Evaluating Greenhouse Gas Emissions from Soil Application of Anaerobic Organic Digestate Compared with Conventional Manure

Guadalupe Abonce, Sharon Zhao, Michael Rodrigues, & Francesca Hopkins
Department of Microbiology

ABSTRACT

The state of California is investing in anaerobic digesters to reduce methane emissions from agriculture. However, little is known about the impact of anaerobic digesters on nitrous oxide (N_2O) and carbon dioxide (CO_2) emissions from soils after land application of digestate. The purpose of this study was to compare soil CO_2 and N_2O emission fluxes from anaerobic digestate treatment in conjunction with manure, manure treatment, and a control group without treatment on agricultural soils from two dairy farms. In addition to comparing treatments and sites, we tested the effects of temperature at either 23°C or 28°C to compare predicted future average temperatures. Soil samples were placed in mason jars with 18 jars per location: three manure treatments x 2, temperatures x 3 replications per treatment, and incubated for six weeks according to the temperature treatment. Soils were watered once a week to maintain 65% water holding capacity. Cavity ring-down spectrometers were used to collect gas emissions in a closed-loop system, and elemental analyzers were used to evaluate soil and treatment nutrient composition. We hypothesized that three main variables — manure, lower temperatures, and soils with low-nutrient content in conjunction with anaerobic digestate would all lead to lower emissions. Anaerobic digestate has been found to reduce greenhouse gas emissions while also being a nutrient-rich energy source. Microbial soil communities are also more active in warmer temperatures, which may increase the production of gas emissions. Overall, the results were inconclusive for either argument.

KEYWORDS: *Greenhouse Gas Emissions; Anaerobic Digestate; Manure; Agriculture; Soil; Incubation*

FACULTY MENTOR

Francesca Hopkins, *Department of Environmental Sciences*

Dr. Francesca Hopkins is an Assistant Professor in the Department of Environmental Sciences. She received her Ph.D. from University of California, Irvine in 2013. During her post-doc, Dr. Hopkins was a part of NASA's Postdoctoral Fellow Program, in 2014, and was announced as one of UC Irvine's Top 50 Graduate and Postdoctoral Scholar Alumni, in 2016. Currently, her research involves anthropogenic greenhouse gas emissions and their contribution to climate change and finding ways to improve our sustainability. Other research areas include trace gas emissions, terrestrial carbon cycle, and isotope biogeochemistry.

Guadalupe Abonce

Department of Microbiology

Guadalupe Abonce is a fourth year Microbiology major. She has been researching under Assistant Professor Francesca Hopkins for three months. She works at Harkins as a playcenter team member. She plans to enroll in the Pathologists Assistants Program in Loma Linda University.

Sharon Zhao

Department of Microbiology

Sharon Zhao is a fifth year Microbiology major. She has been researching under Assistant Professor Francesca Hopkins for over a year. She plans to pursue a career in environmental microbiology with the government and continue research in her field.

Evaluating Greenhouse Gas Emissions from Soil Application of Anaerobic Organic Digestate Compared with Conventional manure

INTRODUCTION

In 2017, California's agricultural industry emitted approximately 8% of the state's total greenhouse gas (GHG) emissions. Most were due to methane (CH_4) and nitrous oxide (N_2O). Compared to 1g of carbon dioxide (CO_2), CH_4 has a global warming potential of 25 g and N_2O has a global warming potential of 298 g over a 100 year time frame8. California's massive dairy industry is a large contributor to GHG; its emissions rose between 2000-2007 and have remained constant every year since[3].

For this study, we examined the impact of supplementing the traditional land application of manure with anaerobic digestate (AD) on soil GHG emissions of CO_2 and N_2O. AD is the decomposition process of organic matter without the presence of oxygen[2]. It is low in carbon, nutrient-rich, and may increase net primary productivity of crops and increase carbon sequestration in soils[3]. AD is thought to have low mineralization activity due to the stabilization of organic matter after the anaerobic process. As a result, this reduces the amount of labile carbon, which could also lower N_2O emissions[16]. Anaerobic digestion of manure may reduce CO_2 and N_2O emissions during field application in comparison to conventional practices.

Figure 1 depicts how carbon is assimilated into the soil through decomposition or sequestration; carbon is also emitted from the soil by root respiration or mineralization[13]. Nitrogen is assimilated into the soil through leaching and decomposition. It is emitted from the soil as excess during nitrification and denitrification[1]. These processes are conducted by soil microbial communities, and the rates of production respond to temperature. Increased temperatures lead to increased microbial activity[13]. However, a consensus is lacking on the temperature sensitivity of soil carbon and nitrogen decomposition after land application of organic amendments[6,18].

In this study, we sampled agricultural soil from two dairy farms in California and amended the soils with manure or a combination of manure and AD. To examine the effects of temperature, we incubated these soils at two different temperatures in the laboratory. Our hypotheses are (1) the more nutrient-rich soil will release more emissions of CO_2 and N_2O, (2) the combination of manure and anaerobic digestate will have less GHG emissions compared to only manure, and (3) the higher temperature setting would release more GHG emissions due to stimulated microbial activity.

METHODS

Materials

The purpose of this procedure was to determine soil and amendment carbon and nitrogen content. Soil samples were taken on-site at two California dairies in Tulare County (Central Valley) and Marin County (San Francisco/Bay Area). Soils were sampled up to 20 cm in depth and air-dried. Afterward, soils were homogenized

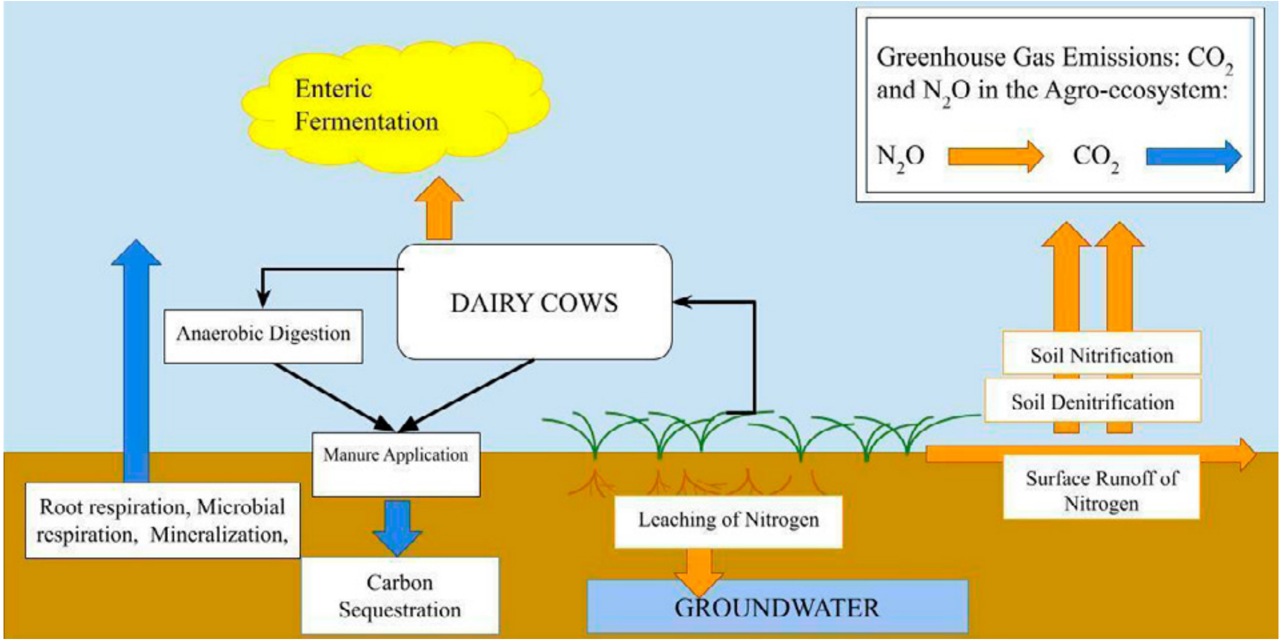

Figure 1. A representation of CO_2 and N_2O molecules circulating the ecosystem with a focus on agriculture and soil.[10]

and sieved to 2 mm. Solid manure was obtained in June 2019 from a Riverside County dairy farm, then dried and grounded to 2 mm. AD was obtained in September of 2019 from the Marin County site. In this experiment, "the anaerobic digestate" treatment is a combination of ⅓ parts digestate slurry from Marin County and ⅔ parts solid manure from Riverside County. In addition, the liquid portion of the AD was used.

We used a Fisher Scientific Flash Elemental Analyzer and a Shimadzu TOC-V to analyze the nutrient content in our soils and manure amendments. The elemental analyzer is used for solid samples, and the Shimadzu TOC-V is used for liquid samples. Soil and solid manure samples were ground to 100 µm and oven-dried for 24 hours at 105°C to remove moisture; only the AD was analyzed for TOC as a liquid. The AD we used was heavily diluted, mostly containing water, which may have affected the calculated values of total carbon and total nitrogen **(Table 1)**.

Experimental Design

Soil samples were placed in mason jars with 18 jars per location (Marin and Tulare): 3 treatments (solid manure, liquid anaerobic digestate + manure, control with no amendment) with 3 replicates. Soils were incubated for 6 weeks at designated temperature groups (23° or 28°). The mason jars were filled with 250 g of air-dried soil and were incubated for 38 days at 65% water holding capacity (WHC) for optimal microbial activity. The amendments were applied to a 560 kg N/ha nitrogen application rate. During the experiment, jars were weighed once a week to determine the amount of water loss. Water was added to maintain a 65% WHC before each measurement.

Soil Emission Measurements

Soil emission fluxes were measured using a Cavity Ringdown Spectroscopy (CRDS) in a closed-loop chamber system. CRDS uses a single-frequency laser and a photodetector to create a continuous traveling light wave. It is able to detect small amounts of light through its three-mirror cavity and emit an amplified signal correlated to the frequency inside the cavity[5,7]. Compared with the traditional method of Gas Chromatography (GC), CRDS overall has performed better than GHG and has a more consistent linear response with CO_2[5]. A chamber system amplifies measurements significantly; therefore, smaller emissions can be detected given low instrumental precision[10].

The three CRDS instruments, on an average of five minutes, had a precision rate as follows: G2308, N_2O < 3.5 ppb +/- 0.05%, G2210i and G2401 for CO_2 < 200 ppb and < 20 ppb of reading, respectively[12]. Given that a shorter enclosure time reduces systematic errors in chambers, the measurement time we used was 10 minutes[5]. Measurements took place frequently for the first two weeks and then biweekly for the remainder of the experiment.

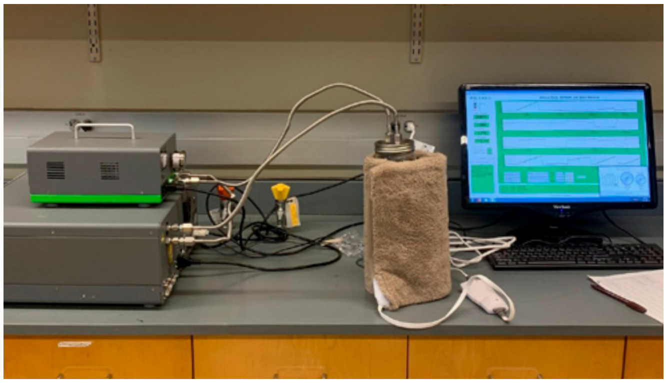

Figure 2. Our set up for measuring an elevated jar and a side view of the CRDS

In order to keep the elevated temperatures consistent, a heating pad was kept on a low setting and wrapped around each elevated mason jar for each measurement. A 12" stainless steel tube collected gas from the jar lid into the CRDS and is represented on the computer screen. A 6" stainless steel tube generated recirculating air inside the jar's headspace. This was generated by a recirculating pump on top of the CRDS. The recirculating pump also has a tube attached to the CRDS **(Figure 2)**.

Flux Measurements & Calculations

The flux measured from the CRDS is in gas units using equation (1), which is then converted into mass concentration (mg/time) using the Ideal Gas Law in equation (2).

Moles of CO_2 or N_2O varied by the slope (ppm/sec) =

End Time - Start Time

Equation 1

PV = nRT

Equation 2

Our calculations yielded the following results: 101325 Pa × 6.697 × 10-4 m³ (headspace) = (mol of CO_2 or N_2O) * 8.314 (R Constant) * 298.15 K

Then, the actual flux (mass/time/area) of both CO_2 and N_2O were calculated using the Closed Chamber System Equation in equation (3), where J is the actual flux (mg CO_2/g soil), V (headspace volume), A (soil area), and ΔC (change in gas concentration) over ΔT (change in time)[9]:

$$J = (V/A) * (\Delta C / \Delta T)$$

Equation 3

We converted J to calculate the cumulative emissions to mass/g soil basis. Using the data from **Table 1**, we normalized emissions by dividing the mass of C/N per g of soil to the final form of mass emitted/ mass C or N. After three replicates of each condition, the cumulative emissions were averaged to calculate the standard deviation and standard error.

For our statistical analysis, we used a paired sample *t*-test for 2-means to make direct comparisons and to find the p-values. For our overarching factors, a statistical analysis of 3-Factor Fixed Analysis of Variation (ANOVA) was done using the XRealStats resource package. Both were done in Excel with an alpha of 0.05.

RESULTS

Normalized Emissions for Carbon Dioxide and Nitrous Oxide

Average fluxes for CO_2 and N_2O were separated into **Figure 3** and **Figure 4.** In the figures, the two locations were separated into two graphs to visualize any statistical significance when using the *t*-test. Both Marin and Tulare had no statistical significance between the ambient and elevated temperatures for both GHGs. The Marin soil also had no statistical significance when comparing manure treatments and AD treatments for both gases **(Figure 3; Figure 4)**.

For Tulare, CO_2 emissions were significantly reduced for ambient temperature AD compared to ambient manure (p-value of 4.7E-02) (**Figure 3**). When analyzing the N_2O emissions for Tulare, statistical significance was seen between elevated temperature manure and elevated temperature AD (p-value of 7.0E-03), with higher N_2O emissions from the manure only treatment (**Figure 4**). However, data referring to N_2O should not be absolute as the standard error was high, indicating large variability in emission rates within a treatment.

Three-Factor Fixed ANOVA

When looking at the overall factors that may influence the fluxes of CO_2, we used ANOVA to identify statistical significance in location (p = 8.4E-11), temperature (p = 2.4E-04), and treatment (p = 7.9E-10). Significance was also seen when location and treatment interacted (p = 4.5E-05) and when all three experimental factors interacted (p = 1.3E-02). However, there was no significant interaction of temperature and location or temperature and treatment alone. For N_2O, the only statistical significance came from treatment (p = 1.9E-03). All other experimental factors and interactions were insignificant.

Carbon and Nitrogen Soil Content Analysis

The results of the elemental analyzer and Shimadzu TOC-V were used to determine the amount of carbon and nitrogen content in soils and amendments **(Table 1)**. Marin County soil had a higher carbon and nitrogen soil content compared with Tulare County soil.

DISCUSSION

In this experiment, we only saw that significant differences in GHG emissions from the Tulare location soils from Marin had no significant treatment effects. Normalized CO_2 emissions were higher

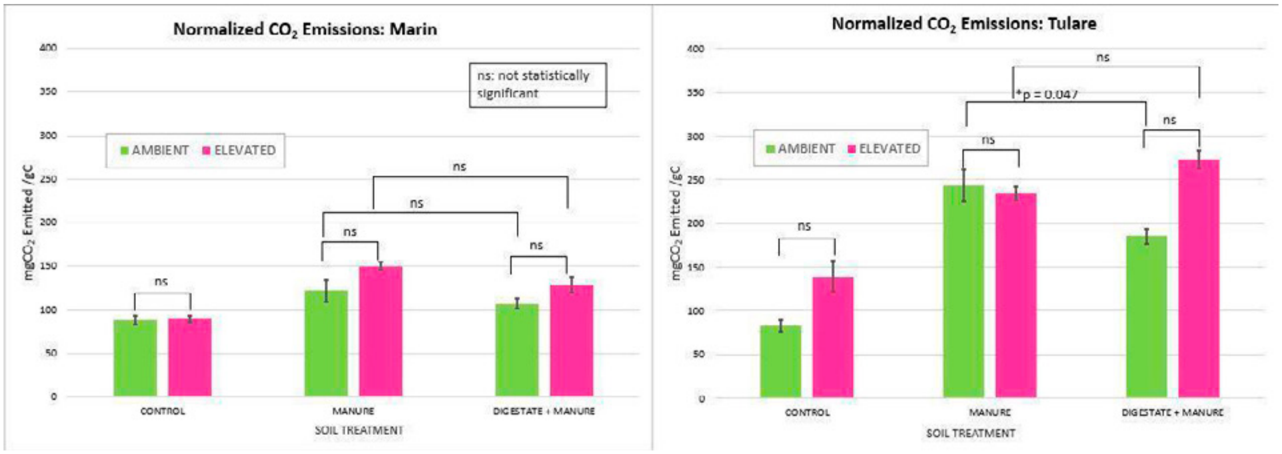

Figure 3. Side by side comparison of Marin County (left) and Tulare County (right) showing temperature differences and different soil amendments affecting average emissions of CO_2.

from Tulare for AD under both temperatures and for manure at the elevated temperature compared to Marin. Thus, the location plays a major role in the stability of carbon and nitrogen in the soil. In **Figure 3**, we saw an increase in CO_2 emissions in Tulare AD, as a result of temperature. This indicates that the interaction between this location and this treatment is sensitive to temperature and will have implications for this site's future soil management when average temperatures are warmer.

N_2O emissions are significantly less from soils amended with manure compared to those amended with AD at elevated temperatures **(Figure 4)**. The lack of statistical significance in N_2O emissions may indicate rapid N_2O loss immediately after application and before measurement, or there might not have been enough AD in our mixture to see a difference. A possible reason for AD having high N_2O emissions is its high concentration of NH_3 and NH_4^+, correlating to the loss of nitrogen through nitrification rather than denitrification activity. There were only statistical differences found between the controls; however, this is likely due to the Marin soil's higher nitrogen content **(Table 1)**. The technology may also be a factor in large standard error values for N_2O. CRDS showed a more consistent linear response to increasing concentrations of CO_2 but not N_2O[5]. Even microbial soil communities will naturally leak NO and N_2O as byproducts. These are dependent on factors that affect microbial growth such as soil type, WHC, and tillage practice[17]. Due to the variable possibilities and large variability between replicates we observed, it is difficult to quantify N_2O emissions.

Since our temperature analysis lacked findings, its effect on CO_2 & N_2O is unclear. A lack of temperature effect is seen in several other studies. For example, researchers in Agriculture and Agri-food Canada saw larger N_2O emissions correlated with an increase of N-fertilizer application and higher daily minimum temperatures; however, annual estimates of N_2O emissions were dependent on the timing of rainfall and snow melts[17]. Another study by Jansen at the University of Iceland discovered that CO_2 and CH_4 emissions did increase with temperature, while N_2O emissions did not vary between temperatures. Instead, NO_2 emissions were driven by water fluctuations[11].

Based on our results, land application of AD does not appear to decrease soil GHG emissions compared to traditional manure applications. However, the production of AD is still beneficial as it captures methane, and the application of AD does not appear to change soil GHG emissions. More research needs to be done

Table 1. Initial amounts of carbon and nitrogen found in Tulare and Marin Counties depicted by percentage.

	Total Carbon*	Total Nitrogen*
Tulare County Soil	1.79%	0.24%
Marin County Soil	4.21%	0.35%
Solid Manure	18.32%	1.71%
Anaerobic Digestate	0.1060%	0.0418%

*The units are in a per mass scale of soil.

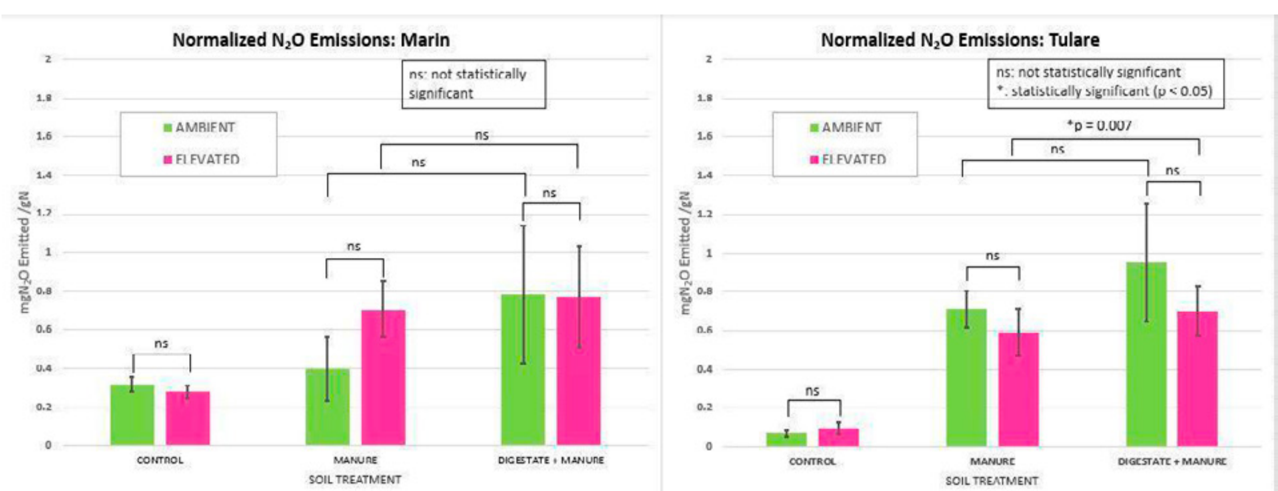

Figure 4. Side by side comparison of Marin County (left) and Tulare County (right) showing temperature differences and different soil amendments affecting average emissions of N_2O.

since a laboratory experiment cannot fully represent a field-scale experiment. In the field, factors can easily alter soil respiration, soil dynamics, and microbial community structure resulting in an impact on GHG emissions[7].

CONCLUSION

The purpose of this study was to compare the effects of GHG emissions from soils amended with a manure treatment and manure + anaerobic digestate treatment on 2 different agricultural soils under different temperature conditions. We used CRDS to collect CO_2 and N_2O emissions and elemental analyzers to measure the nutrient content of soils and amendments. However, findings were lacking in statistical significance and in evidence to support all our hypotheses. Future studies should include more AD, which may reveal differences not currently seen since the AD treatment used here is only ⅔ manure. Furthermore, since the elevated temperature did not show strong differences, it should be set higher. However, this shows that GHG emissions from amended soils are stable in an increased climate. The focus on the properties and factors of a single location would narrow the variety of data and its implications. These results demonstrate the importance of factors such as site-specific management, location, and even how future changes in temperature will affect carbon and nitrogen cycling in the area when considering the land application. The intention of land application should both benefit the soil and result in the reduction of harmful GHG emissions.

ACKNOWLEDGMENTS

We would like to thank our hard-working graduate student and mentor, Michael Rodrigues. Together, we made it through those long hours of measuring gas emissions. We also would like to thank our PI and professor, Dr. Francesca Hopkins, for all her support and guidance. As she has said, the soil is sexy, and we should look more into these hot topics.

REFERENCES

1. Bernhard, A. (2010) The Nitrogen Cycle: Processes, Players, and Human Impact. *Nature Education Knowledge*, 3(10):25.

2. Breunig, G., Amirebrahimi, J., Smith, S., Scown, C. (2019). Role of Digestate and Biochar in Carbon-Negative Bioenergy. *Environ. Sci. Technol.* 53, 22, 12989-12998a. doi: 10.1021/acs.est.9b03763.

3. California Air Resources Board. (2019). California Greenhouse Gas Emissions for 2000 to 2017. *California Greenhouse Gas Emission Inventory: 2000 – 2017*, 1–24. Retrieved from https://ww3.arb.ca.gov/cc/inventory/pubs/reports/2000_2017/ghg_inventory_trend 00-17.pdf

4. CalRecycle (2011). Anaerobic Digestion Program Environmental Impact Report. Retrieved from https://www2.calrecycle.ca.gov/PublicNotices/Documents/1453?opt=dln.

5. Christiansen, J. R., Outhwaite, J., & Smukler, S. M. (2015). Comparison of CO_2, CH_4 and N_2O soil-atmosphere exchange measured in static chambers with cavity ring-down spectroscopy and gas chromatography. *Agricultural and Forest Meteorology*, 211–212, 48–57. DOI:10.1016/j.agrformet.2015.06.004

6. Davidson, E. A., & Janssens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature, 440, 165–173. DOI:10.1038/nature04514

7. Fleck, D., He, Y., Alexander, C., Jacobson, G., & Cunningham, K. L. (Picarro Inc.) (2013). Simultaneous soil flux measurements of five gases — N_2O, CH_4, CO_2, NH_3, and H_2O — with the Picarro G2508. Santa Clara, CA, USA. Application Note 034.

8. Forster, P., V. Ramaswamy, P. Artaxo, et al. (2008): Changes in Atmospheric Constituents and in Radiative Forcing. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the IPCC, S. Solomon et al. (eds.), *Cambridge University Press*, Cambridge, UK, Chapter 2. Retrieved from http://www.cambridge.org/catalogue/catalogue.asp?isbn=9780521705967.

9. Gao, F., and Yates, S. R. (1998), Laboratory study of closed and dynamic flux chambers: Experimental results and implications for field application, J. *Geophys*. Res., 103 (D20), 26115– 26125, DOI:10.1029/98JD01346.

10. Hensen, A., Skiba, U., & Famulari, D. (2013). Low cost and state of the art methods to measure nitrous oxide emissions. *Environmental Research Letters*, 8(2). DOI:10.1088/1748-9326/8/2/025022

11. Jansen, E., Gudmundsson, J., Óskarsson, H. (2008). The effects of land use, temperature and water level fluctuations on the emission of nitrous oxide (N_2O), carbon dioxide (CO_2) and methane (CH_4) from organic soil cores in Iceland. (Master's Thesis) *University of Iceland*, Iceland.

12. Liu, H., Li, J., Li, X., Zheng, Y., Feng, S., & Jiang, G. (2015). Mitigating greenhouse gas emissions through replacement of chemical fertilizer with organic manure in a temperate farmland. *Science Bulletin*, 60(6), 598–606. DOI: 10.1007/s11434-014-0679-6

13. Nottingham, A. T., Bååth, E., Reischke, S., Salinas, N., & Meir, P. (2019). Adaptation of soil microbial growth to temperature: Using a tropical elevation gradient to predict future changes. *Global Change Biology*, 25(3), 827–838. DOI: 10.1111/gcb.14502

14. Picarro. (2018), Gas Concentration Analyzer. Retrieved from https://www.picarro.com/support/documents?doctype=748

15. Rotz, C.A., Montes, F. and Chianese, D.S. (2010), The Carbon footprint of dairy production systems through partial life cycle assessment. *Science Direct*, 93:3 1266-1282. DOI: 10.3168/jds.2009-2162

16. Schouten, S., van Groenigen, J.W., Oenema, O. and Cayuela, M.L. (2012), 'Bioenergy from cattle manure? Implications of anaerobic digestion and subsequent pyrolysis for carbon and nitrogen dynamics in soil'. *Glob. Change Biol. Bioenergy*, 4: 751-760. DOI:10.1111/j.1757-1707.2012.01163.x

17. Smith, W., Grant, B., Desjardins, R. et al. (2004). Estimates of the interannual variations of N_2O emissions from agricultural soils in Canada. *Nutrient Cycling in Agroecosystems*, 68, 37–45. DOI: 10.1023/B:FRES.0000012230.40684.c2

18. Wei, H., Chen, X., He, J., Zhang, J., & Shen, W. (2017). Exogenous nitrogen addition reduced the temperature sensitivity of microbial respiration without altering the microbial community composition. *Frontiers in Microbiology*, 8(DEC), 1–13. DOI:10.3389/fmicb.2017.02382