R-squared value is from the marginal R-squared test. [Note: Data was not collected in Field 1 during 2014. Data collected from some transects during 2015 were excluded during analysis due to severe hailstorm effect on those transects].

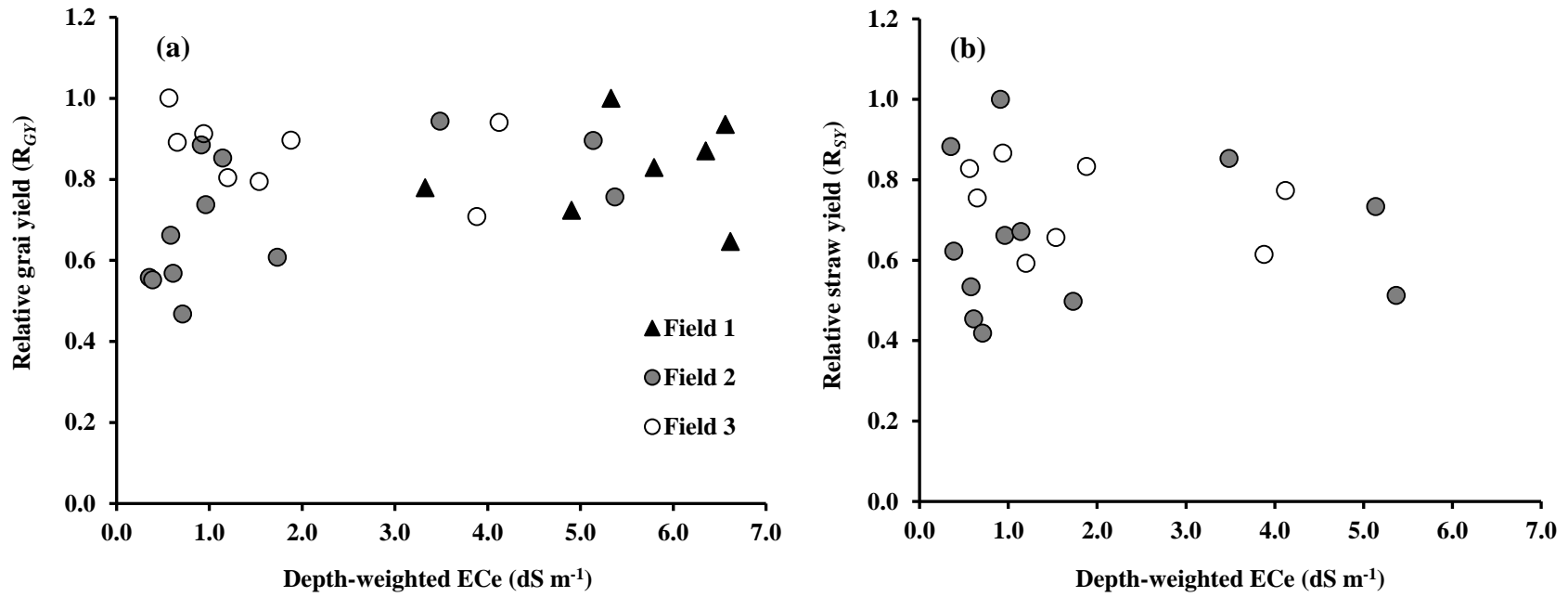


Figure 3.7. Relation of relative grain yield (R_{GY}) and relative straw yield (R_{SY}) of spring wheat with the depth-weighted mean root-zone salinity (ECe). Data was analyzed using linear mixed effects regression with depth-weighted ECe as fixed effect and transects nested within fields as random effects. [Note: Data were pooled across three fields during 2014 and 2015 growing seasons. The yield data was not collected from field 4].

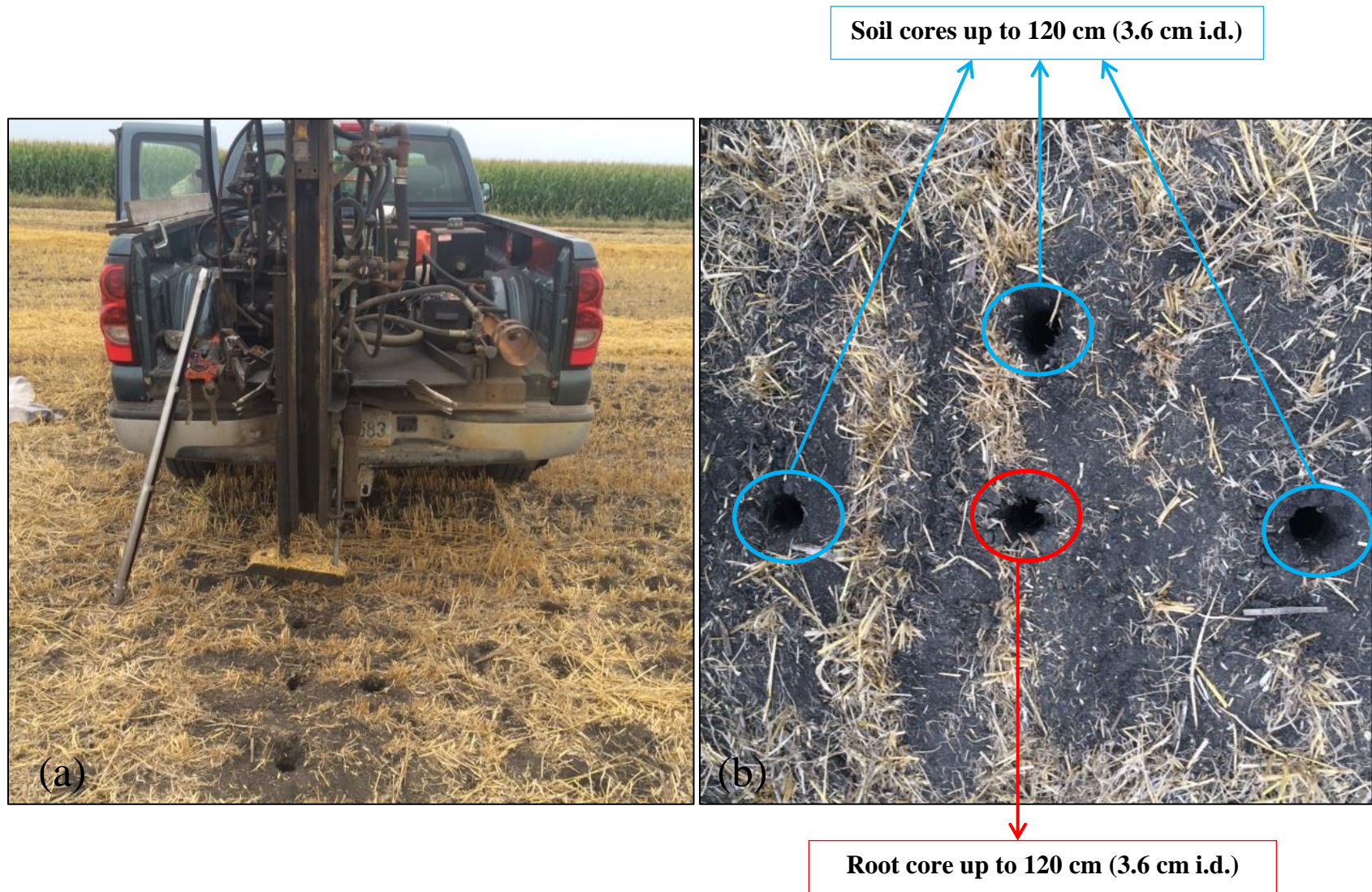


Image 3.1. (a) Truck-mounted hydraulic probe for soil and root cores sampling up to 120 cm depths and (b) Soil and root sampling scheme at each sampling point.



Image 3.2. Response of spring wheat variety Faller to sulfate salts. [Note: Soil salinity decreased from left to right; T₃ (Left) = 9.0 dS m⁻¹, T₂ = 5.5 dS m⁻¹; T₁= 3.0 dS m⁻¹, and T₀ (right) = 0.3 dS m⁻¹].

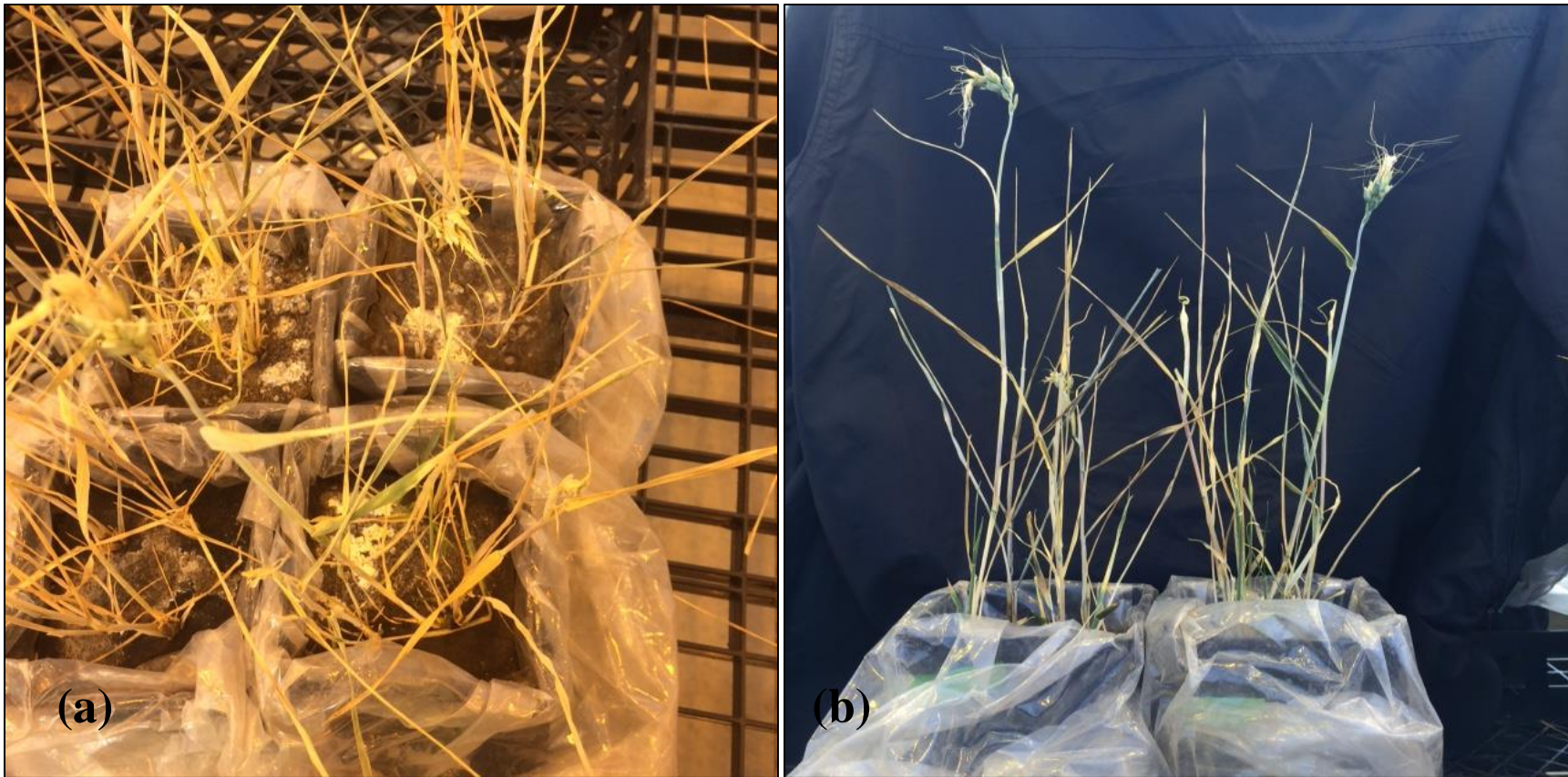


Image 3.3. (a) Capillary rise of sulfate salts on the surface of soil-silica mixes in a greenhouse pot experiment and (b) Severe visible symptoms of salinity on spring wheat when subjected to 15.0 dS m^{-1} (stunted growth, reduced leaf area, senescence of leaf and whole plants, reduced tillers, reduced number of panicles, reduced yield).

CONCLUSIONS

Our results from the first two studies showed the effects of different enhanced efficiency fertilizers (EEF) products on N losses and crop yields under different soil conditions and management practices. Enhanced efficiency fertilizers such as nitrification inhibitors, combination of both urease and nitrification inhibitors i.e. double inhibitors, and controlled release N fertilizers significantly reduced N₂O emissions compared to conventional N fertilizers. Urease inhibitors had no effect on N₂O emissions. Compared to urea, application of double inhibitors also significantly reduced ammonia (NH₃) volatilization and soil water nitrate (NO₃⁻) concentrations below the spring wheat rooting zone. The presence of both urease and nitrification inhibitors delayed urea hydrolysis and also inhibited the nitrification process, thereby synchronizing soil N release with the crop N demands. On the other hand, amending urea with nitrification inhibitor alone increased NH₄⁺ retention in soil for longer duration and resulted in significantly higher NH₃ loss compared to double inhibitor (Chapter 2). These results suggest that the combined application of both urease and nitrification inhibitors would be the best strategy to reduce all possible N losses from urea fertilized soils.

The magnitude of N losses from urea fertilized soils greatly depends upon the pattern and intensity of rainfall in rainfed systems (Chapter 2). Frequent heavy rainfall events elevated soil moisture contents, promoting N₂O fluxes. Higher N₂O fluxes were observed during optimum soil conditions (35-60% soil water filled pore space, soil temperatures >10-12°C, soil nitrate contents >5 mg kg⁻¹ soil). The effectiveness of different EEF products on mitigating N losses and increasing crop yields also depends upon soil (soil texture, soil pH) and management (timing and mode of fertilizer application, tillage and irrigation) factors (Chapter 1). In general, the effectiveness of urease, nitrification or both inhibitors were more evident in coarse-textured than in fine-textured soils and under irrigated than in rainfed systems. Similarly, nitrification

inhibitors were more effective in acidic soils ($\text{pH}<6.5$), whereas double inhibitors in alkaline soils ($\text{pH}>7.5$). Greater effectiveness of double inhibitors was also observed when fertilizers were band-applied rather than broadcasted. All these results suggest that application of either nitrification or double inhibitors might be the most promising N_2O mitigation option among all other available EEF. However, their usage is economically feasible only under specific conditions such as coarse-textured soils, banded fertilizers, and irrigated systems.

Results from the third study indicated variable responses of spring wheat to sulfate-based salinity stress between controlled greenhouse and naturally saline field situations. Under greenhouse conditions, plant roots were subjected to uniform salinity gradient and suffered from salinity stress earlier and for a longer duration. As a result, the negative impacts of salinity were observed in shoot growth, root growth, absolute and relative yields. The threshold sulfate salinity level for kernel and straw yields were 8.2 and 2.9 dS m^{-1} , respectively, with an average decline in kernel and straw yields by 12.0 and 4.9% , respectively, thereafter. Soil salinity in naturally saline fields was highly variable both horizontally and vertically. As a result, the roots of spring wheat were exposed to non-uniform salinity gradient. With lower soil salinity levels in the upper $0\text{-}60$ cm soil depths, root growth of spring wheat was not affected due to which spring wheat may have greater salinity tolerance in naturally saline fields.

In order to ensure global food security for an expanding population in an environmentally sustainable way, future research should be directed towards identifying the best combinations of EEF products, soil conditions, and management practices. The long term effects of the continuous use of EEF year after year in a given site need to be determined in future studies. Similarly, future research on soil salinity should be directed towards understanding the metabolic changes and adaptive strategies of crops to sulfate-based salinity stress. It is also important to

understand the interaction of soil salinity and N management practices on greenhouse gas emissions and crop yields. Moreover, more research focusing on increasing ‘potential yields’ on already productive systems and closing existing ‘yield gaps’ on salt-affected soils should be conducted.

APPENDIX A – FIGURES

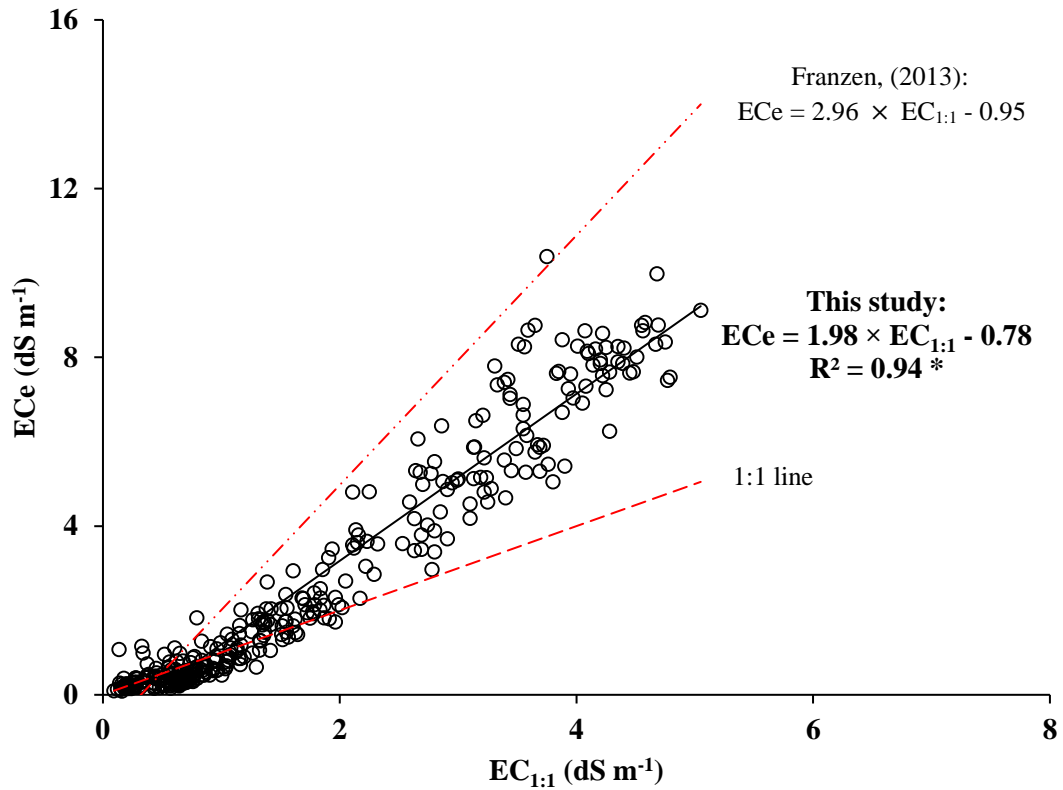


Figure A1. Relationship between EC_e (electrical conductivity of soil determined using saturated paste extract method) and $EC_{1:1}$ (electrical conductivity of soil determined using 1:1 soil to water suspension method) for a fine-textured silty clay loam soil at Richland, ND.

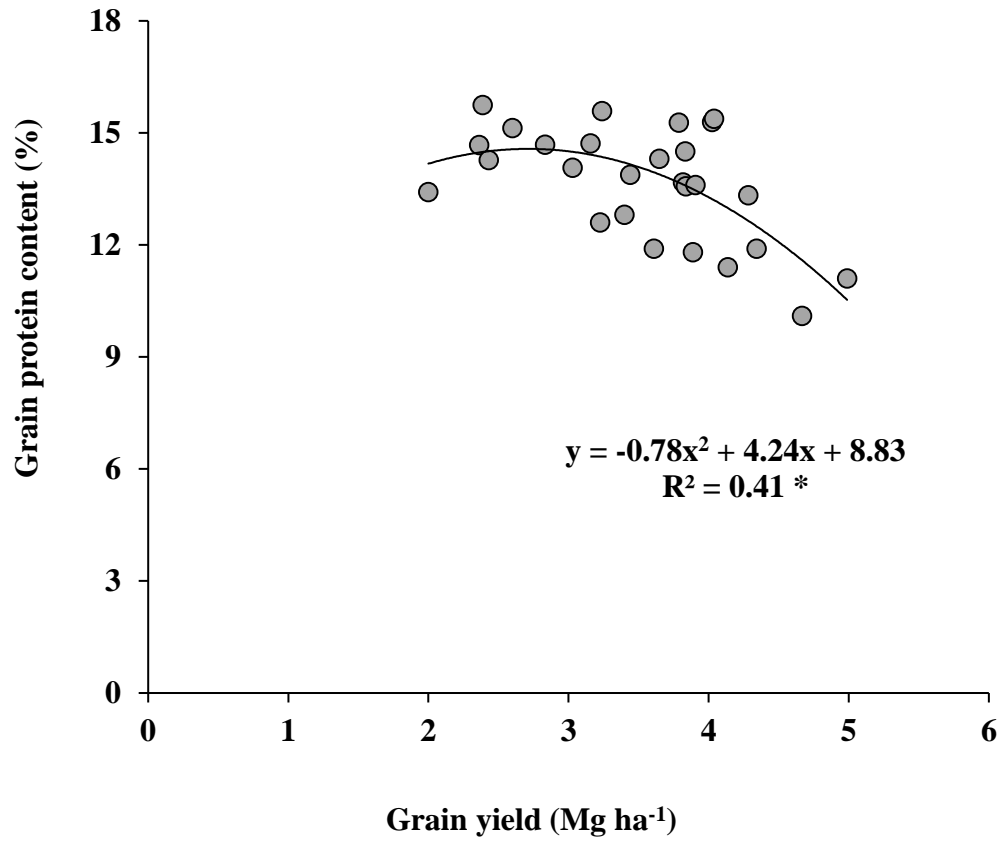


Figure A2. Relationship between grain yield (Mg ha⁻¹) and grain protein content (%) of spring wheat at Richland, ND during 2014 and 2015 growing seasons.

APPENDIX B - BIBLIOGRAPHY

CHAPTER 1 - EFFECT OF ENHANCED EFFICIENCY FERTILIZERS ON NITROUS OXIDE EMISSIONS AND CROP YIELDS IN MAJOR CEREAL SYSTEMS - A GLOBAL META-ANALYSIS

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