

THE EFFECT OF SOIL PH AND TEXTURE ON THE
RATIO OF DENITRIFICATION END PRODUCTS

By

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THE EFFECT OF SOIL PH AND TEXTURE ON THE
RATIO OF DENITRIFICATION END PRODUCTS

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Abstract:

Nitrous oxide (N_2O) is a potent greenhouse gas that contributes to stratospheric ozone depletion and global climate change. Denitrification has two potential end products, N_2O and dinitrogen (N_2), and the ratio of these end-products is controlled by various factors. Soil pH and texture are two factors that have potential biological, chemical, and physical effects on denitrification. This study aims to quantify the influence of soil pH on the ratio of denitrification end-products in Oklahoma soils with different soil textures. Four field soils encompassing three distinct soil textures were incubated in the laboratory under natural pH, more acidic pH (amended with sulfuric acid H_2SO_4), and more basic pH (amended with potassium hydroxide KOH), with an overall, tested pH ranging from 2 to 10. Denitrification end-products were measured in the laboratory using the acetylene inhibition technique and further estimated using a process-based biogeochemical soil model. Both the laboratory and model results showed that soil pH and texture influenced the ratio of the denitrification end-products, here referred to as the N_2O ratio. Generally, as soil pH increased the N_2O ratio decreased, although both lab and model results indicated that this relationship was not strictly linear. Soil texture has an indirect effect on the N_2O ratio, where the results showed the same type of soil has a different N_2O ratio. The clay percentage of the soil has a linear positive correlation with the N_2O ratio based on this study's findings. In conclusion, soil pH is a controlling factor in the ratio of denitrification end-products and warrants further research to sufficiently quantify this nonlinear relationship, particularly when considering its effects in different soil textures.

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CHAPTER I

INTRODUCTION

Greenhouse gases (GHGs) keep the Earth warm by absorbing energy and decelerating the rate at which the energy escapes to space. Without those gases, the temperature of the earth's surface will be -18 C (0 F) rather than the current average temperature of the earth's surface which is 15 C (59 F) (Karl and Trenberth, 2003). Methane (CH₄), carbon dioxide (CO₂), and nitrous oxide (N₂O) are three of the major GHGs, where increasing their concentrations in the atmosphere contributes to climate change and influences life directly and indirectly. With the current GHG emissions (as in table 1) the upper limit of dangerous levels will be reached by 2050 (IPCC 2022). The lifetime of each GHG in the atmosphere and its potential chemical interactions are very important to assess its overall influence on GHGs in the atmosphere. Global Warming Potential (GWP) is a method used to compare the effect of each individual GHG on global warming by measuring the ability of the gas to trap the heat relative to the CO₂ within a time of 100 years. N₂O has a GWP on a molecular basis that is higher than CO₂ and CH₄ with 310 and 16 times respectively over a 100-year period (EPA, 2022). Aside from contributing to global warming, N₂O can also contribute to the depletion of the ozone layer by reacting with oxygen in the stratosphere and producing nitric oxide (NO) (Ravishankara et al., 2009). Furthermore, N₂O has a long lifetime whereas N₂O emitted today could last in the atmosphere for more than 120 years (EPA, 2022).

Table 1 – Annual Global Anthropogenic GHG Emissions (IPCC 2022)

Year	GHG emissions (GtCO ₂ -eq yr ⁻¹)
1970	28.7
1980	35.6
1990	38
2000	42
2010	53
2019	59

N₂O exists in the atmosphere as part of the nitrogen (N) cycle. N cycling is the conversion of N between different chemical forms in the ecosystem. N conversions within soil, plant, and atmospheric systems can be accomplished through biological and/or chemical-physical processes. The biological processes include mineralization, immobilization, nitrification, and denitrification, while the chemical-physical processes involve ammonia (NH₃) volatilization, ammonium (NH₄⁺) fixation, and nitrate (NO₃⁻) leaching (Figure 1). The focus of this work is on denitrification, which will be discussed in more detail below.

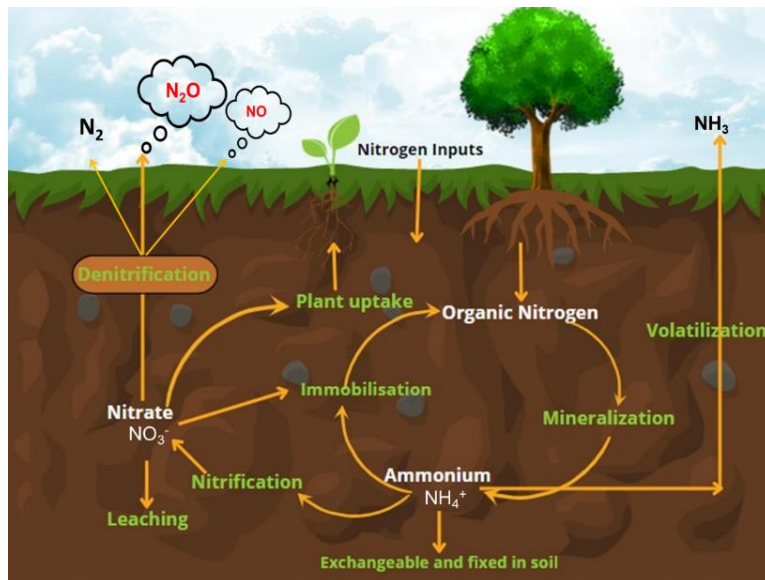


Fig (1) Nitrogen cycle processes within soil, plant, and atmosphere. Green words indicate processes, while white, black, or red indicate different nitrogen compounds/forms.

In natural and agricultural soils, the predominant sources of N₂O emissions are microbial denitrification and nitrification, with 70% of the global N₂O emissions sourced from those two processes (Firestone and Davidson, 1989; Conrad, 1996; IPCC, 2022). Denitrification occurs by denitrifying bacteria and fungi as a reaction to the changes in the oxygen (O₂) concentration in the microorganism's immediate environment. Denitrifying bacteria used the available component of oxidated nitrogen, nitrate, nitrite, nitric oxide, or nitrous oxide, as an electron acceptor to transfer from aerobic respiration to anaerobic respiration when there is a shortage of oxygen (O₂). This process takes place in all terrestrial and aquatic ecosystems besides tropical and temperate soils, in natural and intensively managed ecosystems, in marine and freshwater environments, and in wastewater treatment plants, manure stores, and aquifers. Nitrification is the process by which the conversion of ammonium to nitrate occurs through different types of microorganisms (i.e., bacteria, archaea, fungi). Nevertheless, other microbial processes produce N₂O such as the heterotrophic nitrification in which forms of N are reduced to nitrate, by fungi and heterotrophic bacteria (Blagodatsky et al., 2006; Papen et al., 1989), codenitrification which convert nitrite (NO₂⁻) to N₂O by fungi and bacteria (Kumon et al., 2002; Tanimoto et al., 1992), and dissimilatory nitrate reduction to ammonia by chemoorganoheterotrophic microbes that use the nitrate as an electron acceptor for their respiration under anaerobic conditions (DNRA; Bleakley and Tiedje, 1982; Smith, 1982, 1983; Smith and Zimmerman, 1981) and as illustrated in figure (2).

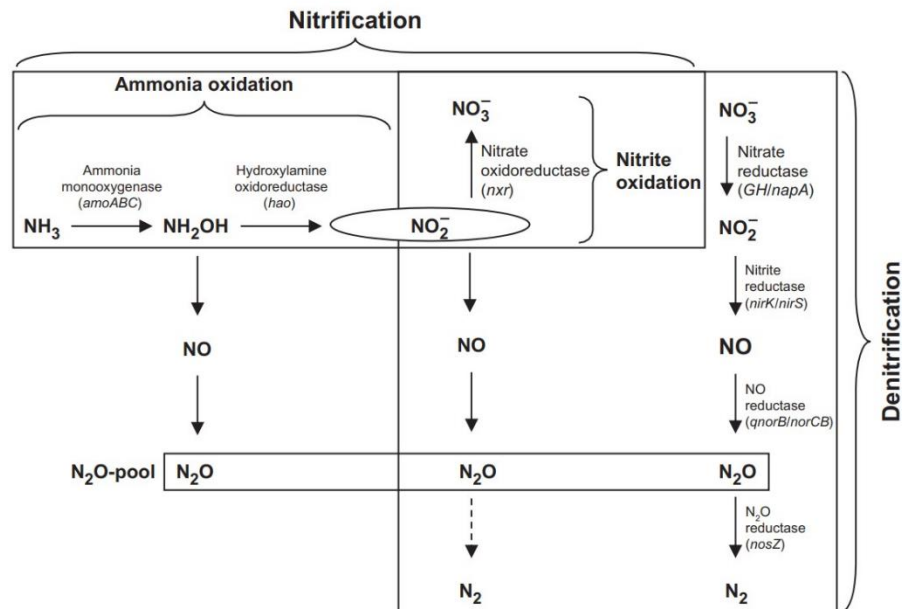


Fig (2) Nitrogen processes that are responsible for N₂O production by (Braker and Conrad, 2011)

The gases produced during denitrification are NO, N₂O, and N₂ depending on the soil conditions and the microbial community. If N₂O leaves the soil before being further reduced to N₂, the denitrification process could be considered incomplete. The N₂O ratio is a term used to measure the completion state of denitrification where the value near or equal to zero means a complete process and the value equal to 1 is more incomplete (more N₂O produced than N₂). The more deficit in oxygen, the better environment for the organisms to convert the nitrate or nitrite to N₂. Besides limited O₂, the ratio of N₂O to N₂ production depends on other factors such as the availability of N oxides, soluble carbon, water-filled pore spaces, temperature, soil texture, and pH (Foltz, M.E.et.al, 2022).

Soil pH can have chemical, physical, and/or biological effects on the denitrification process.

There is evidence that pH directly influences the rate of denitrification and the ratio of the N₂O and N₂ end products (Šimek and Cooper, 2002). Moreover, pH has an indirect effect on the size of the denitrifying community (Čuhel and Šimek, 2011). Denitrification occurs over a broad

range of soil pH values, from 5 to 8 (Weier & Gilliam, 1986; Ramos, 1996; Flessa et al, 1998). On the other hand, experimental results conducted by Šimek & Hopkins (1999), and Šimek et al. (2002) demonstrated that denitrification can hold even at pH below 4 or above 10 when the environmental conditions, the availability of denitrifying microorganisms, soluble C, and oxidized forms of N, are achieved. Several studies have been conducted to test the effect of changing pH on denitrification and N₂O production. Some findings addressed that the denitrification process can be accelerated by increasing soil pH and the dominant end product of the denitrification is N₂ (Ottow et al,1985; Hall et al.,1998) In contrast, experimental results from Koshinen and Keeney (1982) and Waring and Gillian (1983) showed that the availability of these carbon controls denitrification more than the variation of soil pH. Although numerous research has been made to determine whether increasing soil pH reduces the emission of N₂O or not (J. Čuhel and Šimek, 2011; Kaden et al., 2021; Zheng et al., 2022), most of the studies showed a reversed relationship between pH and the N₂O: N₂ ratio (Qu et al., 2014; Sun et al., 2012) . However, limited studies find a statistical relationship between them.

Soil texture plays a role in both factors water-filled pore spaces and soil aeration. Soil aeration has an effect on the all N transformation process specifically denitrification since it is related to the O₂ concentration and the exchangeable gases between soil and the atmosphere. The concentration the O₂ in soils depends on the soil water content and the organic matter that is decomposed by soil microorganisms. When there is rainfall, the soil becomes temporarily anaerobic. The amplitude and duration of the anaerobiosis vary from soil to soil based on the soil type. Fine-textured soils with a higher clay content were reported to remain anaerobic for a longer time than coarse-textured soils due to the greater number of micropores in fine-textured soils (Barton et al., 1999). Therefore, fine-textured soils are more likely to emit N₂O for a longer time than coarse-textured soils following rainfall. Limited studies were conducted to test soil texture's effect on the N₂O: N₂ ratio (Skiba and Ball, 2006), (Ball, 2013), (Hu et al., 2021).

Investigating the influence of environmental factors (e.g., soil pH and texture) on the $N_2O:N_2$ ratio is key to improving mitigation tools for N_2O emissions. The aims of the present study are (a) to quantify the relationship between soil pH and the N_2O ratio, (b) to determine how soil texture influences denitrification and the N_2O ratio, and (c) to utilize DNDC model to predict this relationship.

CHAPTER II

LITERATURE REVIEW

1- The relationship between soil pH and denitrification end products

Overall, the relationship between soil pH and N₂O ratio was mixed in published literature (Table 2). Several studies indicated that soil pH influences N₂O emission (e.g., Jiří Čuhel and Šimek, 2011a; J. Čuhel and Šimek, 2011b; Ha et al., 2015; Kaden et al., 2021; Robinson et al., 2014; Zheng et al., 2022). However, other studies found no effect of pH on denitrification and/or N₂O production (e.g., Čuhel et al., 2010; Šimek et al., 2002). When a relationship was identified, it was sometimes recorded as a strong negative relationship between soil pH and N₂O ratio (e.g., Qu et al., 2014; Samad et al., 2016a, 2016b; Sun et al., 2012).

These discrepancies may be rooted in the variation of the method used and/or the differences in the soil properties.

Table 2 Summaries of the studies related to the relationship between pH and denitrification potential or N₂O ratio (or similar end product ratio).

Study	Objectives *	pH Range	Method	Findings *
Šimek et al. (2002)	<ul style="list-style-type: none"> To assess the relationship between natural soil pH and DEA. To explain the effect of soil pH on the nature of denitrification end products (N₂O, N₂). 	Five soils are similar in texture but differing in pH (4-11) from four sites in South Bohemia, Czech Republic	Acetylene inhibition in the lab	<ul style="list-style-type: none"> The study indicated that there was no relationship between DEA and soil pH, as very high DEA was found both in acid and alkaline soil. The study showed that the soil denitrifies community was adapted to prevailing natural soil pH where they revealed the highest DEA at or near natural soil pH (soil pH range from about 4 to 11 adjusted before DEA determination) The study suggested avoiding the use of optimum pH for denitrification because various denitrification characteristics were found using different methods.
Čuhel et al. (2010)	<ul style="list-style-type: none"> To explore the effect of changes in soil pH on in situ N₂O and N₂ emissions, denitrifying enzyme activity (DEA), and potential N₂O production. 	Soils from the Czech Republic was with pH ranges 5.5-7.6	Field experiment and acetylene inhibition in the lab	<ul style="list-style-type: none"> Soil pH changes the total denitrification activity but not the N₂O production.
Jiří Čuhel and Šimek (2011)	<ul style="list-style-type: none"> To investigate the direct and indirect effect of soil pH on the denitrification rate and N₂O/(N₂O+N₂) ratio. 	Three soils differing in cattle impact from the Czech Republic with 5-7.07 soil pH range.	Acetylene inhibition in the lab	<ul style="list-style-type: none"> The denitrification rate was affected by the long-term pH management. The N₂O/(N₂O+N₂) ratio was induced by the effects of the current pH value on N₂O production and reduction. The structure of the denitrifying community is affected by long term pH management. The proportion of denitrification products was regulated by the short-term effect of soil pH.
J. Čuhel and Šimek (2011)	<ul style="list-style-type: none"> Test the influence of soil pH on N₂O production Test the relation between the denitrifying communities and the ratio of the products N₂O and N₂. 	Three pasture soils differing in cattle impact the Czech Republic 5.3-6.93	Acetylene inhibition in the lab	<ul style="list-style-type: none"> The N₂O/(N₂O+N₂) ratio suggested that relative N₂O production responded similarly to pH in all three soils, even if the soils contained different denitrifying consortia and different intrinsic capabilities to reduce NO₃. The results indicate that changes in soil pH will affect N₂O flux from agricultural fields. The ratio of denitrification products (N₂O and N₂) is pH-specific rather than soil specific. This suggests that manipulations of soil pH can significantly change N₂O fluxes from agricultural soils.

*Stated the objectives related to soil pH and N₂O emissions, ratio, and DEA.

Table 2 (continued) Summaries of the studies related to the relationship between pH and denitrification potential or N₂O ratio (or similar end product ratio).

Sun et al. (2012)	<ul style="list-style-type: none"> Estimate the denitrification rate and N₂/N₂O ratio and control soil properties including the pH and C content. 	Forest and grassland soils from China six soils with a pH range of 4.5-7.4	Acetylene inhibition in the lab	<ul style="list-style-type: none"> Soil pH was strongly correlated with the denitrification rate and N₂/N₂O ratio in both soils.
Qu et al. (2014)	<ul style="list-style-type: none"> None explicitly tied to pH 	pH of 3.7-7.7 for soils from China	Field experiment	<ul style="list-style-type: none"> There is a strong negative correlation between pH and the denitrification ratio. The ratio D/R was largely unaffected by soil pH. The immediate effect of liming acidified soils was lowered N₂O/(N₂O+N₂) ratios. The results provide evidence that soil pH has a high direct effect on potential denitrification. The results are in good agreement with the new understanding of how pH may interfere with the expression of N₂O reductase.
(Robinson et al., 2014)	<ul style="list-style-type: none"> Determine the effects of soil pH change on N₂O emissions. 	pH of 5-6.5 for soils from China	Field experiment	<ul style="list-style-type: none"> The total N₂O emissions were increased when the soil was acidified by the acid treatment. Incomplete denitrification may be caused by the inhibition of the assembly of the N₂O reductase enzyme under acidic conditions.
Ha et al. (2015)	<ul style="list-style-type: none"> Estimate the controlling factors of N₂O and N₂ emissions. 	Different types of soils under flooded conditions with a pH range of 4.3-7 in Japan	Acetylene inhibition in the lab	<ul style="list-style-type: none"> Denitrification (N₂O+N₂) was higher in the neutral soil than that in the relatively acidic soil and the highly acidic soil. N₂O was the main product in the acidic soil while N₂ was the dominant product in the other two soils.
(Samad et al., 2016a)	<ul style="list-style-type: none"> To determine the effect changing methods (extractant type) for determining soil pH has on the observed relationship with N₂O flux 	Soil pH range from 4.5 to 7 from Ireland-Moorepark, Johnstown, Solohead, and New Zealand.	Field experiments	<ul style="list-style-type: none"> A strong negative relationship was detected between soil pH and both N₂O production and N₂O/(N₂O+N₂) ratio.
Samad et al. (2016b)	<ul style="list-style-type: none"> Investigate the relation between pH, community composition, and the N₂O emission ratio (N₂O/(NO+N₂O+N₂)). 	13 temperate pasture soils with 5.5-7 soil pH in New Zealand	Quantitative PCR	<ul style="list-style-type: none"> pH applies a general selective pressure on the entire community and the results changes with emission potential. Soil pH was negatively associated with N₂O emission ratio and positively associated with community diversity and total denitrification gene.

*Stated the objectives related to soil pH and N₂O emissions, ratio, and DEA.

Table 2 (continued) Summaries of the studies related to the relationship between pH and denitrification potential or N₂O ratio (or similar end product ratio).

Kaden et al. (2021)	<ul style="list-style-type: none"> To analyze key controlling factors for soil denitrification potential in a range of floodplain ecosystems. 	Six study areas at four large German rivers with 6-8.2 soil pH range	Acetylene inhibition in the lab	<ul style="list-style-type: none"> There was a strong soil pH effect. Denitrification potential was majorly controlled by soil pH. Mean soil denitrification potentials were higher when soil pH > 7.
Zheng et al. (2022)	<ul style="list-style-type: none"> Examine the influence of soil acidification on N₂O emissions. Estimate the contribution of bacterial and fungal denitrification to N₂O emissions under various pH. 	Soil pH range 6.2-8.7 of soils from China	Acetylene inhibition in the lab	<ul style="list-style-type: none"> N₂O emissions in the acidified soils (pH 6.2 and pH 7.1) are significantly higher when compared with the initial soil (pH 8.7). The pH management should be high to mitigate N₂O emissions, particularly for regions with long-term excessive nitrogen fertilizer likely to acidify the soils.

*Stated the objectives related to soil pH and N₂O emissions, ratio, and DEA.

2- The relationship between soil texture denitrification end products

Soil texture is one of the factors that have been recorded to have an influence on the ratio of denitrification end products. Table 3 illustrates the most related studies that consider the effect of soil texture or type on the denitrification end-products. Overall, there were very few studies that investigated this relationship. The few studies that did explore texture effects had conflicting results. Some studies found that there is a significant relationship between soil texture and denitrification end products (Ball, 2013; Hu et al., 2021a; Maag and Vinther, 1996). In contrast, other studies indicated there is no direct relationship or there is a weak link between texture and denitrification end products (Braker et al., 2015; Skiba and Ball, 2006).

Table 3 Summaries of the studies related to the relationship between soil texture and N₂O ratio.

Study	Objectives*	Soil textures	Method	Findings*
(Maag and Vinther, 1996)	<ul style="list-style-type: none"> determined the amount of N₂O in five different soil types regarding different temperatures and soil moisture contents predicted the effects of temperature and soil moisture on the ratio between N₂ and N₂O in the denitrification process 	Coarse sandy, Loam sand, and sandy loam soils	Acetylene inhibition in the lab	<ul style="list-style-type: none"> Soil texture significantly affected the denitrification loss at varied soil moisture contents. The "N₂: N₂O" ratios were higher in the sandy loam soil than in the coarse sandy soil.
Skiba and Ball (2006)	<ul style="list-style-type: none"> Tested the importance of soil type to the changes in soil aeration. 	poorly drained silty clay loams to freely drained sandy loams	Field experiments using static chambers.	<ul style="list-style-type: none"> The relationship between NO and N₂O fluxes and soil texture is very weak because of the strong link between N fluxes production and soil temperature, moisture, and N oxides concentrations. N₂O emission was affected by the combination of bulk density and clay content.
Ball (2013)	<ul style="list-style-type: none"> Investigate how soil structures affected N₂O emissions 	silty clay and sandy loam soils	Field experiments, static chambers	<ul style="list-style-type: none"> Matric potential, water content, relative diffusivity, air permeability, and water-filled pore space of soil can be used as an indicator for N₂O. Soil type applied a strong influence on GHG emissions, principally through the influences of soil structure and soil wetness. Structure influences moisture content and distribution, compaction status, pore size and continuity, and the distribution of organic residues. These, along with soil temperature, regulate microbial activity creating gases and their emission.
Braker et al. (2015)	<ul style="list-style-type: none"> None explicitly tied to soil texture. 	Loamy and sandy soils	Acetylene inhibition in the lab, PCR	<ul style="list-style-type: none"> The results showed that differences in land use and soil type determined the number of soil characteristics that are relevant for denitrification activity and affected the communities mediating the process indicating that denitrification activity and potential N₂O emissions of soils might indirectly depend on land management and soil type.
Hu et al., (2021)	<ul style="list-style-type: none"> None explicitly tied to soil texture. 	loamy and sandy soils	Acetylene inhibition in the lab	<ul style="list-style-type: none"> There is a high effect of each soil texture, organic matter, and low-level increases in salinity on denitrification.

*Stated the objectives related to soil texture and N₂O emissions, ratio, and DEA.

CHAPTER III

MATERIALS AND METHODS

1- Site description

Four natural grasslands from different parts of Oklahoma state were selected based on access availability and variation of soil texture and natural soil pH that were estimated by using a Soil Web (SW), an interactive map utilized to explore USDA-NCSS soil survey data for locations throughout most of the U.S, (Figure 3). The soil was collected randomly, and three reputations were collected at a depth of (0-20) cm from each location using an auger (2 3/4 in diameter) and excluded from the grass layer. Payne county in Stillwater was the first location with coordinates (36.10148 N, 97.02154 W). The dominant soil in this site is Coyle (85%) and the site is well drained based on SW and covered with crops. The second site is Woods County in Aline, Oklahoma where (36.48580 N,98.67465 W) is the coordinate of that location. The dominant soil in this site is Devol (90%) and the site is well drained too based on SW. This site covered by grass lab. Grant county 1 in Manchester, Oklahoma is the third location selected with coordinate (36.95469 N,98.0723)Theom the same county with different natural pH and texture were the location of the fourth site. The dominant soil in both sites is Krikland (80%) and the sites are well drained based on SW. Both sites covered with natural grass.

2- Soil sampling

The collected samples of each site were mixed and store in 4C for further analysis. Part of each soil were tested at an external laboratory to find some soil properties such us soil texture, pH, ammonium, nitrate, total nitrogen, and soil organic matter as illustrated in table 4. Additionally, soil moisture content was measured by recording the wetting weight and oven dry the samples to record the dry weight and the following equation was used to calculate the soil moisture content.

$$\text{Moisture content} = \frac{\text{wet weight} - \text{dry weight}}{\text{dry weight}}$$



Figure (3) The soil sampling sites location in Oklahoma

Table 4 Soil samples properties obtained from external testing lab (SWAFL)

County	Texture	Natural pH	OM%	NO ₃ ⁻ (ppm)	NH ₄ ⁺ (ppm)	Water Content %
Payne	Loam	7.7	2.95	4.5	3.4	13.4
Woods	Sand	5.4	0.53	1.5	6.5	5.4
Grant 1	Loam	7.8	1.84	9.5	5.3	13.2
Grant 2	Sandy loam	6.3	NA	28.5	7.2	12.6

3- Experimental treatments:

Preliminary experiments were conducted to adjust the soil pH from the natural pH value to the range of (2-10). For each soil type, 25 g of soil samples mixed with 25 mL of deionized water to prepare the soil slurry. The natural soil pH was recorded by using pH probe (Oakton pH/ Ion 700). Soil pH was measured after approximately half an hour of slurry preparation to allow the ions to release in solution. Potassium hydroxide (KOH), a white solid chemical highly soluble in water, was the strong base utilized to increase the soil pH. Different concentrations of KOH mixed with water were added to the soil gradually and the pH of the solution was recorded for each additive to find the exact concentration needed for each change in soil pH. Sulfuric acid (H₂SO₄), a liquid chemical, was the strong acid utilized to decrease the soil pH. By mixing 25 g of soil with different mL of H₂SO₄ diluted in water, the mL of chemical needed to decrease the soil pH was recorded. These recorded additions were used in subsequent pH altered assays described below.

4- Denitrification Enzyme assays:

The denitrification enzyme activity assay (DEA) was applied to determine the denitrification potential and N₂O production potential using variations of the acetylene inhibition technique (Hathaway et al., 2017), amended from (Tiedje et al., 1989). Triplicate samples were prepared by adding 25 g of soil into each 125 mL Wheaton glass bottle sealed with cap and septa. A nutrients solution was prepared by adding 25000 mg of mg D-glucose and 3600 mg of potassium nitrate

(KNO₃) to the 1000 mL of deionized. 5 ml of the nutrient solution were added to reach a goal of approx. 2 mg C and 0.1 mg N per g substrate which provide unlimited nutrients for the soil to achieve an optimal condition for denitrification. Separate solutions were prepared for each pH adjustment using the amount of acid or base calculated in part (2) to adjust the soil pH to the appropriate value. After a short overnight incubation period to get the soil to room temperature (recorded with a thermal probe), the assays were started by adding 25 mL of the solution to each bottle with soil. Soil pH was measured for each sample by using pH probe. The bottles were sealed and flushed with dinitrogen gas for two minutes and over-pressurized for 10 sec. Acetylene gas was injected with 20 mL volume to half of the samples to measure the total denitrification by blocking N₂O converted to N₂. N₂O production was measured with another half of the samples by injecting 20 mL of dinitrogen gas. The incubation time was recorded after shaking the samples for 30 sec. Gas samples were collected from the headspace using 10 mL evacuated vials sealed with gray butyl rubber septa after 2, 3, 4, and 5 hours of incubation time. The incubation period should not exceed 5 h at 20°C that's to avoid any effect of growth of denitrifying organisms on the DEA. The gas samples were stored at room temperature and later analyzed by Gas Chromatography.

4- Gas chromatography (GC):

Gas Chromatography is a powerful tool to specifically analyze one or more gases.

Chromatographic analysis consists of four steps: sample collection, sample injection, sample separation, and sample detection. The collected gas sample is introduced into an inert gas stream called a carrier gas. The carrier gas moves the gas sample through the columns where physical separation of the gases in the sample occurs. The separated gases are directed to a detector which provides an output proportional to their concentration.

The gas samples were analyzed using Agilent 8890 GC System with autosampler and three detectors: a flame ionization detector (FID), a thermal conductivity detector (TCD), and an electron capture detector (ECD). Although detectors were available for other trace gas analysis, a method was developed to only measure N₂O with the ECD with the other detectors offline. Standards were prepared with known concentrations of N₂O using gas cylinder of 10 ppm of N₂O (Table 5), and these standards were included in each run for calibration of the standard curve. Samples were run sequentially after running the standards. The GC software generated an excel sheet containing data of the chromatogram peak area, retention time, and concentration (based on standard curve) for each sample. Concentrations were also checked manually for standards using raw standard peak area data to validate GC software calculations.

Table 5 Concentrations of N₂O & N₂ of standards samples using for GC

Name	N ₂ in ml	N ₂ O in ml	N ₂ O Concentration(ppm)
Std 1	13	2	1.11
Std 2	10	5	3.55
Std 3	7	8	5.56
Std 4	5	10	6.80
Std 5	3	12	7.48
Std 6	0	15	10.16

5- DEA Calculations of N₂O ratio:

1- N₂O concentrations determined from gas samples were adjusted for N₂O dissolved in media using the following equations:

By using the moisture content of each soil type and the wetting weight of each sample we can calculate the dry weight of the soil:

$$W_{Dry} = W_{wet} \times (1 - W\%)$$

Where; W_{Dry} is dry weight, W_{wet} is wet weight, and $W\%$ is water content.

Calculate the Dilution rate from assay bottles to GC vials:

$$r_d = \frac{V_s + V_{N_2}}{V_s}$$

Where; $r_d = \text{dilution rate}$

$V_s = \text{volume of sample taken from assay bottle and added to GC vial}$

, and $V_{N_2} = \text{volume of Nitrogen added to GC vial}$

To find the concentration of the gas in assay bottle: we multiply the dilution rate times the concentration of the gas in ppm from GC:

$$C_h (\text{ppm}_v) = C_v \times r_d$$

Where; $C_h = \text{concentration in assay bottle}$

$C_v = \text{concentration in GC vial}$

Convert the temperature of the incubation room temperature from C to K:

$$T = \text{temperature in Kelvin} = 273.15 + t = 294.65 \text{ in lab}$$

Calculate the concentration of mol N_2O/L by ideal gas law:

$$C'_h (\text{ppm}_v) = C_{h,t_n} + \sum_{i=1}^n \frac{V_{s,t_{i-1}} C_{h,t_{i-1}}}{V_{h,t_{i-1}}}$$

$$V_{N_2O} = \frac{n_{N_2O}RT}{P} \quad V_{tot} = \frac{n_{tot}RT}{P}$$

$$C'_h (\text{ppm}_v) = \frac{V_{N_2O}}{V_{tot}} \times 10^6 = \frac{\frac{n_{N_2O}RT}{P}}{\frac{n_{tot}RT}{P}} \times 10^6 = \frac{n_{N_2O}}{n_{tot}} \times 10^6$$

$$C'_h (\text{mol/L}) = \frac{n_{N_2O}}{V_{tot}} = \frac{C'_h (\text{ppm}_v) n_{tot}}{\frac{n_{tot}RT}{P} \times 10^6} = \frac{C'_h (\text{ppm}_v)}{10^6} \times \frac{P}{RT}$$

Where; $C'_h =$

concentration in assay bottle, corrected to account for gas sampling effect

$n = \text{number of moles in headspace}$

$P = \text{pressure in atmospheres} = 1 \text{ atm}$

$R = \text{ideal gas constant} = 0.082054 \text{ L atm K}^{-1} \text{ mol}^{-1}$

$V = \text{volume in Liters}$

Total moles of N_2O with Bunsen's coefficient (accounts N_2O in solution)

$$M_{N_2O} = C'_h (\text{mol/L}) \times (V_h + V_l \alpha)$$

Where; $V_h = \text{volume of headspace in assay bottle} = 120 \text{ mL} = 0.12 \text{ L}$

$$\alpha = \text{Bunsens coefficient} = 1.3668 - 0.04870T + 0.00068145T^2 = 0.6347502625, \text{ if } T = 21.5^\circ\text{C}$$

M = total number of moles (gas and liquid phase)

V_h = volume of headspace in assay bottle = 120mL = 0.12L

V_l = volume of liquid slurry in assay bottle = bottle volume - V_h = 40mL = 0.04L

Total moles of $\text{N}_2\text{O-N}$ were calculated using the following equation:

$$M_{\text{N}_2\text{O-N}} = M_{\text{N}_2\text{O}} \times \frac{2 \text{ mol N}_2\text{O} - \text{N}}{1 \text{ mol N}_2\text{O}}$$

Convert the Mole of N_2O to total ng of $\text{N}_2\text{O-N}$:

$$w_{\text{N}_2\text{O-N}}(\text{ng}) = M_{\text{N}_2\text{O-N}} \times \frac{14 \text{ g N}_2\text{O} - \text{N}}{1 \text{ mol N}_2\text{O} - \text{N}} \times \frac{10^9 \text{ ng}}{1 \text{ g}}$$

- 2- Calculate the total denitrification potential rate and the total N_2O production potential rate in (ng $\text{N}_2\text{O-N}$ g⁻¹ dry soil h⁻¹) by regressing the amount of N_2O in each vial against time for samples inhabited with acetylene for total denitrification and using samples without acetylene for N_2O production:

$$\text{rate} = \frac{\text{slope} \left(\frac{\text{ng}}{\text{h}} \right)}{\text{dry weight soil (g)}}$$

The slopes were evaluated for linearity. If the rate is non-linear or otherwise poorly fits the time course series, discard the rate due to sampling error.

6- Statistical Analysis:

Triplicate samples were conducted, the mean of each sample was considered the final rate of total denitrification, samples without acetylene, and N_2O production samples with acetylene added. Standard deviations were calculated too. The N_2O ratios were calculated by divided the N_2O production on the total denitrification.

7- Model description and process:

The DeNitrification-DeComposition (DNDC) model is a process-based model established to simulate carbon and nitrogen biogeochemistry in agroecosystems. The model was originally proposed by Li et al. (1992) as a rain event-driven simulation model for N₂O, CO₂, and N₂ emissions from agricultural soils in the U.S. Many developments and improvements were carried out on the DNDC model since the time it was established (Gilhespy et al., 2014). The last version of the model, which is DNDC 9.5, was modified by Li and collaborators in 2013. DNDC model combines both decomposition and denitrification processes to predict carbon (C) and nitrogen (N) movement in agricultural soils (“User’s Guide for the DNDC Model,” 2012).

The model utilizes the basic laws of physics, chemistry, and biology in addition to some empirical equations obtained from statistical analysis of experimental data. There are two modes in this model. The mode utilized in this study is site-related which contains three main parameters, namely climate, soil, and cropping. The required input parameters for which defaults can’t be predicted are location (latitude), weather data (a minimum of daily mean air temperature and precipitation), soil bulk density, pH, and soil organic carbon (SOC) at the surface (0–10 cm) (Gilhespy et al., 2014).

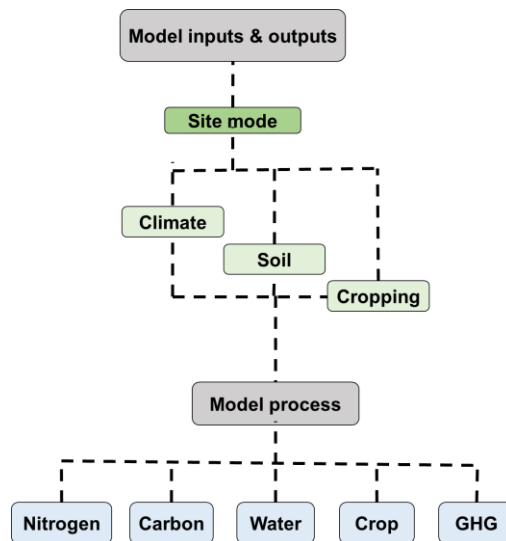


Fig (4) DNDC model operation process.

8- Model inputs parameters:

As described in the previous section, we used the DNDC model to predict N₂O and N₂ emissions and to calculate ratio. N₂O ratio from this model was utilized to be compared with the lab measurements. The input data required by this software were collected from different sources and detailed as follows:

For Climate files, data were extracted from Mesonet (a network of environmental monitoring stations). This data contains measured values of Max and Min air temperature, precipitation, wind speed, radiation, and relative humidity for 11 years from 2011 to 2022 for all fields. The units of the data were converted to match the unit of DNDC. Finally, the data were saved as a text file for each year separately.

One of the model requirements is Carbon concentration (CO₂) at the atmosphere in ppm units that obtained from NOAA, 2022.

Furthermore, Nitrogen concentration in rainfall was calculated by dividing the total nitrogen deposition over an annual precipitation value. This information can be obtained from the National Atmospheric Deposition Program (NADP,2021) website. First, I specified the location of interest to find the site ID and choose the annual data and specify the time intervals. By selecting the water year and deposition in kg/ha, I obtained the report. From the report (Excel file of NTN), the selected columns were “NH₄”, “NO₃”, and “Precipitation (cm)”. The summation of “NH₄” and “NO₃” provided the total nitrogen deposition. The below equation was then used to find N concentration in rainfall:

$$N \text{ conc. in rainfall} = \frac{\text{total N deposition}}{\text{rainfall}}$$

The unit of the obtained nitrogen concentration from the above equation is Kg/ha.cm. Since the DNDC model adopts the unit of mg/l for the nitrogen concentration, it is needed then to convert it when input into the DNDC model.

Some of soil input parameters were obtained from the laboratory measurements of the soil samples such as soil texture, pH, OM, clay percentage. While other parameter that their actual values was not available (e.g., Bulk density, slope) were obtained from the soil survey website USDA,2021. To find the required information, the area of interest (AOI) needs to be identified using the map. Then, from Soil Data Explorer, the necessary information that DNDC required for the soil page was being collected. The collected information was found under soil properties. A data from Miso soil generated by Ochsner were used to find soil conductivity, wilting point, and field capacity that are related to the soil page of DNDC model.

9- Model process:

After collecting the required information for each site, a DNDC files were generated and saved under actual data.

To estimate the relationship between soil pH and N₂O ratio, the whole information was kept constant and only variable of each model run was the soil pH from (2-10) to match that of the laboratory measurements. Then from the results of each run of the model, the value of N₂O and N₂ was extracted from the model at the same day of the soil sampling.

On the other hand, the effect of soil texture on N₂O ratio was estimated by the same technique were all the factors kept constant and consider the change in soil texture and all related parameter such as clay percentage, conductivity, porosity, and field capacity. The parameters related to soil texture were considered by the software itself. Each site was having different crop, tilling, and grazing parameters, but when all those factors taking in account the results were totally

incomparable because of those differences. Therefore, those parameters were ignored in this study to match the situation of the laboratory conditions where there is no effect of those parameters.

9- Model evaluation:

Model evaluation is the process through which we quantify the quality of a system's predictions.

Graphical Analysis Method was used to evaluate the DNDC model performance. Graphical

Analysis Method can be used to visually assess the accuracy of model predictions by plotting a

1:1 graph with measured data on X axis and Modeled data on the Y axis. The graph is separated

into two sections by dashed line and the data will be evaluated based on its location on the graph,

where the data below the line means the model underpredicts the N_2O ratio and the data above the

line means the model is overpredicted the N_2O ratio as illustrated in the below Figure (5).

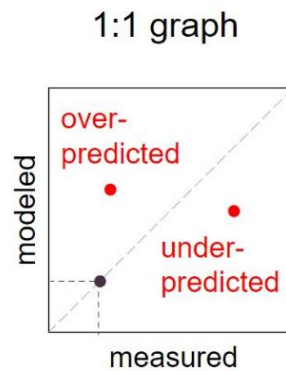


Figure (5) Graphical analysis method of model evaluation

CHAPTER IV

RESULTS AND DISCUSSIONS

Laboratory measurements results:

a) Denitrification potential in soils at natural and modified pHs:

Figure (6) illustrates the relationship between soil pH and the denitrification potential for four soils. For four of the soils, Payne, Woods, Grant 1 and Grant 2, the largest DEA was found at the natural soil pH. This result aligned with other studies, which attributed the observations to the adaptation of the microbial community to the current pH of the soil and thus more activity in the natural soil pH (e.g. Šimek et al., 2002; Šimek and Cooper, 2002). In this study, denitrification potential was highly affected by changing soil pH. Denitrification potential decreased below and above natural soil pH. It is likely that soil pH affects the activity of the microorganism that responsible on the denitrification process since all other factors (e.g., soluble carbon, availability of NO_3^-) were kept constant.

Considering all these soils, Payne County soil has the highest denitrification potential. The Payne soil had the same soil texture and similar natural pH as Grant 1, yet its denitrification potential was still much higher. This difference may be ascribed to the high value of organic matter as compared with other soils. As compared to Grant 1, an otherwise similar soil to Payne, Payne had almost twice as much organic matter. Another potential explanation for Payne having the highest denitrification potential might be associated with the variation of the time between the sampling and the testing of the soils. Payne soils were tested next day of sampling, while other soils,

Woods, Grant 1, and Grant 2 were tested after one, two and three weeks from sampling respectively.

The highest emissions of N₂O of all soils were found under pH ranging from 5 to 6 regardless of the differences in the natural pH of each soil.

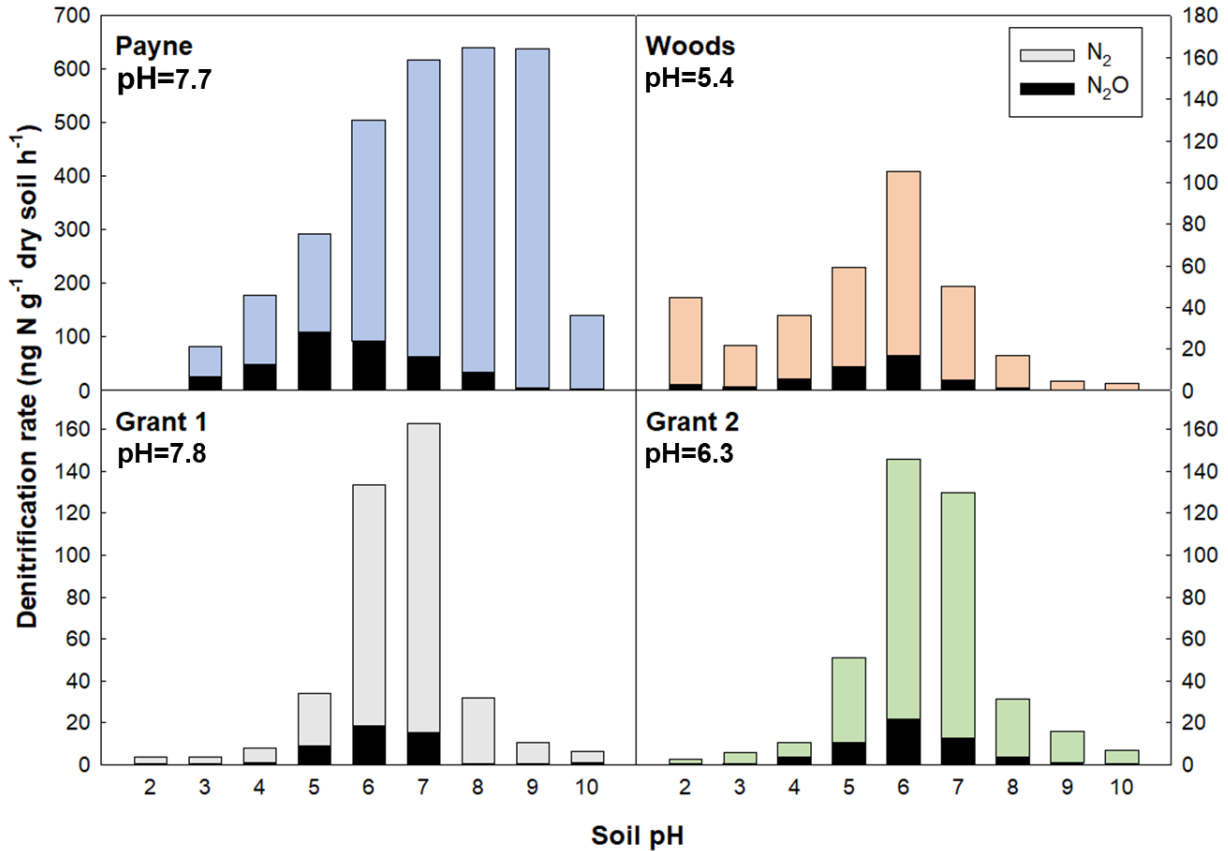


Figure (6) Denitrification potential (expressed as N₂O and N₂ production) in soils Payne, Woods, Grant 1 and Grant 2 determined at native soil pH and modified soil pH; values are means (n=3)

b) The relationship between soil pH and N₂O ratio:

The statistical relationship between different soil pH and the N₂O ratio was obtained from laboratory measurements. To find the best fit equation, the trendline calculator for multiple series was utilized to overcome the problem of having multiple data series with multiple trendlines, four soils, that needed to have one trendline for all. The manual process for combing all trendlines

together was carried out by plotting all four data sets in one figure in excel and then combine all series to have a unified trendline that best fit for them.

Figure (7) showed a non-linear, polynomial, relationship between soil pH and N₂O ratio. This finding has an agreement and disagreement with other studies depending on what range of pH considered. This study's data tended to have a negative linear relationship when the range of soil pH is between 4-8 and was not as highly scattered. This finding has an agreement with (Mukumbuta et al., 2018; Qu et al., 2014)) where they found a negative correlation between those variables. On the other hand, the relationship found by this study is not linear when considering the range of pH from 2 to 10. This expanded pH range had not been tested before so this outcome is novel.

Figure (7) also illustrated that N₂O ratio increased when soil pH decreased. As the N₂O ratio is an indicator of the completion status of denitrification process, where having higher N₂O ratio mean more N₂O production occurs compared with N₂ production. Based on this conclusion, the acidic soils have higher N₂O emissions compared with natural and alkaline soils.

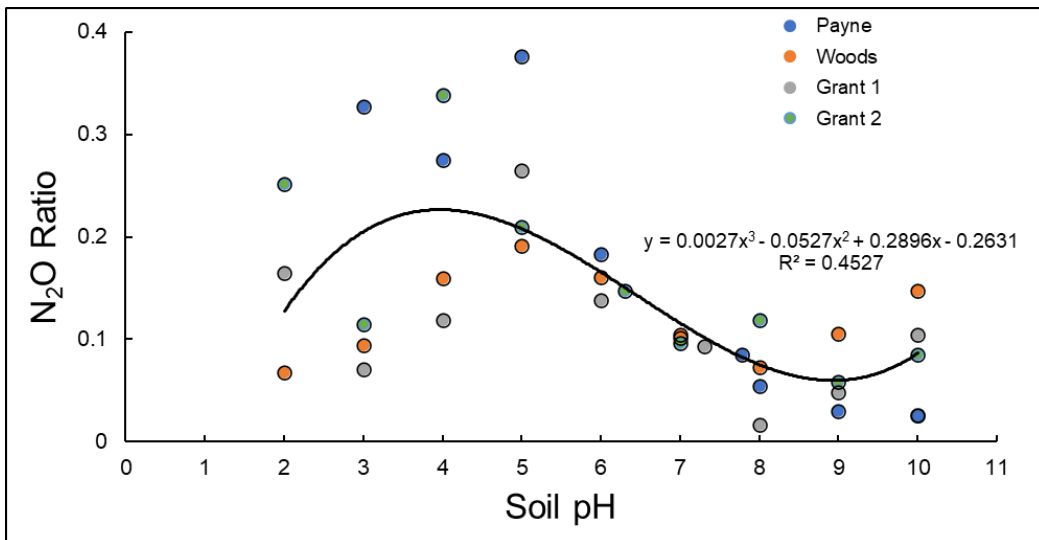


Figure (7) Statistical relationship between soil pH and N₂O ratio obtained from DEA.

c) The effect of Soil texture on N₂O ratio:

To estimate the effect of soil texture on N₂O ratio, three different soil textures (i.e., loam, sand, sandy loam) were tested. The N₂O ratio is varied for each soil and its varied even at the same texture of soil (Figure 8). This finding provides an indicator that there are other factors that lead to this variation in the N₂O ratio. Further scrutiny of the soil texture fractions uncovered that the clay percentage of each of the tested soils were different even among the soil with the same texture. As the clay percentage increased, the N₂O ratio also increased (Figure 9). There is some evidence from field studies that N₂O emissions may be related to clay fraction. Henault et al. (1998) documented much larger N₂O emissions from a soil with high clay content than a freely drained soil. (Skiba and Ball, 2006) also associated the emissions of N₂O to both clay percentage and bulk density of the soil. Although these relationships have been previously identified in the field, to my knowledge this study is the first to document the connection between clay content and the N₂O ratio using DEAs in the lab. Closer investigation of this relationship with different clay fractions is recommended to fully understand the complex relationship between soil texture and the N₂O ratio.

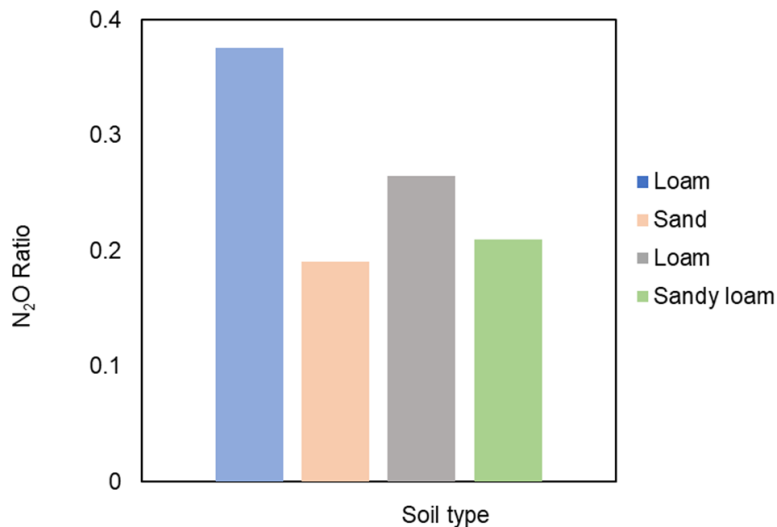


Figure (8) the relationship between soil texture and N₂O ratio obtained from DEA

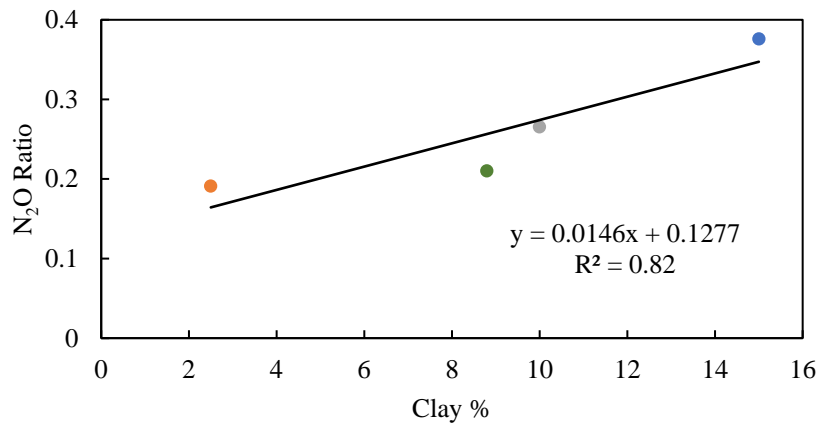


Figure (9) the relationship between Clay percentage and N₂O ratio obtained from DEA

Modeling results:

a) The effect of soil pH on N₂O ratio:

To demonstrate the relationship between soil pH and N₂O ratio that estimated from DNDC model, a varied range of pH 2-10 were utilized. The equation of this figure is a nonlinear, polynomial, which provides an agreement with the lab measurements Figure (10). The curve tends to be linear within the range of pH 5-8, similarly to that of lab measurements. The data scattered below pH of 4 and above pH of 8. The data of loam soil tend to show a linear relationship between the variables, unlike the sandy and sandy loam soil where nonlinear relationship observed that skewed the overall trend to nonlinear. There are differences between the relationship obtained from laboratory measurements illustrated in Figure (7) and the one predicted from the DNDC model Figure (10). these differences are represented by the range of N₂O ratios where N₂O ratios from measured data ranged from 0 to 0.4 while the N₂O ratios from DNDC ranged from 0 to 0.85, and with the shape of the curve too, N₂O ratio from measured data shaped upward trend after reaching pH of 9 and above.

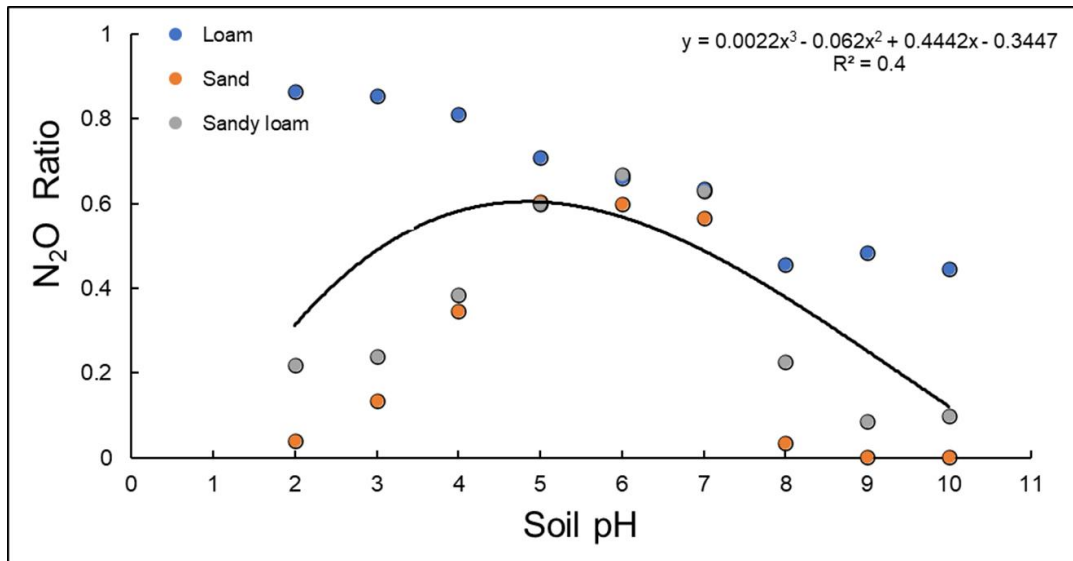


Figure (10) Statistical relationship between soil pH and N₂O ratio obtained from DNDC.

b) The effect of soil texture on N₂O ratio:

Figure (11) demonstrate the relationship between soil texture and N₂O ratio obtained from DNDC model. The figure showed that soil texture has an influence on the N₂O ratio. The model considers the clay content of the soil, and the results showed the same link between soil clay content and N₂O ratio. As the clay content increases, the N₂O ratio increases, too Figure (12).

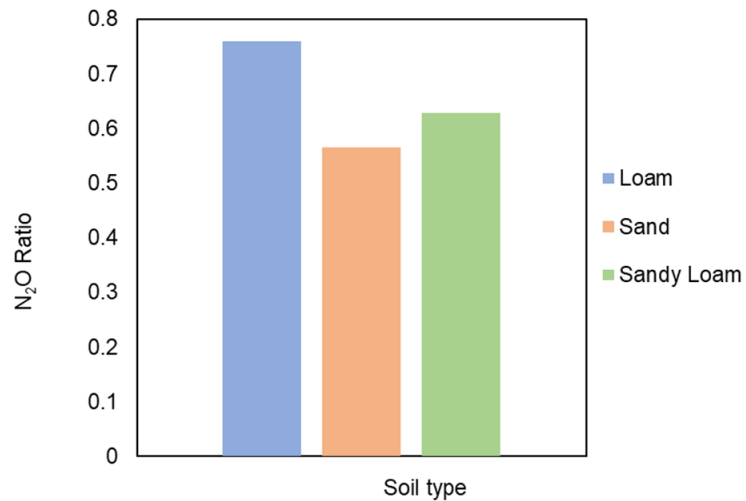


Figure (11) the relationship between soil texture and N₂O ratio obtained from DNDC.

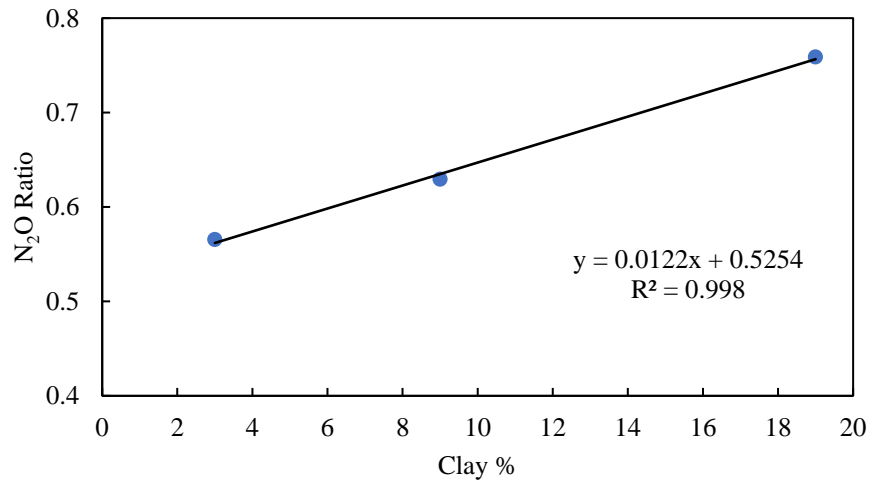


Figure (12) the relationship between Clay percentage and N₂O ratio obtained from DNDC.

c) Model evaluation:

Figure (13) below shows the model performance as a comparison between the modelled and the measured data. Generally, the model overpredicted the N₂O ratio, although in some cases it had good predictions or underestimated the ratio (Figure 13). The over- or under-predictions were not consistent for simulations based on soil texture or pH, so it was difficult to determine the source of this error.

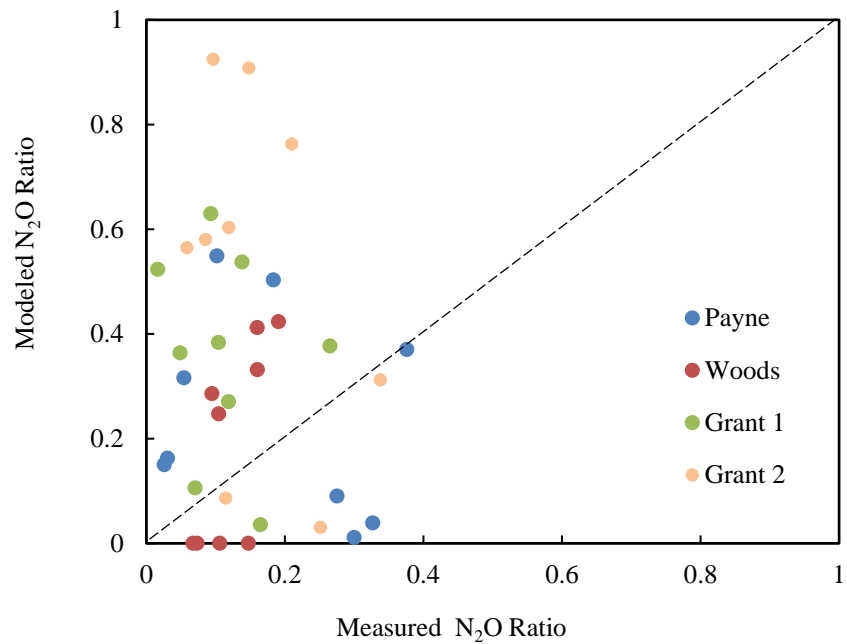


Figure (13) Model evaluation of N₂O ratio from both DEA, measured data, and DNDC, modeled data, for four different soils and under pH (2-10).

CHAPTER V

CONCLUSIONS

In this study, two soil variables, pH and texture, were considered to evaluate their effect on the N₂O ratio and to find a statistical relationship between them. This study targeted a larger pH range than most to increase understanding of the effect of soil pH on the N₂O ratio. We found that the highest denitrification potential in all tested soils were under the natural pH of soil, which varied across samples. Therefore, there is no specific value that can be set as the optimum pH for denitrification. However, over the soil pH range from 4 to 8 the N₂O ratio decreased with pH increase. Therefore, in relatively neutral soils, liming to increase pH slightly may help to mitigate the N₂O emissions. The pH effect on the N₂O ratio could be simulated in DNDC within the limited pH range tested in most studies.

Soil texture is still an area that needs more research. This study did not find clear connections between soil texture and the N₂O ratio. For the few soils tested here, loamy soils had higher potential for emitting N₂O than sandy soils. The strongest texture outcome was an identified relationship with clay percentage of soil, which is linked to the soil texture. Future work should focus specifically on the clay fraction to investigate this relationship further.

Overall, although the large range of pH is a strength of this study, the limited conclusions possible for texture highlighted the difficulty and limitations of considering soil texture effects. More sites with different textures are needed, and specifically soils with the same texture and different clay content will increase the likelihood that statistical relationships can be uncovered.

REFERENCES

- Ball, B.C., 2013. Soil structure and greenhouse gas emissions: a synthesis of 20 years of experimentation. *Eur J Soil Sci* 64, 357–373. <https://doi.org/10.1111/ejss.12013>
- Barton, L., McLay, C.D.A., Schipper, L.A., Smith, C.T., 1999. Annual denitrification rates in agricultural and forest soils: a review. *Soil Res.* 37, 1073. <https://doi.org/10.1071/SR99009>
- Bleakley, B. H., and Tiedje, J. M. (1982). Nitrous oxide production by organisms other than nitrifiers or denitrifiers. *Appl. Environ. Microbiol.* 44, 1342–1348
- Braker, G., Conrad, R., 2011. Diversity, Structure, and Size of N₂O-Producing Microbial Communities in Soils—What Matters for Their Functioning?, in *Advances in Applied Microbiology*. Elsevier, pp. 33–70. <https://doi.org/10.1016/B978-0-12-387046-9.00002-5>
- Braker, G., Matthies, D., Hannig, M., Brandt, F.B., Brenzinger, K., Gröngroft, A., 2015. Impact of Land Use Management and Soil Properties on Denitrifier Communities of Namibian Savannas. *Microb Ecol* 70, 981–992. <https://doi.org/10.1007/s00248-015-0623-6>
- Conrad, R., 1996. Soil microorganisms as controllers of atmospheric trace gases (H₂, CO, CH₄, OCS, N₂O, and NO). *Microbiological reviews*, 60(4), pp.609-640.DOI: <https://doi.org/10.1128/mr.60.4.609-640.1996>
- Čuhel, Jiří, Šimek, M., 2011. Proximal and distal control by pH of denitrification rate in pasture soil. *Agriculture, Ecosystems & Environment* 141, 230–233. <https://doi.org/10.1016/j.agee.2011.02.016>
- Čuhel, J., Šimek, M., 2011. Effect of pH on the denitrifying enzyme activity in pasture soils in relation to the intrinsic differences in denitrifier communities. *Folia Microbiol* 56, 230–235. <https://doi.org/10.1007/s12223-011-0045-x>
- EPA, U. states environmental protection agency, 2022. Inventory of U.S. Greenhouse Gas Emissions and Sinks. (Full, complete Inventory report No. February 15, 2022, 430-P-22-001). U.S. Environmental Protection Agency.
- Firestone, M.K. and Davidson, E.A., 1989. Microbiological basis of NO and N₂O production and consumption in soil. Exchange of trace gases between terrestrial ecosystems and the atmosphere, 47, pp.7-21.
- Flessa, H. Wild, U. Klemisch, M. & Pfadenhauer, J. (1998). Nitrous oxide and methane fluxes from organic soils under agriculture. *European Journal of Soil Science*, 49, pp. 327-335
- Foltz, M.E., A.Alesso, J.L. Zilles., 2022. Factors driving the nitrous oxide ratio: a systematic review of laboratory soil denitrification experiments. submitted 2022, under reviewed.

- Gilhespy, S.L., Anthony, S., Cardenas, L., Chadwick, D., del Prado, A., Li, C., Misselbrook, T., Rees, R.M., Salas, W., Sanz-Cobena, A., Smith, P., Tilston, E.L., Topp, C.F.E., Vetter, S., Yeluripati, J.B., 2014. First 20 years of DNDC (DeNitrification DeComposition): Model evolution. *Ecological Modelling* 292, 51–62. <https://doi.org/10.1016/j.ecolmodel.2014.09.004>
- Hall, J. M., Paterson, E., & Killham, K. (1998). The effect of elevated CO₂ concentration and soil pH on the relationship between plant growth and rhizosphere denitrification potential. *Global Change Biology*, 4(2), 209-216.
- Hathaway, S.K., Bartolerio, N.A., Rodríguez, L.F., Kent, A.D., Zilles, J.L., 2017. Denitrifying Bioreactors Resist Disturbance from Fluctuating Water Levels. *Front. Environ. Sci.* 5, 35. <https://doi.org/10.3389/fenvs.2017.00035>
- Henault C Devis X Page S Justes E Reau R & Germon JC 1998. Nitrous oxide emissions under different soil and land management conditions. *Biology and Fertility of Soils* 26, 199±207.
- Hu, M., Peñuelas, J., Sardans, J., Huang, J., Xu, K., Tong, C., 2021. Denitrification rates in tidal marsh soils: The roles of soil texture, salinity, and nitrogen enrichment. *Eur J Soil Sci* 72, 474–479. <https://doi.org/10.1111/ejss.12956>
- IPCC, 2022: *Climate Change 2022: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. Cambridge University Press. Cambridge University Press, Cambridge, UK and New York, NY, USA, 3056 pp., doi:10.1017/9781009325844.
- Kaden, U.S., Fuchs, E., Geyer, S., Hein, T., Horchler, P., Rupp, H., Scholz, M., Schulz-Zunkel, C., Weigelhofer, G., 2021. Soil Characteristics and Hydromorphological Patterns Control Denitrification at the Floodplain Scale. *Front. Earth Sci.* 9, 708707. <https://doi.org/10.3389/feart.2021.708707>
- Karl, T.R., Trenberth, K.E., 2003. Modern Global Climate Change. *Science* 302, 1719–1723. <https://doi.org/10.1126/science.1090228>
- Koskinen, W. C., & Keeney, D. R. (1982). Effect of pH on the rate of gaseous products of denitrification in a silt loam soil. *Soil Science Society of America Journal*, 46(6), 1165-1167.
- Kumon, Y., Sasaki, Y., Kato, I., Takaya, N., Shoun, H., and Beppu, T. (2002). Codenitrification and denitrification are dual metabolic pathways through which dinitrogen evolves from nitrate in *Streptomyces antibioticus*. *J. Bacteriol.* 184, 2963–2968.
- Li, C., S. Frolking, and T.A. Frolking, 1992a, A model of nitrous oxide evolution from soil driven by rainfall events: 1. Model structure and sensitivity, *Journal of Geophysical Research*, 97:9759-9776.

- Maag, M., Vinther, F.P., 1996. Nitrous oxide emission by nitrification and denitrification in different soil types and at different soil moisture contents and temperatures. *Applied Soil Ecology* 4, 5–14. [https://doi.org/10.1016/0929-1393\(96\)00106-0](https://doi.org/10.1016/0929-1393(96)00106-0)
- Mukumbuta, I., Uchida, Y., Hatano, R., 2018. Evaluating the effect of liming on N₂O fluxes from denitrification in an Andosol using the acetylene inhibition and ¹⁵N isotope tracer methods. *Biol Fertil Soils* 54, 71–81. <https://doi.org/10.1007/s00374-017-1239-4>
- NOAA. (2021). <http://www.esrl.noaa.gov/gmd/ccgg/trends/data.html>.
- NADP.(2021). <https://nadp.slh.wisc.edu/sites/ntn-OK08/>.
- Ottow, J. G. G., Burth-Gebauer, I., & El Demerdash, M. E. (1985). Influence of pH and partial oxygen pressure on the N₂O/N₂ ratio of denitrification. In *Denitrification in the nitrogen cycle* (pp. 101-120). Springer, Boston, MA.
- Papen, H., von Berg, R., Hinkel, I., Thoene, B., and Rennenberg, H. (1989). Heterotrophic nitrification by *Alcaligenes faecalis*: NO₂, NO₃, N₂O, and NO production in exponentially growing cultures. *Appl. Environ. Microbiol.* 55, 2068–2072.
- Qu, Z., Wang, J., Almøy, T., Bakken, L.R., 2014. Excessive use of nitrogen in Chinese agriculture results in high N₂O/(N₂O+N₂) product ratio of denitrification, primarily due to acidification of the soils. *Glob Change Biol* 20, 1685–1698. <https://doi.org/10.1111/gcb.12461>
- Ramos, C. (1996). Effect of agricultural practices on the nitrogen losses to the environment. *Fertilizer Research*, 43, pp. 183-189
- Ravishankara, A.R., Daniel, J.S., Portmann, R.W., 2009. Nitrous Oxide (N₂O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century. *Science* 326, 123–125. <https://doi.org/10.1126/science.1176985>
- Smith, M. S. (1983). Nitrous oxide production by *Escherichia coli* is correlated with nitrate reductase activity. *Appl. Environ. Microbiol.* 45, 1545–1547.
- Šimek, M., Cooper, J.E., 2002. The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years: Soil pH and denitrification. *European Journal of Soil Science* 53, 345–354. <https://doi.org/10.1046/j.1365-2389.2002.00461.x>
- Šimek, M. & Hopkins, D.W. (1999). Regulation of potential denitrification by soil pH in long-term fertilized arable soil. *Biology and Fertility of Soils*, 30, pp. 41-47.
- Šimek, M., Jišová, L., Hopkins, D.W., 2002. What is the so-called optimum pH for denitrification in soil? *Soil Biology and Biochemistry* 34, 1227–1234. [https://doi.org/10.1016/S0038-0717\(02\)00059-7](https://doi.org/10.1016/S0038-0717(02)00059-7)
- Skiba, U., Ball, B., 2006. The effect of soil texture and soil drainage on emissions of nitric oxide and nitrous oxide. *Soil Use and Management* 18, 56–60. <https://doi.org/10.1111/j.1475-2743.2002.tb00050.x>

Smith, M. S., and Zimmerman, K. (1981). Nitrous oxide production by nondenitrifying soil nitrate reducers. *Soil Sci. Soc. Am. J.* 45, 865–871.

Sun, P., Zhuge, Y., Zhang, J., Cai, Z., 2012. Soil pH was the main controlling factor of the denitrification rates and N₂/N₂O emission ratios in forest and grassland soils along the Northeast China Transect (NECT). *Soil Science and Plant Nutrition* 58, 517–525.
<https://doi.org/10.1080/00380768.2012.703609>

Tanimoto, T., Hatano, K.i., Kim, D.h., Uchiyama, H., and Shoun, H. (1992). Co-denitrification by the denitrifying system of the fungus *Fusarium oxysporum*. *FEMS Microbiol. Lett.* 93, 177–180.

Tiedje, J.M., Simkins, S., Groffman, P.M., 1989. Perspectives on measurement of denitrification in the field including recommended protocols for acetylene based methods. *Plant Soil* 115, 261–284. <https://doi.org/10.1007/BF02202594>

United States Department of Agriculture (2016), Natural Resources Conservation Service, Web Soil Survey. [Available at <http://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx>, Last accessed June 2016].

USDA. (2021). (<http://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx> <http://nadp.sws.uiuc.edu/data>, last accessed June 2016].

User's Guide for the DNDC Model, 2012.

Waring, S. A., & Gilliam, J. W. (1983). The effect of acidity on nitrate reduction and denitrification in lower coastal plain soils. *Soil Science Society of America Journal*, 47(2), 246–251.

Weier, K.L. & Gilliam, J.W. (1986). Effect of acidity on denitrification and nitrous oxide evolution from Atlantic Coastal Plain soils. *Soil Science Society of America Journal*, 50, pp. 1202–1205.

Zheng, Q., Ding, J., Lin, W., Yao, Z., Li, Q., Xu, C., Zhuang, S., Kou, X., Li, Y., 2022. The influence of soil acidification on N₂O emissions derived from fungal and bacterial denitrification using dual isotopocule mapping and acetylene inhibition. *Environmental Pollution* 303, 119076.
<https://doi.org/10.1016/j.envpol.2022.119076>

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