SUSTAINABILITY IN AGRICULTURAL PRACTICES: CARBON FOOTPRINT OF AGRICULTURAL GROUNDWATER PUMPING, AND MICROBIAL DENITRIFICATION IN AGRICULTIRAL SOILS

By

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Abstract: This study aims to identify agricultural energy, water, and nutrient management practices that decrease greenhouse gas (GHG) emissions from irrigated agriculture. Irrigation water and agricultural improvements like fertilizer application are required for crop production and increasing crop yield to satisfy food demand of the growing population. However, negative consequences of these activities include production of GHG emissions. To mitigate this global environmental problem, the management methods that minimize agricultural GHG emissions should be identified. Accordingly, the objectives of this work are i) to identify methods that decrease carbon emission of agricultural groundwater withdrawal, ii) to quantify cradle-to-field GHG emission estimations of corn production under different fertilizer and irrigation management, and iii) to investigate the denitrification gene abundance in corn field soils under different application rates of irrigation water and fertilizers.

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CHAPTER I

INTRODUCTION

The principal greenhouse gas (GHG) emissions from human activities include carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and fluorinated gases (EPA, 2022; UNCC, 2022). The global warming potential of N_2O is almost 300 times that of CO_2, and its lifespan is about 114 years, making N_2O emissions of even greater concern (EPA, 2022). Agriculture is the third largest global source of GHG emissions (24% of 2010 global GHG emissions) and the primary source of N_2O (Cocco et al., 2018). A crucial challenge by 2050 is sustaining food security under increasing global water scarcity (Bhattacharyya et al., 2020). With the rising global population, a 60-70 percent increase in food demand between 2009 and 2050 is predicted (FAO, 2009). Significant GHG emissions have raised concerns regarding environmental sustainability and the sustainability and security of world food production to meet the increasing population demands (Perea et al., 2021; Pereira et al., 2019). Raising food production will increase global GHG emissions (Galloway et al., 2003a).

Approximately 17 percent increase in N_2O and CH_4 emissions from agricultural industries have been reported since 1990, accounting for an annual average emission rate of about 60 MT of CO_2 equivalent; 50 percent of which come from agricultural lands and discharges of fertilizer induced N_2O from crop production (Ramzan et al., 2020).

Continuing the rise of GHG emissions above the present level will contribute to global climate change at a higher level than the rates in the twentieth century, change the hydrological cycle, increase global temperature, increase the severity and extent of droughts, raise the frequency, magnitude, and intensity of wildfires, change the precipitation patterns to more intense storms, alter the rates of river sedimentation, and speed up sea-level rises (Bhattacharyya et al., 2020; Bowman et al., 2017; Brar et al., 2019; Flannigan & Harrington, 1988; Hansen et al., 2010; Jolly et al., 2015; Masasi et al., 2017; Sheffield et al., 2012; Trenberth et al., 2003).

Irrigated agriculture worldwide relies heavily on energy resources to extract freshwater and convey it to application sites, especially in arid/semi-arid regions, where large amounts of irrigation water are required to sustain crop production and ensure food security (He et al., 2017). The pumping energy consumption for cropland irrigation using groundwater is expanding worldwide (McGill et al., 2018) and has major environmental consequences, mainly due to GHG emissions (Khan et al., 2014; Pradeleix et al., 2015). That is while, electricity and heat production is the primary source of GHG emissions worldwide and the second source of GHG in the U.S. It emits GHG through burning of coal, natural gas (NG) and oil for generating electricity and heat (EPA, 2022b). Wind and solar energy are known as renewable and clean energies with low environmental impacts contributing to less GHG emission compared to some other types of electricity due to not burning fuel for energy generation. Climate change and decreasing renewable surface water availability has increased rely on groundwater pumping for agricultural irrigation, causing expanding dependency on energy sources to extract groundwater, especially in arid and semi-arid areas (He et al., 2017; McGill et al., 2018). American's groundwater pumping for agricultural irrigation accounts for two-third of the national groundwater withdrawal

(Water in the West, 2013). It contributes to about three million metric tons (MMT) GHG emissions annually (Follett, 2001).

Agricultural soils are also a primary source of agricultural GHG emissions, 20 percent of which includes CO_2 (Mørkved et al., 2006; Sarris et al., 2019; Yadav & Wang, 2017a). Agricultural soils contribute to about 38% and 32% of non-CO_2 GHG emitted from agricultural activities (Kang & Banga, 2013). Denitrification that is an anaerobic N cycling process is known as the main pathway for N_2O emissions that contribute to poor crop consumption of utilized nitrogen fertilizers to agricultural fields through transforming nitrate to N_2 gas (Fan et al., 1997a; Fuller et al., 2016; Nie et al., 2019). Denitrification is significantly influenced by different in-field agricultural management practices and various environmental factors (Enwall et al., 2005; W. Wang et al., 2019; Z. Chen et al., 2015 & Snider et al., 2015). Microbial denitrification is the main cause of nitrogen losses from the agricultural system (Malique et al., 2019). Microbial denitrification is influenced either directly (proximal control) or indirectly (Distal control) by different environmental parameters, and agricultural management practices (Braker & Conrad, 2011; Wallenstein et al., 2006). These parameters include soil structure, temperature, moisture, pH, C:N ratio, calcium, and microbial population, composition and diversity (Braker & Conrad, 2011; Ramzan et al., 2020; Wallenstein et al., 2006). The agricultural management practices that affect denitrification include the water and fertilizer application and tillage (Plaza-Bonilla et al., 2014; Rosa et al., 2014; Xue et al., 2015). The diagnostic genes in the bacterial and fungal denitrification pathway include narG1960, narG, nirKC, cnor, qnor, V, cd, nosZ, nirKF and P450nor (Braker et al., 2010; Delorme et al., 2003; Flanagan et al., 1999; Henry et al., 2006; Higgins et al., 2016; Michotey et al., 2000; Philippot & Hallin, 2005; Wei et al., 2015).

Considering the contributions of agricultural GHG emissions to global warming and climate change, understanding the mechanism and the effects of different agricultural management practices on GHG emission rates is crucial. In confronting the core issues of food demand and environmental concerns, optimizing agricultural practices could result in balanced food and environmental security (Wang et al.,

2022; Wang et al., 2012; Wang & He, 2022; Wang et al., 2019; Wang et al., 2019; Wang et al., 2014). While nutrients and fertilizers can increase crop yields, their negative environmental and economic consequences when they are lost from agricultural farms should not be neglected (Foltz et al., 2019; Galloway et al., 2003b). Studies indicate that some in-field management practices such as nutrient and fertilizer application management could result in decreasing of N_2O emissions and nitrate (NO_3^-) losses from fields (Aldrich, 2015; Bao et al., 2012; Basche et al., 2014; Butterbach-Bahl & Dannenmann, 2011; Decock, 2014; Rafique et al., 2011). So, quantifying and comparing the agricultural GHG emissions from various field management practices and environmental conditions is crucial to distinguish the more sustainable and less environmentally costly farming practices that enhance yield to secure world food production (Benbi, 2018; Yadav & Wang, 2017a). Various components include GHG emissions from groundwater pumping energy generation and combustion, chemical material (e.g., fertilizer and herbicides) production, on-farm human activities, and agricultural soils.

Life Cycle Assessment (LCA) is one of the developed techniques that helps to recognize opportunities to enhance the environmental functioning of products at different steps in their life cycle; inform decision-makers; select the appropriate indicators of environmental functioning, including measurement methods; and improve marketing (ISO 14040:2006). LCA focuses on the environmental aspects and potential environmental effects throughout a product's life cycle from cradle-to-grave. It includes various stages, from acquiring raw material through manufacturing, usage, end-of-life treatment, recycling, and final disposal (ISO 14040:2006). The information established in an LCA study can be applied as part of a more comprehensive decision procedure. The ISO14040 LCA methodology is simply represented in Figure 1.1. SimaPro and GREET-2021 well to pump models could be used to estimate cradle to product GHG emissions from energy generation, fertilizer, herbicide and crop seed production and transportation to application sites.

Several experimental methods (e.g., soil core incubation and an in situ closed chamber) (Fan et al., 1997b) and modeling approaches (e.g., DeNitrification-DeComposition (DNDC), DAYCENT) (Li et

al., 2014; Vogeler et al., 2013) could be adopted to quantify agricultural soils and fields' GHG emissions. GHG prediction models are used to save time and labor required for experimental methods (Yadav & Wang, 2017). The DNDC model is one of the most common simulation computer models for predicting agricultural gas emission (C. Li et al., 1992; C. S. Li, 2000) that has been implemented for predicting gas emission in many studies (Gilhespy et al., 2014) and evaluated against field measures of many agricultural sites and datasets of soil organic carbon changes and GHG fluxes measured worldwide (Abalos et al., 2016; Beheydt et al., 2007; Gilhespy et al., 2014; Giltrap et al., 2010; Li et al., 2014). However, DNDC requires significant data inputs and familiarity with using process-based models. On the other hand, the Intergovernmental Panel on Climate Change (IPCC) offers a simple empirical equation based on N inputs to estimate the direct N_2O emissions from managed soils and can be used when less detailed input data is available (IPCC, 2006). Data on cropland areas, soils, climate/weather, fertilizer types, or other details of in-field agricultural management practices are not required in this methodology. Only the national statistics on fertilizer use, livestock populations, and crop residue management are required for the IPCC methodology (C. Li et al., 1992).

The polymerase chain reaction (PCR) is an enzymatic procedure to detect specific genes within an environmental DNA sample through duplicating specific genes within that DNA to a larger amount which can be detected. A more informative method is real-time quantitative PCR (qPCR). This step is applied for quantifying the amount of a specific gene of DNA in a sample using real-time analysis of its replication. It allows simultaneous screening of genes across a large number of samples.

Considering the mentioned issues regarding GHG emissions and N losses from agricultural production this study aims to identify agricultural energy, water, and nutrient management practices that mitigate GHG emissions and N loses from groundwater-dependent irrigated agriculture. **Accordingly, the objectives of this work are:**

i) **to identify methods that decrease carbon emission of agricultural groundwater withdrawal,**

ii) **ii) to quantify cradle-to-field GHG emission estimations of corn production under different fertilizer and irrigation management, and**

iii) **iii) to investigate the denitrification gene abundance in corn field soils under different application rates of irrigation water and fertilizers.**

The second chapter investigated potential GHG mitigation practices from agricultural groundwater pumping energy. It compared different agricultural groundwater pumping energy demand and supply managements practices to identify the measures that result in higher carbon footprint reduction. Improving overall pump efficiency (OPE) as one of the main factors affecting groundwater pumping energy consumption is considered as the energy demand management practice. The GHG emissions from different energy sources (including electricity mix network, natural gas (NG), wind and solar) have been compared to find out the best energy supply management practice regarding carbon saving. Result shows that GHG emission from energy consumption is related to the source of energy and not always less energy consumption means less GHG emissions. Where NG is used as groundwater energy pumping source, improving OPE through replacing pump with new pump saves more energy than switching to electricity. The electric pumps energy consumption (due to their higher OPE) is less than NG pumps in similar conditions. However, the GHG emission from electric pumps (from the U.S. Central and Southern Pains electricity mix) is higher than from NG pumps. Although, GHG emissions from electricity that is generated using wind and solar plants are very low. The GHG emissions from electricity is very sensitive to the share of different sources of energy (e.g., coal, NG, oil, hydropower, wind, solar) in electricity mix network. For instance, GHG emissions from California mix network is approximately 35% of GHG emissions from the U.S. Central and Southern Pains electricity mix (GREET, 2021).

In the third chapter, the cradle-to-product GHG emissions from in-field and pre-field parameters contributing to gross GHG emissions from corn production in a groundwater-fed irrigated agriculture area with both the NG and electricity pumping energy consumption. Results revealed that in-field GHG emissions contribute the highest (63%) in GHG emissions from corn production. The in-field GHG emissions were mainly associated with agricultural soils, followed by on-site NG combustion from groundwater pumping energy consumption. Fertilizer production, followed by energy generation for groundwater pumping, is the primary source of GHG emissions from pre-field parameters. The sensitivity analysis indicated the highest sensitivity of total GHG emissions from corn production to agricultural soil that is highly sensitive to the emission factor for N_2O emissions from N inputs.

In the fourth chapter, the abundance of various denitrification genes in response to different application rates of water and N fertilizer is investigated. The denitrification gene contribute to lowering the fertilization efficiency through either nutrient N consumption by the denitrification bacteria or denitrification pathway that results in GHG emissions. In addition to fertilizer and irrigation regime, denitrification community size could be affected by different distal and proximal factors, including soil pH, organic C, soil moisture, soil texture and calcium (Bowen et al., 2018; Braker et al., 2010; Bru et al., 2010; Carson et al., 2010; Kandeler et al., 2006; Z. Li et al., 2020). The results of this study show the abundance of individual denitrification genes responses differently to fertilizer and water application. While narG abundance did not result in high correlations with water application, cnorB2 abundance correlated significantly positive with water application. The nirKC abundance had no correlation to high correlation in different fields. The narG community size correlated negatively low to moderate with the fertilizer application. The correlation between nirKC and cnorB2 differed negatively (between low and high) for different fields. However, cnorB2 abundance was more sensitive than nirKC to different fertilizer application. The results indicates that the denitrification gene abundance response to fertilizer application cold be affected by other factors. These factors include soil pH, organic C, soil moisture, soil texture and calcium (Bowen et al., 2018; Braker et al., 2010; Bru et

al., 2010; Carson et al., 2010; Kandeler et al., 2006; Z. Li et al., 2020). So, these parameters should be considered when analyzing denitrification gene abundance in response to fertilization and irrigation treatments.

The results indicate the importance of targeting agricultural soil when planning to reduce agricultural GHG emissions from crop production since most of the agricultural GHG emissions are emitted from agricultural soil. Due to the high contribution of the total GHG emitted from on-site NG combustion and energy production for groundwater pumping, pumping energy management practices should be adapted to decrease total GHG emissions from agricultural crop production. Besides, the effects of water and fertilizer application on denitrification communities should be estimated along with the influence of other parameters that affect these genes.

Multi-objective optimization models can be hired to estimate the optimum application rate of irrigation water and fertilizer regarding reducing GHG emissions and nutrient losses from groundwater-fed irrigated crops. The results are valuable for energy and agricultural managers and decision-makers, and farmers regarding sustainable agriculture and the low GHG footprint of crop production in the study areas and similar regions in the world.

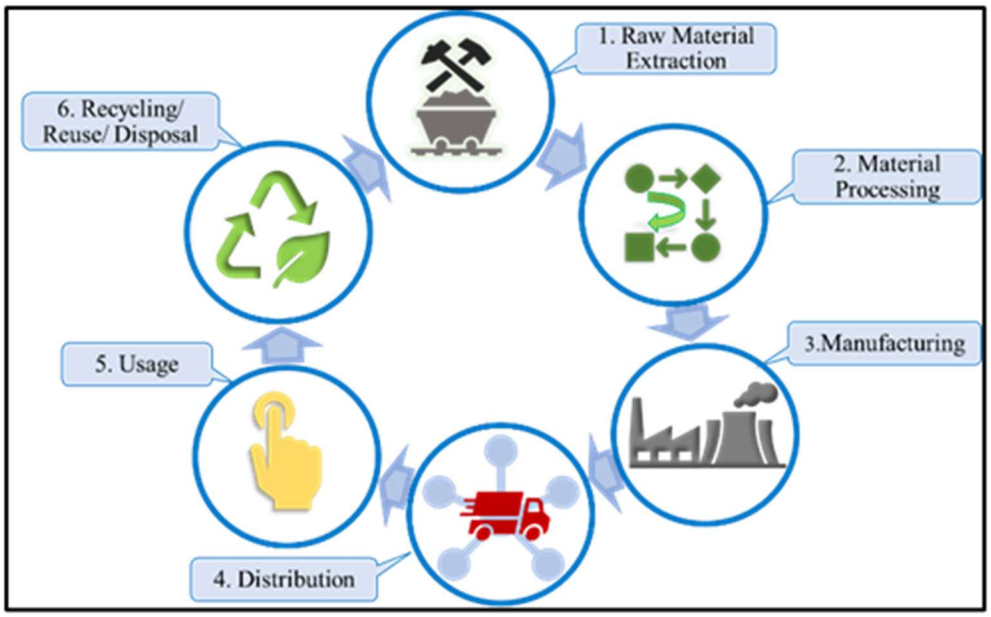

Figure 1. 1: ISO14040 LCA Methodology

CHAPTER II

GREENHOUSE GAS EMISSIONS OF AGRICULTURAL GROUNDWATER PUMPING WITH ENERGY DEMAND AND SUPPLY ANALYSIS

Abstract

Irrigation water is required for crop production and increased crop yield to satisfy global food demand. However, irrigation has negative impacts as well, including the production of greenhouse gas (GHG) emissions from groundwater pumping energy. To lessen this environmental problem, management methods that minimize agricultural GHG emissions from groundwater pumping should be identified. This work aims to identify measures that decrease agricultural groundwater withdrawal GHG emissions. A comparison among different energy supply and demand management for groundwater pumping is made to identify the most effective measure. The results show the current average annual energy consumption for electric and gas pumps are up to 35,635 and 310,502 (kWh $year^{-1}$), with GHG emissions of 25.4 and 63 (ton CO_2-eq $year^{-1}$), respectively.

By replacing the current electricity mix with solar and wind energies, the GHG emissions will decrease by about 23.5 and 25 (ton CO_2-eq $year^{-1}$), respectively. Improving the overall pump efficiency (OPE) of electric pumps will save up to 10,312 (kWh $year^{-1}$), which equals saving about 6.84 (ton CO_2-eq $year^{-1}$) GHG emissions. Improving the OPE of natural gas combustion pumps will save up to 40,183 (kWh $year^{-1}$) energy and 7.61 (ton CO_2-eq $year^{-1}$) GHG emissions. Replacing natural gas combustion with electric pumps will decrease energy consumption by up to 227,075 (kWh $year^{-1}$), which saves only 2.79 (ton CO_2-eq $year^{-1}$) GHG emissions. The reason is the higher GHG emissions from producing electricity compared to producing and on-site combustion of natural gas, even though the OPE of electric pumps is much higher than natural gas combustion pumps. Replacing natural gas with solar and wind energy will save 55.1 and 58.4 (ton CO_2-eq $year^{-1}$), respectively. These values will be significant when applied to all the agricultural groundwater pumps in the study areas and multiply the study's timeline. It indicates the importance of energy management regarding sustainable agriculture globally.

Keywords: Life Cycle Assessment, Solar Energy, Wind Energy, Irrigation, Groundwater, Pumping Energy, Carbon footprint.

2.1 Introduction

A crucial challenge by 2050 is sustaining food security under increasing global water scarcity (Bhattacharyya et al., 2020). With the rising global population, a 60-70 percent increase in food demand between 2009 and 2050 is predicted (FAO, 2009). Raising food production will increase global GHG emissions (Galloway et al., 2003). Significant greenhouse gas (GHG) emissions from agricultural food production have raised concerns regarding sustainability of the environment and secure world food production to meet the increasing population demands (Perea et al., 2021; Pereira et al., 2019). Continuing the rise of GHG emissions above the present level will contribute to global climate change at a higher level than the rates in the twentieth century (Brar et al., 2019; Masasi et

al., 2017), change the hydrological cycle, increase global temperature, increase the severity and extent of droughts, raise the frequency, magnitude, and intensity of wildfires, change the precipitation patterns to more intense storms, alter the rates of river sedimentation, and speed up sea-level rises (Bhattacharyya et al., 2020; Bowman et al., 2017; Flannigan & Harrington, 1988; Hansen et al., 2010; Jolly et al., 2015; Sheffield et al., 2012; Trenberth et al., 2003).

Electricity and heat production is the main source of greenhouse gas (GHG) emissions worldwide and the second source of GHG in the United States (U.S.) (EPA, 2022a). About 61% of electricity in the U.S. in 2021 was generated from fossil fuels, mainly natural gas (NG) and coal, 19% from nuclear, and 20% from renewable energy sources (EIA, 2022). Wind and solar contributed to about 9.2 and 2.8% of the total electricity generation of the country, respectively (EIA, 2022). Carbon dioxide (CO_2) emissions from electricity generation differ based on the source of energy (e.g., renewable, fossil fuel, etc.) and types of fossil fuel used to generate electricity (e.g., burning coal to generate electricity emits more CO_2 than natural gas or oil). Hence, using renewable energy sources rather than fossil fuels for energy generation and increasing the share of total electricity generated from wind, solar, hydro, and geothermal source, and specific biofuel sources is considered as an opportunity to reduce GHG emissions associated with electricity generation (EPA, 2022a).

The agriculture sector was accounted for about 10-12% of total the estimated GHG emissions worldwide in 2005 (Terry Barker et al., 2007). Irrigated agriculture worldwide relies heavily on energy resources to extract freshwater and convey it to application sites, especially in arid and semi-arid regions, where large amounts of irrigation water are required to sustain crop production and ensure food security (He et al., 2017). The pumping energy consumption for cropland irrigation using groundwater is expanding worldwide (McGill et al., 2018) and has major environmental consequences, mainly due to GHG emissions (Khan et al., 2014; Pradeleix et al., 2015). American's groundwater pumping is estimated to be about 110-117 billion cubic meters per year (Alley, 2010;

Smith et al., 2011); two-thirds of that is used for irrigation (Water in the West, 2013). The annual carbon emissions from agricultural groundwater pumping in the U.S. are about three million metric tons (MMT); 46 percent from electric pumps, followed by 32 and 19 percent from diesel and natural gas pumps, respectively (Follett, 2001). This reliance on groundwater pumping and concerns about agricultural pumping energy consumption and GHG emissions are not unique to the U.S. and are also reported in countries such as Australia, China, India and Iran (Acharya et al., 2015; Karimi et al., 2012; Qiu et al., 2018; Rajan et al., 2020; Shah, 2009; J. Wang et al., 2012). Studies reported that enhancing pumping efficiency could decrease pumping energy consumption and GHG emissions from groundwater-fed agriculture (Luc et al., 2006; Mora et al., 2013; Patle et al., 2016).

The main factors affect pump efficiency include operating conditions such as the total dynamic head (TDH) (based on groundwater level depth) and the pump condition. Any aberrations from optimum conditions result in deviation from design parameters considered to select the most efficient pump that will cause declining system efficiency and increasing GHG emissions. An increase in TDH due to declining the groundwater level is a deviation from optimum conditions. It mainly happens in groundwater-fed agricultural areas with high amounts of groundwater withdrawal that cause significant groundwater level declines. Declining groundwater levels, often tied to irrigation, have led to increases in groundwater pumping energy consumption and agricultural GHG in many areas in the world (C. Li et al., 2013, 2013; Mehata & Taghvaeian, 2020; Singh Dhillon et al., 2018).

The state of Oklahoma (U.S.) has been experiencing similar challenges. The energy consumption of 6,530 agricultural groundwater pumps cost Oklahoma agricultural producers more than 21.5 million U.S. dollars (USD) in 2018, which accounts for approximately 104 USD per hectare (Mehata & Taghvaeian, 2020). Electricity and natural gas were the main sources of groundwater pumping energy providing irrigation water for 46 and 39 percent of total irrigated areas in Oklahoma in 2018, respectively (Mehata & Taghvaeian, 2020). The shares of energy sources for

groundwater pumping in Oklahoma and in the U.S. are represented in Figure 2.1. The Oklahoma Panhandle has experienced considerable groundwater decline over the past decade due to drought, decreased recharge rates, and mainly increased groundwater withdrawal for crop irrigation (OWRB, 2018). Therefore, focusing on energy consumption efficiencies can positively impact sustainable agricultural production in Oklahoma.

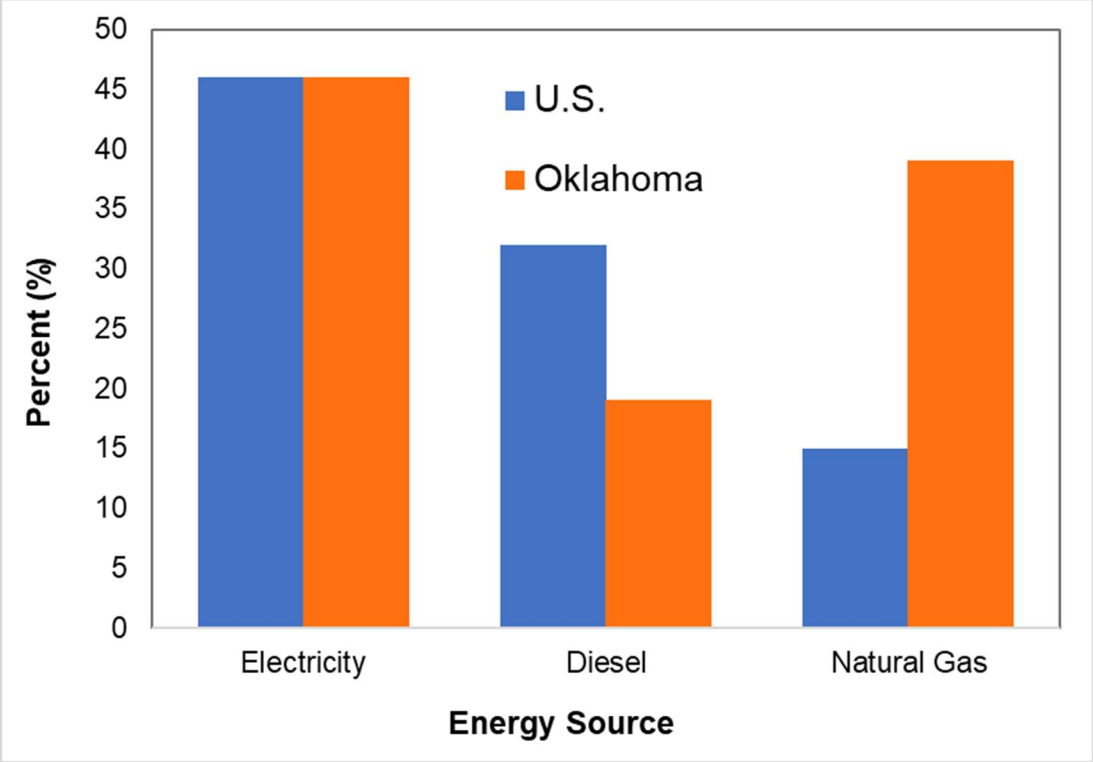

Figure 2. 1: Energy Sources of Agricultural Groundwater Pumping in the U.S. and in Oklahoma (Follett, 2001; Mehata & Taghvaeian, 2020)

This work aims to identify methods that decrease the carbon emissions of agricultural groundwater withdrawal. This study specifically aims to (i) conduct a life cycle GHG emissions assessment under the current and achievable OPEs and (ii) investigate the GHG emissions of irrigation pumping plants under current and alternative electricity mixes. This work will help decision-makers such as Oklahoma agricultural producers, water managers, and environmental experts from insight into the existing and potential GHG footprint of irrigation pumping in the study areas and the

alternatives for reducing GHG emission. Moreover, the results will be applicable to similar areas regarding agro-climatological and groundwater resources conditions.

2.2 Study Area:

The study area includes Rush Springs Aquifer (RSA), located in west-central Oklahoma, and a part of Ogallala Aquifer (OGA) underlying Texas county located in the panhandle of Oklahoma (Figure 2.2). About 44 percent of water use in Oklahoma is provided from groundwater, and the OGA serves as the largest groundwater resource in the state (Oklahoma Water Resources Board, 2014c).

With regards to the RSA, the Oklahoma Water Resources Board (OWRB) had issued 1780 groundwater permits to property owners allowing more than 1.23 million m^3 of extraction from approximately 2,467 wells (GMAP, 2014), as of December 31, 2013. The primary water user of RSA is the agriculture sector (GMAP, 2014). OWRB has designated RSA as an aquifer with a moderate vulnerability level, and the rates of permit extraction from this aquifer are not to exceed 0.61 m^3 per m^2 per year (GMAP, 2014). The groundwater level in the RSA has declined by more than 3 meters from 2001 to 2017 (Khand et al., 2017), which equates to about 0.19 meters per year. Overall average depth to groundwater in RSA is estimated to be about 18 m (OWRB, 2018).

OGA underlies eight states in the U.S.: Colorado (CO), Kansas (KS), Nebraska (NE), New Mexico (NM), Oklahoma (OK), South Dakota (SD), Texas (TX), and Wyoming (WY). This aquifer provides water for about 30% of all groundwater irrigation in the U.S. The groundwater discharge in this aquifer is at a faster rate than its natural recharge (McMahon_2011_Water and Energy Interactions). Since 1940 excessive groundwater pumping has led to a 30-60 m decline in groundwater table levels in parts of this aquifer in northern Texas, the panhandle of Oklahoma, and southwest Kansas (Y. Zhou et al., 2020). The overall average rate of groundwater table decline of the OGA is 4.6-6.1 (m) between 2009 and 2019. However, the rate of decline ranges between 3 (m) and 4.6 (m) from 2014 to 2019 with a 0.9-1.8 (m) decline in 2018 alone (OWRB, 2018). So, the

average annual groundwater decline of the OGA, Texas county Oklahoma is considered as 1.1 m. The overall average depth to groundwater in OGA is estimated to be about 55.2 m (OWRB, 2018).

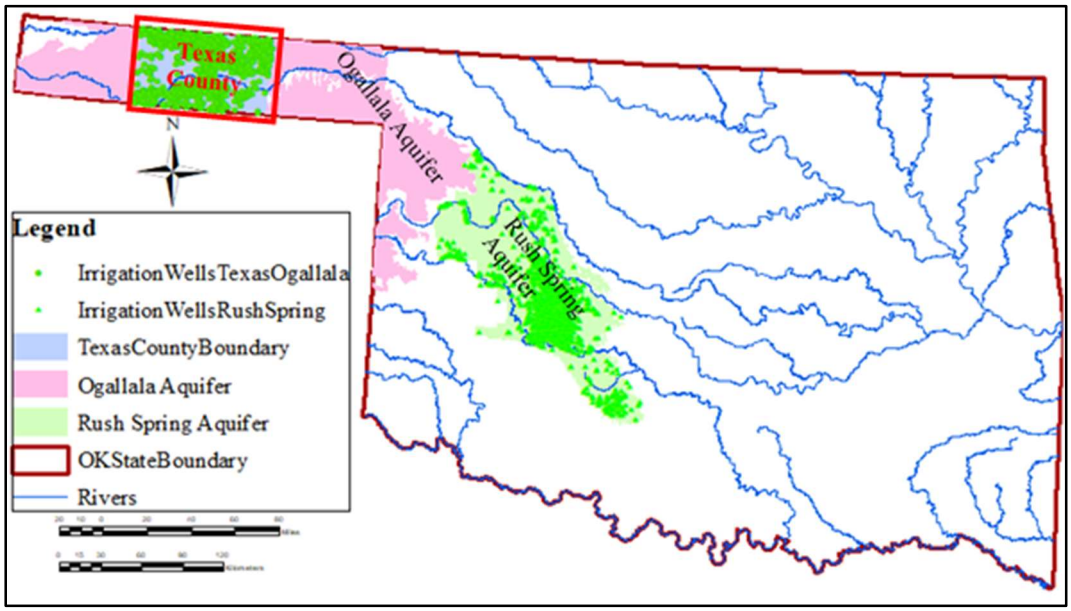

Figure 2. 2: Study Area and Irrigation Wells

2.3 Material and Methodology:

This study compares the potential reduction in GHG emissions from agricultural groundwater pumping through energy demand management versus energy supply management and among different energy supply managements. The pumping energy demand management is through improving the OPEs to the Nebraska Pumping Plant Performance Criteria (NPPPC) level by replacing existing pumps with new pumps. The current natural gas and/or electric pumps are supposed to be replaced with generic deep well vertical turbine pump. Energy supply management is considered as supplying different types of energy including natural gas, the current U.S. Central and Southern Pains electricity mix, solar energy and wind energy. For the aim, the energy consumptions for electric and natural gas pumps are calculated based on different scenarios. One scenario is to replace the current energy mix with clean energy for electric pumps. Other scenarios will estimate annual energy consumption based on the current groundwater level and OPE; current

groundwater level and improved OPE (through replacing pump); dropped groundwater level in 20 years (constant current OPE) and current OPE and dropped groundwater level and decreasing current OPE in 20 years and considering 5% electricity transmission, and distribution (T & D) loses (EIA). The saved electric energy for each pair condition (Scenarios) is calculated. Then the related annual saved GHG emissions are calculated based on the energy type used in each condition. It is assumed that OPEs of new pumps will decrease to the current OPEs.

2.3.1 Groundwater level Data

Data related to groundwater levels in the study area are obtained from the U.S. Geological Survey (USGS) and OWRB websites, and through direct communication with these agencies (OWRB, 2022; USGS, 2022). The groundwater level data includes that from observation wells, monitoring wells and agricultural wells. The groundwater level data downloaded from OWRB and USGS were examined for outliers (e.g., zero and negative values), and missing data. After data cleaning, the data set were examined by statistical distributions to figure out which data set fits better a normal distribution. So, their mean could be a good representative of the average groundwater levels of agricultural wells in the study area.

2.3.2 Energy Consumption

The energy consumption for a generic electric pump will be calculated based on the averages in the study area (OWRB, USGS, and OSU Data). The following equation will be used to estimate the pumping energy consumption of irrigation wells:

$$Energy = \frac{2.73 * D * V}{OPE * (1 - T_1) * 1000} \qquad (2.1)$$ (Karimi, 2012)

Where energy is energy consumption (kWh), D is lifting height or total dynamic head (m), V is groundwater discharge volume (m^3), OPE is overall pumping plant efficiency, and T_l is transmission and distribution loss (only in case of electric pumps, otherwise 0).

The energy consumptions for both the electric and natural gas pumps were evaluated based on the current average OPEs in the study area and the OPEs suggested by NPPPC for each type of pump (Ross, 1997). The energy consumption was also estimated by replacing natural gas pumps with electric pumps. It was assumed that the OPEs will gradually decrease to reach the current levels in the study area in 20 years of operating the pumps. The groundwater discharged volume for each pump was calculated based on the annual water requirement of cotton within an area under irrigation with a center pivot and considering the irrigation efficiency (Warren et al., 2019). It has been assessed considering once per year cotton growing and leaving the lands fallow for the rest of the year.

2.3.3 GHG Emission from Groundwater Pumping

Replacing the current low efficient pumps with the new ones could improve the OPE to the desired (NPPPC) level. In this strategy, the life cycle carbon footprint of manufacturing a deep well vertical turbine pump was estimated based on the life cycle GHG emissions from a water distribution pump extracted from a related LCA study (Jocanovic et al., 2019). The result was used to estimate the net carbon footprint of groundwater pumping resulted from improving OPEs.

After estimating the energy consumption of the pumps using equation 1, the GHG emissions from generating the consumed energy by pumps were estimated using the GREET well to pump (WTP) model (GREET.NET version 2021, Argonne National Laboratory, Argonne, IL, USA). The GREET is a well-established LCA tool. It is basically a transportation LCA tool for modeling vehicle emissions based on different energy sources (e.g., gas, biofuels, natural gas, electricity, etc.). It can provide a good approximate of well to wheel (WTW) and well to pump (at the stationary) LCA of fuels. WTP technique of the GREET provides an examination of the life cycle of fuels and energy production from extraction and processing (the "well") of raw materials (e.g., coal or crude oil) to storage (the "pump"). The GREET WTP model can be applied alone for

stationary irrigation pumping plants, and no additional modification is required. The CO_2 emissions from "U.S. Central and Southern Pains" Utility Mix category was considered to represent the electrical grid generator composition for Oklahoma. The result was used to estimate the total carbon footprint of extracting required groundwater for irrigation of a hectare of corn (m^3) in RSA.

In the case of natural gas pumps, on-site GHG emissions due to the engine combustion process should be added to the GHG emissions from producing natural gas (extraction, transmission, and distribution). The life cycle GHG emissions for stationary natural gas (from raw material extraction to end-use combustion) were estimated using the GREET-2021 WTP model. The CO_2 emissions from "NA (North American) NG from Shale and Conventional Recovery as Stationary Fuel" category was considered to represent the carbon footprint of NG production. The stationary engine emissions were estimated based on the US Environmental Protection Agency (EPA) Greenhouse Gas Inventory Guidance (EPA, 2016). The methodology may be given as follows:

$$\text{Emissions} = \text{Fuel} * \text{HHV} * \text{EF} \qquad (2.2)$$

Where "Emissions" is the mass of CO_2, CH_4, or N_2O emitted, fuel is the mass or volume of fuel combusted, HHV is the fuel heat content (higher heating value) in units of energy per mass of fuel, and EF is the emission factor of CO_2, CH_4, or N_2O per energy unit. The HHV and EF values reported in EPA, 2016 for natural gas combustion will be used in this research. This research only focuses on CO_2 equivalent, and the emission factor of CH_4 and N_2O are not considered. Based on EPA, 2016, the total GHG emissions from combustion CO_2 equivalence factors of 25 will be adapted.

2.3.4 GHG Emission from Wind and Solar Energy

The utility-scale solar and wind electricity generation in Oklahoma, U.S., in 2021 are reported at 0.0860 and 33.388 billion kilowatt-hours, respectively (EIA). Although no direct GHG emission is associated with solar and wind power industries by burning fossil fuels to generate electricity, they

do contribute to other GHG emissions during all parts of their life cycle, including materials production, manufacturing, construction, transportation, maintenance, and decommissioning activities. The life cycle GHG emissions from solar and wind energy applied to this study were extracted from the previous studies. For the aim, more than 72 and 76 papers related to GHG emission estimations from solar and wind electricity generation (including more than a hundred case studies for each energy type), respectively, were studied to find the average GHG emissions from these energies (e.g., Ardente et al., 2008; Constantino et al., 2018; Garrett & Rønde, 2013; Guezuraga et al., 2012; Kommalapati et al., 2017; Ozoemena et al., 2016, 2018; Pacca et al., 2007; Raadal et al., 2011, 2014). The GHG emissions rated in the solar and wind industries had differed significantly to date from one study to another due to various scopes of LCAs. Most reviewed studies reported that the manufacturing phase contributed the greatest GHG emissions and overall impacts. Installation, transportation, maintenance, and acquisition of raw materials ranked second through fifth. Two studies estimated the average GHG emissions from solar (Hsu et al., 2012) and wind (Dolan & Heath, 2012) energy based on harmonizing the GHG emission data represented in several other studies. Hence, the estimated average GHG emissions for solar and wind energy from these two studies were applied to this research. The beneficial life of solar panels and wind turbines that ranged between 20 and 30 years in different cited studies. Then, the GHG emissions to generate the energy required in different scenarios were estimated for solar and wind energy.

2.3.5 Life Cycle GHG Emission from producing a Deep Well Vertical Turbine Pump

It was assumed to replace current low efficient pumps with generic deep well vertical turbine pumps to improve pumping efficiencies. No study was found related to the life cycle GHG emission assessment of a deep well vertical turbine pump, and no responses from the companies that manufacture these types of pumps were received. A study was found about the LCA evaluation of pump units in water distribution systems (Jocanovic et al., 2019). Although the pump units in Jocanovic et al. 2019 study were smaller than deep well vertical turbine pumps, since the materials

are similar, the result of that study was used to estimate the GHG emissions from a deep well vertical turbine pump. The primary materials for manufacturing a deep well vertical turbine pump include bronze, steel, and iron.

Using average reported values the total pump weight was estimated at 3328 (kg) and the total life cycle GHG emission of a pump, considering maintenance and repairment and recycling at the end of life, was estimated to be at 3384.56 kg CO_2-eq (Jocanovic et al., 2019). We assumed that the ratios of the materials used to manufacture a deep well vertical turbine pump compared to the materials for manufacturing a water distribution system pump equal the ratio of the weights of these pumps. This assumption was applied to estimate life cycle GHG emissions from a deep well vertical turbine pump.

2.4 Scenarios

Different scenarios have been assumed to estimate groundwater pumping energy consumption and GHG emissions. Groundwater level was assumed to decline in both the aquifers over time. In RSA, the current low efficiency pumps were replaced with new pumps with NPPPC level of efficiency. In OGA more conditions were considered, including replacing the current low efficient NG pumps with new NG pumps, switching to electricity (current electricity mix) and replacing NG pumps with new electric pumps (current electricity mix), and replacing NG pumps with new electric pumps and switching to clean (solar or wind) energy.

The first scenario (S1) assumed the OPEs were equal to the current average level that would not change during the study's timeline and the second scenario (S2) assumed that the OPEs would decrease over time. Scenario three (S3) assumed that the OPEs were improved to the NPPPC level by replacing pumps with new ones but OPEs would decrease gradually to reach the existing OPE levels in 20 years. In this scenario, GHG emissions from manufacturing a pump were also included

in the estimation. Replacing NG pumps with electric pumps in OGA was considered as the fourth scenario (S4).

2.5 Results and Discussions:

2.5.1 Groundwater level:

Among groundwater level data sets downloaded from OWRB and USGS, the agricultural wells data were found to be fitted to normal distributions. So, the mean of the data set could be a good representative of the average groundwater levels of agricultural wells in the study area. These data sets included 1262 irrigation wells with an average of 57 m depth to groundwater in OGA and 1523 irrigation wells with an average depth to groundwater of 19.1 m in RSA. These results were in accordance with the values reported in OWRB (2018) and Handa (2019). The average groundwater levels in RSA and OGA were estimated to reach to 22.7 m and 79 m in 20 years.

2.5.2 Groundwater Energy Consumption Parameters

The average overall pump efficiencies are estimated at 46.9% for electric pumps in RSA and 14.8% for natural gas pumps in OGA (Handa et al., 2019). To satisfy NPPPC standards, the OPE should be raised to 66% and 17% for accurately designed and properly maintained electricity- and natural gas-driven pumping plants, respectively (Ross, 1997). Replacing natural gas pumps with electric pumps that was described in methodology means improving the OPE from 14.8% to 66%).

The total net water requirement of cotton is 762 mm, approximately 60 percent of that is provided from precipitation, and the rest should be supplied through irrigation (Warren et al., 2019). We assumed each pump provides water for a center pivot with a covering area of about 48.6 ha. So, considering once per year cotton growing and leaving the lands fallow for the rest of the year and 65% irrigation efficiency, each pump is required to extract at least 285 thousand m^3 per year or 5.7 million m^3 during 20 years of its life cycle.

2.5.3 GHG Emission from Electricity and Natural Gas

Based on the GREET-2021 WTW model, the total CO_2 emissions from electricity generation for the "U.S. Central and Southern Plains Mix" was 712.3 g CO_2 kWh $^{-1}$. The CO_2 emissions from producing the stationary natural gas based on the "NA (North American) NG from Shale and Conventional Recovery as Stationary Fuel" category from GREET 2021 WTW model was estimated from GREET-2021 WTW model at 20.95 g CO_2 kWh $^{-1}$. Adding the on-site GHG emissions due to the engine combustion process, the total carbon footprint of natural gas energy consumption was 202 g CO_2 kWh $^{-1}$.

2.5.4 Wind and Solar Energy GHG Emission

The GHG emission from solar energy ranged from 5 to 345 g CO_2 eq kWh $^{-1}$, and from wind energy was estimated to be between 1.7 and 123.7 g CO_2 eq kWh $^{-1}$ (e.g., Ardente et al., 2008; Constantino et al., 2018; Garrett & Rønde, 2013; Guezuraga et al., 2012; Kommalapati et al., 2017; Ozoemena et al., 2016, 2018; Pacca et al., 2007; Raadal et al., 2011, 2014). Two studies estimated the average GHG emissions from solar (Hsu et al., 2012) and wind (Dolan & Heath, 2012) energy based on harmonizing the GHG emission data represented in several other studies. Hence, the estimated average GHG emissions for solar (52 g CO_2 eq kWh $^{-1}$) and wind (12 g CO_2 eq kWh $^{-1}$) energy from these two studies are applied to this research. The beneficial life of solar panels and wind turbines, which ranged between 20 and 30 years in different mentioned studies, was assumed to be 20 years in this research.

2.5.5 Life Cycle GHG Emission from a Deep Well Vertical Turbine Pump

Considering the 3328 kg and 1107 kg weight of a deep well vertical turbine pump and a water distribution system pump, respectively, the GHG emission of a deep well vertical turbine pump was approximately estimated at 10,175 kg CO_2-eq. The beneficial life cycle of a pump was considered 20 years.

2.5.6 Estimated Energy Consumption and GHG Emissions

The results show that the second scenario used the highest average annual groundwater pumping energy in both study areas accounted for 35,635 (kWh year $^{-1}$) with 25.4 (ton CO_2-eq year $^{-1}$) in RSA and OGA, respectively (Table 2.1). The least GHG emissions were associated with wind energy followed by solar energy. Estimated GHG emissions for the S3 and S4 scenarios include life cycle GHG emissions from a deep well vertical turbine pump. The fourth scenario shows even though groundwater pumping energy consumption drops to 104,215 (kWh year $^{-1}$) due to higher OPE of electric pumps compared to NG pumps, the GHG emissions from current electricity mix were not decreased in this scenario.

Table 2. 1: Average Annual Energy Consumption, and GHG Emissions from Irrigation Groundwater Pumping in Study Area.

Energy Type	Study Area	Scenario	OPE_1 (%)	OPE_2 (%)	D_1 (m)	D_2 (m)	E_{Ave} (kWh)	GHG_{ave}	GHG_{Solar}	GHG_{Wind}
								(ton CO_2-eq)		
Electricity	RSA	S1	46.9	46.9	19.1	22.7	29,181	20.8	1.52	0.35
		S2	46.9	33.3			35,635	25.4	1.85	0.43
		S3	66	46.9			25,322	18.55	1.83	0.81
Natural gas	OGA	S1	14.8	14.8	57	79	285,822	58	-	-
		S2	14.8	12.9			310,502	63	-	-
		S3	17	14.8			270,319	55	-	-
Electricity		S4	66	46.9			104,215	74.7	4.85	1.51

OPE_1: OPE at the beginning of the Study
OPE_2: OPE after 20 years operation
D_1: Groundwater depth at the beginning of the Study
D_2: Groundwater depth in 20 years
E_{Ave}: Average annual energy consumption
GHG_{Ave}: Average annual GHG emissions from current energy types
GHG_{Solar}: Average annual GHG emissions from solar energy
GHG_{Wind}: Average annual GHG emissions from wind energy

2.5.7 Comparison of the Energy and GHG Saved Through Different Scenarios

Comparison of energy consumption through different scenarios revealed that in RSA, the highest amount of energy GHG (10,312 kWh year $^{-1}$) and consequently (6.84 ton CO_2-eq year $^{-1}$) would be saved through replacing the current pumps with decreasing OPE over time with a new pump (Table

2.2). In OGA, replacing NG pumps with decreasing OPE over time by electric pumps results in the most energy saving (227,075 kWh year $^{-1}$). Replacing NG pumps with decreasing OPE over time with new NG pumps will save 227,075 kWh year $^{-1}$. However, among all scenario altering in OGA, replacing NG pumps with decreasing OPE over time with new Ng pumps with higher OPE decreases GHG the most. The reason is that although OPE of electric pumps is much higher than NG pumps, the GHG emissions per kWh energy of the NG category applied to this study are much lower (less than 30%) than the electricity mix category.

Table 2. 2: Comparison of the Average Annual Saved Energy and GHG Emissions Through Improving OPEs by Replacing Pumps

Study Area	Energy Type	Scenario	Energy Saved (kWh year $^{-1}$)	Saved GHG (ton CO_2-eq year $^{-1}$)
RSA	Electricity	S1 to S3	3,859	2.2
		S2 to S3	10,312	6.8
OGA	Natural Gas	$S1$ to $S3_{NG}$	15,503	2.6
		$S2$ to $S3_{NG}$	40,183	7.6
	Electricity	$S1_{NG}$ to $S3_{Electric}$	202,395	-2.2
		$S2_{NG}$ to $S3_{Electric}$	227,075	2.8

The GHG emissions saving through different groundwater pumping energy supply management (switching from NG to electricity, switching to solar, and switching to wind energy) and pumping energy demand management (improving the OPE through replacing pumps) was conducted (Table 2.3). In the case of NG energy, improving the OPE resulted in more reduction in GHG emissions than switching from NG to electricity, in OGA. Results show in both study areas switching to clean energy could save more GHG than improving the OPE of the pumps. The highest GHG saving in both study areas could be met through energy supply management measures, especially switching to wind energy. The most GHG reduction among all scenarios was related to switching from NG to wind energy (61.2 ton CO_2-eq year $^{-1}$) in OGA. The saved GHG emissions were estimated

considering the life cycle GHG emissions from wind energy generation and a deep well vertical turbine pump. However, the energy demand management practice applied to this study is easier to be conducted than applied energy supply management practices. Because it can be applied to one single pump that could be decided by its owner alone. The GHG emissions from solar and wind energy are reported to have a significant negative correlation with the size of plant (Alsaleh & Sattler, 2019) that could considerably affect the results. Some social practices and financial support may be needed to encourage groundwater energy customers to replace the pumps and switch to a new type of energy.

Table 2. 3: Comparison of the Average Annual Reduction in GHG Emissions Through Different Energy Management Practices (ton CO2-eq year -1)

Energy Type	Study Area	Scenario	Improving OPE Through Replacing Pumps	Switching from NG to Current Electricity Mix	Switching to Solar Energy	Switching to Wind Energy
Electricity	RSA	S1	2.2	-	19.3	20.4
		S2	6.8	-	23.5	25.0
		S3	-	-	16.7	17.7
Natural Gas	OGA	S1	2.6	-2.2	52.9	56.2
		S2	7.6	2.8	57.9	61.2
		S3	-	4.8	55.1	58.4

It should be considered that the estimations in this study are based on GHG emissions from the "U.S. Central and Southern Pains" Utility Mix for electricity and "NA (North American) NG from Shale and Conventional Recovery as Stationary Fuel" for natural gas. These estimations could be applied to areas with similar mix sources of electricity generation. For example, based on GREET-2021, the GHG emissions from this electricity mix are estimated at 712.3 g kWh $^{-1}$, while it is reported to be at 415.3 g kWh $^{-1}$ from the average U.S. mix and 267.4 g kWh $^{-1}$ from California mix. The differences among GHG emissions from different electricity mixes are due to different source shares, especially clean sources of energy (e.g., hydropower, wind, and solar).

The amount of energy consumed to pump the groundwater, and consequently, GHG emissions from groundwater pumping depends on the lifting height of pumping, which changes over time; the volume of groundwater pumped; and the types of pumping devices (e.g., age, efficiency). A well's basic depth differs widely across regions and is often in flux, particularly in aquifers with a fast decline in groundwater table level. So, in-field water management practices that decrease groundwater withdrawal requirements and groundwater level decline could be hired to mitigate groundwater declines and GHG emissions. These measures could include applying deficit irrigation, growing lower water-use crop species, and improving the efficiency of irrigation water systems.

Many factors are associated with the significant differences among GHG emissions from solar and wind energy estimated in different studies. These factors include different estimation methods; different plant sizes (e.g., GHG emissions per kWh energy generated from solar plants were found to have a negative correlation with turbine size, reflecting economies of scale (Alsaleh & Sattler, 2019); different module efficiency; climate (e.g., wind characteristics and sunny hours); production methods for solar panels and wind turbines' materials (e.g., silicon and panels (Hagedorn & Hellriegel, 1992); and recycling (not considering recycling materials in the decommissioning stage could be the major reason for the difference in GHG emissions resulting from various studies (Ozoemena et al., 2018). It is suggested that standardizing assessment practices could reduce uncertainties in life cycle studies (Lenzen & Munksgaard, 2002).

2.6 Conclusion:

The estimations of this study show the importance of energy demand and supply management in reducing GHG emissions from agricultural groundwater pumping. Results indicate that the effectiveness of energy management practices in reducing GHG emissions depends on the type of energy used for groundwater withdrawal. In the case of natural gas energy, groundwater pumping

energy demand management, through improving OPE, will save more energy than switching to the electricity mix applied to this study. Nevertheless, switching to clean energies (wind and solar) saves significantly higher amounts of carbon than just improving OPE.

Wind energy shows the least amount of GHG emissions from pumping energy consumption for all the estimated conditions. Solar energy has the second ranking among wind, current electricity mix (U.S. Central and Southern Plains Mix), and NG ("NA (North American) NG from Shale and Conventional Recovery as Stationary Fuel" Plus Stationary Combustion) energy. So, increasing the share of wind energy that could be a reliable and sustainable source of energy for regions like Oklahoma with a high potential of wind could save thousands of tons of GHG emissions from agricultural groundwater pumping in Oklahoma during the 20-30 years life cycle of wind turbines. The energy demand management measure could be applied to as small scale as an agricultural well. But the GHG emissions from solar and wind energy have a negative correlation with the size of plants, and smaller plant scales contribute to higher GHG emissions (Alsaleh & Sattler, 2019). Hence, carbon footprint estimations for small power plants are suggested to be conducted before any investment. Further studies are required to focus on the feasibility of developing wind energy in the study area regarding economic assessment, other potential environmental aspects and required infrastructures. In-field water demand management practices (e.g., deficit irrigation and improving the efficiency of irrigation systems (Karimi et al., 2012)) could be considered to mitigate GHG emissions from agricultural groundwater pumping while protecting the aquifers.

The results of this study could be modified and applied on a global scale to identify opportunities to reduce carbon emissions of groundwater pumping in groundwater-dependent agricultural production systems through renewable energies (new technologies) or in-field management.

CHAPTER III

COMPARISION OF LIFE CYCLE GREENHOUSE GAS EMISSIONS OF VARIOUS PRE-FIELD AND IN-FIELD FACTORS THROUGH IRRIGATED CORN PRODUCTION

Abstract

This study aims to identify agricultural irrigation water, and nutrient management practices that affect greenhouse gas (GHG) emissions from groundwater irrigated agriculture. Irrigation water and agricultural improvements like fertilizer application are required for crop production and increasing crop yield to satisfy food demand of the growing population. However, negative consequences of these activities include production of GHG emissions. To mitigate this global environmental problem, the management methods that minimize agricultural GHG emissions should be identified. Accordingly, this work aims to estimate the cradle-to-product GHG emissions of corn production under various rates of fertilizer and irrigation water application in Oklahoma, U.S. This study specifically identifies the categories that contribute most to total GHG emissions, and the effects of in-field agriculture management practices on GHG emissions.

The results show the average total GHG emission from corn production is 271.46 g CO_2-eq kg^{-1} corn, 63.24 percent of that is associated with in-field GHG emissions. Agricultural soils, with an average of 88.77 g CO_2-eq kg^{-1} Corn are the driving factor contributing to total GHG emissions from corn production through nitrification and denitrification processes. On-site natural gas combustion for agricultural groundwater pumping, fertilizer production, and energy production for groundwater pumping are the next most influential parameters on total GHG emissions. Diesel production for the agricultural vehicles' fuel and seed and herbicides production contribute the least in GHG emissions from corn production. So, optimizing the application rate of irrigation water and fertilizer will assist the most to reduce GHG emissions from groundwater irrigated crops. The results are valuable for agricultural managers and farmers regarding sustainable agriculture and low GHG footprint of crop production in the study areas and similar regions in the world.

Keywords: Life cycle assessment, Greenhouse gas emission, Sustainable agriculture, Food and environmental security, Corn, Groundwater, Energy consumption.

3.1 Introduction:

Among different principle greenhouse gas (GHG) emissions from human activities the global warming potential of nitrous oxide (N_2O) is almost 300 times that of carbon dioxide (CO_2) and its lifespan is about 114 years, making N_2O emissions of even greater concern (EPA, 2022b). Worldwide, agriculture is the third largest source of GHG emissions and the main source of N2O (Cocco et al., 2018b). Since 1990, about 17 percent increase in N_2O and methane (CH_4) emissions from agricultural industries, accounting for an annual average emission rate of about 60 MT of CO_2-equivalent, has been reported. Approximately half of which come from agricultural lands and crop production and discharges of fertilizer-induced N_2O from crop production (Ramzan et al., 2020b). Hence, it is important to evaluate GHG emissions from agricultural soil when planning mitigation strategies for making environmental efficiency and economic planning possible.

As one approach, there is a need to understand the effects of different agricultural management practices and climate on GHG emission rates. A variety of factors including soil structure, temperature, moisture, microbial population, pH, C:N ratio, and several in-field management practices significantly affect the rates of N_2O emissions from agricultural activities (Foltz et al., 2019a; Ramzan et al., 2020b). Regarding their impacts on soil microenvironments, agricultural management practices (e.g., tillage, fertilization management, water regime) have an essential role in GHG emissions (Plaza-Bonilla et al., 2014; Rosa et al., 2014; Xue et al., 2015).

In confronting the core issues of food demand and environmental concerns, optimizing agricultural practices could result in balanced food and environmental security (Wang et al., 2022). While nutrients and fertilizers can increase crop yields, their negative environmental and economic consequences when they are lost from agricultural farms should not be neglected (Foltz et al., 2019a; Galloway et al., 2003b). Studies indicate that some in-field management practices such as nutrient and fertilizer application management could result in decreasing of N_2O emissions and nitrate (NO_3^-) losses from fields (Aldrich, 2015; Bao et al., 2012; Basche et al., 2014; Butterbach-Bahl & Dannenmann, 2011; Decock, 2014a; Rafique et al., 2011). So, quantifying and comparing the agricultural GHG emissions from various field management practices and environmental conditions is crucial to distinguish the more sustainable and less environmentally costly farming practices that enhance yield to secure world food production (Benbi, 2018; Foltz et al., 2019).

Several experimental and modeling approaches could be adopted to quantify agricultural soils and fields' GHG emissions. GHG prediction models are used to save time and labor required for experimental methods (Yadav & Wang, 2017). The Intergovernmental Panel on Climate Change (IPCC) offers a simple empirical-equation based on N inputs to estimate the direct N_2O emissions from managed soils and can be used even when minimal input data is available (IPCC, 2006). A life cycle assessment (LCA) modeling approach can be applied to estimate the life cycle GHG

emissions and carbon footprint of various pre-field and in-field factors contribute to agricultural productions.

This research aims to quantify cradle-to-product GHG emission estimations of corn production under different fertilizer and irrigation management. It should be noticed that the absorbed CO_2 by the crop is ignored in the estimation, and the values represent the gross carbon footprint on corn production. This study specifically aims to (i) estimate the total GHG emissions of corn production under various rates of fertilizer and irrigation water application in Oklahoma, (ii) identify the categories that contribute most to total GHG emissions, and (iii) quantify the effects of in-field agriculture management practices on GHG Emissions.

3.2 Study Area

The study area is an interactive program titled "Testing Ag Performance Solutions" (TAPS) that is operated in Texas county, Panhandle, Oklahoma (Figure 3.1). TAPS has been developed by Oklahoma State University's Division of Agricultural Sciences and Natural Resources to help irrigated corn and cotton producers to improve water use efficiency and management. The program provides the opportunities for farmers to examine research-based improved technologies and strategies at OSU Ag Research sites in the Oklahoma Panhandle before any investigation and spending their money in similar systems. The field is operated with one center pivot irrigation system, although individual plots can be irrigated and fertilized at different rates, which are remotely controlled. In the year of consideration (2020), there were fourteen different fields that could be included in this analysis. Each field had different rates of irrigation water, corn hybrid, seeding rate, herbicide, and fertilizer application and therefore, different yield (Table 3.1).

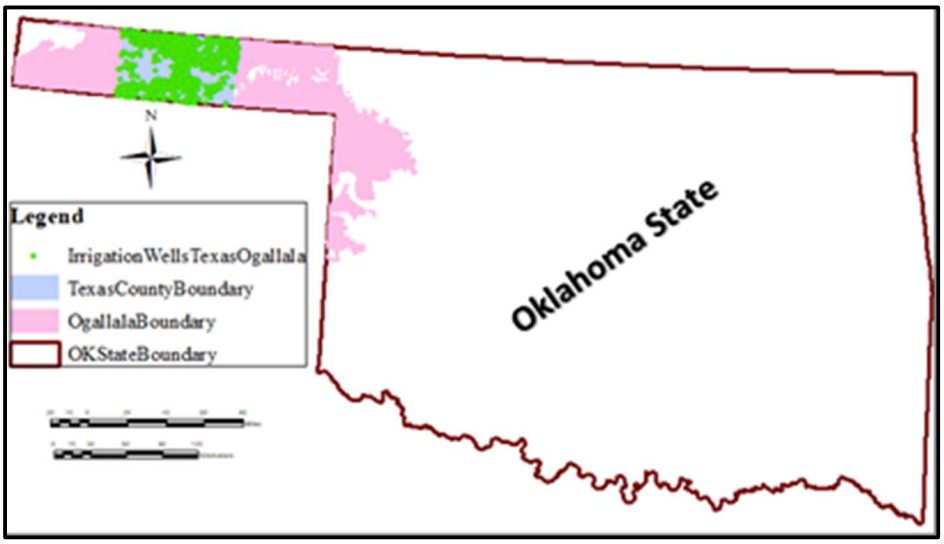

Figure 3. 1: Texas County, Oklahoma Panhandle, OK

Table 3. 1: Application Rate of Irrigation Water, Herbicide, Fertilizer and Seed in Each Field

Field ID	Total Water Applied (m^3 ha^{-1})	Fertilizer Applied (kg ha^{-1})	Herbicides (kg ha^{-1})	Seeding Rate (kg ha^{-1})	Corn Yield (kg Corn ha^{-1})
1	6,662	247	3	14	14,150
2	6,236	235		13	14,921
3	7,201	269		14	14,910
4	6,007	230		15	13,709
5	6,998	247		14	13,737
6	7,709	269		15	14,910
7	7,264	342		11	14,435
8	6,121	303		14	14,095
9	6,845	471		13	14,399
10	5,258	213		13	7,880
11	6,803	297		13	15,189
12	5,512	296		13	9,594
13	6,502	280		12	14,732
17	6,519	191		13	14,934

3.3 Material and Methodology:

3.3.1 Energy Consumption

The energy consumption for natural gas and electric pumps was estimated as explained in chapter II. The irrigation water for the corn fields was provided through groundwater pumping from three wells, one electric and two NG pumps. About 75 percent of the groundwater pumping energy for the study fields is natural gas (NG), and 25 percent is electricity. The irrigation water applied to each corn field will be adopted as groundwater discharge (m^3). The groundwater level in the area is 57 meters (estimated in chapter II), and the lifting height is estimated at about 60 meters.

3.3.2 Total Life Cycle GHG Emissions

A carbon footprint was adopted to estimate the total GHG emissions from corn production under different application rates of irrigation water and fertilizer. The life cycle GHG emissions were estimated for different pre-field and in-field parameters. Total GHG emissions from each TAPS field will be calculated as the sum of the pre-field and in-field GHG emissions.

3.3.3 Pre-Field GHG Emissions

Pre-field GHG emissions include life cycle GHG emissions from generating agricultural groundwater pumping energy (electricity and natural gas), fuel production for agricultural vehicles, and producing and transportation of seeds and chemical production (e.g., fertilizers and herbicides). SimaPro and Greet-2021 models were applied to estimate different components of pre-field life cycle GHG emissions.

3.3.3.1 GHG Emissions from Energy Production for Groundwater Pumping and Fuel Production for Agricultural Vehicle

The life cycle GHG emissions from producing the energy required for groundwater pumping were estimated as explained in chapter II. GHG emissions from fuel production for agricultural vehicles were estimated using the GREET-2021 well to pump (WTP) model (GREET.NET version 2021, Argonne National Laboratory, Argonne, IL, USA). "Conventional Diesel from Crude Oil for US Refineries Main Output: Conventional Diesel" category from GREET-2021 was considered as diesel used for agricultural vehicles fuel.

3.3.3.2 GHG Emissions from Producing Seed and Chemical Production

GHG emissions from fertilizer and seed production and transportation of these materials to the agricultural site were estimated using the SimaPro model. The fertilizer type that was used in the site was Ammonia, anhydrous, liquid. So, GHG emissions from the "Ammonia, anhydrous, liquid {RNA}| market" category that is for the fertilizer production in Northern America were applied to this study.

It was assumed that GHG emissions from corn seed production were approximately equal to GHG emissions from maize seed production. Hence, GHG emissions from seed production were estimated based on the GHG emissions from "Maize seed, for sowing {GLO}| market for | APOS, U" using the SimaPro model. GHG emissions from herbicide production is estimated based on the "Glyphosate {GLO}| market for | APOS, U" category in the SimaPro model.

The transportation was assumed to be through roads. So, GHG emissions for transporting fertilizer, herbicides, and seeds were also estimated based on "Transport, freight, lorry 3.5-7.5 metric ton, euro6 {RoW}| market for transport, freight, lorry 3.5-7.5 metric ton, EURO6 | APOS, U" category in SimaPro.

3.3.4 In-Field GHG Emissions

The in-field GHG emissions include GHG emissions from agricultural soils, natural gas combustion from groundwater pumping, and several on-farms human activities (e.g., preparing the land, planting, applying chemical materials, harvesting).

3.3.4.1 GHG Emissions from Agricultural Soils

GHG emissions from agricultural soil for each TAPS field, under combinations of different rates of irrigation water and fertilizer application, will be estimated using emission factor approaches as specified in IPCC guidelines (De Klein et al., 2006). The Tier 1 IPCC model was used to estimate direct N_2O fluxes for each field based on the applied amount of fertilizer (De Klein et al., 2006). The model was specified by eliminating non-relevant terms to this site. The specified Tier 1 IPCC model after simplification that was applied here is:

$$N_2O_{direct} = (F_{SN} + F_{CR}) \times EF_1 \quad (3.1)$$

where N_2O_{direct} is the direct N_2O emissions from managed soils per year (kg N y^{-1}), F_{SN} is the amount of synthetic fertilizer applied to the field (kg N y^{-1}), F_{CR} is the amount of N in crop residues (corn) returned to the soils (kg N y^{-1}), and EF_1 is the emission factor for N_2O emissions from N inputs (kg N (kg N input)$^{-1}$)(De Klein, et al., 2006). The IPCC default value of EF_1 is 0.01 with an uncertainty range between 0.003 and 0.03 that is applied to this study.

3.3.4.2 GHG Emissions from Groundwater Pumping Natural Gas Combustion

The natural gas pumps also contribute to on-site GHG emissions regarding the engine combustion process. The life cycle GHG emissions for stationary natural gas (from raw material extraction to end-use combustion) was examined using the GREET-2021 WTP model. The stationary engine emissions were estimated as is explained in chapter II.

3.3.4.3 GHG Emissions from On-Farm Human Activities

Life cycle GHG emissions from agricultural vehicles fuel include GHG emissions from producing Diesel fuel and the GHG emissions from fuel combustion. The GHG emissions from diesel combustion that were considered as GHG emissions from on-farm human activities were estimated using GREET-2021 WTW model for "Conventional Diesel from Crude Oil for US Refineries Main Output: Conventional Diesel" category. The average fuel consumption for on-farm human activities based on the data reported in Lazarus, 2000.

3.3.5 Uncertainty Analysis

The uncertainties contributed to the estimations include the uncertainty regarding EF_1 with a range between 0.03 and 0.003. Other uncertainty in estimating the average total GHG emission from corn production is related to various application rates of irrigation water and fertilizer that result in different GHG emission estimated from each field. A Monte-Carlo uncertainty simulation with 100,000 runs per year and treatment based on variation in model inputs was adopted in R to analyze the uncertainties associated with the estimations. A uniform distribution was assumed for EF_1 based on the minimum and maximum of its range. The rest of the parameters were assumed to fit normal distributions with different means and standard deviations. The uncertainty was estimated using variability in the inputs provided by field managers at the TAPS site (Table 3.1).

3.3.6 Sensitivity Analysis

Sensitivity analysis was done by varying different parameters within their ranges of uncertainty, with up to 100,000 Monte Carlo runs per year per treatment. Spearman's Rank correlation was used to find the correlation coefficient (rho) between various parameters (EF_1, F_{CR}, F_{SN}, Corn yield) and GHG emissions from agricultural soil for each field. The same method was applied to find the correlation coefficient (rho) between various parameters (EF_1, F_{CR}, F_{SN}, corn yield, natural gas, diesel, pumping energy, herbicide, seed, and fertilizer production, diesel and natural gas

combustion, and agricultural soil GHG emissions) and total GHG emissions from corn production for each field. The average correlations were used to assess the overall sensitivity of total GHG emissions and GHG emissions from agricultural soil to each parameter. Average correlations were calculated by taking the absolute value of each treatment's rho value (-1,1) and averaging them for a total of 14 different treatment combinations.

3.4 Results and Discussion:

The life cycle GHG emissions from various factors that are used for the total GHG emissions from corn production were extracted from SimaPro and GREET-2021 or estimated based on EPA Greenhouse Gas Inventory Guidance (Table 3.2).

Table 3. 2: GHG Emissions from Various Factors Based on SimaPro, GREET-2021 or EPA Greenhouse Gas Inventory Guidance

Parameter	GHG Emissions	Unit
Fertilizer Production [S]	2545.89	kg CO_2-eq ton^{-1}
Corn Seed Production [S]	1971.14	
Pesticide Production [S]	11002.89	
Transportation [S]	132.69	kg CO_2-eq km^{-1}
Electricity Generation [G]	712.3	g CO_2-eq kWh^{-1}
Natural Gas Production [G]	20.94	
On-site Natural Gas Engine Combustion[E]	185.76	
Diesel Production [G]	534.2	kg CO_2-eq lit^{-1}
On-site Diesel Combustion[E]	2673.2	
On-farm Fuel Consumption of Vehicles	36.85	lit ha^{-1}

[S]: Extracted from SimaPro
[G]: Extracted from GREET2021
[E]: EPA Greenhouse Gas Inventory Guidance

Total average GHG emissions from the 14 corn fields ranged from 213 to 360 (average 271 ± 46) g CO_2-eq kg^{-1} corn (Figure 3.2 & Table S1). The differences among per hectare GHG emitted from various fields were due to different rates of irrigation water, fertilizer, and corn seed application and different corn yields. The maximum GHG emissions per kilogram of corn production was estimated from field 10, which had the lowest corn yield (7,880 kg corn ha^{-1}). The minimum GHG

emission came from field 17, which had the lowest fertilizer application (191 kg ha^{-1}) and the second highest corn yield (14,934 kg Corn ha^{-1}). This trend is to be expected, as decreased fertilizer application rates are tied to decreases in N_2O emissions and overall GHG emissions (Decock, 2014b). Considering the range of uncertainty, total GHG emissions from each individual field could vary from -24 % to +56% due to different values of EF_1 (e.g., minimum of 0.003, average of 0.01, and maximum of 0.03). These extreme uncertainty ranges reveal the importance of accurate estimation on EF_1 when calculating agricultural GHG emissions.

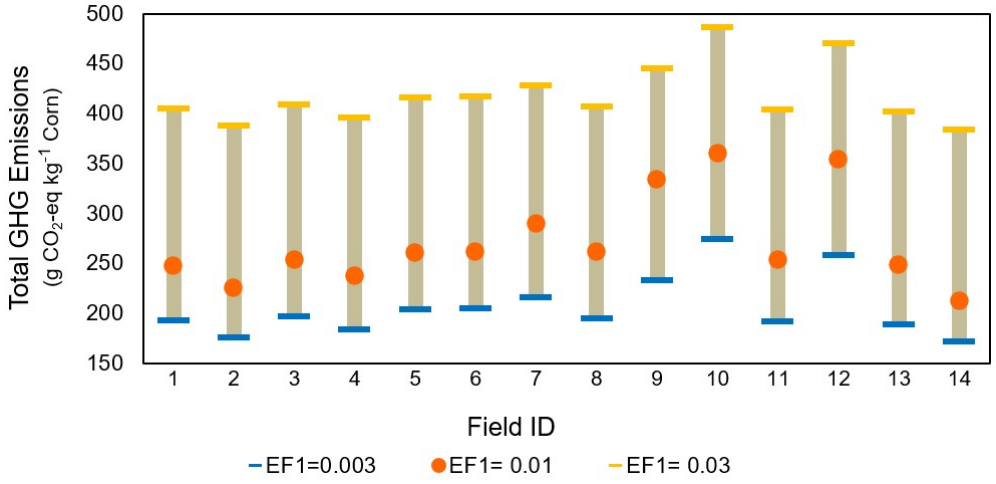

Figure 3. 2: Total GHG Emissions per kg Corn Production from Each TAPS Field

3.4.1 Comparison of Various Sources of GHG Emissions from Corn Production

Estimated average GHG emitted from each pre-field/in-field parameter (Figure 3.3 & Table S1) indicates that agricultural soils, with an average of 89 g CO_2-eq kg^{-1} Corn, contribute the most (33%) to the total GHG emissions from corn production. Denitrification and nitrification processes are two main pathways resulting in nitrogen losses and N_2O emissions from agricultural soils (Fan et al., 1997a; Fuller et al., 2016; Malique et al., 2019; Nie et al., 2019). Denitrification (nitrate to N_2 gas, often anaerobic), and nitrification (ammonium to nitrate, usually aerobic), are substantially

affected by agricultural management practices (e.g., fertilization, irrigation) and environmental factors (e.g., soil pH, temperature) (Foltz et al., 2019; Ramzan et al., 2020). Against other parameters that mainly emit CO_2, most of the GHG emissions from agricultural soils are N_2O (IPCC, 2006), with a global warming potential of about 300 times of CO_2 (EPA, 2022b). This relationship is consistent with previous studies that have reported agricultural soils as a primary source of agricultural GHG emissions (Mørkved et al., 2006; Sarris et al., 2019; Yadav & Wang, 2017a).

Research on GHG emissions from various N fertilizers reported the highest N_2O emissions from agricultural soil induced by anhydrous ammonia than other commonly applied synthetic N fertilizers (Breitenbeck & Bremner, 1986). As the considered fields applied anhydrous ammonia, it is possible contributions from agricultural soils could be decreased when applying best management practices (BMPs) for fertilizer. Studies suggest BMPs for N fertilizer application considering the source of N, rate, timing, and placement in addition to other in-field practices (e.g., cropping and tillage practices) could lower GHG emissions from corn production (Decock, 2014; Snyder et al., 2009).

Besides agricultural soil, other influential parameters on total GHG emissions in this study include on-site natural gas combustion for agricultural groundwater pumping (28%), fertilizer production (20%), and energy production for groundwater pumping (15%). This is consistent with a carbon footprint study from China, where fertilizer, electricity consumption for irrigation, and agricultural films were identified as the main factors contributing to carbon emissions (Huang et al., 2022). Improving overall pump efficiencies to decrease agricultural pumping energy consumption and switching the pumping energy from natural gas to electricity, specifically wind energy, where possible, could also reduce agricultural carbon footprint.

The lowest portion (0.55%) of the total GHG emissions was associated with diesel production for agricultural vehicles (1.5 g CO_2-eq kg^{-1} Corn), followed by seed (0.77%) and herbicide (0.81%) production. The GHG emission from on-site diesel combustion from the agricultural vehicle is estimated at an average of approximately 7.5 g CO_2-eq kg^{-1} Corn.

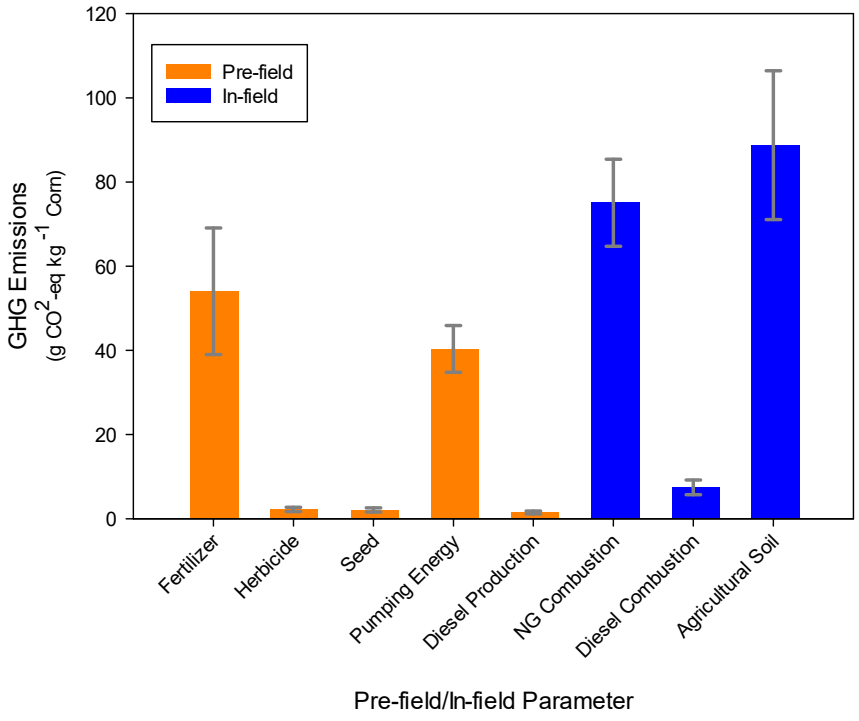

Figure 3. 3: Average (± STDEV) GHG Emitted from Each Pre-field / In-field Parameter of Corn Production

Model uncertainty in average GHG emissions from corn production was estimated using Monte Carlo simulations based on uncertainty in EF_1 and variation of the GHG emitted from different pre-field and in-field parameters at each of the 14 TAPS fields. The model results show approximately 25 percent uncertainty in estimating total GHG emissions from corn production.

3.4.2 Comparison of Pre-field and In-field GHG Emissions from Corn Production

The highest GHG emissions from corn production (average of 171 g CO_2-eq kg^{-1} Corn or about 63%) are associated with in-field GHG emissions (Table 3.3). In-field emissions include those from

agricultural soils and on-site natural gas combustion, which are the primary sources of GHG emissions from corn production.

Table 3. 3: Pre-Field, In-field and Total GHG Emissions from Each TAPS Field (g CO2-eq kg^{-1} Corn)

Field ID	Pre-Field	In-Field	Total
1	90	158	248
2	81	144	225
3	92	161	253
4	86	151	237
5	94	166	260
6	95	167	262
7	108	182	290
8	97	165	262
9	130	204	334
10	135	225	360
11	93	160	253
12	136	219	354
13	91	158	249
17	74	138	213
Mean	100	171	271
Min	74	138	213
Max	136	225	360
STDEV	20	27	46

Corn yield had a strong positive polynomial relationship with water application ($R^2 = 0.96$, Figure 4) while it has a weak relationship with fertilizer application ($R^2 = 0.04$). Regarding the historical drought information represented by National Integrated Drought Information System (NIDS), 2020 was a dry year with a drought starting in May (NIDIS, 2022). The drought can be the reason for crop yield's strong dependency on the irrigation water application rate. A study in the rainfed corn belt of the U.S. between 2003 and 2014 related a significant corn yield reduction due to the severe hydrological drought that resulted in excessive water depletion in agricultural soils in 2012 (W.

Zhou et al., 2020). Corn yield is reported to have a significant response to water and nutrient applications (Mandal et al., 2010). The difference between the results of crop yield response to fertilizer application between that study and the current research could be because of applying different types of fertilizer and using of organic manure along with the applied fertilizer in the mentioned study.

The regression between GHG emissions and most effective parameters (e.g., the water, fertilizer and seeding rate and corn yield) was examined and the following equation is found among these parameters (Figure 3.4).

$$GHG = 376.28 + 0.017 \times W + 0.375 \times F - 0.711 \times S - 0.023 \times Y \quad (3.2)$$

W: Water rate application, F: Fertilizer rate application, S: Seeding rate, Y: Corn yield

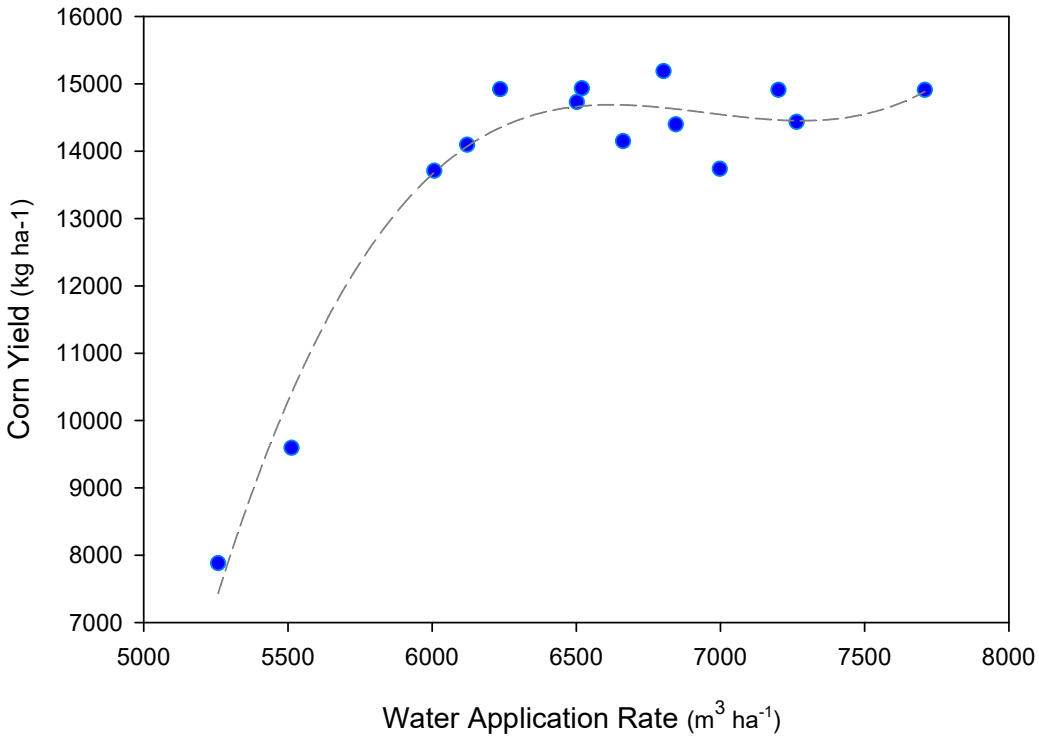

Figure 3. 4: Relation Between Corn Yield and Application Rate of Water

3.4.3 Sensitivity Analysis

Sensitivity analysis revealed that GHG emissions from agricultural soil are least sensitive to F_{CR} and most sensitive to EF_1 (Table 3). This outcome was to be expected as agricultural soils are the dominant source of total GHG emissions, so their sensitivities would be linked. The total GHG emission from corn production is the least sensitive to herbicide, seed, and diesel production.

Table 3. 4: Spearman's Rank (rho) Correlations for Estimated Total GHG Emissions and GHG Emissions from Agricultural Soils Versus Various Parameters Across 14 Treatments

Variable	GHG Emissions from Agricultural Soil	Total GHG Emissions
F_{CR}	0.0705	0.0676
F_{SN}	0.2845	0.2768
EF_1	0.8984	0.8706
Corn Yield	-0.2611	-0.2553
NG Combustion	-	0.1162
Diesel Combustion	-	0.0212
Fertilizer Production	-	0.1790
Herbicide Production	-	0.0041
Seed Production	-	0.0043
Pumping Energy	-	0.0610
Diesel Production	-	0.0060
Agricultural Soil	-	0.9705

These results indicate the importance of targeting agricultural soil when planning to reduce agricultural GHG emissions from crop production. Due to the high sensitivity of GHG emissions from agricultural soils and crop production to EF_1, accurate estimation of EF_1 is essential to reduce the uncertainty of these GHG estimations. Sustainable agriculture could be planned through a multi-objective optimization model that targets maximizing crop yield and minimizing GHG emissions.

3.5 Conclusion:

The main portion of GHG emissions from corn production is associated with in-field activities; most of it is emitted from agricultural soil, followed by natural gas combustion from groundwater pumping. Among pre-field parameters, energy production for groundwater pumping is the driver GHG emitter, followed by fertilizer production. So, when planning to reduce the GHG emissions from corn production, these parameters, especially GHG emissions from agricultural soil, could be targeted to achieve the optimum result. An optimization model is suggested to estimate optimum fertilizer and water application rates to maximize crop yield and minimize GHG emissions. The source of N fertilizer could also affect the rate of GHG emitted from agricultural soils.

The total cropping system emission is highly influenced by the agricultural soil component of the model which is highly sensitive to the EF_1. It indicates the importance of accurate measurement of EF_1 and GHG emissions from agricultural soils when estimating GHG emissions from corn production to reduce the uncertainties.

This work brings insight to agricultural managers and farmers on how the application rates of fertilizer and irrigation water will impact the existing and potential GHG footprint of corn production in the study areas and similar regions in the world.

CHAPTER IV

MICROBIAL DENITRIFICATION IN AGRICULTIRAL SOILS

Abstract

Denitrification is known as the primary pathway for nitrous oxide (N_2O) emitted from agricultural soils. Furthermore, it reduces the utility of nitrogen (N) fertilizer applied to crops since during denitrification, the bacteria transform the applied N to the gaseous forms of N which are loss to the atmosphere. The higher the denitrification gene abundance, the higher potential that nutrient will be taken away from crops by denitrifying bacteria. So, finding the optimum application rates of fertilizer and water that minimize the growth of denitrification community abundance can assist in mitigating nutrient loss from cropping systems. Other parameters may also affect denitrification community size, including soil texture, pH, moisture, and organic C. However, the aim of this study is to evaluate the influences of various fertilizer application regiments and water application rates on the abundance of denitrification genes.

4.1 Introduction

Microbial denitrification is the critical driver causing nitrogen losses from the agricultural system (through transforming the applied nitrogen (N) to the gaseous form of N and poor crop consumption of utilized N fertilizer (Fan et al., 1997b; Fuller et al., 2016b; Malique et al., 2019; Nie et al., 2019b). Denitrification (nitrate to N_2 gas, often anaerobic) is substantially affected by several agricultural management practices (e.g., fertilization, irrigation water) and environmental factors (e.g., soil pH, temperature).

4.1.1 Denitrification

Denitrification is a microbial anaerobic pathway in which N oxides are used as alternative electron acceptors in the absence of oxygen (O_2) for the aerobic process. Denitrification pathway (bacterial and/or fungal) includes nitrate (NO_3^-) reductase to dinitrogen gas (N_2) with the obligatory intermediates nitrite (NO_2^-), nitric oxide (NO) (gas), and N_2O (gas) (Figure 4.1). The first denitrification step is nitrate reductase (NO_3^- to NO_2^-), encoded by the functional genes napA and narG (Braker & Conrad, 2011). The nitrite reduction (reduction of NO_2^- to NO), known as the primary step in denitrification, is encoded by the functional genes nirK and nirS due to either being catalyzed by a copper or cytochrome cd1 nitrite reductase, respectively (Philippot et al., 2007). Subsequently, nitric oxide reductase (the reduction of NO to N_2O) is encoded by norB genes (Braker & Conrad, 2011). NO_2^- and NO reductions can also be caused by ammonia oxidizers, which results in an N_2O formation in the procedure called nitrifier-denitrification (Kool et al., 2011). The reductions of NO_2^- and NO are more likely to be occurred as detoxifying mechanism due to nitrosative stress (Philippot et al., 2007). However, nitrifier denitrification is considered an independent N_2O emission pathway (Kool et al., 2011).

The last step in the denitrification pathway, the nitrous oxide reductase (reduction of N_2O to N_2), is encoded by the functional gene nosZ and nosZ-II (a recently found uncommon gene) (Sanford et al., 2012; Jones et al., 2013). The N_2O reductase is the only recognized biological sink process for N_2O. It is highly sensitive to O_2 availability and soil pH. Constricted functionality of the N_2O reductase results in an incomplete denitrification and N_2O formation (Butterbach-Bahl et al., 2013).

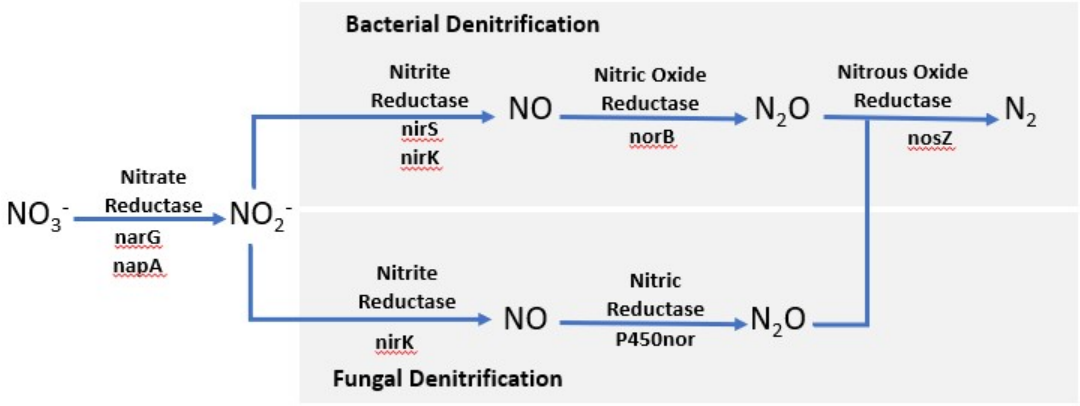

Figure 4. 1: Bacterial and Fungal Denitrification Pathway (Ma et al., 2019)

The growth rates of denitrifiers, the microbes which make N_2O or N_2, are related to the reduction of N oxides (Philippot et al., 2007). Denitrification is a modular pathway, emphasizing the importance of influence of community composition for N_2O emissions (Graf et al., 2014). It means not all microbes involved always have the entire set of enzymatic systems and hence fulfill only a subset of steps within denitrification pathway (Zumft, 1997). Approximately one-third of denitrifiers are recognized to lack the functional gene nosZ making them a source of N_2O (Philippot et al., 2011). Many atypical nosZ functional genes cannot be referred to as typical denitrifiers because of their phylogenetically widespread occurrence and lack of antecedent denitrification genes. Nevertheless, they can contribute to the sinking of N_2O due to their generic capability to nitrous oxide reductase (Jones et al., 2014).

4.1.2 Influencing Factors on Denitrification Genes in Managed Soils

N_2O emissions from managed soils mostly happen due to altering biogeochemical conditions and have significant temporal and spatial variability (Butterbach-Bahl et al., 2013). Several factors affect N_2O emitted from agricultural soils, either directly (proximal control) or indirectly (distal control) (Braker & Conrad, 2011; Wallenstein et al., 2006). Proximal controls are the environmental conditions and resources that affect instantaneous denitrification rates resulting in the kinetics of denitrification at particular times by affecting the metabolism of an existing microbial community. They can include soil pH, availability of C and N species, oxygen (O_2) availability, calcium, and moisture. O_2 is an important characteristic as denitrification occurs only when O_2 availability becomes scarce and bacteria communities switch to nitrate and nitrite for their terminal electron acceptors. The O_2 availability in soils significantly depends on the water content of soil-filled pore spaces (Braker & Conrad, 2011; Wallenstein et al., 2006). Due to the influence of soil aeration on the dominant nitrogen cycling process, O_2 availability is likely to be the primary driver for N_2O emissions on regional scales (Jungkunst et al., 2006). Higher water contents of soil result in less O_2 availability for anaerobic processes like denitrification and anammox (Butterbach-Bahl et al., 2013). However, aerobic, and anaerobic N cycling processes could happen within very small soil particles, and denitrification can occur even at about 80% water-filled pore soil (Butterbach-Bahl et al., 2013). Nitrification and nitrifier-denitrification are the main sources of N_2O emissions at water-filled pore soil below 70% (Kool et al., 2011). NO_3^- mainly affects the denitrification rates as a proximal control. However, its long-term direct effects on denitrifier communities are less. Soil moisture is likely to make leaching of NO_3^- - N (Bowen et al., 2018). The nirK abundance is suggested to have a significant positive correlation with soil moisture while abundance of nirS is not (Bowen et al., 2018). Not surprisingly, the abundance of denitrification

genes is also reported to be sensitive to rainfall events as dentrifiers grow as a result of the favorable redox conditions (Snider et al., 2015).

Studies reported significant changes in abundance of narG genes in response to application of N fertilizer (Li, Tang et al., 2016; Ma et al., 2016). The N availability and speciation can influence the magnitude of N_2O emitted from agricultural soils. Ammonium (NH_4^+) contributes to several levels of magnitude lower (103-106) N_2O emissions than NO_3^- (Braker & Conrad, 2011; Canfield et al., 2010). N_2O emissions from agricultural soils often happen after N fertilization and have an exponentially positive relationship with the application rate of N fertilizer. Hence, fertilization rate is suggested to be the best single parameter for estimating N_2O emissions from soil, indicating the importance of applying the efficient rate of N fertilizer in agricultural soils (Shcherbak et al., 2014).

The community size and composition of denitrification genes, mostly narG and nirS, are significantly sensitive to long-term fertilization, and nirK, nosZ, and qnorB are less sensitive, respectively (Chen et al., 2012). However, no significant statistical differences in diversity of narG genes due to various fertilizer treatments is reported (Li, Tang et al., 2016; Ma et al., 2016). Besides, it is indicated that it is poorly understood whether there is significant changes in abondance of narG, nirS and nosZ due to applying nitrogen fertilizer alone (Chen et al., 2012). However, various denitrifiers have different responses to different fertilization regimes. For example, the community composition of narG genes is very sensitive to mineral fertilizers, and less influenced by rice straw, plant residues (roots) and secretions left in the soil. In contrast, nosZ is significantly influenced by rice straw, and qnorB is not highly affected by fertilizer regime (Chen et al., 2012). The abundances of narG, nirS, nirK, and nosZ genes do not change significantly due to N gradient changes (W. Ma et al., 2016; Miller et al., 2008). The lack of the sensitivity of the genes may were because the genes were not much limited by N in the studied lands (W. Ma et al., 2016).

Due to the requirement of organic C as electron donors in heterotrophic N cycling processes (e.g., denitrification), C availability is another proximal control of N_2O emissions that limits the denitrification rate (Wallenstein et al., 2006). Hence, denitrification can be significantly influenced by any factor that affects C mineralization rates (Saggar et al., 2013). These factors include root exudation, incorporation of crop residues, and temperature. If NO_3^- concentrations are not limiting, a positive relationship between N_2O emissions from denitrification and the availability of C also can be assumed (Bhandral et al., 2007; Saggar et al., 2013). Shortage conditions of NO_3^- in adequate availability of C, are reported to cause N_2O reduction reduces N_2O emissions and the ratio of N_2O/N_2O+N_2 product (Miller et al., 2009; Senbayram et al., 2012). A high correlation is reported between the amount of organic carbon in soil and the quantity of denitrifier genes (Kandeler et al., 2006).

Soil pH is another factor affecting N_2O reduction and the abundance (Bru et al., 2010), composition (Lauber et al., 2009) and diversity (Fierer & Jackson, 2006) of microbial community. Low soil pH impedes N_2O reduction (Baggs et al., 2010; Saggar et al., 2013). It inhibits the correct bending of the nitrous oxide reductase enzyme in *Paracoccus denitrificans*, and high N_2O emissions are reported to be related to dysfunctional nitrous oxide reductases (Bergaust et al., 2010). Furthermore, low soil pH is reported to increase fungal denitrification, increasing N_2O emissions due to fungi commonly lacking genetic capability for N_2O reduction (Saggar et al., 2013). A positive relationship between pH and the abundance of nirS gene, even due to narrow changes in pH levels in the soil environment, can be expected (Bowen et al., 2018). Studies reported higher sensitivity of nirK to pH changes than nirS (Bowen et al., 2018). The soil pH and moisture are reported to have a negative correlation with narG abundance and positive correlations with the abundance of nirK, norB (Z. Li et al., 2020), some versions of nosZ (Bowen et al., 2018; Zhou et al., 2011) and nosZ-II but not with nosZ-I gene abundance (Bowen et al., 2018).

Distal controls that influence N_2O emissions indirectly are less dynamic. Soil texture is one of the distal controls that affect N_2O emissions through a significant effect on soil pore space and soil hydrology (Wallenstein et al., 2006). Hence, N_2O emissions from clay dominant soils (with higher water-holding capacity than sandy soils) are less than sandy soil. High clay contents also relate to enhanced organic C stocks and N retention that affect N_2O emission (Gaines & Gaines, 1994; Grüneberg et al., 2013). The abundance and diversity of bacterial communities are also reported to be affected by soil texture (Carson et al., 2010). Because N cycling is primarily controlled by microbial activities, microbial community characteristics (e.g., the size, metabolic activity, and structure of the functional gene communities) contributing to N_2O emissions and reduction is another distal control for N_2O emissions (Wallenstein et al., 2006).

In addition to bacterial pathways, the abundance of Archaea amoA, nirS, and nosZ genes under agricultural soil conditions have a substantial positive correlation with nitrification/denitrification activities which could be helpful to improve the methods applied to mitigate N_2O emissions from agricultural soils (W. Wang et al., 2019). Several studies reported a relationship between denitrifier gene abundances and N_2O emissions (Chen et al., 2015; Morales et al., 2010; Tatti et al., 2014). It is suggested that a rapid increase in N_2O production typically corresponds with a substantial rise in the quantity of nitrifier and denitrifier genes (Snider et al., 2015). However, other studies reported denitrification activity is not related to the abundance of denitrifier community (Miller et al., 2008). The abundance of functional genes cannot be references alone to comprise a measure of microbial activity and efficiency of enzymatic reactions and several factors can affect it (Bier et al., 2015). Therefore, other studies reported the abundance of functional genes has no significant relationship with the rate of N_2O emissions (Dandie et al., 2011; Henderson et al., 2010). Nevertheless, the composition of functional communities can significantly influence their functionality. The abundance of denitrification genes has a negative relationship with soil depth (Shen et al., 2017; S.

Wang et al., 2019). Soil temperature influence the composition and abundance of denitrification genes (Braker et al., 2010).

The $N_2O/(N_2O+N_2)$ product ratios in annual and perennial cropping systems are significantly influenced by the population composition of N_2O-reducing bacteria (Domeignoz-Horta et al., 2015). In consequence, soil management practices may affect microbial communities and size. These management practices include all soil-related practices applied to crop growing that change the environmental conditions and nutrient availability in agricultural soils. Tillage, crop residue incorporation, crop rotation, fertilization, and other chemical application (e.g., pesticides) are some soil management practices that can influence microbial communities in various ways. Denitrifier communities' composition and size are influenced by land use intensity and fertilization regime (Hallin et al., 2009; Meyer et al., 2013; Tatti et al., 2014). Microbiological soil management through hiring the mentioned management practices is reported as the most likely method for N_2O emissions mitigation (Thomson et al., 2012). However, the influence of various soil management practices on N_2O emissions and functional gene communities affecting N_2O emission rates is not fully identified. The main challenge regarding the influence of various introduced soil management practices on mitigating N_2O emissions is the complex nature of N_2O-causing and decreasing processes (Venterea et al., 2012).

As mentioned, processes cause changes in the rates if the N_2O emissions are sensitive to a variety of factors and their spatial and temporal variability. The factors' spatial and temporal variability often impede the reliable application of studies outcomes on N_2O emissions for other places and times (Venterea et al., 2012). However, decreasing the application rate of N fertilizer is reported to mitigate N_2O emissions regardless of the pedoclimatic conditions (Venterea et al., 2012). Optimizing the application rate of N fertilizer to decrease N_2O emissions while preventing lowering crop yield is required to secure and sustain agricultural production (Thomson et al., 2012).

In this study, denitrification bacteria occurring in agricultural soil in an experimental field growing corn under various irrigation water and fertilizer application rates have been investigated through the corn-growing period. Community analysis and genes which are involved in the denitrification pathways were analyzed using amplicon high-throughput sequencing and real-time quantitative polymerase chain reaction (qPCR) assays. The study investigated the response of the abundance of denitrification genes in corn field soils under different irrigation water and fertilizers application rates. The abundances of different genes involved in the bacterial denitrification pathway were examined to determine if they are affected by different fertigation treatments. The increasing quantity of the genes means more consumption of the applied fertilizers by denitrifier genes, which leads to fewer N fertilizers available for crops.

4.2 Study Area

Like chapter three, the study area was an interactive program titled “Testing Ag Performance Solutions” (TAPS) that is operated in Texas County, Panhandle, Oklahoma. However, the values applied to this chapter were related to TAPS 2022. The fields were operated with one center pivot irrigation system, although individual plots could be irrigated and fertilized at different rates, which were remotely controlled. In the year of consideration (2022) and for this study, there were 24 different plots representing 8 treatments replicated 3 times. The eight treatments received different amounts of irrigation and either different nitrogen fertilizer applications before planting and/or during the growing season.

4.3 Material and Methods:

4.3.1 Sample Collection

Soil samples were collected from the study area at five dates. It included one pre-planting sampling and four times sampling through the corn growing period. For the pre-planting sampling, samples were taken randomly from across the study field. During the four sampling periods between sampling and harvest, sampling was done from all three replicates of the eight different treatments. The sampling dates were Mar 25, 2022 (Pre-planting), May 11, 2022, July 14, 2022, Aug 16, 2022, and Oct 25, 2022. The field was planted on May 12 and harvested on October 27th. The corn was approximately 1 m tall on July 14 and 2 m tall on August 16th. A total of 114 samples (18 pre-planting samples plus 4 sampling from 24 fields under 8 treatments replicated 3 times) were collected in 50 ml tubes and stored in an ice box. Also, 96 samples (4 sampling from 24 fields under 8 treatments replicated 3 times) were collected in 2 ml cryogenic vials and kept in ice and liquid nitrogen to be transferred to the laboratory for DNA, RNA, PCR, and qPCR analysis and stored in the freezer at -20°C until DNA and RNA extraction. All the soil samples were taken from the soil surface at a depth of 5 to 10 cm.

4.3.2 DNA Extraction

DNA was extracted and washed from 250 mg aliquots of all soil samples using the DNEasy Power Soil Kit (Qiagen) following the protocol that was provided by the manufacturer's instructions. The extracted DNA was then quantified using Quantus^{TM} fluorometer spectrophotometry (Promega) The applied protocol is represented in Appendix I). The DNA was frozen at -20 °C until further analysis.

4.3.3 Primer Design

Studies reported that the diagnostic genes in the bacterial and fungal denitrification pathway include narG1960, narG, nirKC, cnor, qnor, V, cd, nosZ, nirKF and P450nor (Braker & Tiedje, 2003; Delorme et al., 2003; Flanagan et al., 1999; Henry et al., 2006; Higgins et al., 2016; Y. Ma et al., 2019; Michotey et al., 2000; Novinscak et al., 2016; Philippot et al., 2002, 2007, 2011; Wei et al., 2015). A total of thirteen primer pairs targeting these genes with a high coverage for recovering the genetic diversity within each target group were used from previous studies (See Table 4.2). These primer pairs had a high degeneracy and may be prone to amplify non-targeted genes. Thus, for a diverse microbial community (such as agricultural soils) they are not suitable for qPCR directly. Thus, the approach was as follows: 1. these primers were used to amplify the denitrification pathway genes present in the field; 2. these amplification products were then sequenced so that the specific sequences of dominant pathway genes can be recovered; (3) specific qPCR primers were then designed based on those recovered sequences, and (4) several qPCR assays could be used in parallel to quantify the dominant specific denitrification pathway genes through all samples.

The initial PCR experiments were applied to a combination of the DNA extracted from pre-planting soil samples from the TAPS field. The PCR products of the primer pairs that had good results were sent to Molecular Research DNA (Shallowater, Texas) laboratory for amplicon sequence analysis of functional genes.

To design the primers, sequences obtained from sequencing were aligned using Molecular Evolutionary Genetics Analysis (MEGA). Sequences were analyzed with BLASTn and BLASTp (NCBI GenBank) to confirm their function as expected (NIH, 2022). Sequences which did not match the expected nitrogen cycling genes were excluded from further analysis. Then Phylogenetic Analysis and tree clustering (Construct/Test neighbor-joining Tree) were applied to analyze

diversity of obtained sequences. For any closely related group of genes (i.e. those that were highly homologous), a primer pair was designed to target that group. Primers were also designed for genes which were not highly similar to other genes obtained from the sequences. Then primers were designed using primer-blast (NIH, 2022). Primer blast is an online primer designing tool for making primers that are specific to the input PCR template. Primer pairs were then analyzed in MEGA against the collected sequences, and primers that offer the most specificity for the gene (or closely related groups of genes) were chosen for qPCR assay development. Accordingly, primer pairs were designed for a total of fifty-two specific genetic targets covering the dominant denitrification genes in this field.

4.3.4 Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is an enzymatic procedure to detect specific genes within an environmental DNA sample. PCR uses short DNA sequences (oligonucleotide primers) that are defined by the user, and their sequences are complementary to target regions of genes identified to encode for particular microbial functions (e.g., denitrification genes). The DNA sample is denatured to generate single-stranded DNA (template DNA) to which the oligonucleotide primers can bind. Then nucleotide bases are added to the end of each primer by the enzyme DNA polymerase. Then the enzyme DNA polymerase adds nucleotide bases to the end of each primer and extends the primer based on the pattern of the template DNA. So, a new double standard DNA is produced. The DNA samples for each target gene by the oligonucleotide primers get enriched by repeating the process for a number of cycles. Each cycle of PCR involves producing two new double-stranded DNAs from each DNA molecule present. Hence, every cycle of PCR theoretically doubles the amount of DNA. Consequently, after two cycles, DNA concentration rises by 2^3-fold; after three cycles, a 2^3-fold rise; and after N cycles, PCR creates a 2^N-fold rise in the target DNA. PCR steps are simply represented in Figure 4.2. The applied PCR protocol to this work is described

in Appendix I. The PCR products were then quantified using Quantus™ fluorometer spectrophotometry (Promega).

4.3.5 Cleaning Up the DNAs/ or PCR Products

It is typically necessary for downstream use to purify DNAs from PCR reactions. It facilitates removing enzymes, nucleotides, primers and buffer components. Spin columns containing a silica matrix were used to purify DNAs/or PCR products. DNA can be selectively bound in the existence of chaotropic salts. While other reaction components (e.g., enzymes, nucleotides, detergents, and primers) will not bind well or will be removed during the cleaning up process. After all, the DNA/ PCR products were washed from the columns under low-salt conditions and were ready for demanding downstream applications. The PCR Cleanup Kit (Qiagen) was used according to manufacturer protocols.

4.3.6 Quantitative Polymerase Chain Reaction (qPCR)

Most PCR applications are utilized to duplicate DNA to a larger amount. It is required for running gel electrophoresis and most types of DNA sequencing. However, it limits the informativeness of PCR. Hence, PCR is often considered a step in another measurement procedure rather than a measurement tool on its own. Real-Time Quantitative PCR (also known as qPCR) is applied to find out the amount of a specific section of DNA in a sample in real-time. This method allows simultaneous screening of DNA (or nucleic acids) sections. The applied qPCR protocol to this work is described in the appendix I.

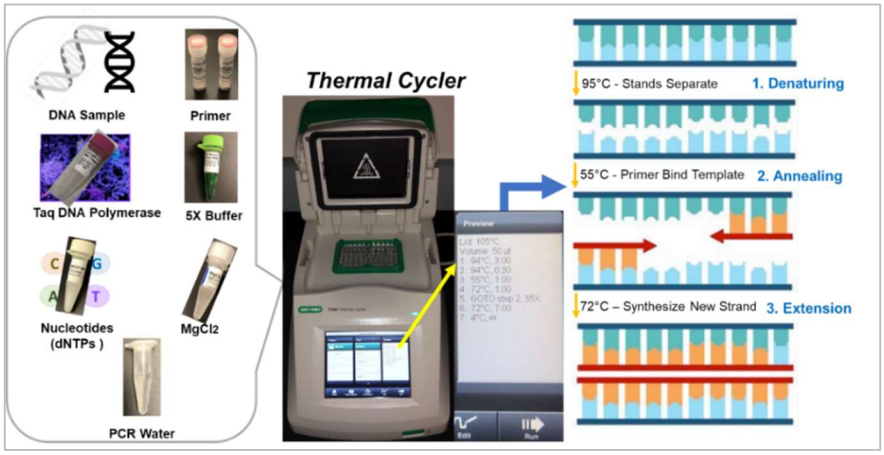

Figure 4. 2: Polymerase Chane Reaction (PCR) Steps

The qPCR analyses were conducted using Bio-Rad CFX Manager software on a CFX Connect Real-Time System (Bio-Rad Laboratories). Thermal cycling conditions for each primer pair included an initial cycle of 95°C for 3 min followed by 40 cycles of 95°C for 15 s, 59°C for 30 s, followed by a melting curve analysis (Figure 4.3). The thermal cycling conditions for all primer pairs were the same.

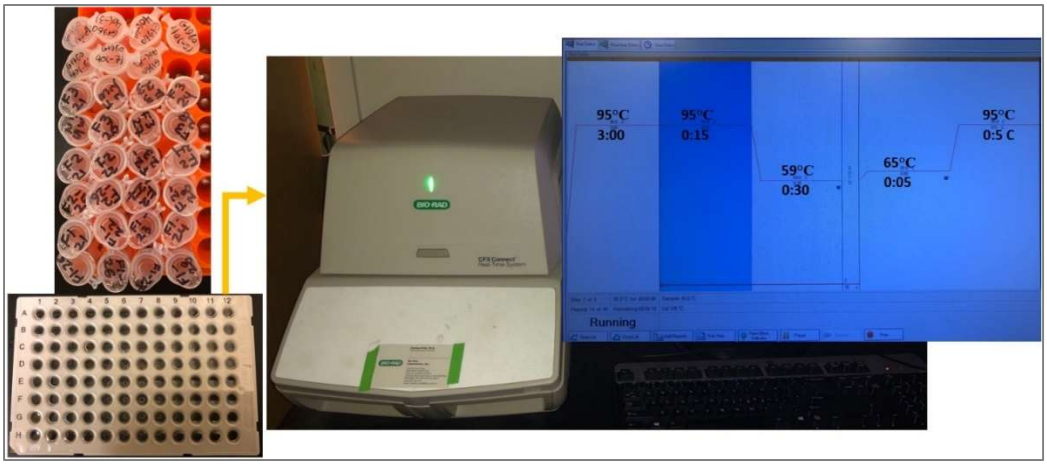

Figure 4. 3: Real-time Polymerase Chane Reaction (qPCR) Steps

The quantification results (amplification and standard curve) were examined, and the standards' outliers were removed from the plates. NTPCs were checked out to ensure they were low enough; otherwise, they were removed. After each complete run, a melting curve analysis was performed to ensure that primer dimers were not amplified, and that the amplification was specific. Then, the melt curve that did not follow the dominant trend and those with more than one peak (hump) were removed. Each sample was analyzed with qPCR in triplicate; the triplicates were log10 transformed and averaged. The regression results were checked for the efficiency, slope, and R^2 values. After assuring about the quality of the results, the quantitative analysis of the results was conducted in Excel.

4.3.7 Climatic Data and soil temperature

Climatic data and soil temperature, including daily rainfall, maximum, minimum, and average daily air/and soil temperature, were downloaded from the Mesonet website for Eva station (EVAX – Eva), which is the closest station to the study area (Mesonet, 2022). The data were checked out in excel for any significant changes or event between and before sampling date that may affected the abundance of specific genes.

4.3.8 Statistical Analysis

A two-way analysis of Variance (ANOVA) with a 95% confidence interval (α=0.05) was applied to result to find out any significant relationship between the abundances of individual genes with fertigation (water and fertilization) treatment and with dates. For a more detailed assessment multi correlation of the abundance of each individual gen and target group was examined with the fertilizer application rate, water application rate, and the date. The correlations were also done based on the summation of water and fertilizer application to the dates of samplings.

4.4 Results and Discussion:

The eight different amounts of irrigation and nitrogen applied to 24 plots (8 plots replicated 3 times) were as presented in the Table 4.1.

Table 4. 1: Applied treatments to TAPS-2022 Fields

Field ID	Treatment	Fertilizer (Kg N/ha)				Water (mm)			
		13-Apr	21-Jul	29-Jul	3-Aug	11-May	14-Jul	16-Aug	25-Oct
F21	Low Water High N	235	0	0	0	0	140	165	227
F22	High N High water	235	0	0	0	0	165	165	227
F23	High Water Zero N	0	0	0	0	0	165	165	227
F24	Zero Water High N	235	0	0	0	0	70	0	19
F25	Zero Water Zero N	0	0	0	0	0	70	0	19
F26	High Water	112	0	34	0	0	165	165	227
F27		112	34	34	0	0	165	165	227
F28		112	67	34	34	0	165	165	227

No significant daily rainfall was reported for the nearby Eva station on the Oklahoma Mesonet weather monitoring network on the days directly leading up to the sampling dates (Tables A2.2). The maximum air temperature was highest on July 14, the day the second sampling from specific fields was done (sampling was done during the highest temperatures of that day) (Tables A2.3). The minimum temperature on the sampling day in October (25^{th}) was the lowest throughout the corn-growing season up to the date (Tables A2.5). These results were used to analyze the qPCR results. Because the abundance of denitrification genes were reported to have positive correlations with the soil temperature (Braker et al., 2010) and rainfall events (Snider et al., 2015) through increasing soil moisture as explained in the introduction.

The results of quantifying DNA with Quantifluor (Promega) represent various abundances of genes' DNA for samples from different fields and dates. The differences could be due to the environmental changes and the various treatments applied to the fields on different sampling dates.

A total of thirteen primer pairs including include narG1960, narG, nirKC1, nirKC2, cnor, qnor, V, cd, nosZ, nirKF and P450nor, with the highest coverage in each target gene were used from

previous studies as listed in Table 4.2. PCR experiments of seven (narG1960, cnorB2, qnorB2, nirKC1, nirKC2, narG, and nosZ) out of the thirteen primer pairs tested had good amplifications with the field soil DNA. After high throughput sequencing of the amplification products and analysis of the sequences, a total of fifty-two primer pairs were designed to more specifically amplify individual genes (or closely related groups of genes) covering these seven genes (Table A2.7-A2.13). The results of the quantification of samples DNA and PCR products represented a reasonable amount of DNA (ng/µl) for all samples and most the target genes (Tables A2.14 & A2.15).

Table 4. 2: Primer Pairs for with High Coverage in Amplification of Target Genes on Denitrification Pathway that Were Applied to PCR

Pathway		Forward_Name	Reverse_Name	Product Length (bp)	Target Group	Coverage in Target Group (%)	Reference
NO3 to NO2-		narG1960f	narG2650r	650	narG	99.6	Philippot et al., 2002
		narGf	narGr	1008	narG	99.6	Delorme et al., 2003
		V16F	V17R	1002	napA clade I-III	95.8	Flanagan et al., 1999
Bacterial denitrification	NO2- to NO	nirKC1F	nirKC1R	487	nirK clade I	89.3	Wei et al., 2015b
		nirKC2F	nirKC2R	458	nirK clade II	76.7	Wei et al., 2015b
		cd8F	cd2R	369	nirS clade I	91.5	Michotey et al., 2000
	NO to N2O	cnorB2F	cnorB7R	454	cnorB	81.9	Braker and Tiedje, 2003
		qnorB2F	qnorB7R	637	qnorB	43.2	Braker and Tiedje, 2003
N2O to N2		nosZF	nosZR	1433	nosZ clade I	93.6	Delorme et al., 2003
		nosZ2F	nosZ2R	267	nosZ clade I	64.8	Henry et al., 2006
Fungal denitrification	NO2- to NO	nirKfF	nirKfR	480	fungal nirK	88.1	Wei et al., 2015a
	NO to N2O	p450nor394F	p450nor1008R	614	p450nor	85.3	Higgins et al., 2016
		P450nor1F	P450nor1R	660	p450nor	85.3	Novinscak et al., 2016

The quantitative assessment of qPCR results indicates seasonal changes of denitrification genes for all the individual examined genes. However, the patterns are not the same for all the targeted genes and each one resulted to an individual response to water and N fertilizer application rates and time. The least abundances of narG1960: OTU36, OTU40, OTU49 and OTU83 and narG: OTU37 were observed in May. The highest abundance of target genes specified to narG1960: OTU49 and

OTU83 were shown in July based on another study (Braker et al., 2010) could be a response to increased soil temperature on that day compared to other sampling dates. However, the abundances of narG1960: OTU40 and OTU14 were the highest for samples of October. While qPCR of narG: OTU14 and narG: OTU37 shows the least and highest abundances of these specific genes in July and October, respectively. The abundance of nirKC1-OTU 43 and nirKC2-OUT 13 in most the fields was the highest in October. However, the highest abundance of this gene if fields 21 and 26 was shown in July. The nirKC1-OTU 126 abundance had a different pattern with the highest abundance occurring in August. The abundance of nirKC1-OTU 126 in F23 (with zero N applied) was the highest in October. The abundance of nirKC2-OTU 12 was different from field to field during the growing period and showed no specific pattern. The changes of genes' abundances within the corn-growing period for each field also did not show any common pattern of the changes in various genes abundances.

The Analysis of Variance (ANOVA) results indicated that the P-values for the date for all the targets and specific genes were lower than the α value (Table 4.3). Hence, the changes in specific genes' abundances over time were statistically significant throughout the corn-growing season. Due to P-value, the only specific genes with significant (P-value < α) reactions to treatments included narG1960-OTU36, narG1960-OTU83, and nirKC2-OTU13. The p-values for the interaction between "Field" (that represented different fertigation treatments) and "Date" (representing sampling date and growing period) were statistically significant for none of the specific genes but narG1960-OTU83. That means there were no significant changes in specific genes' abundances regarding the interaction of the corn-growing period (sampling date) and various fertigation treatment practices (various fields) for none of the target genes but narG1960-OTU83.

Table 4. 3: The Summary of ANOVA Results Showing the Changes of the Specific Genes Abundance Due to Applied Fertigation Treatments (Field) and Sampling Date

Target	Specific Gene	*P-value*		
		Field	Date	Interaction
narG1960	OTU 14	0.381	3.77E-30	0.321
	OTU 36	0.002	8.07E-22	0.880
	OTU 40	0.140	7.65E-12	0.777
	OTU 49	0.212	1.75E-58	0.646
	OTU 83	0.006	6.87E-16	0.007
narG	OTU 37	0.065	1.14E-11	0.183
nirKC2	OTU 13	1.18E-07	9.65E-05	0.996
cnorB2	OTU 6	0.193	1.09E-30	0.845

Different genes responses were different to water application (Table 4.4). The abundance of cnorB2 genes had significant positive correlations with water application to all the fields. While, there were no significant correlations between the abundance of narG and nirKC genes with water application for most of the fields. The narG community size has weak negative correlation with water application to all the fields. A negative correlation between narG abundance and soil moisture is reported by another study (Z. Li et al., 2020). The only field with a strong correlation between the size of nirKC community and water application was F22. The results are not completely in accordance with other study that suggested a significant positive correlation between nirK abundance and soil moisture (Snider et al., 2015; Z. Li et al., 2020). The difference between this field and other studied fields was the high water and high fertilizer application rate that the whole amount of fertilizer was applied at once, while planting corn. The reason for the differences between the results of this study regarding the correlation between denitrification community abundance and water fertilizer may be because most the sampling were done when the fields moisture were very low.

Table 4. 4:Correlation Between Denitrifier Genes Abundances with Water Application

Target Gene	F21	F22	F23	F24	F25	F26	F27	F28
narG	-0.26	-0.33	-0.32	-	-	-0.35	-0.34	-0.29
nirKC	0.00	0.81	0.20	-	-	0.33	0.21	0.27
cnorB2	0.88	0.77	0.83	-	-	0.74	0.77	0.87

Most the target gene abundances had negative correlations with individual fertilization events (Table 4.5). The narG target genes had no significant sensitivity to moderate negative correlations with fertilization events in all the fields. None of the genes' abundances was highly sensitive to fertilizer application in fields F27 and F28. It was the same for narG and nirKC in fields F26 and F24, but not for cnorB2. The reason might be because of graduate fertilization and high water application rate to fields that could cause N leaching (Bowen et al., 2018). For field F24 that was treated by high fertilizer and very low water rate, low water content of soil may be the reason of low correlation. Because denitrifier bacteria are anaerobic, while low soil moisture results in higher amount of available O_2 (Braker & Conrad, 2011; Butterbach-Bahl et al., 2013; Wallenstein et al., 2006). The abundances of nirKC and cnorB2 were negatively sensitive to fertilizer application in fields F21 and F22. The fertilizer application for both the fields was the same, just less water had been applied to field F21. Several studies reported denitrifier genes' abundances are not affected by fertilizer application (W. Ma et al., 2016; Miller et al., 2008). Another study indicated that it is poorly known whether there is significant changes in abundance of narG, nirS and nosZ due to applying nitrogen fertilizer alone (Chen et al., 2012).

Table 4. 5: Correlation Between Denitrifier Genes Abundances with Fertilizing

Target Gene	F21	F22	F23	F24	F25	F26	F27	F28
narG	-0.48	-0.46	-	-0.35	-	-0.26	-0.21	0.03
nirKC	-0.76	-0.94	-	-0.02	-	-0.37	**0.29**	-0.07
cnorB2	-0.83	-0.84	-	-0.83	-	-0.65	-0.26	-0.05

Various reasons might be involved in the negative correlation between denitrification genes abundances and fertilizer rates in this study. The sampling schedule did not exactly follow water and fertilizer application timing as all the last three fertilizations on field F28 and the two latest fertilizations on field F27 were done between the July and August sampling. Besides, sampling was not conducted according to certain interval timing from fertigation. So the results are likely to be affected by the sampling schedule. Furthermore, the abundance of denitrification genes could be affected by different distal and proximal factors, including soil pH, N, organic C, soil moisture, soil texture and calcium (Bowen et al., 2018; Braker et al., 2010; Bru et al., 2010; Carson et al., 2010; Kandeler et al., 2006; Z. Li et al., 2020). So, these parameters should be considered when analyzing denitrification gene abundance in response to fertilization and irrigation treatments.

This result is based on the data that has been obtained as of the date of writing this. More of the individual genes are being analyzed with the assays developed. The soil samples from the studied fields will be sent to a lab for chemical measurement of pH, N and organic C. Results will reveal whether the denitrification community sizes have been influenced by these factors. The results could be used in qPCR results assessment to determine if they are in conformity with other studies.

4.5 Conclusion:

The response of denitrification genes to fertilizer and water application rates differed from different genes. No strong positive correlation between denitrifiers abundance and fertilizer application was estimate. It is likely that the changes of the abundance of denitrification genes do not increase in response to fertilizer application alone. the denitrifiers abundance could be affected by different distal and proximal factors, including soil pH, N, organic C, soil moisture, soil texture and calcium (Bowen et al., 2018; Braker et al., 2010; Bru et al., 2010; Carson et al., 2010; Kandeler et al., 2006;

Z. Li et al., 2020). So, these parameters should be considered along with fertilizer application rate when analyzing denitrification gene abundance in response to fertilization and irrigation treatments.

The result can reveal the influence of fertigation treatments along with other likely influential parameters on changing the abundance of denitrification genes. It can be used to mitigate N fertilizer wasting through denitrification when planning for sustainable agricultural productions.

CHAPTER V

CONCLUSSION AND FUTURE WORK

The results of this study revealed how the existing and potential GHG emission from groundwater-fed irrigated agricultural systems can be affected by fertilizer, irrigation water and agricultural energy management. Results indicated that the agricultural groundwater pumping management practices could be affected by the type (e.g., electricity, natural gas) and source (e.g., coal, natural gas, oil, wind, solar) of pumping energy. Furthermore, it suggested that when estimating the abundance of denitrification genes more parameters than the application of N fertilizer alone, should be analyzed.

Detailed conclusions from each part of the study are listed below.

- The main portion of GHG emissions (63%) from corn production is associated with in-field activities; most of it is emitted from agricultural soil, followed by natural gas combustion from groundwater pumping.
- The GHG emissions from agricultural soil is highly sensitive to the emission factor for N_2O emissions from N inputs (EF_1).
- Among pre-field parameters, energy production for groundwater pumping is the driver GHG emitter, followed by fertilizer production.
- Even though, due to their higher overall pump efficiency, electric pumps consume less energy than NG pumps to extract an equal volume of groundwater from similar conditions. Nevertheless, NG pumps produce less GHG emissions than electric pumps using the U.S. Central and Southern Pains electricity mix.
- In the case of natural gas energy, groundwater pumping energy demand management, through improving OPE, will save more energy than switching to the electricity mix applied to this study.
- The GHG emissions from the electricity mix depend on the share of various sources of electricity (e.g., the Carbon footprint of the California energy mix is about 35% of that of the U.S. Central and Southern Pains electricity mix).
- Switching to clean energies (wind and solar) saves significantly higher amounts of carbon than just improving OPE for either NG or current electricity mix.
- The abundance of denitrification genes is influenced by more parameters than N fertilizer application alone.

When planning to reduce the GHG emissions from corn production, GHG emissions from parameters with the highest effects, especially from agricultural soil, could be targeted to achieve the optimum result. The total cropping system emission is highly influenced by the Agricultural

soil component of the model which is highly sensitive to the EF_1. It indicates the importance of accurate measurement of EF_1 and GHG emissions from agricultural soils when estimating GHG emissions from corn production to reduce the uncertainties.

Further studies are required to focus on the feasibility of developing wind energy in the study area regarding economic assessment, other potential environmental aspects and required infrastructures. In-field water demand management practices (e.g., deficit irrigation and improving the efficiency of irrigation systems could be considered to mitigate GHG emissions from agricultural groundwater pumping while satisfying crop yield and protecting the aquifers. A multi-objective optimization model is suggested to estimate optimum fertilizer and water application rates to minimize GHG emissions and nutrient losses while satisfying the crop yield.

The results of this study could be modified and applied on a global scale to identify opportunities to reduce carbon emissions from groundwater-dependent agricultural crop production. This work brings insight to agricultural managers and farmers on how fertilizer, irrigation water and agricultural energy management practices can impact the existing and potential carbon and N footprint of crop production in the study areas and similar regions in the world.

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APPENDIX I

PROTOCOLS

AI-1- Polymerase Chain Reaction (PCR) Protocol

The purchased primers were provided as 100µM concentration from Thermofisher and roughly 250 µl volume. After identifying the forward and reverse primers for each target gene, the primers were diluted to 25 µM concentration. So, 75 µl of PCR water was added to 25 µl of each primer to dilute them. The next step was amplifying the specific genes for the assays designed above. Amplifications of target genes were done with PCR with the sample DNA and without (for negative control). The following recipe was applied to prepare each reaction.

- 2 µl of 25 µM MgCl2
- 10 µl of 5X Buffer
- 2.5v µl of 10 µM dNTPs
- 1.25 µl of 25 µM "Forward Primer"
- 1.25 µl of 25 µM "Reverse Primer"
- 0.25 µl of taq DNA polymerase
- 30.95 µl of Clean DNAs/RNAs free water (PCR water)

49 µl of the PCR reaction for each target was added to 1 µl of DNA extracted from field samples (mixed) for positive, and nothing was added for negative control. The PCR reactions (positive & negative) were run using a standard thermocycler protocol (Lozano-Sardaneta et al., 2020). Then the PCR products were run on an agarose gel. As a size marker, the first well on agarose gels was filled with 100 bp DNA ladder (Promega). Thus, the amplification product could be identified for having the right size. The gels were run using a PureGel Electrophoresis System, model PG-ES03, with an AC100-120V, 1A, 50/60HZ input for 20 min (e.g., Figure AI-1). Agarose gels were then stained with GelRed and imaged in a molecular imager machine. To serve as qPCR standards, the PCR product should show a clear single band of the right size (usually 70-200 bp) without any band in the negative control (e.g., Figure AI-2).

When a correctly sized, and clean looking, PCR product resulted, the PCR amplification was cleaned, quantified, and diluted to serve as qPCR standards.

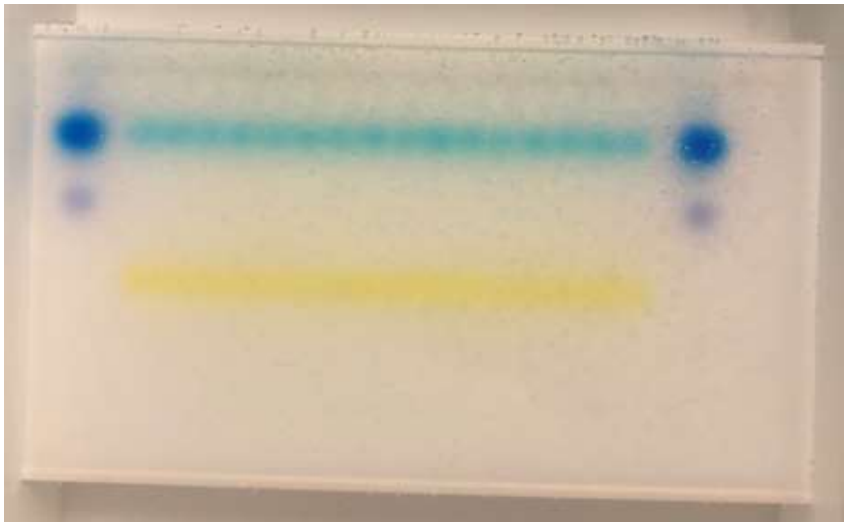

Figure A1. 1: An Example of an Agarose Gel After Running in an Electrophoresis Machine

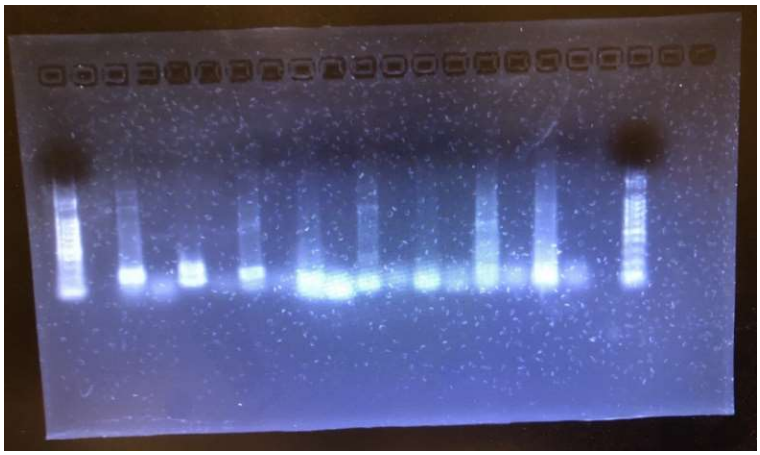

Figure A1. 2: An Example Result of Molecular Imager Machine. The smaller smear in the negative control is a factor of primer dimer formation.

AI-2- Quantitative Polymerase Chain Reaction (qPCR) Protocol

The real-time PCR master mix (MM) was carried out in a volume of 9 µl and contained:

- 5 µl of 2X SYBR green (iTaq Universal SYBR green Super Mix; Bio-Rad, USA),

- 0.15 µl of each forward and reverse primer,
- 0.5 µl of Bovine Serum Albumin (BSA), and
- 3.2 µl of PCR water.

The applied qPCR plates included 96 wells in twelve columns and eight (A to H) rows. Nine µl of the qPCR master mix were added in each well plus 1 µl of soil sample DNA, diluted PCRs (as standards), or PCR water (as blank). Three independent quantitative PCR assays were performed for each gene, and the three replicate DNA extractions from each soil sample (i.e. three 'technical replicates', and three 'experimental replicates'). Three no-template controls (NTCs) were run for each quantitative PCR assay. All primer pairs standard assays (-2 to -8) were also run with the mixed DNAs extracted from soil samples collected from TAPs fields before corn planting.

AI-3- Quantifying DNAs and PCRs Using Quantus™ fluorometer

The following process was applied to quantify the amount of double-stranded DNA in DNA extracts or PCR products.

- Zero control: 100 µl of the working solution was added to 100 µl of 1X TE buffer in a 0.5 ml tube.

- Standard: 100 µl of the working solution was added to 98 µl of 1X TE buffer and 2 µl of the DNA standard (100ng/ µl) in a 0.5 ml tube.

- Samples: 100 µl of the working solution was added to 98 µl of 1X TE buffer and 2 µl of DNA extract or PCR product in a 0.5 ml tube.

- Vortex: The tubes were vortexed quickly to mix the contents and then contributed in a dark place at room temperature for 5 minutes.

- Calibration: The Quantus™ fluorometer spectrophotometry was calibrated using the zero control (as a blank) and standard solution (as a standard) and saved.

- Reading the quantities: The machine was checked using the standard and zero control. It should read the standard at 100 and the zero control at zero or close. Then the rest of the samples were read, and the measures were recorded. The reading process was repeated three times, and the averages were used as the concentration of the original samples.

It is important not to label the tubes on the wall and ensure no bubbles in the bottom of the tubes when reading to avoid wrong results.

APPENDIX II

TABLES AND FIGURES

Table A2. 1: GHG Emitted from Various Factors at Different TAPS Fields (g CO2-eq kg $^{-1}$ Corn)

Field ID	Pre-Field GHG Emisions From Corn Production						In-Field GHG Emissions				Total GHG Emissions
	Fertilizer	Pesticide	Seed	Pumping Energy	Diesel Production	Total Pre-Field GHG Emissions	NG Combustion	Diesel Combustion	Agricultural Soil	Total In-Field GHG Emissions	
1	45.30	2.06	2.05	39.01	1.39	89.82	72.60	6.96	78.54	158.10	247.92
2	41.00	1.96	1.83	34.62	1.32	80.73	64.44	6.60	73.46	144.50	225.23
3	46.90	1.96	1.95	40.01	1.32	92.13	74.47	6.61	80.21	161.29	253.42
4	43.57	2.13	2.15	36.30	1.44	85.59	67.56	7.19	76.64	151.39	236.98
5	46.66	2.13	2.05	42.20	1.43	94.47	78.54	7.17	80.18	165.90	260.37
6	46.90	1.96	2.07	42.83	1.32	95.08	79.72	6.61	80.21	166.54	261.62
7	61.56	2.02	1.57	41.69	1.36	108.21	77.60	6.83	97.12	181.54	289.76
8	55.81	2.07	2.06	35.98	1.40	97.32	66.96	6.99	90.60	164.55	261.87
9	84.98	2.03	1.89	39.39	1.37	129.66	73.30	6.84	123.98	204.13	333.78
10	70.25	3.71	3.46	55.28	2.50	135.20	102.89	12.50	109.53	224.92	360.12
11	50.83	1.92	1.79	37.11	1.30	92.95	69.06	6.49	84.67	160.22	253.17
12	80.17	3.04	2.84	47.59	2.05	135.70	88.58	10.27	119.94	218.79	354.49
13	49.44	1.98	1.60	36.57	1.34	90.93	68.06	6.69	83.17	157.91	248.84
17	33.17	1.96	1.82	36.17	1.32	74.43	67.31	6.60	64.47	138.38	212.81
Mean	**54.04**	**2.21**	**2.08**	**40.34**	**1.49**	**100.16**	**75.08**	**7.45**	**88.77**	**171.30**	**271.45**
Min	**33.17**	**1.92**	**1.57**	**34.62**	**1.30**	**74.43**	**64.44**	**6.49**	**64.47**	**138.38**	**212.81**
Max	**84.98**	**3.71**	**3.46**	**55.28**	**2.50**	**135.70**	**102.89**	**12.50**	**123.98**	**224.92**	**360.12**
STDEV	**15.05**	**0.51**	**0.50**	**5.55**	**0.35**	**19.71**	**10.33**	**1.74**	**17.67**	**26.58**	**46.22**

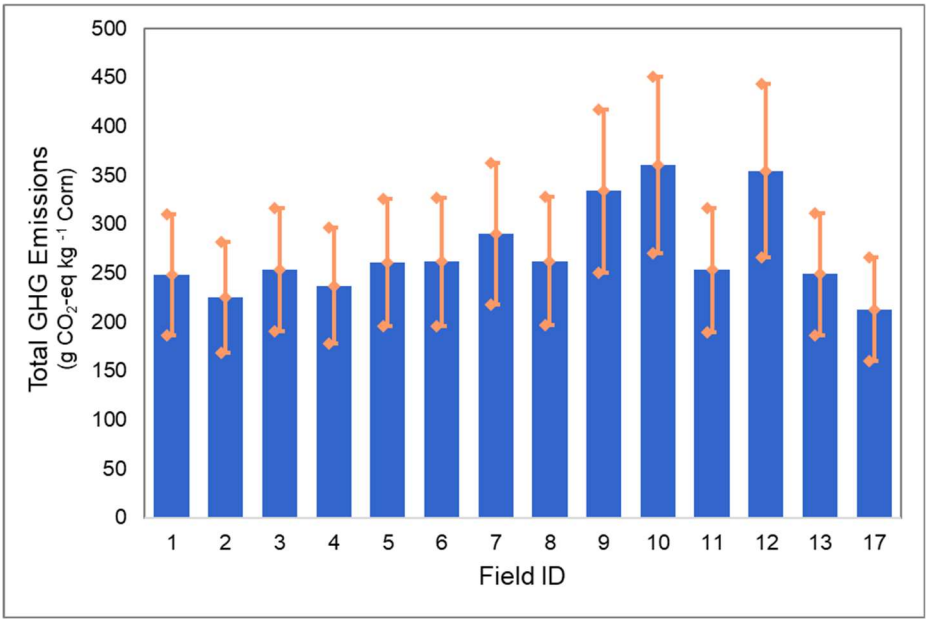

Figure A2. 1: Uncertainty Ranges of Total GHG Emissions from Different TAPS Fields

Table A2. 2: Daily Rainfall in Eva Station from Jan to Oct 2022 (mm)

DAY	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct
1	0.00	0.00	0.00	0.00	-	0.00	11.18	0.00	0.00	0.00
2	1.27	0.00	0.00	0.00	-	0.00	33.53	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	-	5.84	2.54	0.00	0.00	0.00
4	0.00	2.29	0.00	0.00	0.25	0.00	0.00	0.00	0.00	10.92
5	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.78
6	0.00	0.00	0.00	0.00	0.00	5.59	1.02	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00	40.13	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	38.10	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25
10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.51	0.00
11	0.00	0.00	0.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	1.52	0.00	0.00	0.00	0.00	2.29	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16	0.00	0.00	0.00	0.00	1.02	0.00	0.00	11.68	0.00	0.00
17	0.00	0.00	1.02	0.00	0.25	0.00	0.00	9.65	0.00	0.00
18	0.00	0.00	0.00	0.00	2.29	0.00	0.00	0.00	0.00	0.00
19	0.00	0.00	0.00	0.00	0.00	4.06	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.25	0.00	0.00
21	2.29	0.00	4.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22	0.00	0.00	0.76	2.29	0.00	0.00	0.00	0.00	0.25	0.00
23	0.00	0.00	0.00	1.27	4.32	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	18.29	0.00	0.00	0.00	0.00	0.51
25	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.00
26	1.52	0.00	0.00	-	0.00	1.78	0.76	0.00	0.00	0.00
27	0.00	0.00	0.00	-	0.00	7.62	6.86	0.25	0.00	0.00
28	0.00	0.00	0.00	-	0.00	0.00	2.03	0.00	0.00	0.00
29	0.00	0.00	0.00	-	0.00	0.00	9.40	1.27	0.00	0.00
30	0.00	0.00	0.00	-	0.00	0.00	0.25	0.00	0.00	0.00
31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Monthly	5.08	4.06	6.60	3.56	26.42	103.38	70.10	23.11	0.76	13.46

Table A2. 3: Maximum Daily Temperature in Eva Station, May to Oct 2022 (°C)

Day	May	Jun	Jul	Aug	Sep	Oct
1	-	18.41	32.89	35.60	34.77	31.50
2	-	19.60	30.11	38.02	35.75	29.90
3	-	26.56	34.42	38.38	33.71	29.35
4	11.56	32.67	34.82	36.30	33.55	18.78
5	21.76	30.38	36.21	39.30	34.79	18.67
6	31.33	33.44	29.75	39.13	35.91	23.78
7	37.83	30.62	32.29	38.81	37.60	12.56
8	32.64	23.83	32.47	32.10	36.87	17.22
9	37.02	31.20	35.51	35.67	32.53	24.46
10	35.57	32.60	37.72	34.78	15.81	26.21
11	34.95	38.27	36.21	34.81	23.45	29.70
12	36.45	41.33	30.36	36.21	33.54	25.37
13	27.67	39.26	35.63	37.19	35.37	21.36
14	32.97	38.61	38.59	38.04	30.50	28.93
15	31.45	30.11	40.53	37.64	33.65	25.22
16	30.34	37.27	40.48	31.48	35.40	16.20
17	37.96	35.12	36.67	23.88	36.22	17.91
18	32.02	34.49	38.46	30.22	37.63	18.47
19	37.74	34.74	40.63	33.76	35.22	27.84
20	22.10	33.78	34.92	27.64	36.68	27.08
21	11.97	31.37	35.88	26.90	34.31	30.18
22	20.29	29.48	39.43	29.15	14.50	31.64
23	21.18	35.64	40.04	31.01	33.51	29.25
24	9.92	38.70	36.14	32.00	31.67	15.76
25	20.79	29.39	36.43	32.79	26.83	19.48
26	28.82	20.29	39.29	33.81	30.06	21.43
27	36.92	16.49	34.61	34.39	31.38	17.20
28	39.28	31.69	28.21	37.79	29.98	16.61
29	36.05	34.77	23.22	36.69	34.02	19.99
30	31.95	37.66	30.17	32.98	31.47	20.54
31	27.11	-	33.22	33.29	-	23.51

Table A2. 4: Average Daily Temperature in Eva Station, May to Oct 2022 (°C)

Day	May	Jun	Jul	Aug	Sep	Oct
1	-	14.09	25.62	25.42	24.08	20.83
2	-	15.02	23.00	27.76	25.03	19.14
3	-	18.27	26.43	28.97	24.78	19.53
4	8.79	22.16	27.27	27.55	23.01	15.21
5	11.83	22.60	28.15	29.66	23.53	14.62
6	18.64	23.97	23.67	30.02	24.64	14.92
7	23.00	21.67	24.01	28.84	25.69	9.71
8	21.96	18.32	25.04	24.34	24.88	10.60
9	26.96	21.43	26.31	25.06	22.65	13.72
10	21.98	24.59	28.24	24.83	13.23	15.52
11	25.09	27.49	27.48	25.16	14.73	18.63
12	25.27	29.76	23.78	25.54	19.86	15.02
13	18.44	32.57	26.84	26.36	23.92	10.87
14	21.37	29.26	28.46	27.33	22.78	17.28
15	20.20	23.78	31.03	28.69	23.22	14.33
16	21.22	28.01	31.43	24.40	23.10	11.14
17	25.22	27.21	28.43	19.27	25.51	7.82
18	22.66	27.28	28.40	21.54	27.27	7.43
19	24.42	26.22	30.49	24.44	26.94	11.79
20	15.09	25.27	28.60	21.34	27.84	14.61
21	7.64	22.88	26.54	19.95	22.91	16.33
22	11.74	22.21	29.20	20.30	11.56	20.20
23	12.23	26.42	30.30	21.23	20.78	22.17
24	8.48	28.81	28.79	21.92	20.96	8.32
25	12.41	23.45	26.48	22.64	17.77	7.53
26	17.30	17.13	27.31	23.93	18.37	9.95
27	24.60	13.48	25.52	24.93	20.41	10.75
28	28.49	20.92	22.96	25.53	19.08	8.99
29	26.48	26.39	19.62	25.78	21.21	9.00
30	23.14	28.45	22.62	24.09	21.28	10.41
31	18.87	-	25.01	24.27	-	11.13

Table A2. 5: Minimum Daily Temperature in Eva Station, May to Oct 2022 (°C)

Day	May	Jun	Jul	Aug	Sep	Oct
1	-	9.36	18.15	16.83	13.45	10.42
2	-	9.73	17.14	17.13	14.17	8.23
3	-	13.63	18.89	19.62	17.04	8.93
4	6.96	11.51	19.32	18.90	12.99	13.01
5	3.22	16.55	18.51	18.21	11.71	10.63
6	2.77	14.27	18.71	19.85	14.25	8.19
7	9.58	12.04	14.81	19.63	14.29	6.02
8	13.29	10.90	18.69	18.64	11.97	6.89
9	16.49	15.55	15.90	15.81	11.72	5.54
10	8.38	15.93	16.75	14.53	11.40	5.71
11	14.42	16.40	16.07	14.48	6.96	9.80
12	12.79	18.54	18.55	12.85	5.91	5.38
13	8.18	26.60	16.87	13.86	10.12	1.73
14	7.82	21.50	15.58	14.56	15.44	7.14
15	8.07	15.59	18.62	18.82	14.74	3.73
16	8.50	16.63	22.92	17.83	10.86	4.08
17	15.16	15.42	19.16	16.90	12.88	-1.21
18	14.75	17.58	15.40	13.30	16.09	-2.32
19	10.49	18.83	18.91	15.49	17.85	-2.11
20	7.28	16.12	22.12	16.99	18.46	4.28
21	1.09	16.50	18.10	14.67	11.36	4.08
22	0.27	16.20	17.44	12.92	9.37	7.81
23	7.01	16.07	17.12	11.30	9.45	14.36
24	7.32	18.15	20.64	10.88	12.50	-0.62
25	6.37	16.76	18.85	10.71	9.32	-2.64
26	4.82	14.66	16.65	11.78	7.81	-2.96
27	9.41	10.53	19.87	14.58	8.98	4.59
28	15.77	8.91	19.44	13.29	9.63	1.77
29	14.27	16.81	16.98	17.05	8.73	-0.52
30	13.15	19.01	17.95	16.04	9.28	1.11
31	11.62	-	16.91	15.22	-	0.26

Table A2. 6: Average Soil Temperature in Eva Station, May to Oct 2022 (°C)

Day	May	Jun	Jul	Aug	Sep	Oct
1	-	19.38	26.04	26.66	27.03	23.15
2	-	18.42	24.94	27.49	27.05	22.77
3	-	19.06	25.14	28.36	27.08	22.39
4	14.57	20.76	25.10	29.09	26.58	20.75
5	15.59	22.89	25.98	29.55	26.62	19.32
6	17.13	24.21	25.07	29.76	27.07	18.16
7	18.99	22.48	25.58	29.97	27.22	16.15
8	20.02	18.32	26.21	29.47	27.01	15.71
9	20.93	20.13	26.42	29.18	26.99	16.49
10	21.17	21.66	26.86	28.98	23.51	17.11
11	22.68	23.61	26.91	28.98	22.63	18.11
12	23.16	24.33	26.19	29.02	23.82	17.62
13	21.57	24.95	26.48	29.17	25.00	16.17
14	22.12	25.32	27.37	29.47	24.82	16.49
15	22.28	24.99	28.22	29.96	25.20	16.35
16	22.66	25.22	28.71	29.63	25.56	15.52
17	23.44	25.19	29.19	25.49	26.09	14.50
18	24.08	25.42	28.82	24.87	26.68	13.73
19	23.49	25.66	29.52	25.49	27.01	13.81
20	22.66	24.24	29.37	24.47	27.56	14.76
21	19.34	24.55	28.73	24.01	27.16	15.47
22	18.32	24.38	29.41	24.03	23.24	16.13
23	18.52	24.87	30.25	24.34	23.78	17.96
24	15.81	25.69	30.56	24.82	24.61	15.73
25	15.23	25.64	30.05	25.21	23.66	13.33
26	17.97	22.84	30.31	25.34	22.92	12.92
27	20.29	20.34	29.42	26.37	23.47	14.22
28	21.88	21.49	26.64	26.42	22.69	14.20
29	21.99	24.22	24.34	27.26	22.84	13.16
30	22.24	25.34	24.76	26.64	22.77	13.48
31	21.57	-	26.01	26.82	-	13.04

Table A2. 7: Designed Primer Pairs for narG1960 Target Genes

No.		Target	Primer	Product Length
1	narG1960	NarG1960_Otu16F	CATTCTGGTACCTCGCCACC	162
		NarG1960_Otu16R	GATCGAACGTGGGGTAGCTC	
2		NarG1960_Otu17F	CGCTACGAGACGCTCAAGAT	196
		NarG1960_Otu17R	GGATAGCCTTGTCGGGATCG	
3		NarG1960_Otu36F	CGATCCGGACAAACCCAAGA	160
		NarG1960_Otu36R	CTTCTGTGTGCCCATTTCGC	
4		NarG1960_Otu40F	TGTGGCGCTCGAATATCCTC	187
		NarG1960_Otu40R	AAGTCGAGCGTGACCAGAAG	
5		NarG1960_Otu14F	CAGTGGCGTTATGAGACCGT	195
		NarG1960_Otu14R	ATCCTTGGGCTCGAGCTTTG	
6		NarG1960_Otu6F	CAGTTCCGCTACGACCAGTT	91
		NarG1960_Otu6R	CGATCAGATCTGCGGTGCT	
7		NarG1960_Otu49F	TACGAGAAGCTGGGGATGGA	84
		NarG1960_Otu49R	GATCATCGAGCCCCGATACG	
8		NarG1960_Otu83F	TGGTATATGCACACCGACCAG	148
		NarG1960_Otu83R	GAGGGTAGAACGGCATCCAG	

Table A2. 8: Designed Primer Pairs for nirKC2 Target Genes

No.		Target	Primer	Product Length
1	nirKC2	nirKC2_Zotu1F	CTTCTACACCGTCGGCAAGT	89
		nirKC2_Zotu1R	ACAACACGTAGGTCGGGTTC	
2		nirKC2_Zotu2F	TCCTAGTGGAACCGGAAAGC	113
		nirKC2_Zotu2R	TGAAAACGCCTGAAGTCCCG	
3		nirKC2_Zotu16F	GTGGACCGCGAGTACTTCAT	115
		nirKC2_Zotu16R	CGTACTCGGGCTTCTCTTCC	
4		nirKC2_Zotu13F	TGCAGGAGAACGTGCAAACT	84
		nirKC2_Zotu13R	CATAGTTGCCGGGGACTTCT	
5		nirKC2_Zotu20F	CGTGCATTTCGGCTTCAAGA	92
		nirKC2_Zotu20R	TAAAGTCGACCGCCTTGTCC	
6		nirKC2_Zotu18F	CAAATACCGCGAGAAAGGCG	125
		nirKC2_Zotu18R	TCTTCACCTTGAGGGCGTTC	
7		nirKC2_Zotu30F	CGAACCTGGTCTCGAGCTTT	87
		nirKC2_Zotu30R	GGTCTGCACGTTCTCCTGAT	
8		nirKC2_Zotu12F	AGCCACGACAAGCTGATGAA	97
		nirKC2_Zotu12R	CAACCTTGGCTTTCAAGGGC	

Table A2. 10: Designed Primer Pairs for nirKC1Target Genes

No.		Target	Primer	Product Length
1	nirKC1	nirKC1_Zotu66F	CCAAGGGACTTATCCCGACC	183
		nirKC1_Zotu66R	CAAATGAGCCGCCTACCCAT	
2		nirKC1_Zotu43F	AAAGGCAACCCGCTCAAGTA	71
		nirKC1_Zotu43R	TCGTCGCGCGGAATGTAATA	
3		nirKC1_Zotu142F	CTACATCGGCGAGCAGGATT	142
		nirKC1_Zotu142R	ATGTCCGAATAGGCGTCACC	
4		nirKC1_Zotu139F	GAAGGACCATCAGGGCAAGG	95
		nirKC1_Zotu139R	TGAACTTCCCGTTCTCGTCG	
5		nirKC1_Zotu45F	AGGCCGGGAACTACAAGAAG	100
		nirKC1_Zotu45R	CCGTTGAAGACGATGTGGGT	
6		nirKC1_Zotu71F	CGACGAGAAGGGCAAGTTCA	178
		nirKC1_Zotu71R	AGAATGGACGATCAGCACGG	
7		nirKC1_Zotu126F	GGTGTTCTACATCGGCGAGA	71
		nirKC1_Zotu126R	ACCCGTACGCCTTGAAGTTT	
8		nirKC1_Zotu68F	ACTGATCGTCCATTCGCAGG	91
		nirKC1_Zotu68R	GGCGAATTTGCCTTCTTCCC	

Table A2. 9:Designed Primer Pairs for qnorB2 Target Genes

No.		Target	Primer	Product Length
1	qnorB2	qnorB2_Zotu10F	TGGCGTTGATGGTTCGAGTG	121
		qnorB2_Zotu10R	GTCAGTCCGGCACCGTAAAA	
2		qnorB2_Zotu132F	TGGCTGTTTTTGATGCTCCG	75
		qnorB2_Zotu132R	CAGTGCGACGAGTTGCTTTT	
3		qnorB2_Zotu24F	AGCAGGACTTCTCTTGTGGC	88
		qnorB2_Zotu24R	GGCGACTAGTTGCTTCTGCT	
4		qnorB2_Zotu1F	CACGATCATCTTTCTCGGCG	172
		qnorB2_Zotu1R	GAACAATCCTTCGTGCCTCG	
5		qnorB2_Zotu112F	GAAAACAGGCGAGCAGAAGC	112
		qnorB2_Zotu112R	CATCGAGAGATGCGAGTGCT	
6		qnorB2_Zotu1098F	GGCATTATCGGGACGTGTCA	200
		qnorB2_Zotu1098R	GCAACCGCGACGAAAAAGTA	
7		qnorB2_Zotu2F	CACCTGCCACCACCTGTATT	190
		qnorB2_Zotu2R	GGCAACGGCGACAAAGAAAT	
8		qnorB2_Zotu21F	CCTGTTTGCGGGACTCTTGA	163
		qnorB2_Zotu21R	CGTATGCTGTCCCCATGTCA	
9		qnorB2_Zotu12F	ATCGTACCGTTGTTGCTGGT	75
		qnorB2_Zotu12R	CTTCAACCATGGTCTGGCCT	

Table A2. 11: Designed Primer Pairs for narG Target Genes

No.		Target	Primer	Product Length
1	narG	narG_Otu187F	TTCAAGCAGATGGAGGAGCG	170
		narG_Otu187R	ATGGTGAACGGGGAATAGCG	
2		narG_Otu34F	TCTTCATGCTCTCGCTGTCC	87
		narG_Otu34R	AGTCGTTGTCCTTGATGCCC	
3		narG_Otu37F	GGTCAAGGACTGGAAGACCG	103
		narG_Otu37R	GAAGCGCTTGTGGATGTTGG	

Table A2. 12: Designed Primer Pairs for cnorB2 Target Genes

No.		Target	Primer	Product Length
1	cnorB2	cnorB2_Zotu20F	ACTGGTGATGGCGTCAATCC	79
		cnorB2_Zotu20R	CAGCCATTTGTCCACCACCT	
2		cnorB2_Zotu1F	TGGTGCATCTTTGGGTGGAA	86
		cnorB2_Zotu1R	GTCGACACCGGTGATCTTGA	
3		cnorB2_Zotu5F	CTCGCCTTCCTGCTCATCAA	194
		cnorB2_Zotu5R	GCGAAGAAGGGGAGGACTTC	
4		cnorB2_Zotu21F	GACTGAAGACAGGCAAGCGA	74
		cnorB2_Zotu21R	ATTCCGGTCTAAACGTCGCA	
5		cnorB2_Zotu13F	GCTCATCATGGCCTCGATCC	136
		cnorB2_Zotu13R	GATCGATCCGATCCACTGCC	
6		cnorB2_Zotu6F	GTTCTCGCCTTCCTGGTCAT	169
		cnorB2_Zotu6R	AGAAGATGCTGCCGATCCAC	
7		cnorB2_Zotu7F	TCGGCACGGGACATCACTAT	137
		cnorB2_Zotu7R	GCGACGGCGGTTAATTACG	
8		cnorB2_Zotu28F	TCTTCGCCATGGTCGTCTTC	155
		cnorB2_Zotu28R	GTTCACCCAGCTCAGCGTAT	

Table A2. 13: Designed Primer Pairs for nosZ Target Genes

No.		Target	Primer	Product Length
1	nosZ	nosZ_Otu4F	CCAGCTCCCCAATCAAGCGA	142
		nosZ_Otu4R	AGGGACGAGTACTTCTTGGGA	
2		nosZ_Otu25F	TGGAGTACGCCAACGACATC	143
		nosZ_Otu25R	CACCTCGTCCATGTTGGTGA	
3		nosZ_Otu20F	ATCGTCCATGACAACCCCAC	196
		nosZ_Otu20R	TGTACACCCGGACCTTGTTG	
4		nosZ_Otu1503F	CCGAGATGATGGGGAACGAG	88
		nosZ_Otu1503R	TCCTCGAAATCACCCTTCGC	
5		nosZ_Otu1F	GACGGCAAGTGGCTGATTTC	199
		nosZ_Otu1R	CCCAGATCGAGATCGGGTTG	
6		nosZ_Otu1596F	GATCCCGCCCAGTACCGG	105
		nosZ_Otu1596R	CATCATGTCGGCCAACGAG	
7		nosZ_Otu1774F	CAACTCCGAGAAGGGCGTCA	120
		nosZ_Otu1774R	GCGCCCGAGGTTGAAGAC	
8		nosZ_Otu37F	CGACCAGCTCGTTGACATCT	98
		nosZ_Otu37R	TCGAGGAGTGCACGATTGTC	

Table A2. 14: Abundances of Genes' DNAs in Different Fields Through Corn Growing Period (ng/ηl)

Field ID	5/11/2022	7/14/2022	8/16/2022	10/25/2022
F1-21	45.7	35.0	90.7	341.7
F1-22	66.3	38.7	85.3	222.7
F1-23	52.0	30.0	52.7	251.3
F1-24	54.0	16.3	84.3	477.3
F1-25	23.7	30.0	87.0	246.7
F1-26	52.3	56.0	142.0	210.0
F1-27	47.0	28.0	78.0	285.0
F1-28	49.3	31.0	86.3	189.3
F2-21	36.7	38.7	52.7	250.7
F2-22	49.7	59.7	79.7	113.0
F2-23	24.0	71.0	49.3	232.0
F2-24	27.7	37.3	43.3	196.7
F2-25	36.3	32.0	71.3	394.7
F2-26	38.3	43.0	75.7	216.0
F2-27	32.0	62.0	59.0	166.0
F2-28	34.3	37.0	60.3	227.0
F3-21	91.3	17.0	91.3	253.0
F3-22	54.7	40.0	59.0	211.0
F3-23	88.0	37.0	71.0	208.0
F3-24	71.7	28.3	89.0	199.7
F3-25	74.0	29.3	93.3	243.3
F3-26	56.0	37.7	51.0	163.7
F3-27	58.0	49.7	61.3	160.0
F3-28	95.0	51.7	74.7	267.0

Table A2. 15: Abundances of Genes' DNAs Resulted from Quantification of PCRs (ng/ηl)

No.	Specific Target	DNA (ng/ηl)
1	NarG1960_Otu16	1.60
2	NarG1960_Otu36	0.75
3	NarG1960_Otu40	1.33
4	NarG1960_Otu14	1.06
5	NarG1960_Otu49	1.41
6	NarG1960_Otu83	0.64
7	NarG_Otu37	7.83
8	nirKC2_Zotu13	3.28
9	nirKC2_Zotu12	4.45
10	nirKC1_Zotu43	3.02
11	nirKC1_Zotu126	2.19
12	qnorB2_Zotu1	4.45
13	qnorB2_Zotu12	0.01
14	nosZ_Otu25	4.58
15	nosZ_Otu1503	2.23

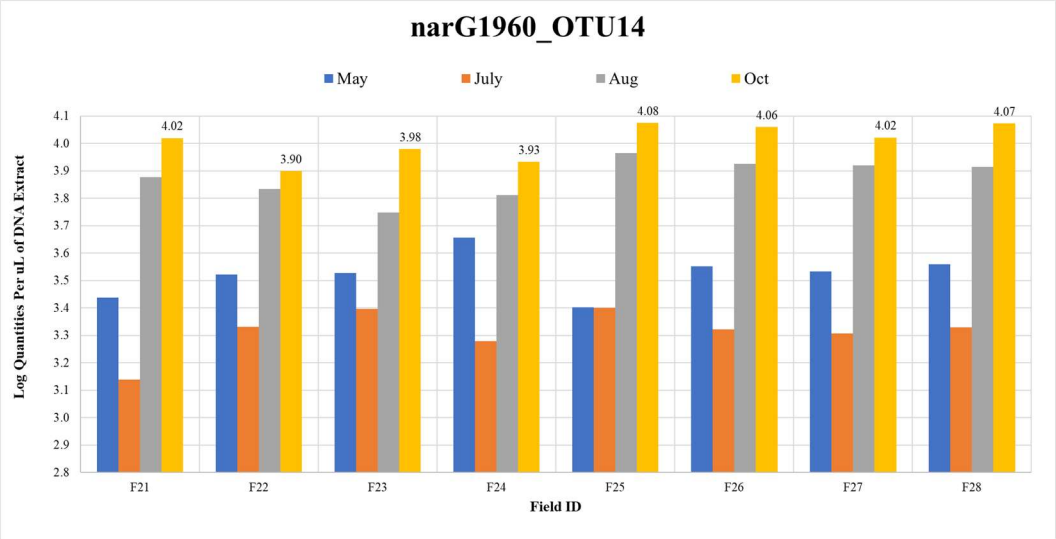

Figure A2. 2: Date, and Fertilizer and Irrigation Water Application Rates Effects on Changes of narG1960-OTU14 Genes' Abundance

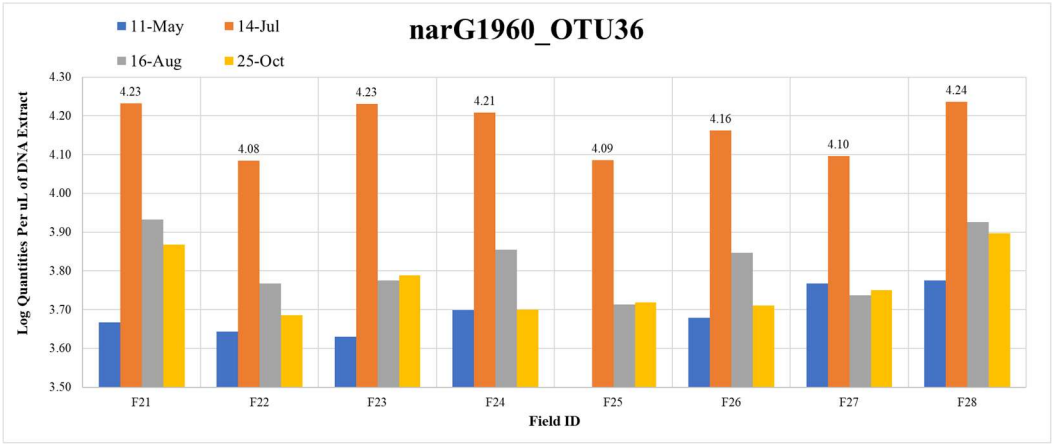

Figure A2. 3: Date, and Fertilizer and Irrigation Water Application Rates Effects on Changes of narG1960-OTU36 Genes' Abundance

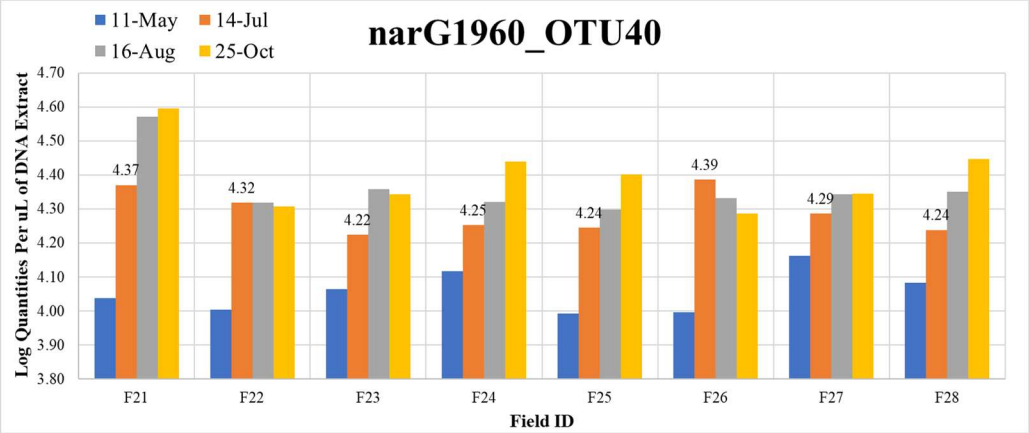

Figure A2. 4: Date, and Fertilizer and Irrigation Water Application Rates Effects on Changes of narG1960-OTU40 Genes' Abundance

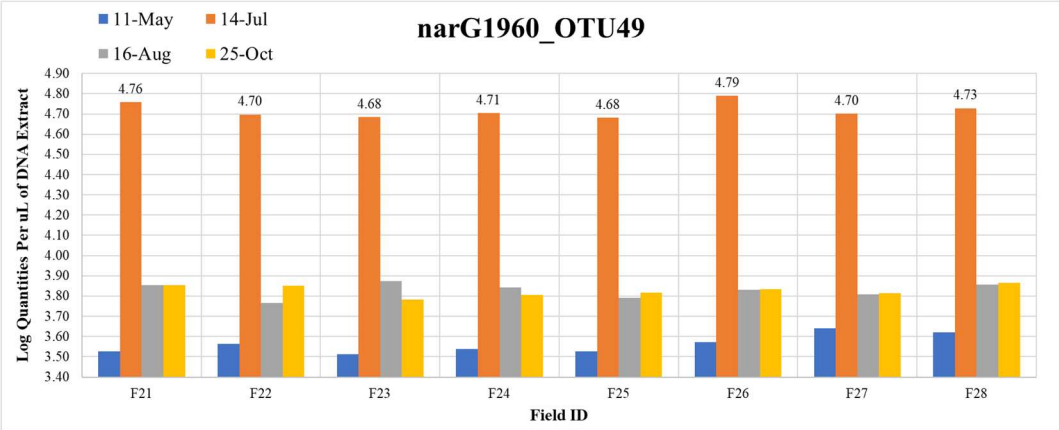

Figure A2. 5: Date, and Fertilizer and Irrigation Water Application Rates Effects on Changes of narG1960-OTU49 Genes' Abundance

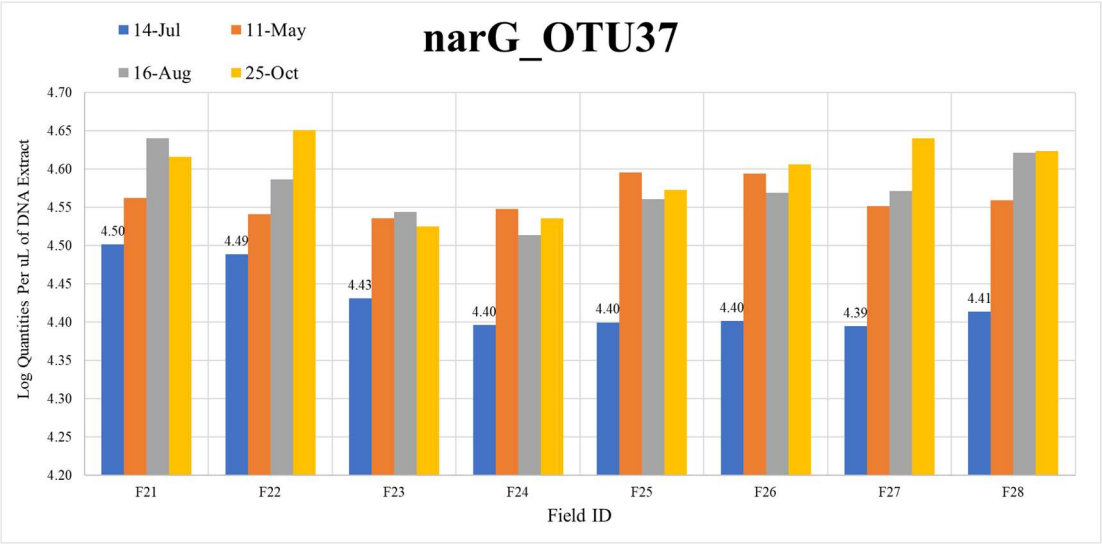

Figure A2. 6: Date, and Fertilizer and Irrigation Water Application Rates Effects on Changes of narG1960-OTU83 Genes' Abundance

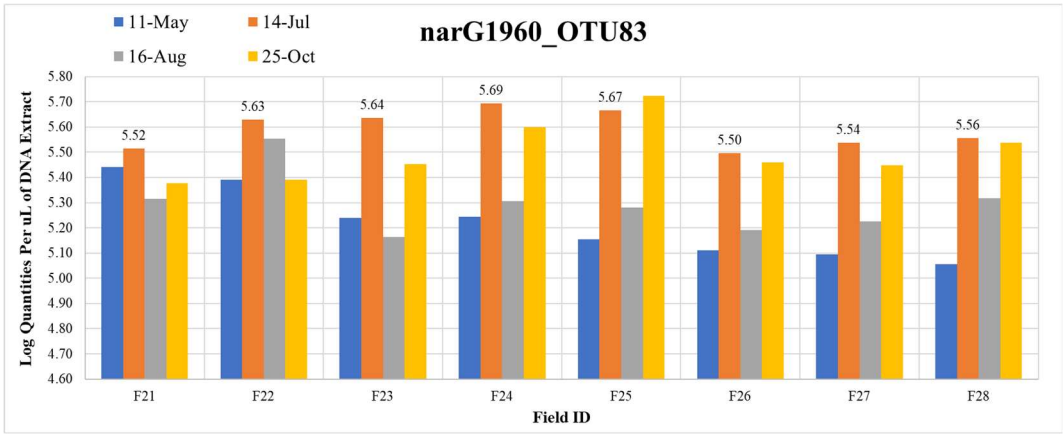

Figure A2. 7: Date, and Fertilizer and Irrigation Water Application Rates Effects on Changes of narG-OTU37 Genes' Abundance

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