Washington College

Department of Environmental Studies and Science

N_2O Flux Analysis of Agricultural Land and Restored Native Habitat

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Washington College Honor Code

I pledge my word of honor that I have abided the Washington College Honor Code while completing this assignment.

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Abstract

Nitrous oxide (N_2O) is a naturally occurring greenhouse gas (GHG) and emissions of N_2O have been drastically increased over the past century. This is due to anthropogenic forcings primarily from the agricultural sector through fertilization of soils and animal waste. Increases in soil moisture, temperature, decreases in pH, and decreases in O_2 within the soil are known drivers contributing to enhanced fluxes of N_2O from soils via microbial denitrification. To mitigate the effects of N_2O full understanding of its causes and effects must be defined. Nitrous oxide flux was examined three times over the winter of 2014-2015 between current agricultural lands and restored native grasslands on the Eastern Shore of Maryland. Samples were taken from flux chambers and were examined in relation to nmol N_2O/ mL of gas and time step in hours. Flux was calculated in terms of μmol N_2O-N m^{-2} h^{-1}. Over the three sampling dates no consistent trend was observed in the grasslands, but a gradual rise in overall flux was noticed within the agricultural field. No statistical significance was noted between the flux from either test area (p-value of 0.3439). While higher soil moisture typically predicts higher rates of flux, the data gathered show a contradictory negative relationship between soil moisture and average flux. Random hot spots of N_2O flux were measured most likely due to background synthetic nitrogen from previous fertilizations, animal waste from wild game and irregular melting of ice layers beneath the soils during data sampling.

1. Introduction

1.1 Atmospheric Effects of N_2O

Nitrous oxide (N_2O) is both a naturally occurring gas and one that is anthropogenically produced often as a byproduct from both nitrification and denitrification within soil. This gas is of particular concern as N_2O is a strong greenhouse gas with a Global Warming Potential (GWP) of 310 over a 100 year temporal range (EPA, 2014). Nitrous oxide could potentially be more detrimental than carbon dioxide (CO_2) in terms of climate change as one molecule of N_2O holds the same amount of energy over a 100 year period as 310 molecules of carbon dioxide. N_2O has a 114 year lifetime on average within the atmosphere therefore, the gas produced now will effect multiple generations in the future (EPA, 2014). The lifespan of this molecule is not only damaging due to the warming it may cause, but it is also reactive once the gas enters the stratosphere (Ravishankara et al., 2009).

Stratospheric ozone (O_3) is relied on by humans for its ability to protect us from ultraviolet radiation, which may cause skin damage and potentially cancer (Marionnet et al., 2014). Unfortunately, N_2O is a stratospheric ozone depleter (Ravishankara et al., 2009). While nitrous oxide is very stable within the troposphere, once it reaches the stratosphere it reacts through nitrogen oxide catalyzed processes (Ravishankara et al., 2009). The Ozone Depletion Potential (ODP) is a metric to determine the effectiveness of a compound at destroying ozone. Current estimates give nitrous oxide a 0.017 ODP which is comparable to HCFC-123 which has an ODP of 0.02 (Ravishankara et al., 2009). While the latter compound is banned, N_2O is naturally occurring making it very hard to control.

1.2 Contributing Factors to the Rise of N_2O

Anthropogenic forcings are the large reason for the majority of the N_2O that is within the Earth's atmosphere. Of these there are two major categories, biogenic and abiogenic forcing (Mosier et al., 1998). Abiogenic refers to the nitrous oxide formed from the combustion of a nitrogen rich material, which worldwide is not a large contributor. However, biogenic refers to the enhanced nitrous oxide production by bacteria in fertilized fields (Mosier et al., 1998). Globally, it is estimated that about 40% of annual nitrous oxide emissions are anthropogenically caused whereas 75% of which is due to agriculture and soil management practices (EPA, 2014). Some examples of these factors include the utilization of synthetic fertilizer, the use of animal waste as fertilizer and crop residue that is returned to the field after harvest (Mosier et al., 1998). Peak N_2O-N flux are often observed immediately following both fertilization of fields and rain events (Tesfai et al., 2015).

These factors contribute to nitrous oxide within the atmosphere due to the conditions in the soil. N_2O is produced by both nitrification and denitrification. However, it has been observed that within the soil in this region N_2O flux is due to denitrification (Fox, 2011). Denitrification is the process of reducing NO_3^- to N_2. However, incomplete denitrification often yields nitrite (NO_2^-), nitric oxide (NO) and N_2O. If the soil becomes limited in oxygen the rate of denitrification will slow down. When this occurs, more N_2O is yielded (Fox, 2011). In addition, higher temperatures typically produce higher nitrous oxide fluxes (Knowles, 1982). This is why much higher concentrations are seen during the summer months; however, because of this little research has occurred during cooler months. Furthermore, pH has been shown to have an effect on N_2O flux. As pH increases denitrification rates increase as well (Knowles, 1982). Conversely, low pH thus

slows the rate enhancing the likelihood of incomplete denitrification. Therefore, nitrous oxide reductase, the enzyme that reduces nitrates to N_2O, is inhibited causing the N_2O flux to increase (Knowles, 1982).

1.3 Purpose

By better understanding the processes that occur in nature, humans can find solutions to the growing problem of N_2O, especially in relation to Climate Change. This worldwide problem must be faced immediately, yet can only be done once full understanding of the gases sources and magnitude of fluxes are realized. Once this is achieved, the mitigation of N_2O may begin. In recent years, many agricultural best management practices (BMP's) have been employed. These are often utilized in order to stop nitrogen contamination of waterways as well as to reduce land erosion. An increasing amount of agricultural lands today are beginning to shift towards restoration of land for local fauna habitat. Chino Farms on the Eastern Shore of Maryland, has dedicated much land to the restoration of quail habitat (Small, D., personal communication, November, 2014). Natural quail habitat has been removed from a large portion of the area due to agricultural practices and natural disasters (Small, D., personal communication, November, 2014). These habitat areas may have unintended benefits similar to other BMP's already being applied nationwide. However, little research has been completed and it is unclear if there are consequences to the employment of such practices.

Within the proposed area of study of Chino Farms there is noted a relatively long frost period. Therefore, there is potential for the ground to freeze during a winter season for 180- 220 days which may impact the output of nitrous oxide flux from the soil (USGS, 2014). Within previously studied areas buffer strips slowed runoff, induced nitrogen uptake and enhanced denitrification (Mankin et al., 2007). Therefore, while groundwater nitrate is reduced N_2O flux

may unintentionally increase. The objective of this research is to better understand the relationships between these factors. While high N_2O is often seen on fertilized farm land, it is also plausible that this flux may also be observed on restored land directly adjacent to the farmland. The hypothesis was tested that due to the use of fertilizers on the current farm land a greater flux of nitrous oxide will be seen within the agricultural land and a lower flux would be measured from the restored native grasslands of Chino Farms.

Commented [I1]: I only removed this because it makes it sound as if chino farms is being irresponsible with fertilizer application, which I am sure isn't the case.

2. Materials and Methods

2.1 Study Area

The area of interest is located within a 228 acre plot on Chino Farms in Queen Anne's County on the Eastern Shore of Maryland. Chino Farms receives a mean annual precipitation of 106.68 – 121.92 cm. and the soils are characterized as well drained (USGS, 2014). Much of this land has undergone extensive land restoration from agricultural land to native habitat (Small, D., personal communication, November, 2014). The plot of land where data was collected is separated into two main areas. Location one is still utilized as an agricultural plot for corn currently and soy as an alternate annual crop (Fig. 1 and 2). A cover crop of radishes was planted throughout the sampling area for the winter to help prevent soil erosion. The farm land is fertilized using synthetic nitrogen and lime is applied to the field roughly every 2 – 3 years (Small, D., personal communication, November, 2014). Location two is a strip of restored native grasses to the Eastern Shore of Maryland (Fig. 1 and 2). During the time of study, the grassland consisted mostly of winter grasses with some additional woodier shrubs within the center of the strip (Table 1).

Study Area

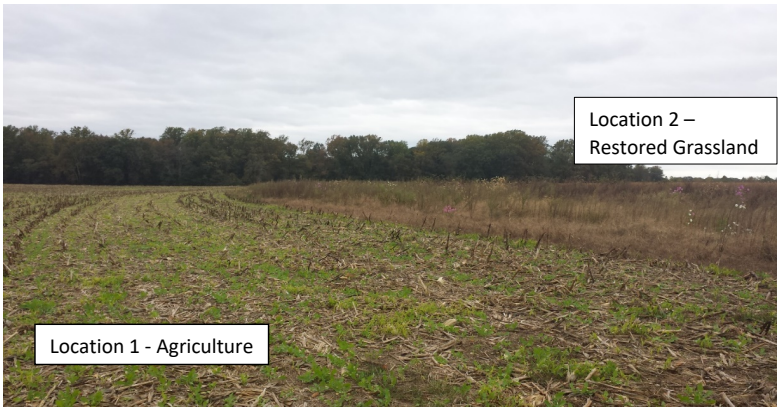


Fig. 1. Photo taken in November, 2014 shows Location 1 where the agricultural land has a radish cover crop while Location 2 depicts restored grassland quail habitat.

Native Grasses Restored to the Area

Common Name	Scientific Name
Broomsedge Bluestem	*Andropogon virginicus*
Little Bluestem	*Schizachyrium scoparium*
Sideoats Grama	*Bouteloua curtipendula*
Partridge Pea	*Chamaecrista fasciculate*
Eastern Purple Coneflower	*Echinacea purpurea*

Table 1. Grasses planted in location 2. A standard mix of wildflower seeds was also planted to create the native quail habitat (personal communication, April 5, 2015). These perennial grasses ranged in size from about 0.5 – 2 m in height and consist of both bunch and prairie grasses.

The area is a habitat for native fauna as there are clear signs of wildlife within this area. A wide variety of birds was seen consistently during sampling and large game trails were visible

throughout the entirety of the sample area. These trails were likely due to native white tail deer in the area and it is important to note that on different days different sample areas fell within these visible game trails.

2.2 Soil Composition

Throughout the entire sample area there are three soil types; downer sandy loam (DoB), Ingleside sandy loam with 0 – 2 percent slopes (IgA) and Ingleside sandy loam with 2 – 5 percent slopes (IgB), (Web Soil Survey 2012, Fig. 2).

Soil Composition within the Sample Area

Fig. 2. The image depicts the soil composition by area as provided by the USDA Web Soil Survey. It is important to note that this imagery is pre- habitat restoration. On the left is still agricultural land (Location 1) and through the middle where there is a slight curvature in the satellite imagery from irrigation is now restored habitat (Location 2).

Each of these soil types are near identical and are only differentiated largely from the percent slope of the area. The soil has been graded high for agriculture use due to both proper aeration of the soil as well as nutrients present (USGS, 2014).

2.3 Preparations and Equipment Analysis

Static chambers were used to measure N_2O flux from the soil surface. The chambers were composed of 5-gallon buckets with the bottoms cut off. Air tight lids were applied to the tops. Within the lid a rubber septum was applied to extract samples from the chamber. A thermometer was also placed within to monitor changes in temperature and a small plastic tube was placed in order to allow air flow in cases of extreme pressure changes due to weather or a buildup of heat within the chamber (Fig. 3 (a)). Within the bucket, markings were made for the first 10 cm to determine insertion depth (Fig, 3 (b)).

Chamber Design

(a) **(b)**

Fig. 3. (a) Chamber implanted within the agricultural land. On top is a 12 mL exetainer vial, a 20 mL sampling syringe, a thermometer to measure air temperature and a lolly-pop thermometer to measure chamber temperature. (b) The inside of an implemented chamber showing the depth markings.

Prior to sampling, 52 12 mL Exetainer vials (Labco Limited; United Kingdom) were evacuated to remove all gases. These served as the holding vials for the sampled gases until the samples were analyzed. Two 5TM time domain reflectometry (TDR) probes (Decagon Devices; Pullman, WA) were used to measure the soil moisture (m^3/m^3) and temperature (ºC) for each time of data acquisition.

2.4 Field Analysis

To begin, two researchers placed six chambers randomly within each of the selected test areas (Fig.4). One person was assigned to each area and took samples from that particular site each day sampling occured to maintain consistency. The lid was removed from each chamber for installation by hammering into the ground through the utilization of a rubber mallet and large piece of wood until the base of the chamber was roughly 5 cm into the soil. This depth was determined to be deep enough so that the chamber was secure within the soil, but is also not too deep where it may disturb or compact the soil (Fox, R., personal communication, November, 2014). At this point GPS data was taken for each chamber location so that every sample from each day would occur at the exact spot to reduce error (Fig. 4). Chambers D and M were visibly located within game trails.

GPS Location of Chambers

Fig. 4. Pins H, I, K, L, O and N represent chambers placed within the agricultural land (Location 1). Pins A, C, D, E, G and M represent chambers within the restored habitat (Location 2). This imagery is taken prior to the land restoration.

Before collecting any data from the chambers, a standard air sample was taken. To do this, each researcher simultaneously took a 20 mL air tight syringe and pulled in 10 mL of air. The sample was thus pushed out to ensure the minimization of error due to previous gas within the syringe. After this had been completed, 17 mL of air was taken into the syringe and was

pushed out to 15 mL. The sample was then injected into a vacuum sealed 12 mL Exetainer vial (Labco Limited; United Kingdom). The vials were over-pressurized with the sample to ensure that in the event of a leak, gas would be lost out and the sample would not be contaminated. Upon completion the time and vial number was recorded.

Data sampling for each chamber now began. On the first approach to the chambers the lids were still not on. To initiate sampling, the air tight lid was quickly placed on each chamber upon approach making sure all holes were plugged and that a thermometer was placed into the lid. The first sample was taken just as the standard was; however, now the first 10 mL was collected and pushed back out through the rubber septum inside the chamber to mix potential concentration gradients inside the chamber. Moisture level and temperature within the soil was recorded via the utilization of a 5TM TDR moisture and temperature probe (Decagon Devices; Pullman, WA) along with the air temperature in and outside of the chambers. The time of each sample and the vial number per sample was recorded.

A sample from the same chamber was taken about every 20 minutes until four samples were retrieved per chamber. Upon completion any final observations were taken along with one final air sample. This air standard was completed in the exact manner as the first standard had been taken.

2.5 GC Analysis

Before samples were examined, standards were run through a Shimadzu Gas Chromatograph (GC). Each injection, both standards and samples, were run with an injection volume of 1 mL. The program runs for 7 minutes and takes the sample through an Electron Capture Detector (ECD, N_2O) and a Flame Ionization Detector (FID). The N_2O peak was around 5.800 minutes +/- .005.

To analyze the data a standard curve was created. A linear regression was plotted according to injected nmol N_2O-N on the y-axis and the peak area on the x-axis (Fig. 6). Barometric pressure was also accounted for on the day of injection as it effects the injection volume introduced into the GC.

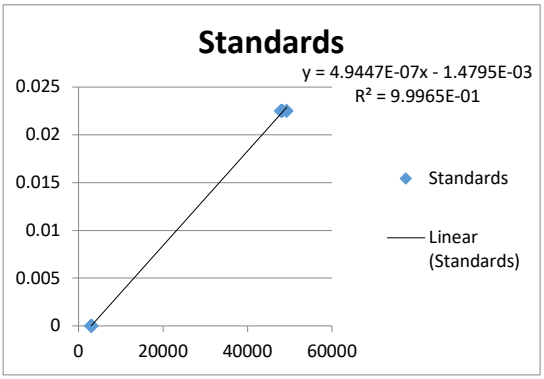


Fig.5 An example of a linear regression of standards run on March 27, 2015.

Percent coefficient of variation of the standards was calculated for each day to ensure the peak areas were within a viable range of one another for use.

$$\%CV = \frac{STDEV(peak\ area)}{Avg\ (peak\ area)} * 100 \quad (1)$$

The time of sampling recorded in the field was used to calculate a time step. The time steps were plotted against nmol N_2O/ mL of gas for each sample (Fig. 6 (a) and (b)). This data was obtained using the standard curve equation attained from the standard calculations as seen plotted on Fig. 5. The peak area obtained from the GC-2014 was entered in for x in order to

solve for y (absolute nmol in the injection volume). Absolute nmoles in the injection volume were divided by injection volume at STP ~0.78 mL to give final concentrations in nmol/mL.

2.6 Flux Calculation

Nitrous oxide flux into the atmosphere was calculated according to equation (2).

$$N_2O\ Flux = \frac{\frac{dN_2O}{dt} * Vb}{Ab} * 1000 \quad (2)$$

N_2O flux was measured in ($\frac{\mu mol}{m^2 d}$), where $\frac{dN_2O}{dt}$ is the slope of nitrous oxide within the chamber ($\frac{mmol}{hm^3}$) (Fig.5), V_b is the volume (m^3) of the chamber and A_b is the surface area of the ground (m^2) which is covered by the chamber. Multiplying the entirety of the equation by 1000 converts the units from mmol to µmol. Each chambers volume was determine separately by filling them with a known volume of water. A relationship was also calculated in order to determine the volume of the chamber based on average insertion depth (Fisher & Fox; unpublished). The depth was estimated by averaging 4 insertion depths each time a chamber was placed within the field.

2.7 Statistics

Statistics were calculated using Microsoft Excel. Significant flux was determined by regressing nmol N_2O/ mL of gas versus time step. Chamber data was determined to be statistically significant if the r^2 value per chamber per day was at least 0.800. All other data was omitted and reported as zero flux.

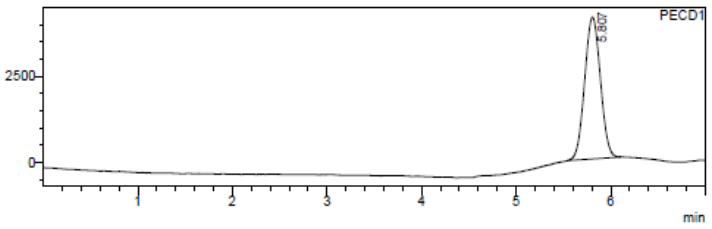
Statistical analysis of N_2O flux from locations 1 and 2 was done in order to determine if data was significantly different. A t-test was utilized through Microsoft Excel and was applied to check if the mean flux per day was reliably different from one another. P-values were considered significant if below 0.05. The average and standard deviation per day and per test area was determined using Microsoft Excel functions.

3. Results

3.1 N_2O Chamber and Sampling Data

On average N_2O retention time was about 5.800 minutes with a typical peak area between 46000 and 52000 (Fig.6). Any data outside this area was omitted and was deemed contaminated possibly due to improper injection into the GC, poor seal within the storage vial, or an unknown gas.

GC Analysis Report of Random Sample on Day 3



PECD1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	5.807	47390	4112	0.371	ppm		Nitrous Oxide
Total		47390	4112				

Fig. 6. An example of a GC ECD1 analysis report. The graph visually depicts the retention time (5.807) and the area of the peak (47390).

On day one the final concentrations of N_2O in the chamber after the first hour of deployment ranged from 0.0300 nmol N_2O/ mL of gas to 0.0411 nmol N_2O/ mL of gas. An example of a chamber which showed a statistically significant regression was chamber M within the grassland. At time-0 concentration in the chamber was at 0.0348 nmol N_2O/ mL of gas at time-1 0 .0373 nmol N_2O/ mL of gas, at time2 0.0390 nmol N_2O/ mL of gas and at time-3 the concentration increased to 0.0411 nmol N_2O/ mL of gas.

On day two the final concentrations of N_2O ranged from a much less 0.0083 nmol N_2O/ mL of gas to 0.0133 nmol N_2O/ mL of gas. An example of a chamber which showed a statistically significant regression was chamber L within the farm field. At time0 concentration was at 0.0109 nmol N_2O/ mL of gas at time1 0 .0109 nmol N_2O/ mL of gas, at time2 0.0124 nmol N_2O/ mL of gas and at time3 the concentration increased to 0.0127 nmol N_2O/ mL of gas.

On day three the final concentrations increased dramatically from two days prior (Fig.8). Concentrations of N_2O in the chamber ranged from 0.0207 nmol N_2O/ mL of gas to 0.0336 nmol N_2O/ mL of gas. An example of a chamber which showed a statistically significant regression was chamber K within the farm field. At time0 concentration was at 0.0295 nmol N_2O/ mL of gas at time1 0 .0301 nmol N_2O/ mL of gas, at time2 0.0326 nmol N_2O/ mL of gas and at time3 the concentration increased to 0.0333 nmol N_2O/ mL of gas.

It was often seen that many chambers fluctuated within the sampling period. Chambers such as in Fig. 7 (b) showed a positive linear regression except for a drop in amount of N_2O measured in nmol/mL gas on the second or third sample time. This occurred randomly on all days of testing as well as in both sample areas.

nmol N2O-N/ mL of Gas Plotted Over Time

(a) **(b)**

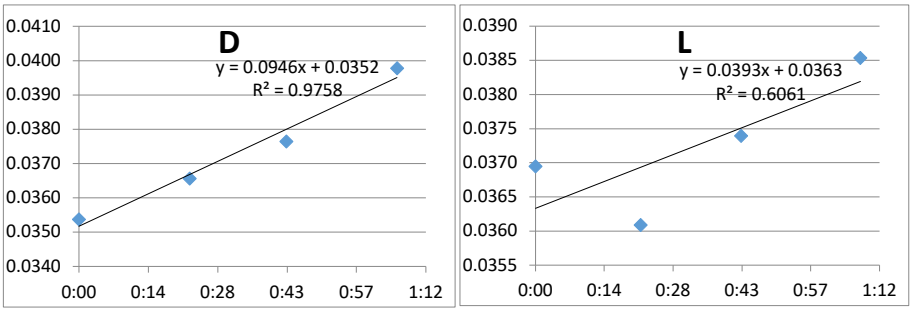


Fig.7. (a) Results from chamber D on day 1 of sampling (11/5/2014). This chamber was located within the restored quail habitat and showed a statistically significant flux over time. (b) Results from chamber L on day 1 of sampling (11/5/2014). Chamber L was located within the agricultural field and does not show a statistically significant flux. While there is a positive regression, a decrease in N_2O concentration is seen on sample two yielding an R^2 value of only 0.6061.

3.2 N_2O Flux Comparison

On day one of sampling (11/5/2014) the data produced 2 chambers of flux within the grasslands with 4 chambers of flux within the agricultural land. Higher overall flux was seen within the grassland, but significant variability among the chambers was observed (Fig. 8). The grassland produced a mean flux of 10.9731 +/- 14.4427 µmol N_2O-N $m^{-2}h^{-1}$ while the agricultural field produced 4.1463 +/- 7.4331 µmol N_2O-N $m^{-2}h^{-1}$.

On day two (2/4/2015), the data displayed 2 chambers of flux within both areas of research although the agricultural land had a higher overall flux (Fig. 8). The grassland produced a mean flux of 2.5243 +/- 4.4196 µmol N_2O-N $m^{-2}h^{-1}$ while the agricultural land showed a mean flux of 7.1938 +/- 7.5901 µmol N_2O-N $m^{-2}h^{-1}$.

Day three (2/8/2015), was characterized by only one area of flux within the grassland but revealed 3 chambers of flux within the agricultural land. The mean flux for the grassland was

3.0232 +/- 7.4053 µmol N_2O-N $m^{-2}h^{-1}$ while the mean within the agricultural land showed a mean flux of 9.2097 +/- 10.0663 µmol N_2O-N $m^{-2}h^{-1}$ (Fig.8).

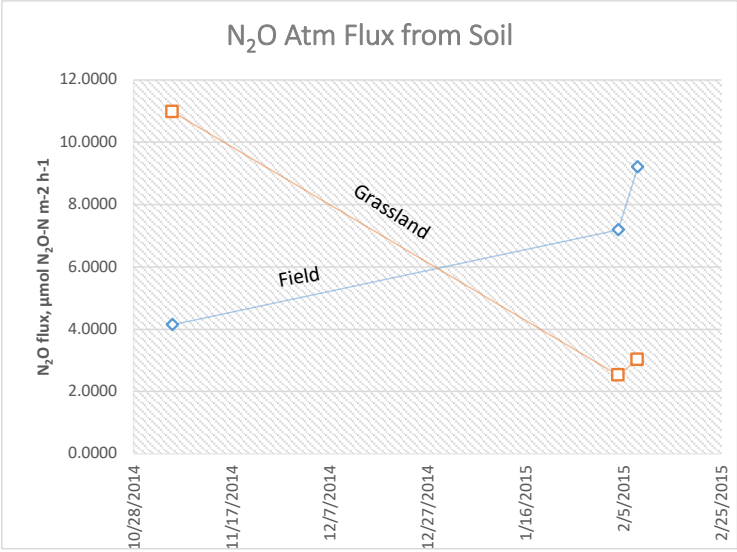


Fig.8. The data shows average total flux per day over time between the two sample areas. While the grassland (squares) initially had less chambers showing fluxes on day 1, the total overall flux was much greater. As winter progressed the agricultural field (diamonds) produced greater fluxes while the restored grassland produced a lower total flux.

While the grassland concentrations were initially greater, over time the mean flux became less (Fig. 8). Conversely, the agricultural area depicted an increase as time went on. Figure 9 shows the same mean flux over time along with the standard deviation bars. The standard deviation for each mean is greater than the mean in all cases. This shows a large variability in flux. No significant difference was observed between the sites ($p = 0.344$).

Rather than a clear pattern of flux, localized hot spots were observed in all locations leading to the vast standard deviations. While many chambers saw no flux, areas such as chamber D on day 1 had a 22.45 µmol N_2O-N $m^{-2}h^{-1}$ flux. This phenomena was not localized to just one location. Chamber K within the agricultural location showed no N_2O flux on day 1 and 2; however, revealed a 25.22 µmol N_2O-N $m^{-2}h^{-1}$ flux on day 3.

A small spike in overall N_2O flux was seen in both areas between the second and third day of sampling. This corresponded with an overall thaw. Prior to these days the daily temperature did not exceed the freezing point. During day 2 the average air sampling temperature was 12.8 ºC yet a layer of ice was noticeable about 4 cm beneath the surface. On day 3 the average air temperature was 14.5 ºC with no layer of ice present beneath the surface.

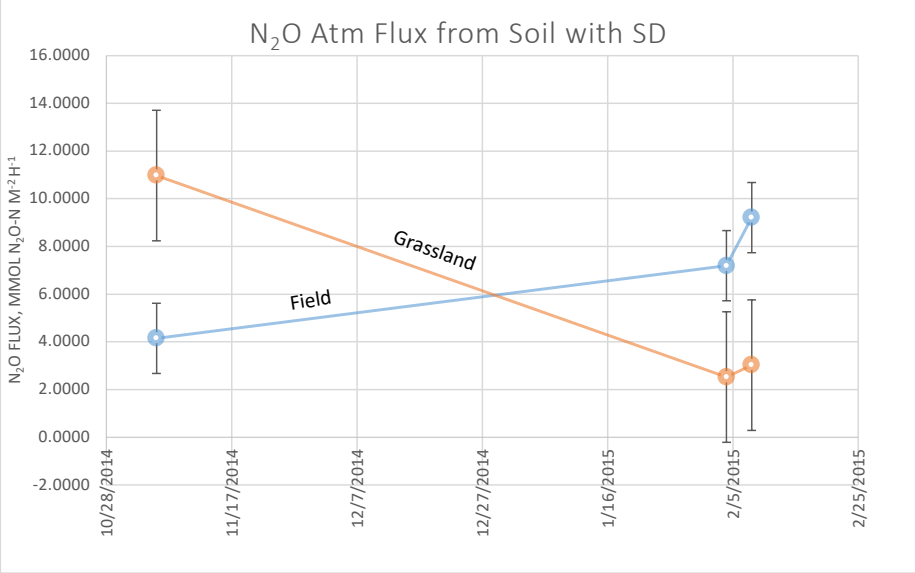


Fig. 9. The data shows that the standard deviations for each data point were greater than the actual results themselves yielding statistically insignificant data overall.

3.3 Soil Temperature and Moisture

Over the course of each study area and day, no relationship was observed between changes in soil temperatures and overall flux. The data shows days where temperature increased and there was a statistically significant flux such as within chamber D on day 2 of sampling (Table. 3 (b)). The data also shows that N_2O flux had occurred on days whereas the temperature of the soil fluctuated and even dropped during the chamber deployment such as with chamber H on day 1 (Table 2 (a)) and chamber M on day 3 (Table. 4 (b)).

Agriculture Day 1 (a)

Chamber ID	Time 0	Time 1	Time 2	Time 3
H				
Soil Temperature (°C)	17.1	19.1	18.2	18.3
Soil Moisture (m^3/m^3)	0.194	0.208	0.204	0.194
I				
Soil Temperature (°C)	18.8	19.2	18.7	18.3
Soil Moisture (m^3/m^3)	0.144	0.152	0.152	0.153
K				
Soil Temperature (°C)	19.7	19.3	18.6	17.4
Soil Moisture (m^3/m^3)	0.183	0.176	0.154	0.145
L				
Soil Temperature (°C)	20.4	19.1	18.1	17.0
Soil Moisture (m^3/m^3)	0.161	0.186	0.186	0.173
N				
Soil Temperature (°C)	20.5	19.0	18.4	16.9
Soil Moisture (m^3/m^3)	0.167	0.171	0.190	0.178
O				
Soil Temperature (°C)	20.8	18.7	17.3	17.0
Soil Moisture (m^3/m^3)	0.212	0.200	0.220	0.192

Grassland Day 1 (b)

Chamber ID	Time 0	Time 1	Time 2	Time 3
A				
Soil Temperature (°C)	18.0	19.8	20.2	20.0
Soil Moisture (m^3/m^3)	0.129	0.110	0.111	0.145
C				
Soil Temperature (°C)	20.0	20.3	20.6	19.2
Soil Moisture (m^3/m^3)	0.110	0.115	0.121	0.149
D				
Soil Temperature (°C)	21.5	21.0	19.5	19.6
Soil Moisture (m^3/m^3)	0.127	0.121	0.150	0.118
E				
Soil Temperature (°C)	19.7	19.7	19.0	18.9
Soil Moisture (m^3/m^3)	.120	.125	.142	.143
G				
Soil Temperature (°C)	20.3	19.4	18.8	18.3
Soil Moisture (m^3/m^3)	0.119	0.139	0.116	0.117

M				
Soil Temperature (°C)	20.7	20.3	19.6	17.9
Soil Moisture (m^3/m^3)	0.118	0.119	0.122	0.133

Table 2. Change in soil moisture and temperature over the course of day 1 between the farmland (a) and the grassland (b). The hi-lighted information depicts chambers which showed flux.

Agriculture Day 2 (a)

Chamber ID	Time 0	Time 1	Time 2	Time 3
H				
Soil Temperature (°C)	16.8	10.5	11.0	9.5
Soil Moisture (m^3/m^3)	0.105	0.143	0.168	0.217
I				
Soil Temperature (°C)	14.5	10.6	12.4	9.4
Soil Moisture (m^3/m^3)	0.150	0.119	0.207	0.204
K				
Soil Temperature (°C)	13.1	9.0	9.7	12.3
Soil Moisture (m^3/m^3)	0.088	0.127	0.125	0.181
L				
Soil Temperature (°C)	14.6	8.7	11.3	8.8
Soil Moisture (m^3/m^3)	0.135	0.186	0.187	0.235
N				
Soil Temperature (°C)	11.1	8.4	13.2	9.5
Soil Moisture (m^3/m^3)	0.143	0.161	0.160	0.142
O				
Soil Temperature (°C)	11.3	8.6	11.5	11.3
Soil Moisture (m^3/m^3)	0.148	0.194	0.211	0.208

Grassland Day 2 (b)

Chamber ID	Time 0	Time 1	Time 2	Time 3
A				
Soil Temperature (°C)	11.1	10.7	13.8	13.5
Soil Moisture (m^3/m^3)	0.143	0.163	0.165	0.160
C				
Soil Temperature (°C)	10.0	11.2	11.9	12.5
Soil Moisture (m^3/m^3)	0.165	0.173	0.188	0.185
D				
Soil Temperature (°C)	10.0	10.8	10.9	12.0
Soil Moisture (m^3/m^3)	0.160	0.236	0.236	0.237
E				
Soil Temperature (°C)	8.5	12.5	12.2	11.5
Soil Moisture (m^3/m^3)	0.133	0.143	0.155	0.178
G				
Soil Temperature (°C)	8.4	10.7	12.2	12.0
Soil Moisture (m^3/m^3)	0.208	0.198	0.162	0.180
M				

Soil Temperature (°C)	8.2	11.1	10.9	10.1
Soil Moisture (m^3/m^3)	0.145	0.174	0.200	0.190

Table 3. Change in soil moisture and temperature over the course of day 2 between the farmland (a) and the grassland (b). The hi-lighted information depicts chambers which showed flux.

Agriculture Day 3 (a)

Chamber ID	Time 0	Time 1	Time 2	Time 3
H				
Soil Temperature (°C)	12.1	10.4	8.10	11.6
Soil Moisture (m^3/m^3)	0.126	0.137	0.141	0.141
I				
Soil Temperature (°C)	11.2	9.40	8.80	10.8
Soil Moisture (m^3/m^3)	0.141	0.155	0.178	0.152
K				
Soil Temperature (°C)	10.6	9.10	10.5	10.8
Soil Moisture (m^3/m^3)	0.151	0.161	0.184	0.214
L				
Soil Temperature (°C)	8.80	9.90	8.80	11.3
Soil Moisture (m^3/m^3)	0.178	0.185	0.202	0.210
N				
Soil Temperature (°C)	9.30	9.10	10.6	10.3
Soil Moisture (m^3/m^3)	0.214	0.187	0.165	0.176
O				
Soil Temperature (°C)	10.9	9.90	11.2	11.3
Soil Moisture (m^3/m^3)	0.132	0.137	0.138	0.138

Grassland Day 3 (b)

Chamber ID	Time 0	Time 1	Time 2	Time 3
A				
Soil Temperature (°C)	12.3	10.3	9.60	9.90
Soil Moisture (m^3/m^3)	0.181	0.196	0.201	0.193
C				
Soil Temperature (°C)	12.2	11.4	11.2	11.2
Soil Moisture (m^3/m^3)	0.175	0.174	0.152	0.170
D				
Soil Temperature (°C)	10.3	10.7	11.4	11.7
Soil Moisture (m^3/m^3)	0.239	0.219	0.185	o.211
E				
Soil Temperature (°C)	10.0	9.50	11.2	10.7
Soil Moisture (m^3/m^3)	0.204	0.227	0.185	0.204
G				
Soil Temperature (°C)	11.2	10.5	11.2	10.7
Soil Moisture (m^3/m^3)	0.165	0.172	0.154	0.166

M				
Soil Temperature (°C)	12.5	11.1	11.2	11.3
Soil Moisture (m^3/m^3)	0.141	0.170	0.178	0.180

Table 4. Change in soil moisture and temperature over the course of day 3 between the farmland (a) and the grassland (b). The hi-lighted information depicts chambers which showed flux.

No relationship was evident between changes in soil moisture overtime and N_2O flux. The data did show a negative relationship between average soil moisture measured in m^3/m^3 and N_2O flux from soil. The average of the soil moisture was taken for each chamber which was compared to flux per day (Fig. 10). Within the agriculture field as the average soil moisture decreased, N_2O flux increased (Fig. 10).

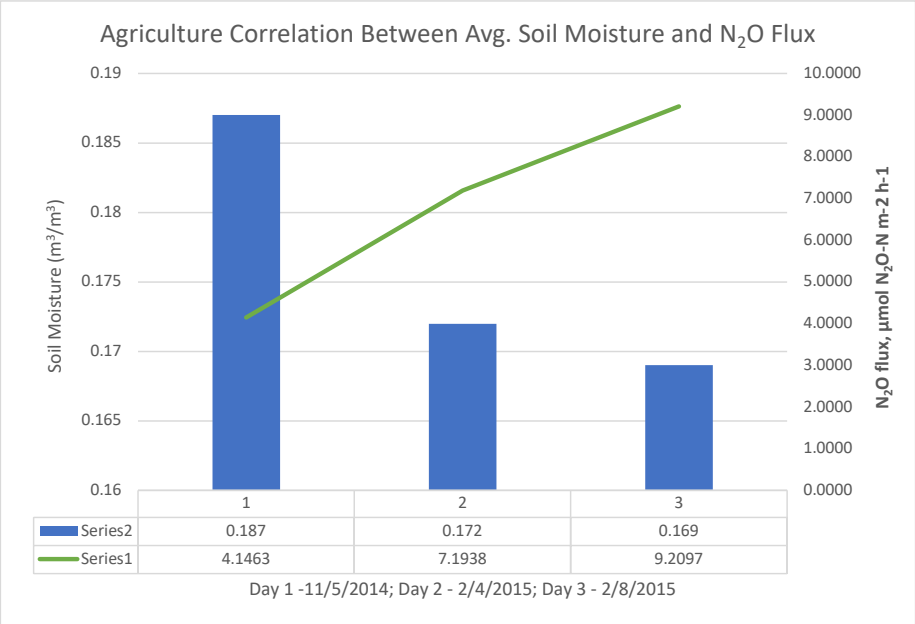


Fig. 10. Series 2 represents the average soil moisture content per day per chamber flux within the agricultural land. Series 1 represents the N_2O flux over the course of the study period within the agricultural land.

As within the agricultural land, the grassland showed the same negative relationship. Average soil moisture was determined from each chamber which showed flux per day. As moisture content within the soil decreased between the sampling dates, the N_2O flux for that corresponding day increased. Between day 1 and 2 the moisture within the soil increased from 0.125 m^3/m^3 while the N_2O flux dropped from 10.9731 mmol N_2O-N $m^{-2}h^{-1}$ to 2.5243 mmol N_2O-N $m^{-2}h^{-1}$. From day 2 to day 3 the flux increased from 2.5243 mmol N_2O-N $m^{-2}h^{-1}$ to 3.0232 mmol N_2O-N $m^{-2}h^{-1}$ while the moisture content decreased from 0.184 m^3/m^3 to 0.167 m^3/m^3 (Fig. 11).

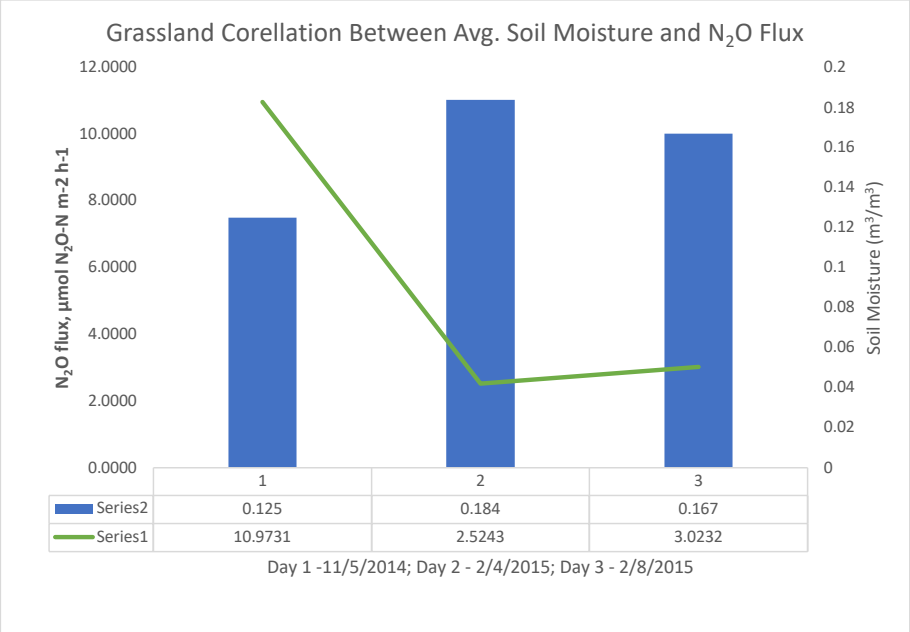


Fig. 11. Series 2 represents the average soil moisture content per day per chamber flux within the grassland. Series 1 represents the N_2O flux over the course of the study period within the grassland. Note: the left and right y-axes have been flipped in comparison to figure 10.

The data consistently depicts this relationship with no discrepancies. However, it is important to note that the N_2O flux data was not statistically significant, and the absolute changes in soil moisture were small.

Average temperatures for the entire time of sampling of both areas were taken and compared with the changes in flux per day. The data suggests a positive relationship with temperature and nitrous oxide flux within the grassland (Fig.12). Within the agricultural field, this relationship does not seem to be present. While concentrations do increase together on days

two and three, on day one the temperature was the highest yet the smallest overall flux was seen (Fig. 13).

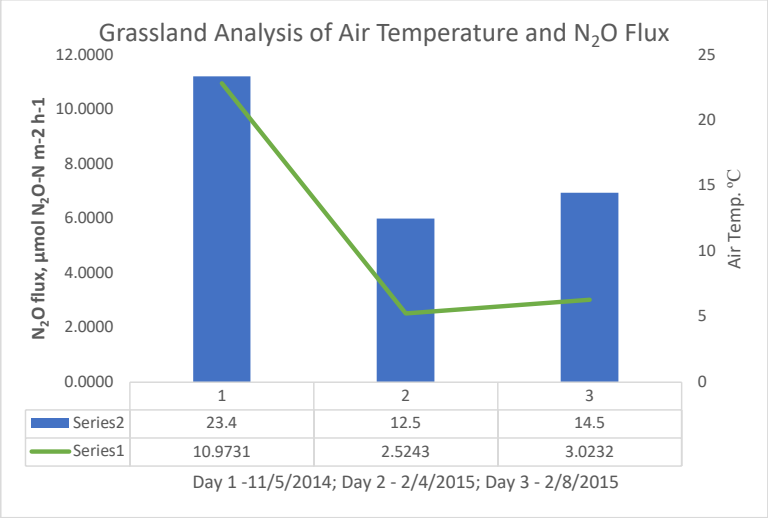


Fig. 12. Series 1 depicts the nitrous oxide flux per day within the grassland. Series 2 depicts the average air temperature per day within both sample areas as there was no major difference between the sites.

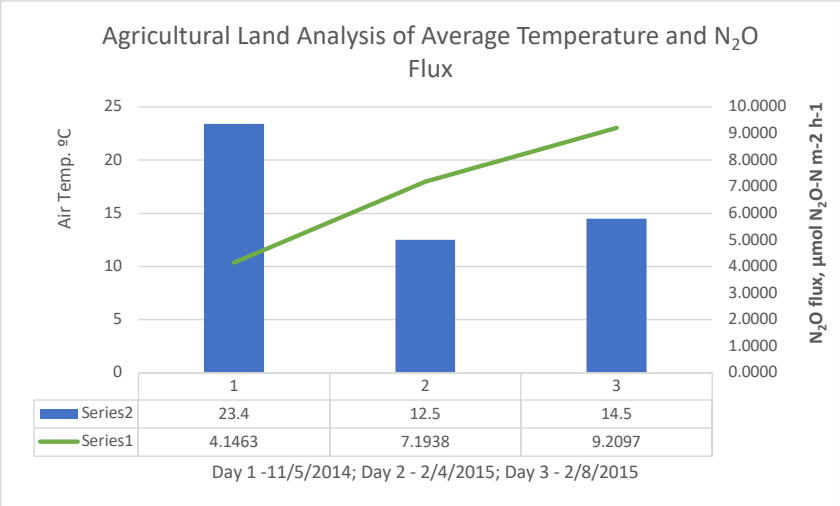


Fig. 13. Series 1 depicts the nitrous oxide flux per day within the agricultural land. Series 2 depicts the average air temperature per day within both sample areas as there was no major difference between the sites. Note: Left and right y-axes has been flipped in comparison to Figure 12.

4. Discussion

4.1 Outcome

It was hypothesized that there would be significantly larger nitrous oxide flux from the agricultural field in comparison to the restored quail habitat. However, the data shows no statistically significant difference and the hypothesis was not supported. Sporadic hot spots were recorded each day samples were taken within each area of study. These could have been from areas of high carbon density, low oxygen content, high nitrate presence or even from low soil pH (Mosier et al., 1998; Knowles, 1982). There was no significance on what chambers reported the flux as this changed per sample day. Data obtained during day one showed a higher flux within the grassland while on days two and three of study the farmland produced higher flux. There was

a trend within the agricultural field for increasing nitrous oxide flux over the course of the study yet the grassland tended to fluctuate more. This could possibly be due to the presence of more animals producing waste or the breakdown of corn stubble.

Unexpected negative relationships were made between average soil moisture and average N_2O flux. Traditionally, as soil moisture content increases, flux increases as well. Within both sample areas where flux occurred greater soil moisture corresponded with a lower N_2O flux (Fig. 10 and 11). Wet soil typically produces an anaerobic environment as less O_2 pockets are available within the soil (Knowles, 1982). This slows down the rate of denitrification which provides a mechanism for incomplete denitrification typically yielding larger nitrous oxide flux (Knowles, 1982). One possible answer is that the area is classified as well drained (USGS, 2014). Tesfai et al (2015) showed that within poorly drained soils significantly more nitrous oxide is emitted due to higher soil moisture and shallow groundwater table than within well drained soils. Perhaps there is a threshold for the amount of moisture content needed within well drained soil before a noticeable positive correlation is seen. It is feasible that the moisture content was not appreciably different between sampling days to make a difference or due to the large standard deviation of the nitrous oxide flux results to produce the opposite relationship.

Commented [I2]: Wouldn't it favor denitrification since denitrification is an anaerobic process?

When analyzing average daily temperature and nitrous oxide flux there was a positive relationship for all points except for one outlier. Typically, as temperature rises microbial activity rises as well as nitrous oxide flux (Knowles, 1982). While within the grassland this was true, within the farm field the highest average temperature which occurred on day one was accompanied by the lowest observable flux in this location. One likely source for this was that it was the opposite of a hot spot, a cold spot, for nitrous oxide production. While microbial life speeds up the process it needs nitrate to first break down. This area could have been lacking a

large carbon density or simply lacked sufficient nitrate within the area. It is likely that if more data was taken overtime this lone outlier would become more independent.

4.2 Flux Interpreted

Although the difference in nitrous oxide flux between the two areas of study showed no significant difference, the results were not uncommon. Liversley et al (2013) measured changes in nitrous oxide flux in remnant forests, pasture land and viticulture land through all four seasons and found no statistically significant difference in N_2O flux. Each of these soils had drastically different drainage rates as well as different carbon densities within, yet no differences were noted. Chino Farms consisted of all of the same soil with a new natural quail habitat created in recent years. It is plausible that the lack of difference in nitrous oxide flux between the farm field and restored quail habitat is common during the winter months. While soil composition may change microbial activity (Cowan et al., 2015) this is negligible within Chino Farms as the soils are nearly identical within the entire study area.

It is believed that due to the sporadic nature of the presence of hot spots inside both study areas of Chino Farms there may be multiple causes. The first source is the possibility of the background presence of fertilizers within the soils. As months have gone by the synthetic fertilizers applied have most likely been removed through the uptake by plant material and through the process of denitrification. However, in some instances as rain washes the synthetic nitrogen away, often natural sinks may form whether it be from a riparian buffer zone or due do a drop in surface soil depth (Lin et al., 2009). If this occurs then this could account for the isolated large spikes in nitrous oxide flux witnessed. Rain events occurred between each time of sampling as well. This could be the cause of the seemingly sporadic movement of the localized N_2O hot spots as the nitrogen is washed away and moved to a new location.

N_2O flux hotspots may also be due to animal waste. As many signs of life such as game trails, several birds and even fox dens were recognized within the area the notion that there would be large amounts of waste within this region is plausible. While no studies have been done specifically on these animals, other studies have shown a direct increase in flux due to animal waste. One study of yak urine patches yielded nitrous oxide flux 2.1 – 3.7 times greater than the soil around them (Lin et al., 2009). This study is similar to the study area of Chino Farms as these were not wide spread grazing animals but rather small pockets which produced sporadic hot spots. Cowan et al (2015) studied grazed land in Scotland that showed similar results. Large nitrous oxide fluxes were found on both urine and manure patches of the grazing animals. Only 1.1% of the field was covered by animal waste yet it accounted for 55% of the daily N_2O flux (Cowan et al., 2015). As more wildlife enters both the farm field and grassland each year, it is possible that the impact of animal waste on nitrous oxide flux will increase. The role of game trails in nitrous oxide flux in restored habitats is worth further study.

The increase of N_2O flux in both areas of study from day two to day three is believed to be from a thawing event. On day two of study there was a noticeable ice layer roughly 4 cm below the surface. On day three this layer was no longer there. Nitrous oxide among other greenhouse gases tend to build up beneath layers of ice underneath the soil surface until released upon thawing (Furon et al., 2008). This may also account for some of the patchy nature of the detected hot spots. Thawing may have occurred at different rates in different areas due to changes in thickness of ice or changes in direct sunlight. Chambers placed on areas that thawed quicker would produce a higher rate of flux.

4.3 Future Challenges

While the results concerning differences in nitrous oxide flux between the agricultural land and restored habitat land were negligible and not statistically significant, this does not mean the effects of N_2O are negligible as well. Low levels of flux were expected as research was conducted during the winter months. Due to freezing, slowed rates of microbial activity, and lack of applied fertilization, N_2O fluxes are typically lesser in winter months (Knowles, 1982, Mosier, et al., 1998). The presence of so many irregular yet high flux inducing hot spots in both areas lead to many new opportunities of research within the area.

Precipitation may have a large effect on transferring synthetic fertilizer throughout the region. As some chambers reported flux one day and not the other, it is important to understand why this is. If similar studies occur immediately after rain events, perhaps a map of the movement of nitrogen within the area may be made. If nitrogen is being trapped within the soils and not entering the water supply this may be a good thing as it would improve water quality and lessen instances of eutrophication. However, if these sinks trap too much nitrogen the possibility for an increase in nitrous oxide could overall be worse as the gas has a global warming potential of 310 (EPA, 2014). As it is determined that much of the apparent flux is due to the presence of game animals, a study into the animal density should also be completed. If the populations of the organisms and their population growth rates are better understood humans can thus enhance their understanding of the organisms' impact on the environment.

Unanticipated results were seen in terms of moisture content within the soil and nitrous oxide flux. Typical results show a positive correlation between the two; however, within both the farm field and the grassland a negative relationship was observed. Understanding anomalies such as this is crucial in understanding the impact of nitrogen on the environment. If there is one area

which acts in this manner then it is plausible that other areas of similar output exist. pH was not studied and perhaps may have had an effect. It is known that low pH causes higher nitrous oxide flux (Knowles, 1982). However, because this was not studied there may have been changes in pH within hot spot areas that was overlooked.

4.4 Conclusion

Nitrous oxide and other greenhouse gasses are increasingly more important to study as climate change and its effects begin to be felt globally. Large quantities of research is currently being undertaken for methane (CH_4) and carbon dioxide (CO_2), yet less study has occurred for nitrous oxide (N_2O) which has a greater energy holding capacity than CO_2 by a factor of 310 (EPA, 2014). While no statistically significant difference was seen between the farm field and the grassland, there is still a cause for concern. Large hot spot fluctuations occurred randomly in both locations and were relatively unpredictable. If these are not understood than humans are not able to understand all of the effects causing nitrous oxide flux into the atmosphere from soil. Continual research must be done to understand these unexpected results. Each year nitrous oxide emissions due to anthropogenic causes increase, yet humans still are not able to understand the contributing factors to increases in flux. As shown from this study, seasonal thaws cause a release of nitrous oxide. What is concerning is that each year as the planets temperature rises, more thawing occurs in permafrost areas (Schuur, et al., 2015). Greenhouse gases have been trapped in these regions along with high densities of carbon which is known to increase N_2O flux. A troubling positive feedback loop may thus already be underway. As the permafrost thaws more each year, the more GHG raise total climate temperature. The more global temperature rise the more permafrost thaws (Schuur et al., 2015). Continual research on nitrous oxide must be

done so that humans can mitigate and stop this and other feedback loops from growing out of control.

New methods to produce higher yielding crops must be discovered. Global demand for food is increasing every year as population continues to grow exponentially. Consequently, more nitrogen based fertilizers is thus demanded. Nitrogen based fertilizer efficiency is currently very low (Reay et al., 2012). For every 100 units of N used in agriculture only 17% is utilized by humans via crop ingestion, dairy output and meat consumption (Reay et al., 2012). During the 1990's of the global 16 Tg N_2O-N yr^- 50 % was anthropogenically forced. It is predicted that by 2030 global N needed by current practices would be between 100 – 135 Tg N_2O-N yr^{-1} which may offset CO_2 abatement efforts implemented by humans globally (Reay, et al., 2012). It is imperative that nitrous oxide flux is continued to be studied as its causes and effects are projected to only increase with the years to come.

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