The Effects of Different Soil Characteristics on the Nitrous Oxide Fluxes in California Central Coast Organic Strawberry Farms

A Thesis Presented By:

Isaac Medina

To the Environmental Analysis Program

Pomona College

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Abstract

Real-time trace fluxes of nitrous Oxide (N_2O), a powerfully potent greenhouse gas and ozone depleting substance, were measured in soils of organic strawberry farms along with basic soil characteristics thought to influence the production and/or consumption of N_2O. These factors included: nitrate concentrations, and soil particle distributions among others. None of the measured variables had significant corollary effects with nitrous oxide fluxes (NOFs) as determined via regression analyses, indicating overall that the interplay between environmental and biotic factors is highly complex and dependent on many intersecting factors.

1. Introduction

Understanding the factors that control the productive and consumptive processes of nitrous oxide (N_2O), a powerful greenhouse gas (GHG) is a key issue in the ongoing effort to find ways to mitigate the risks global climate change. According the United Nation's Intergovernmental Panel on Climate Change, the evidence of anthropogenic forcing of the climate system is both overwhelming and unequivocal. Increasing concentrations of GHGs in the atmosphere due to human activity are driving an unprecedented change in the climate system such that we are certain the last three successive decades have seen the highest average mean surface temperatures since 1850 and were probably the warmest period on Earth in the last 1400 years (IPCC 2013).

1.1 Earth's Climate System and Radiative Forcing from GHGs

Nitrous oxide like other GHGs is just one piece of a complex system that regulates Earth's relatively stable climate. It begins with the uneven distribution of solar energy received across the surface and atmosphere of the Earth. The atmosphere most directly facing the sun receives an average of 1,360 watts/m^2 but because the surface of the earth is not flat, regions closer to the poles receive less energy. Thus, the amount of energy hitting the atmosphere averaged over surface area is approximately 340 watts/m^2 (Wen et al. 2013, 6281-6289). The Earth's climate system works to redistribute the incoming energy through processes like evaporation, convection and emission and together these processes drive the Earth's climate including all ocean and wind currents, precipitation, etc. The climate system in essence attempts to balance the energy budget received from the sun by moving it around, and most importantly by also radiating the energy back into space in the form of heat and infrared radiation (Marshall and Plumb 2008).

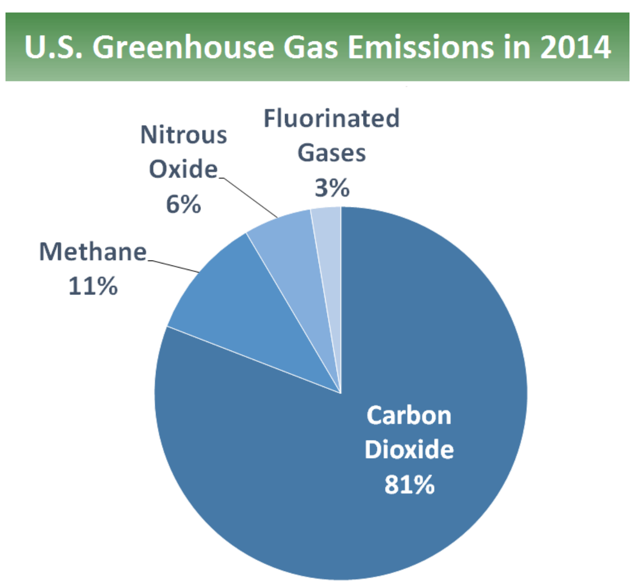
The atmosphere and surface of the Earth together absorb 71 percent of the incoming solar radiation from the sun (23 and 48 percent respectively), the rest is immediately reflected back into space by albedo effects (Kushnir 2000). Some of the energy absorbed by the surface is radiated as thermal energy, which is lower in wavelength than the ultra-violet/visible light form in which it first came in from the sun. In the process of being emitted back into space, this lower wavelength infrared energy radiated from the surface is now absorbable by GHGs in the atmosphere such as N_2O. GHGs absorb and re-emit this energy in all directions, including back to the surface which causes warming. Without the natural greenhouse effect the Earth's temperature would be approximately 30℃ cooler (IPCC 2013).

The concept of radiative forcing takes into account the components of the climate system and their net positive or negative contribution to amount of energy retained in the system. Radiative forcing is, therefore, the change in the balance between radiation coming into the atmosphere and radiation going out. Positive radiative forcing leads to warming on the surface of the Earth, and negative forcing leads to overall cooling on the surface. GHGs play a role in positive forcing by trapping thermal energy. The potential of a unit mass of a species of GHG to cause warming and positively influence climate forcing is measured by their global warming potential (GWP). GWPs are calculated over a specific time interval to account for how long the species stays in the atmosphere. The GWP is also calculated in relation to Carbon Dioxide (which has a standardized GWP of 1).

1.2 Addressing Two Problems with N_2O Emissions: Climate Change and Ozone Depletion

Many GHGs, such as nitrous oxide, have GWPs much higher than carbon dioxide, but they are not necessarily present in large enough concentrations to contribute significant radiative forcings. N_2O emissions, however, are the third largest source of anthropogenic GHG emissions accounting for 6 percent of the total in 2014 (EPA 2014) (see chart below). Additionally, nitrous oxide is a significant GHG because it has a whopping GWP of 298 over a 100 year time period (EPA 2014).

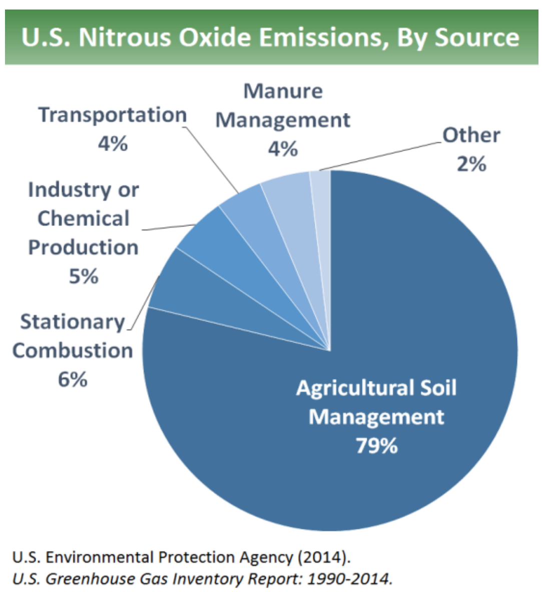
N_2O is not only a powerful GHG but it has also been shown to be the current and potential future's most dominant ozone depleting substance, having an ozone depleting potential comparable to some fluorinated hydrocarbons (HFCs) (Ravishankara, Daniel, and Portmann 2009, 123-125). These characteristics of N_2O coupled with the fact that it remains active in the atmosphere for about 118-131 years (Fleming *et al.* 2011), make it a significant challenge to address.



U.S. Environmental Protection Agency (2014).
U.S. Greenhouse Gas Inventory Report: 1990-2014.

1.3 Sources of N_2O

N_2O fluxes from soils are largely the result of microbial processes that mediate denitrification and nitrification (FIRESTONE MK and DAVIDSON 1989). Human activities such as adding synthetic fertilizer to cropland increases the amount of total nitrogen available for nitrification and denitrification, and ultimately the amount of nitrous oxide emitted (EPA 2006). By far agricultural soil management practices account for the greatest amount of anthropogenically produced N_2O gas (EPA 2014) see chart below.



N_2O emissions are projected to increase by 5 percent between 2005 and 2020, driven largely by increases in emissions from agricultural activities (EPA 2014). According to the National Oceanic and Atmospheric Administration the rate increase of N_2O concentrations on a decadal scale has been between 0.73 ± 0.03 ppb yr^{-1} (NOAA-GMD). Researchers estimate that food production will be responsible for up to 80% of the increase in N_2O emissions because of the addition of synthetic fertilizer to more land (Davidson 2009) (Park et al 2012).

The UN's Food and Agriculture Organization projects that feeding the 9.1 billion people expected to be alive in 2050 will require raising overall food production by 70 percent (FAO.org). This increase includes the acreage used to farm organic produce. In California organic acreage is expected to comprise between 20 and 60 percent of all agricultural cropland by 2025. Already it is over 405,000 acres, nearly half used for fruit/vegetable production (USDA 2016). Of particular importance to the fruit market, are California's strawberry farms which take up nearly 40,000 acres alone. As the nation's leading producers of strawberries, harvesting over 2.3 billion pounds in 2014, California's strawberry farms are a sizable portion of total land use dedicated to farming (California Strawberry Commission).

1.4 Our Study

The highly complex nature of physical and chemical interactions between biotic and abiotic factors in soil make N_2O emissions hard to characterize as they can be highly spatially and temporally variable (Clayton et al., 1997). Wanting to get a better look at how different soil characteristics might interact with the production of N_2O we investigated and characterized N_2O fluxes from soils in different organic strawberry farms located in California's Central Coast region. Specifically, we looked at U.S. Department of Agriculture (USDA) certified organic farms. These organic farms were all similar in that they comply with the strict standards set forth by the U.S.D.A. regulating and or banning the use of synthetic fertilizers, pesticides, antibiotics and genetically modified seeds (EPA 2015).

At each farm we measured real-time trace N_2O fluxes from soils, as well as a variety of potentially influential soil characteristics such as: soil basal respiration (CO_2) rates ($ppm \cdot day^{-1}$), soil water infiltration rates (seconds), total nitrite and nitrate nitrogen concentrations (ppm). We also performed a particle size analysis on soil samples collected on each farm to classify the soils by type and quantify the percentage of sand, silt and clay particles in the soils at each farm.

2. Materials and Methods

Overview

We collected soil samples from 25 organic strawberry farms in the California Central Coast, primarily in the Pajaro and Salinas Valleys. The team also measured a variety of soil characteristics including nitrate ($N0_3^-$) and nitrite (NO_2^-) nitrogen concentrations, soil respiration, and infiltration. Soil GHG fluxes were measured from 5 random points along a 30 meter transect using an automated closed dynamic chamber technique (Pumpanen et al. 2004, 159-176), coupled with a Picarro G2508 trace gas analyzer.

2.1 Locations for Soil Sampling and Field Measurements

Using a standard AMS soil probe, we extracted soil cores of 30 cm length (~1.59 cm diameter) from 5 random points along predetermined 30 meter transect that cut across a central strawberry block at each farm. A total of 30 soil cores were collected from each farm, 6 cores from the area around each of the 5 random points on the transect. In soil beds where strawberries are grown the amount of available nitrogen from fertilizer can vary significantly by depth, usually concentrating at the surface of the soil where it is released from drip tape irrigation lines. Thus, we separated the mass of each soil core based on the depth at which the soil was located. Only soil 0-15 cm from the surface (half the length of the soil core) was used in our measurements since this was the soil most closely associated with rootzone of the strawberry plants and most affected by fertilization. The soil cores were homogenized into a single slurry for each farm by mixing for at least 3 minutes. Chunks of clay were broken up as best as possible rocks and debris were excluded.

2.2 Greenhouse Gas Flux Measurements

Real-time trace greenhouse gas fluxes of CO_2, N_2O, CH_4 and NH_4 were measured near each of the 5 random points on the transect using a closed automated dynamic chamber technique (Pampanen *et al*. 2004). We used 5 automated soil chambers developed by Eosense Inc, the EosAC soil flux chambers coupled with a Picarro G2508 cavity ring-down spectrometer for gas analysis. More information about this technique, the hardware used and general information about cavity ring-down spectroscopy can be found Appendix B. Each chamber was placed within the strawberry plots between 0 and 3 meters away from one of the previously determined 5 random points on the transect. This was done in order to avoid sampling soil gas fluxes near areas that had been disturbed by our soil probes.

A complete installation of each soil flux chamber included the following processes: first circular openings were cut into the strawberry bed's plastic covering (or mulch), secondly gas chamber collars were placed no more than 2.5 cm into the soil (or as deep as the soil allowed), thirdly gas chambers were secured on top of the collar and a tight seal between the soil, collar, and gas chamber was confirmed. Lastly we attached 30 meter long Teflon tubing and electrical cables to each chamber connecting them to the gas analyzer.

Each chamber took turns sequentially measuring soil GHG fluxes for 15 minutes, with a 30 second delay between each new chamber measurement. A full cycle of measurements consisted of measuring soil GHG fluxes at each chamber (1-5) for 15 minutes including all 30 minute delays in between measurements (no less than 77 minutes total).

<u>Time Intervals for GHG flux Measurements</u>

GHG Flux measurements were taken at 3 specific time intervals, with the goal being to capture at least one cycle of measurements before, during and after an irrigation event in the strawberry plots being measured. This was done because multiple research suggests that peak N2O fluxes are associated with fertilization and irrigation events (Venterea et al., 2010). Since the goal of our study is to deduce which factors might be most heavily responsible for N_2O emissions from agricultural soils we measured fluxes during these critical periods in soil water content availability to assess if they are significant drivers of N_2O emissions.

Due to the logistical challenges associated with coordinating the capturing of data during irrigation events, sometimes we were able to capture a full cycle or more of pre-irrigation data for soil GHG fluxes, while on other farms set up of the instrumentation may have only allowed for collecting less than a full cycle's worth of pre-irrigation data.

2.3 Soil Textural Analysis

Soil samples collected for textural analysis were processed according to the American Society for Testing and Material's standard test method for particle size analysis of soils (ASTM-D422). This a Bouyoucos type hydrometer method that entails steeping a known amount of soil in 100 mL (50 g/L) Sodium Hexa-metaphosphate solution and 250 mL of distilled water overnight to disaggregate soil particles. The slurry is then diluted up to 1 liter in a cylinder, after which the cylinder is capped and inverted back and forth for 30 seconds no less than 2 times per second. The soils are now suspended in solution and a 151H type hydrometer is used to take readings at predetermined intervals of time. Our readings were taken at 0.5, 1, 3, 10, 30, 60, 90, 120, and 1,440 minutes after suspending the soil. Hydrometer readings were then worked up in R according the equations in the ASTM-D422 and soil texture profiles were generated for each farm (See Appendix A).

2.4 Nitrate and Nitrite Quick Tests

Total nitrite and nitrate concentrations were determined in triplicate for each soil sample collected using nitrogen test strips. This involved filling a 50 mL centrifuge tube up the to the 30 mL mark with a dilute calcium chloride solution (6 g of $CaCl_2$ / gallon of distilled water) and then adding the soil to be tested into the tube until the solution level reached the 40 mL mark. The centrifuge tubes were then capped and shaken vigorously for one minute each after which they were allowed to settle for 10 minutes. The nitrate test strips were then dipped into the solution to determine nitrate and nitrite nitrogen concentrations in ppm.

2.5 Soil Infiltration Measurements

Basic soil infiltration measurements were taken in triplicate using 3 additional random points along the 30 meter transect at each farm. We employed the use of a single ring infiltrometer technique using 1-gallon aluminum paint buckets (with the bottom side cut out). Each infiltrometer was driven 8cm into the soil as levelly as possible using a rubber mallet and wooden plank. A 6" square splash guard was laid out inside the infiltrometer and 65 mL of water was dispensed above the splash guard. A stopwatch was started after quickly (but carefully) removing the splash guard to ensure all the water hit the surface at around the same time. We recorded the time it took for the water level to be flush with the soil in minutes.

2.6 Data Processing and Analysis

To determine whether irrigation had an effect on N_2O fluxes from soil a Student's t-test was performed in Rstudio (RStudio Inc. 2015, Boston, MA USA), comparing average N_2O fluxes before and after irrigation. To check if any of the variables we measured had a significant effect on N_2O fluxes multiple regression analysis was performed.

3. Results

3.1. Summary of Measudrements by Farm and Data Distributions

The average concentration nitrate nitrogen across all farms was 11.19 (ppm) and ranged from 0.17 to 40 (ppm), with a standard deviation of 9.8 ppm (Figure 1). Average CO_2 flux due to basal respiration from the soils ranged from 3.40 to 73.25 (ppm) (Figure 2).9

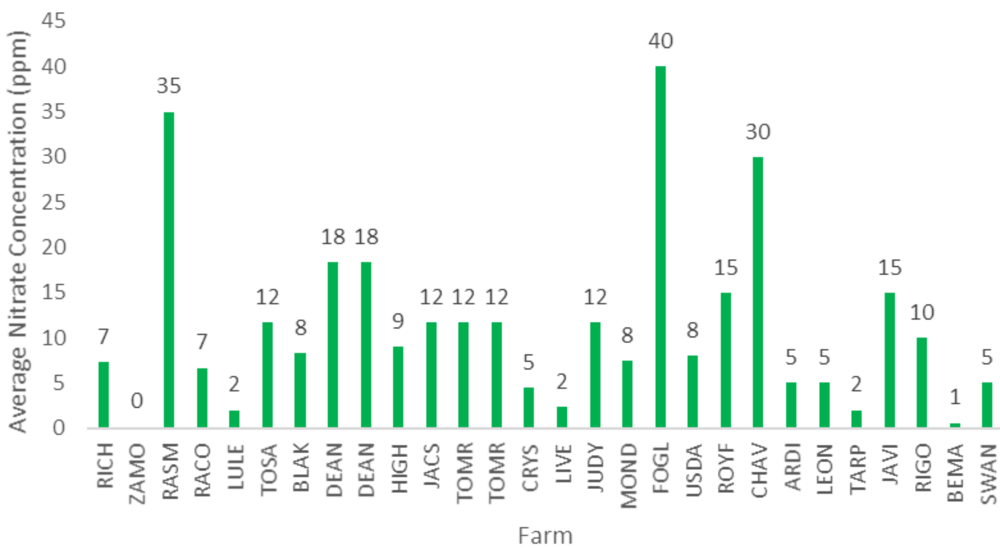


Figure 1. Average Concentrations of Nitrate (ppm) by Farm.
Note: results have been rounded to the nearest ones place for easy visualization of the data.

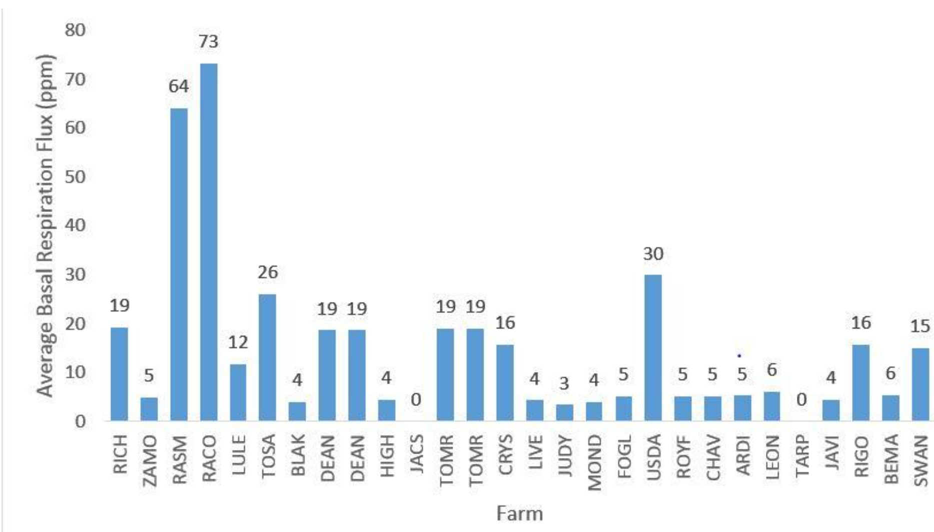


Figure 2. Average Basal Respiration Flux (CO_2) by Farm.
Note: results have been rounded to the nearest ones place for easy visualization of the data

Soil water infiltration rates were measured in triplicate. The average time it took for 65 mL of water to completely permeate into the ground across all farms was 122.8 seconds, and ranged from 3.7 to 865.6 seconds with a standard deviation of 194.5 seconds (Figure 3). The results of our soil nitrate, respiration, and infiltration measurements are summarized in Table 1.

Table 1. Summary Data of Basic Soil Measurements

	Nitrate Concentration (ppm)	CO2 Respiration (ppm)	Infiltration Time (sec)
Mean	11.19	15.69	122.75
Min	0.17	3.40	3.7
1st Quartile	5.00	4.95	27.71
Median	8.67	8.89	41.5
3rd Quartile	12.5	18.95	140.8
Max	40.00	73.25	865.6

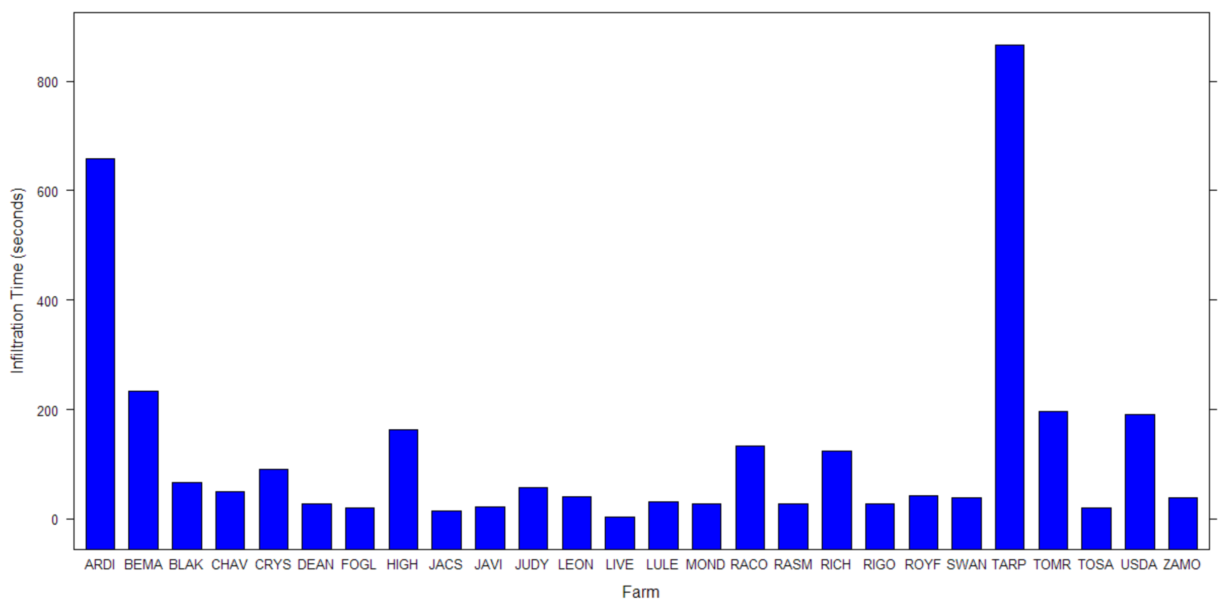


Figure 3. Average Infiltration Test Time by Farm.

3.2. Soil Nitrous Oxide Fluxes

Soil nitrous oxide fluxes (NOFs) during all time periods (before, during and after irrigation) ranged from -0.28 to 29.9 nmol/m^2 /sec (Table 1). The average NOF across all farms was similar but some farms such as LEON, RACO, RASM, RIGO and ROYF had high amount of variability, with some NOF readings well above the mean (Figure 4). Table 2 summarizes

NOF data by time period across all farms. Histograms of NOFs by time period measured (before, during and after irrigation) show a right skew tendency among our data (Figure 5).

Table 2. Summary of Nitrous Oxide Fluxes by Time Period Across all Farms

	Overall N_2O Flux	N_2O Flux Before	N_2O Flux During	N_2O Flux After
Mean	7.08E-02	4.98E-01	6.04E-01	6.95E-01
Median	1.15E-01	1.02E-01	1.80E-01	1.43E-01
Min	-2.84E-01	-4.75E-02	-6.09E-02	-2.84E-02
Max	2.99E+01	2.47E+00	2.88E+00	4.40E+00

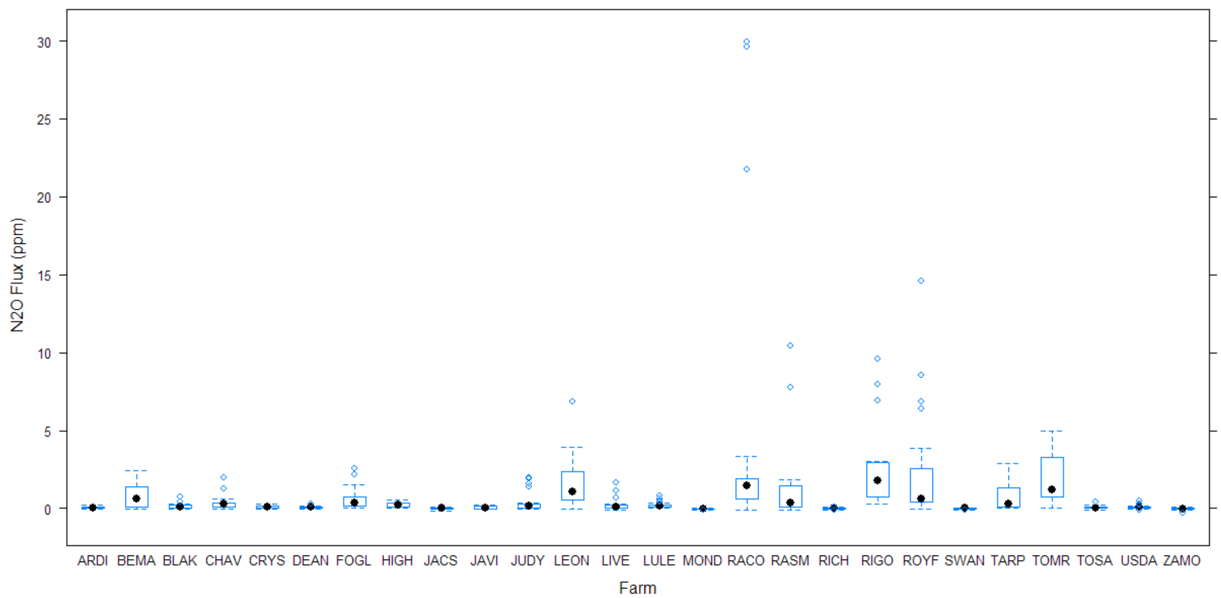


Figure 4. Box Plot of Average Overall Nitrous Oxide Fluxes (NOFs) by Farm.
Box plot show similar mean fluxes across farms, but high amount of variability at certain farms such as RACO, RASM, RIGO and ROYF.

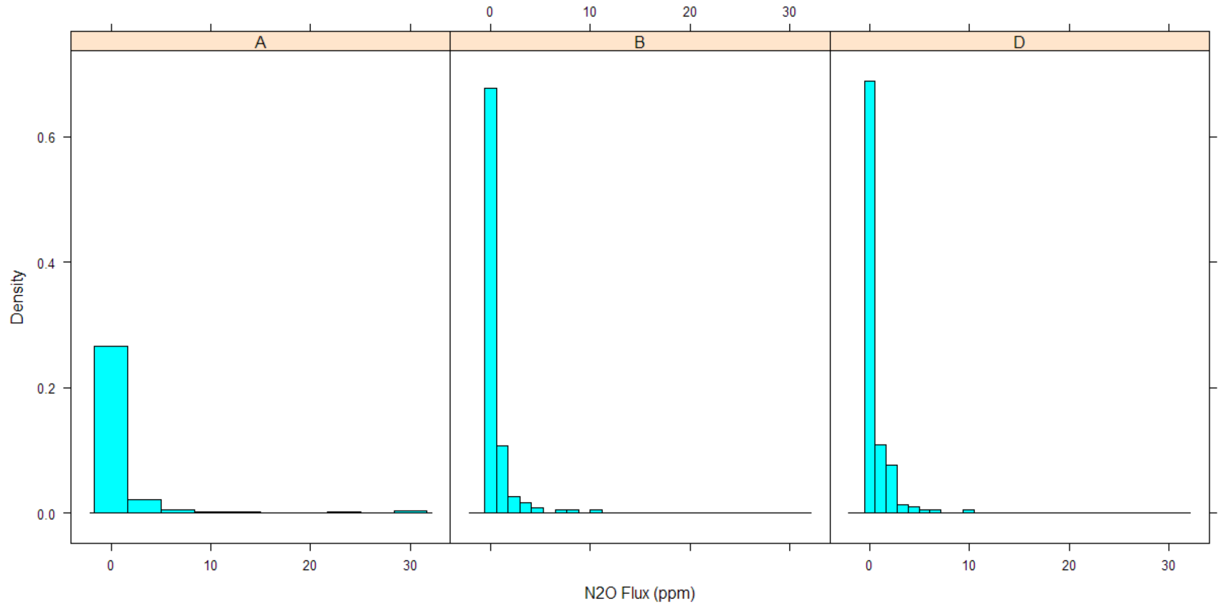


Figure 5. Histograms of Nitrous Oxide Fluxes by Time Period.
In all cases (B = before, D = during, A = after irrigation) the distribution of N_2O Fluxes are right skewed.

3.3. Soil Texture Classifications by Farm

Calculated soil percentages were plotted in a USDA soil texture triangle using Rstudio for visualization and classification of the soils. The great majority of farms fell into the Loamy Sand and Sandy Loam categories while only a few were Loam and Clay Loam soils (Figure 5). Only a couple farms had Silty Clay and Silty/Clay Loam soils (one in each category) (Figure 5). Appendix A at the back of this document gives the texture classifications and soil texture percentages of sand, silt and clay particles for each farm.

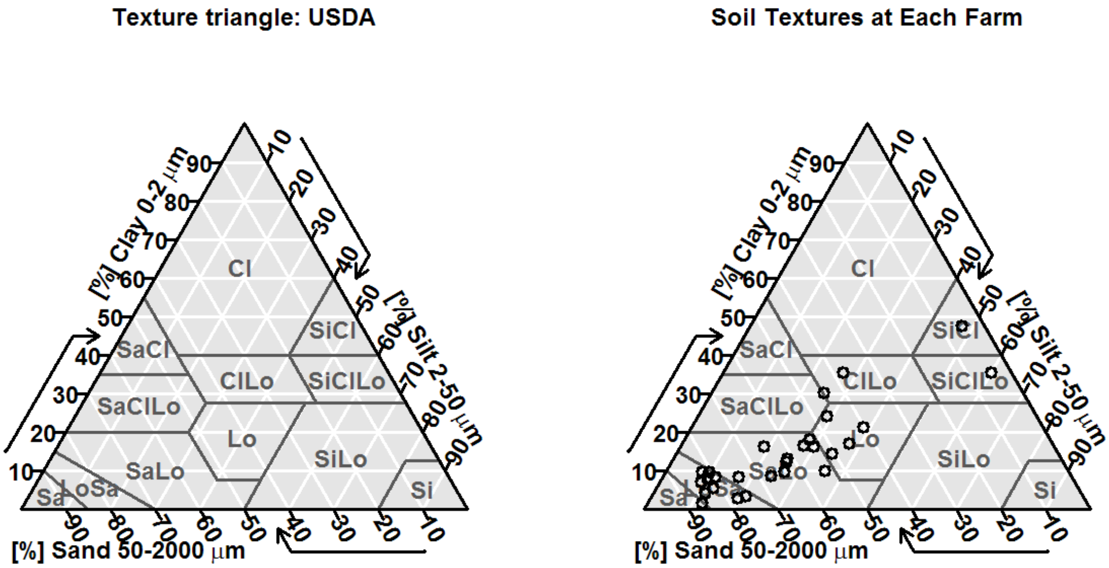


Figure 5. USDA Soil Texture Triangles with Classifications and Soil Texture Percentages by Farm. The triangle on the left is an empty for easy reading of the different soil classificaitons. The triangle on the right displays our soil texture percentages by farm. Most farms were either Sandy Loam or Loamy Sand.

3.4. Statistical Analysis

T-test Results

A one tailed paired Student's t-test was performed comparing average N_2O fluxes before and after irrigation to determine whether irrigation had an effect on N_2O fluxes. T-test results were negative, irrigation had no significant effect on N_2O fluxes from soil (p-value = 0.1138, df = 26).

Multiple Regression

Results of the multiple regression analysis comparing percent sand and silt, and other measured soil characteristics such as respiration rate, nitrate concentrations and infiltration rates are summarized in Table 3. Overall, none of the variables we measured significantly explained the variation in N_2O fluxes. The multiple r^2 coefficient was 0.1098 indicating poor fit of the model (p-value = 0.7948, df=19) and the F statistic was 0.469.

Table 3. Multiple Regression Model Results by Soil

Variables	St. Error	t value	Pr(>|t|)
Sand	2.94E+00	-0.662	0.516
Silt	-2.28E-02	-0.802	0.433
Respiration Rate	-4.47E-02	0.872	0.394
Average [Nitrate]	-4.02E-03	-0.186	0.855
Average Infiltration Rate	7.36E-04	0.218	0.83

4. Discussion

As mentioned in the introduction, agricultural production is expected to increase significantly in the future to keep up with the expanding human population. Because agriculture is largest anthropogenic driver of N_2O emissions, which cause positive radiative forcing and depletion of the ozone, mitigating the effects of N_2O emissions in the future requires a robust knowledge of the factors in agricultural systems that lead to higher N_2O fluxes (NOFs). The present study was an attempt to characterize important soil characteristics responsible for increased NOFs in order to think about potential land management practices that could be used to mitigate them.

Pinpointing the important soil characteristics involved in NOFs has proven to be more challenging than expected. Our results suggest that the interplay between environmental factors (such as the soil characteristics we measured) and other factors (such as microbial biological pathways) regulating NOFs in organic strawberry farms are highly complex. In that sense our findings are consistent with the literature in the field of agricultural N_2O emissions research but certain trends we expected to see based on the literature are either obscured by variability in the data or missing altogether.

For example, none of the variables we measured seemed to have significant predictive power on the level of NOFs; as evidenced by lack of statistically significant correlations in our multiple regression (see section 3.4). Although we expected that certain variables might have less of an impact on the soil's microbiological potential to generate N_2O it was surprising that we saw no correlation between certain variables and NOFs, such as the measured amount of nitrate concentrations.

The vast array of literature in this field of research upholds the idea that human activities strongly influences the source of N2O. In particular, that use of nitrogen fertiliser in agriculture is the main source of nitrogen for nitrification and denitrification (Opdyke et al., 2009) and that fertilized soils are primarily responsible for the historic increase in N_2O (Park et al., 2012). And multiple previous studies by other researchers posit that synthetic fertilizer and added manure are responsible for increases in N_2O emissions from agriculture (Syakila & Kroeze, 2011). This makes sense when looking at a simple conceptual model for understanding NOFs -the hole in the pipe model or "HIP" model (Figure 6) (FIRESTONE MK and DAVIDSON 1989).

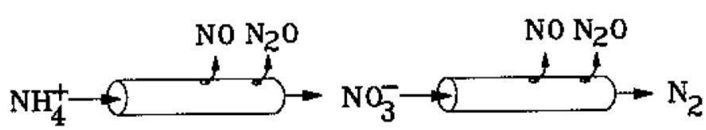


Figure 6. Hole in the Pipe Model proposed by Firestone and Davidson.
A conceptual model for N_2O and NO emissions that shows two basic levels of regulation via nitrification and denitrification. The 1st level of regulation is the flux of N through process "pipes" and the 2nd is the size of the "holes" in the process pipes through which trace N-gases "leak".

The HIP model shows two pipes; each one representing a biological process that fixes nitrogen to the soil (a.k.a. biological nitrogen fixation or BNF). The pipe on the left represents "nitrification" whereby microbes convert ammonium into nitrate that is then biologically available for plants to use. The pipe on the right represents “denitrification” whereby certain soil microbes convert nitrates in the soil back into its nonreactive form nitrogen gas, N_2 (g). Along these processes (which are both natural parts of the global nitrogen cycle) there is the potential for bacteria to fail to convert the initial substrate fully into either nitrate (NO_3^-) or nitrogen gas (N_2), thus creating other species of nitrogen gas such as N_2O. This is represented by “holes” in the pipes which leak out nitrogen gases like N_2O.

What the HIP model demonstrates very well is the two levels of regulation that exist in the creation of N_2O gas. That is, that the amount of N_2O produced will depend on how much N (nitrogen) is flowing through the “pipes” (i.e. how much nitrogen is available for BNF in agricultural systems or present in the soil) and what the size of the “holes” in the pipes are (the inherent biological activity of the soil microbes which mediate nitrogen conversion processes, such as bacterial enzyme kinetics).

Globally, humans have altered the amount of nitrogen available to partake in the processes of nitrification and denitrification, especially through the commercial production fertilizers or ammonia by the Haber–Bosch process which converts unreactive nitrogen gas into reactive nitrogen species which is used to make fertilizer. Thus, we can think of increasing industrial agricultural practices as increasing the flow of N through the pipes of the HIP model. The effect of increased nitrogen flowing through agricultural systems on NOFs cannot be understated. In fact, most of the increases in N_2O emissions the past 20 years which resulted in 6.9 (2.7 to 11.1) TgN (N2O) yr–1 (IPCC 2013) are attributed in some form to the production of reactive nitrogen in industrial processes. Even models used to estimate direct N2O emissions from managed soils by the IPCC predict that 1% of applied N fertilizer is emitted as direct N2O emissions (De Klein et al., 2006).

For these reasons, it was surprising that we did not notice an effect of increased nitrate nitrogen concentrations on measured NOFs. However, this is not to say that nitrate concentrations were not an influencing factor as the rest of our results, generally, point out that there was a high amount of variability from farm to farm. Thus, finding statistically significant correlations even between variables which are known to be highly correlated may have been difficult through all the variability. To make this point, Figure 7 below illustrates soil texture triangles with bubble plots of nitrate concentrations (left) and a bubble plot of soil infiltration rates (right). The size and dark hue of the bubbles in each triangle is proportional to the concentration of nitrate or the infiltration time (i.e. the bigger and darker the more nitrate or infiltration time was larger). Nitrate concentrations are obviously independent of the soil particle size distributions, since they are controlled directly by farmers. Thus, the high amount of variability in the size of the bubbles in the triangle on the left is expected. Although the triangle with the bubble plot for infiltration time (right) shows less variability in the size of the bubbles, it still lacks to show a clear trend in infiltration rates, and this is surprising because soil infiltration rates are known to be highly dependent on particle size.

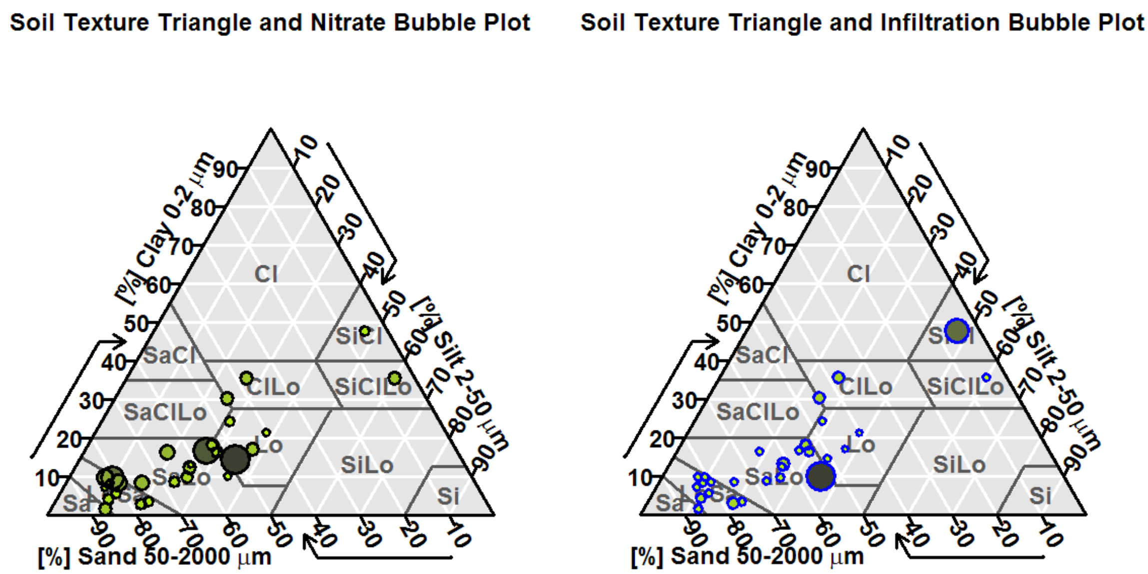


Figure 7. Soil Texture Triangles with Bubble Plots for Nitrate Concentration (left) and Infiltration (right). Nitrate concentrations show high variability between each type of soil class which is expected. Infiltration rates which generally are highly dependent on soil class don't show as much variability, but still lack a clear trend.

Linear regression models testing for an effect of percent clay on water infiltration rates confirmed no statistically significant correlations. This was true also for the simple regression of nitrate concentrations vs. nitrous oxide fluxes after irrigation.

Going back to the results of our multiple regression; one critique could be that you generally want to assume constant variance and normal distribution of error when using a multiple regression model. The above results illustrate that this was not the case in our study, and overall this helps to understand why we might have failed to see correlations where we expected them.

If for some reason(s) we failed to detect increases in NOFs due to increased nitrate concentrations because of the variance of measurements from farm to farm, the negative results of our paired t-test are less surprising. This is because according to the hip model nitrate concentrations are more “proximal” controllers of the biological pathways that lead to N_2O emissions. Meaning that they more closely regulate how much N_2O can leak from the pipes than outside environmental factors which are “distal” controllers such as the amount of water present in the soil from irrigation. Our t-test was one tailed, because we assumed that irrigation events would lead to increased NOFs based on literature such as (Davidson et al. 2000, 667) who proposed that when water filled pore space reaches 60% saturated capacity, the enzymatic activities of microbes who use anoxic conditions for denitrification get ramped up, leading to increases in NOFs. We failed to detect a significant difference between NOFs measured before and after irrigation, although here too we must be cautious of our results. T-tests generally also make many of the assumptions used in multiple regression. One of these assumptions is that the data is normally distributed and this was not the case in our study as histograms of our data show a clear right skew amongst NOF measurements (Figure 5).

Generally, when studies produce “negative” results; meaning that no statistically significant effects are found between the predictor and response variables (i.e. between soil characteristics and NOFs); there are three possible explanations. The first is that no true correlation truly exists between the variables and responses measured. The second is that the response variable is affected, perhaps more strongly, by other variables that were not measured or accounted for. And lastly the possibility of “bad” data collection exists as well; meaning that perhaps measurement collection was done in an irregular or irreproducible manner from farm to farm.

The first concern, that no true correlation exists between the predictor and response variables can be addressed with literature. As we stated above, we expected to find positive correlations between variables such as nitrate concentrations and irrigation on NOFs, because literature and theory posit that they exist and have been documented by other researchers (Venterea et al., 2010). Thus, we can sort of rule this possibility out, but not entirely. The highly variable measurements from farm to farm suggest that while the variables we measured might be correlated to NOFs according to the literature, there must be other factors controlling NOFs as well. This conclusion is supported by the literature which theorizes a host of potential factors influencing NOFs (Figure 8 and 9).

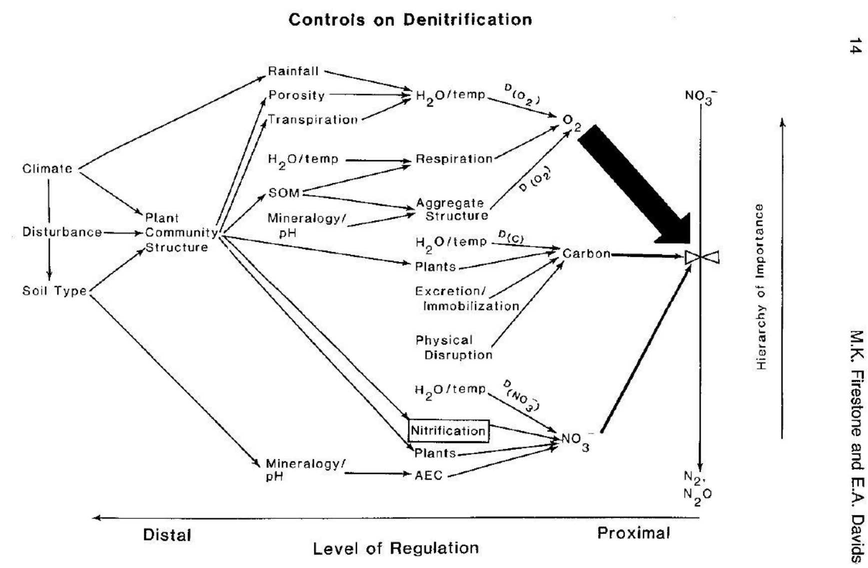


Figure 8. Diagram of the major factors regulating denitrification in soils

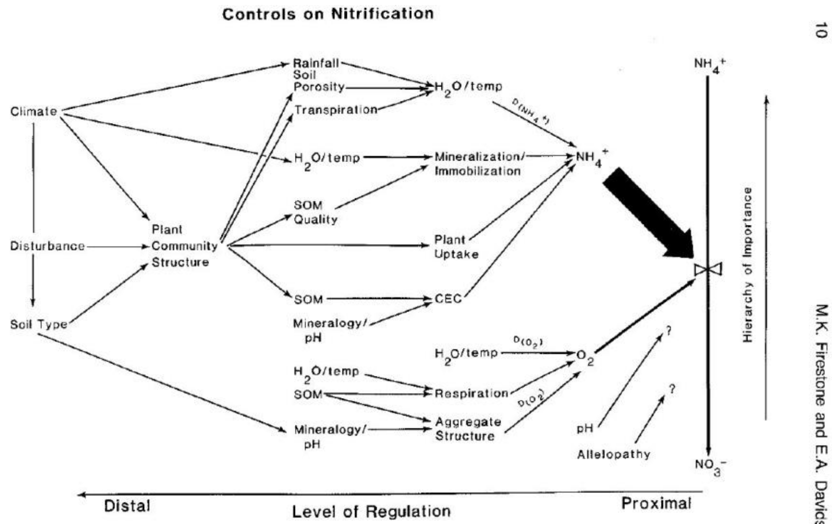


Fig. 2—A schematic diagram of the major factors regulating nitrification in soil (example given for rain forest soils).

Figure 9. Diagram of the major factors regulating nitrification in soils

The factors described in the above diagrams were thought to play an influential role even way before the bacterial metabolic processes of nitrification and denitrification were understood. However, more recent advances in the field of genetics and bacterial enzymatics point out that other factors besides environmental (distal) controllers are highly influential in creating conditions for bacteria to produce NOFs. We now have a significant amount of understanding of biological pathways and genes needed for bacteria metabolize nitrate substrates and produce NOFs. But these same studies also find that environmental factors such as the amount available oxygen, pH, temperature, and water are highly influential in determining biological activity of AOB (anaerobic oxidizing bacteria) which produce NOFs by chemodenitrification (Hu, Chen, and He 2015, 729-749). None of the factors these more recent studies find important were directly measured in our study.

These findings present future directions our project can take. Perhaps by measuring soil pH at each flux measuring chamber we can tease our more of the factors causing variability in our measurements. Additionally, it’s important to note that most of the literature stating that increased nitrous oxide emissions are due to fertilizer use, speak directly of synthetic fertilizer. This is important since the use of synthetic forms fertilizer on organic farms is banned. This complicates the forms of available nitrogen to soil bacteria as farmers use “natural” sources like concentrated fish meal solutions and so forth. There exists high amounts of variability in the forms of nitrogen available in different types of fertilizers and getting a sense of what kind of fertilizer the farmers at each strawberry farm are using could also account for some of the variability in our measurements.

Some organic farmers are also more likely to compost and this could be a significant influencer of NOFs. Some papers report that the amount of available nitrogen locked up in organics or organic nitrogen is highly important as a source of nitrogen for heterotrophic denitrifiers (bacteria which use organic nitrogen as an energy source)(Parkin 1987, 1194-1199). This is implicated in studies that used stable isotopes of nitrogen to deduce that heterotrophic denitrifiers preferentially get their nitrogen from organic sources such as compost (Zhu-Barker, Doane, and Horwath 2015, 57-65). Thus a potential future study would account for the rates at which compost is added into the soils at each farm if at all.

5. Conclusion

Although we did not find any statistically significant correlations between the soil characteristics we measured and nitrous oxide fluxes (NOFs), we did find evidence that the interplay between environmental/abiotic factors and biotic factors affecting nitrous oxide generation in soils is highly complex. We used novel technology to measure accurate real-time trace GHG fluxes from organic strawberry farms in California’s central coast which we summarized and report above. Because of the documented spatial and temporal variability of

NOFs across the globe, our reported values serves as a data set which can be incorporated into higher level models and meta-analyses trying to predict global N_2O emission from agricultural soils.

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Appendix A

Soil Texture Percentages and USDA Classification by Farm

Farm Code	Sand	Silt	Clay	Soil_Class
TOMR	37.7	26.9	35.4	Clay Loam
JACS	45.7	37.3	17.1	Loam
LIVE	40.1	38.5	21.4	Loam
FOGL	50.9	34.8	14.3	Loam
SWAN	47.0	28.9	24.1	Loam
ZAMO	83.7	9.2	7.1	Loamy Sand
RACO	84.4	11.4	4.2	Loamy Sand
LULE	81.8	10.0	8.2	Loamy Sand
DEAN	82.4	8.0	9.6	Loamy Sand
CRYS	75.6	21.0	3.4	Loamy Sand
USDA	77.6	19.4	3.0	Loamy Sand
CHAV	80.7	9.7	9.6	Loamy Sand
LEON	81.7	12.7	5.6	Loamy Sand
JUDY	86.4	12.0	1.6	Sand
RICH	54.0	29.8	16.3	Sandy Loam
RASM	56.0	27.5	16.6	Sandy Loam
BLAK	63.6	26.5	9.9	Sandy Loam
HIGH	54.0	27.8	18.2	Sandy Loam
MOND	67.2	24.1	8.7	Sandy Loam
ROYF	74.8	16.8	8.5	Sandy Loam
TARP	54.6	35.4	10.1	Sandy Loam
JAVI	64.9	18.7	16.3	Sandy Loam
RIGO	62.0	25.6	12.4	Sandy Loam
BEMA	61.5	25.3	13.2	Sandy Loam
ARDI	5.0	47.4	47.7	Silty Clay
TOSA	4.6	60.0	35.4	Silty Clay Loam

Appendix B.

The technique entails inserting electronic chambers into the ground connected to a gas analyzer and vacuum pump which can circulate the air and analyze the ratios of greenhouse gases. It is called a dynamic chamber technique because, the chambers open and close in between measurements in order to allow accumulated GHGs to flow out, thus re-establishing a sort of pseudo steady-state gas diffusion (not to be confused with other techniques that actually measure steady-state gas fluxes since the accumulation of GHGs in the chamber once it is closed will slow down the diffusion process from the soils). Increasing concentrations of the GHGs are plotted with time and linear regression is performed to determine the mean flux within the time frame measured

Cavity ring-down spectroscopy utilizes the unique infrared absorption spectrum of gas-phase molecules to quantify the concentrations and isotopic compositions of H_2O, CH_4, CO_2, N_2O, and other molecules (Picarro 2015). It measures the decay rate of absorption, rather than absolute absorption of the infrared waves. By measuring the strength of the absorption, the concentrations of each gas can be determined. But in conventional infrared spectrometers, trace gases provide far too little absorption to measure, limiting sensitivity to parts per million at best. However, cavity ring-down spectroscopy avoids this sensitivity by using a path length of several kilometers, allowing gas to be monitored in seconds or less at the parts per billion level, and in some cases, parts per trillion. We used a multiplexer in order to switch between different gas chambers, located along the transect in the focal strawberry block. All machinery was set up in the back of the car, so that we did not need to lift the heavy machinery out into the field.

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