ABSTRACT

KNIGHT, ALEXANDRA MARIE. Greenhouse Gas Emissions in Long Term Agricultural Production Systems. (Under the direction of Drs. Samuel Reberg-Horton and Wesley J. Everman).

Global estimates of greenhouse gas emissions indicate that agriculture accounts for approximately 10 percent of emissions. The gases carbon dioxide (CO_2), methane (CH_4) and, nitrous oxide (N_2O) make up roughly 97% of contributions to climate change. Agricultural practices largely contribute CH_4 and N_2O with row crops contributing most of this N_2O. Cropping management practices contributed approximately 75% of N_2O emissions. One reason N_2O is of greatest concern is that N_2O has approximately 12 times the energy holding capacity, or global warming potential, of CH_4 and 300 times that of the baseline gas, CO_2. Research was conducted in Goldsboro, NC (35.38743 N, -78.03442 W) at the Cherry Research Farm, Center for Environmental Farming Systems in long-term cropping systems from April 2013 to September 2015 to estimate nitrous oxide (N_2O) emissions over time. Emissions were measured using the static chamber method in six long-term cropping systems including: conventional tilled (CT), conventional till-hay (CTH), conventional no-till (CNT), organic tilled (OT), organic till-hay (OTH), and organic minimum till (OMT). In a separate study, weedy and weed-free conditions were compared in these six systems. In a final study, the input of combinations of urea ammonium nitrate (UAN) and herbicides of 2,4-D, atrazine, chlorimuron, dicamba, flumioxazin, glyfosinate, glyphosate, isoxaflutole, mesotrione, nicosulfuron, paraquat, pendimethalin, and *s*-metolachlor were compared on a bare soil. Within cropping systems, those systems with greater inorganic nitrogen, or conventional systems, were predicted to emit greater nitrogen. However, it appeared that each system had dates with the greatest N_2O emissions and, no

single system continuously had the greatest N_2O emissions. When comparing weedy and weed-free treatments, weedy plots were anticipated to mitigate N_2O by removing free nitrogen from the soil. Regarding weedy and weed-free treatments, results varied with organic and conventional system type. In those systems managed organically, nitrogen was the limiting factor in denitrification. Those plots which were weed-free had more free nitrogen and emitted more N_2O. Within conventionally managed systems, carbon was the limiting factor in denitrification. Therefore, in those plots with weeds, weeds served as a carbon source for denitrification and promoted N_2O emissions. The final study was predicted to show greater N_2O with greater nitrogen applied and herbicides were predicted to show minimal differences in N_2O. Results indicated greater N_2O is shown with greater nitrogen applied. Additionally, sulfonylureas have the potential to mitigate N_2O while chloroacetamide herbicides have the potential to promote N_2O emissions.

Greenhouse Gas Emissions in Long Term Agricultural Production Systems

by
Alexandra Marie Knight

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APPROVED BY:

Dr. Samuel Reberg-Horton Committee Co-Chair	Dr. Wesley J. Everman Committee Co-Chair
Dr. Shuijin Hu	Dr. David Jordan

Dr. Nancy Creamer

DEDICATION

Dedicated to my sister, and best friend, whose support, encouragement, and love has made this endeavor and all others possible.

BIOGRAPHY

Alexandra Knight grew up on a beef cattle farm in east central Ohio in the town of Cadiz. Here, her family raised black Angus cattle, Polypay sheep and grew hay. Alexandra was born the eldest daughter of Anthony and Mary Knight and had a love for agriculture instilled in her at a young age. In 2007, Alexandra graduated with honors as Valedictorian of Harrison Central High School. The following year, she finished her 14th and final year in 4-H and received her American FFA Degree. During Alexandra's undergraduate career she was a member of the Science Club, the student founder of the Walsh University Chapter of the Sigma Zeta Honor Society, and a member of the University Honors Program. Alexandra also held a two year research internship at the Ohio Agricultural Research and Development Center where she worked under the direction of Dr. Parwinder Grewal in Entomology. She completed her senior honors thesis titled, "Active Carbon as a Useful Predictor of Urban Soil Quality and Plant Growth" under the direction of Drs. Jennifer Clevinger at Walsh University and Dr. Parwinder Grewal at Ohio State University. She graduated in the spring of 2011 with University Honors and Magna Cum Laude with a B.S. in Biology and minors in Chemistry and Environmental Studies. Alexandra completed her M.S. degree under the direction of Dr. Wesley Everman in June of 2013 with a thesis entitled “Impact of Nitrogen Source, Rate, and Weed Removal Time on Nitrogen Availability to Corn.” Following completion of her M.S., Alexandra began her Ph.D. in the Crop Science Department under the direction of Drs. S. Chris Reberg-Horton and Wesley Everman.

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NITROUS OXIDE OUTPUT BASED ON WEED MANAGEMENT IN AGRICULTURAL SYSTEMS

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CHAPTER 1: IMPACT OF LONG TERM PRODUCTION SYSTEMS ON GREENHOUSE GAS EMISSIONS

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Knight, Alexandra M.[1], Wesley J. Everman[1], Samuel Reberg-Horton[1], Shuijin Hu[2], David Jordan[1], and Nancy Creamer[3]

[1]Graduate Student, Assistant Professor, Associate Professor, Professor North Carolina State University Department of Crop Science. [2]Professor North Carolina State University Department of Plant Pathology. [3]North Carolina State University Department of Horticultural Science.

Abstract

Row crop management practices contribute the greatest percentage of global N_2O emissions emitted by agriculture. These emissions have an energy holding capacity 12 and 300 times that of the greenhouse gases CH_4 and CO_2, respectively. Tillage and fertilizer application have been shown to be large contributors to N_2O production. Research was conducted in Goldsboro, NC (35.38743, -78.03442) in a long-term cropping systems trial from April 2013 to September 2015. Emissions were measured using the static chamber method in six long-term cropping systems including: conventional tilled (CT), conventional till-hay (CTH), conventional no-till (CNT), organic tilled (OT), organic till-hay (OTH), and organic minimum till (OMT). A significant system x collection date interaction demonstrated that different systems have the greatest emissions on different dates dependent on field events. Annual emissions showed no differences in the more conservative

estimation of trapezoid + decay integration. Decay integration alone, showed greater seasonal and annual emissions in organic systems over that of the conventional.

Introduction

Global estimates of greenhouse gas emissions indicate that agriculture accounts for approximately 10 percent of these emissions. The gases carbon dioxide (CO_2), methane (CH_4) and, nitrous oxide (N_2O) make up roughly 97% of what is contributing to global warming (Environmental Protection Agency 2015; IPCC 2007). Agricultural practices largely contribute CH_4 and N_2O with row crops contributing most of the N_2O. Crop management practices of fertilizer, tillage, irrigation, and burning of crop residues contribute approximately 75% of N_2O emissions in the U.S. (Environmental Protection Agency 2015). N_2O is of greatest concern primarily because N_2O has approximately 12 times the energy holding capacity of CH_4 and 300 times that of CO_2 (Environmental Protection Agency 2015; IPCC 2007) and is trending upward over time. This general rise in greenhouse gas emissions became pronounced after the start of the Industrial Revolution in the 1750's. An increase of 18.2% in N_2O emissions was observed between 1990 and 2013 (Environmental Protection Agency 2015). Today, the annual net increase in N_2O emissions is between 0.6 and 0.9 ppb yr^{-1}. These levels are expected to continue rising with the expected increase in global nitrogen fertilizer use, particularly in developing countries (Cavigelli et al. 2012).

N_2O is produced in agricultural systems through denitrification, chemo-denitrification, and nitrification in the form of nitrifier denitrification or hydroxylamine oxidation (Bateman and Baggs 2005; Venterea et al. 2012; Environmental Protection Agency 2015; Thangarajan et al. 2013). Bacteria, fungi, archaea, and yeast conduct denitrification in anaerobic soils and are considered to be the main contributing factor of N_2O emissions (Thangarajan et al. 2013; Bateman and Baggs 2005).

While each of these factors contributes to N_2O emissions, a number of factors determine whether denitrification and nitrification occur and to what degree these processes contribute N_2O. Oxygen supply, temperature, soil pH, water content, nitrogen and carbon sources, and organic matter can impact N_2O emissions (Thangarajan et al. 2013). Higher levels of O_2 supply lead to a more aerobic environment and less N_2O production (Thangarajan et al. 2013). Both an increase in temperature and soil pH will promote N_2O production by increasing microbial activity. Water content is different in that below a 60% water filled pore space (WFPS) nitrification will be the primary process conducted and therefore a means of N_2O production. When above 60% WFPS, ideal conditions for denitrification are present and therefore N_2O production occurs. N_2O production occurs when neither diffusion of substrates nor diffusion of oxygen is restricted (Bateman and Baggs 2005; Chirinda et al. 2010). However, above a WFPS of 80%, most N_2O will go through complete denitrification and become N_2. Denitrification depends on carbon and nitrogen content in the soil as carbon serves as an electron donor and nitrogen (mostly NO_3^-) an electron acceptor. With NO_3^- as the primary electron acceptor, previous studies have demonstrated it is one of the most important predictors in N_2O emissions (Thangarajan et al. 2013; Ruser et al. 2006; Strong and Fillery 2002).

In addition to the effects of the soil environment on N_2O emissions, larger scale factors such as climate and management practices impact N_2O emissions (Thangarajan et al. 2013). Climate determines whether N_2O emissions are primarily contributed by nitrification or denitrification. In arid to semi-arid environments, nitrification will occur readily and is the primary driver of N_2O production due to limited moisture in this environment. In a more humid or temperate climate, denitrification will drive N_2O production as moisture levels are ideal for this process (Thangarajan et al. 2013). Additionally, an increase in temperature will result in an increase in N_2O emissions. This can be noted both seasonally and daily. On a seasonal basis the greatest emissions are noted in the summer while N_2O emissions also rise with daily temperature (Parkin and Venterea 2010). Research has shown agricultural management practices of rotation, crop, fertilizer, tillage, and pesticides all impact N_2O emissions.

Studies have shown different N_2O emission levels with different crops grown. One study showed that those systems in continuous row crops had greater NO_3^- concentrations than those which had pasture worked into the rotation (Perdomo et al. 2009). Another study showed the greatest emissions in a conventionally-tilled soybean and a no-till rye treatment (Baggs et al. 2003). This was attributed to the incorporation of plant residues in the case of the soybeans and availability of readily degradable carbon in an anaerobic environment in the case of the rye. In another study, greater N_2O emissions in corn rotations compared to soybean due to the nitrogen applications linked with a corn rotation (Parkin and Kaspar 2006).

Differences have been noted not only between crops but also between management types of organic and conventional. A study comparing systems in five European countries showed that differences in organic versus conventional systems were location dependent (Petersen et al. 2006). Research in the countries of Italy, Finland, Denmark, and the United

Kingdom all showed conventional systems had greater N_2O emissions than the organic systems. However, in Austria the organic systems showed greater N_2O emissions than the conventional. The authors mention that Austria had greater rainfall than the other countries during this season and, the treatments in Austria had only been in place for two years prior to measuring gas emissions. Both of these factors may have contributed to the differing results in emissions in Austria (Petersen et al. 2006). Another study was able to tie greater N_2O emissions to greater levels of inorganic nitrogen in the soil on a system by system basis (Burger et al. 2005). Research mentioned while N_2O emissions were greater in conventional systems on an area basis, this was not the case when quantified on a per yield basis (Flessa et al. 2002). No statistical differences between emissions of N_2O in organic versus conventional systems were noted in another study (Chirinda et al. 2010).

Fertilizer regimes have also been evaluated with those systems where nitrogen fertilizer was added resulting in greater N_2O emissions when compared to the unfertilized. The fertilizers resulting the greatest N_2O emissions were synthetic fertilizers such as ammonium nitrate (Baggs et al. 2003) while other research showed organic fertilizers to have greater N_2O emissions than synthetic fertilizers (Kaiser and Ruser 2000). Additionally, the way in which a fertilizer is applied can impact N_2O emissions. Surface applied fertilizers emitted two to seven times greater N_2O than fertilizers which have been tilled into the soil (Baggs et al. 2003). This was a result of an ideal denitrification situation due to anaerobic conditions with mineralized carbon and inorganic sources of nitrogen (Baggs et al. 2003). Greater N_2O emissions have been shown in banded applications over that of broadcast applications (Deng et al. 2015). In addition to early season fertilizer applications, the degradation or incorporation of crop residues has also shown to increase N_2O emissions (Baggs et al. 2003).

Some research has investigated the impact of the combination of fertilizers and tillage. In a study by Venterea et al. (2011) tillage and nitrogen fertilizer sources were compared in corn systems which had been in place for over 17 years. Nitrogen fertilizer treatments included a polymer coated urea, conventional urea, and a urea injected with an enzyme inhibitor. N_2O emissions were quantified on a per area basis, grain yield, grain nitrogen levels, and total aboveground nitrogen. No differences were noted between treatments when N_2O emissions were quantified on a per area basis. No-till treatments were also quantified by grain yield, grain nitrogen levels, and total aboveground nitrogen showing 52%, 66%, and 69% greater N_2O emissions than the tilled, respectively. Regarding fertilizer treatments, there was no statistically significant differences. However, the combination of conventional tillage and polymer coated urea emitted the greatest N_2O followed by no-till combined with conventional urea or polymer coated urea. The lack of statistical significance between fertilizer treatments is likely due to fertilizers being applied several weeks after emergence and at a critical growth stage for the corn crop. This provided less opportunity for microbiota to transform nitrogen with nitrification and denitrification before plant uptake (Venterea et al. 2011).

While incorporating fertilizers with tillage can decrease N_2O emissions, tillage has often caused increases in N_2O emissions (Omonode et al. 2011; Baggs et al. 2003; Goglio et al. 2013; Jin et al. 2014). In one study where the authors noted a general trend of increased nitrous oxide emissions with tillage, plots using the chisel plow showed the greatest emissions, followed by a mold board plow and the least emissions in a no-till system (Omonode et al. 2011). Denitrification tends to be more rapid in conventionally tilled soils compared to those fields not tilled. Two factors are suspected to enhance denitrification including the tillage supplying carbon and nitrogen to microorganisms within the soil and promoting decomposition and mineralization of residues thereby promoting denitrification and the production of N_2O (Goglio et al. 2013; Bateman and Baggs 2005). Fields with recent conversion to no-till practices have demonstrated greater emissions than those with conventional tillage (Cavigelli et al. 2012; Benckiser and Schnell 2007; Grandy et al. 2006). This initial increase in N_2O emissions was a result of higher water content, increased bulk density, decreased diffusion of oxygen, and greater carbon due to increased soil organic matter decomposition on the surface (Cavigelli et al. 2012). Fields where no-till conversion occurred less than ten years prior showed greater N_2O emissions than conventional till (Benckiser and Schnell 2007; Grandy et al. 2006). However, those fields where no-till had been in place between ten and twenty years showed no difference in N_2O emissions in conventionally tilled systems (Benckiser and Schnell 2007). Other studies have shown no statistical difference between emissions in gases with different tillage management practices (Parkin and Kaspar 2006; Robertson et al. 2000; Smith et al. 2012).

While many agricultural systems likely have practices both promoting and mitigating N_2O emissions, the most influential processes vary by climatic regions. In the southeastern U.S., and North Carolina in particular, the sandy soils and humid environment are suspected to lead to emission events in which N_2O rapidly spikes. However, little research has been done on N_2O emissions in the southeastern U.S. This research aimed to fill the void in N_2O

emission data in the southeastern U.S. The three objectives of this research were to: (i) investigate differences in N_2O emissions for three conventional and three organic cropping systems, (ii) detect annual fluxes for these six systems and (iii) determine which cropping system is the best at mitigating N_2O emissions. Identifying best management practices for mitigating N_2O emissions in North Carolina could be applicable to the southeastern U.S. and other similar climatic regions of the world.

Materials and Methods

Research was conducted annually in Goldsboro, NC at the Cherry Research Farm, Center for Environmental Farming Systems (35.38743 N, -78.03442 W) from April 2013 to September 2015. A total of six long-term cropping systems were used. Most of these six systems were established in 1996 and consist of conventional no-till, conventional tilled, conventional crop-hay, organic minimum till, organic full till, and organic crop-hay system (Table 1). The study was organized in a randomized complete block design and blocked according to soil textured classification. Block 1 consisted of a Tarboro loamy sand (Typic Udipsamments), block 2 consisted of a Wickham sandy loam (Typic Hapludults), and block 3 consisted of a combination of State loam (Typic) and Tarboro loamy sand (Typic Udipsamments).

Plot size was 8 rows for a width of 6.0 meters with static gas chambers placed between rows 6 and 7. Gas chambers were made of stainless steel and measured 1211 cm^2 with a volume of 13.48 L. Gas samples were taken within 24-48 hours following a rainfall event of ≥ 1.25 cm. These gas samples were collected at time 0, 10, 20, and 30 min.

following chamber lids being water sealed to capture gas emissions over time (Parkin and Venterea 2010). These 5 mL gas samples were collected in N flushed crimp top vials and taken between 9:00am and 1:00pm to capture the average daily temperature. N_2O emissions will rise with increasing temperature. This is the result of higher temperatures leading to greater microbial activity and therefore greater rates of denitrification. Collecting samples during the day's average temperatures, results in a more accurate calculation of the day's emissions. Sampling during the daily high would result in an overestimation of daily N_2O emissions and, sampling during the daily low temperature would result in an underestimation of daily N_2O emissions (Parkin and Venterea 2010). Gas chromatography was used to measure content of N_2O for each chamber. A multipoint standard curve was used to determine concentration of N_2O within each vial.

Chamber data were analyzed through the HMR program in R statistical software based on previous research (Pedersen et al. 2010). The manual operation for this program was used in which the user can view the program's selection of HMR or linear for a particular chamber. Chamber data were used based on program recommendations and data was calculated to reflect N_2O emissions per area and unit time.

Values from the HMR program were log transformed and run through a repeated measures analysis in PROC MIXED using SAS 9.4. Within the repeated measures analysis, multiple adjustments were used for the variance-covariance matrix. Models of spatial power law (SP(POW)), heterogeneous compound symmetry (CSH), unstructured (UN), a separate residual per system, a CSH with yearly grouping, and a general mixed model with date as a split-plot factor were all tested. A best fit model was selected based on Akaike Information

Criterion (AIC), Bayesian Information Criterion (BIC), and Akaike's Information Criterion Corrected (AICC) values. A mixed model analysis using date as a split-plot factor was a better fit than models tested within repeated measures analysis.

Annual flux was calculated using the trapezoid method, or linear interpolation, in which data points were connected with a straight line and the area under these points was considered annual flux. A second method was used in which N_2O emissions considered baseline, or ambient, used linear interpolation. Sampling events resulting in values greater than baseline used a decay function (N_2O flux = A $e^{(-Bx)}$) to integrate from the sampling time value of N_2O emissions back to a baseline or ambient value. Previous research has indicated that real-time N_2O data follows the patterns of peak N_2O emissions following a decay function when declining. However, during portions of the season when N_2O is at ambient values, a linear interpolation seems appropriate. In this previous research, spatial variability was present for decay function following a peak N_2O event. However, the "B" value showed little variability between chambers (Figure 1). This indicates that while spatial variability may be present in the quantity of N_2O produced in a field, the pattern which the data will follow in N_2O emission decline is well defined. The "B" value used in this formula was 0.0384 as previous research at this site determined this decay in real-time data while, the "A" value used was that detected with the static chamber method (Ross 2016). Seasonal fluxes were calculated in the same two forms but focused on data from April through September of 2013, 2014, and 2015.

Cumulative fluxes were analyzed in PROC MIXED using a one-way ANOVA for cropping system. Seasonal fluxes were analyzed in PROC MIXED using a two-way ANOVA of cropping system and year.

Results

Individual Dates

Within the repeated measures analysis, the mixed split-plot approach was the best fit of any repeated measures design. The main effect of date was significant ($p \leq 0.01$) as was the interaction of system x date ($p \leq 0.01$). System differences were noted on 14 dates in 2013, 15 in 2014, and 8 in 2015 (Table 2). These dates and significance are listed in Table 2. Across years, the majority of significant dates were between April and September, with 78, 73, and 62% of significant events for 2013, 2014, and 2015, respectively.

Seasonal and Multi-Year Flux

The two-way analysis for trapezoidal seasonal (April-September) flux indicated that there was a main effect of year ($p \leq 0.05$) (Table 3). 2014 and 2015 results indicated a greater seasonal flux of 1366 mg N_2O m^{-2} in 2015 with a corn crop and of 533 mg N_2O m^{-2} in 2014 with a soybean crop. 2013 had a corn crop and showed the least amount of emissions. The main effect of system was also significant ($p \leq 0.05$) as was the interaction of system within year ($p \leq 0.05$). While looking at seasonal differences with the trapezoid method (Figure 5), 2015 showed the greatest differences in systems ($p \leq 0.10$) with N_2O fluxes totaling between 813 and 2417 mg N_2O m^{-2} for this time period (Figure 6). The conventional till-hay rotation showed the least N_2O emissions in this year while the OMT showed the greatest overall emissions. The organic systems all showed greater emissions than the conventional during 2015.

Annual flux calculations using the trapezoid method indicated cumulative fluxes for this experiment between 2431 and 4397 mg N_2O m^{-2}, with the CNT system emitting the least and the OMT emitting the most. Flux differed for systems at a $p \leq 0.10$ (Figure 6).

Using the combined approach of trapezoid and decay function, a significant main effect for year was observed ($p \leq 0.01$). This seasonal flux was 193, 312, and 458 mg N_2O m^{-2} for 2013, 2014, and 2015, respectively. Seasonal differences were not observed among the six cropping systems ($p \leq 0.05$) with values of between 256 and 347 mg N_2O m^{-2} for April through September.

Cumulative fluxes also did not show differences between the six cropping systems ($p \leq 0.05$). Cropping system cumulative fluxes had values of 1442, 1627, 1686, 1710, 1808, and 1972 mg N_2O m^{-2} for CNT, CTH, CT, ONT, OT, and OTH, respectively.

Discussion

Individual Dates

The main effect of date was significant ($p \leq 0.01$). Summer months had overall greater emissions than that of winter months as higher temperatures promoted microbial activity and denitrification (Thangarajan et al. 2013; Bhandral et al. 2007). Greater emissions during summer months was demonstrated by the fact that 60% or greater of significant dates were between April and September for each year.

The interaction of system and date indicated that different cropping systems had the greatest emissions depending on the date. In 2013, significant differences were noted in cropping system shortly after planting and fertilizer application (Figure 2, April 21). The April 30 sampling event closely aligned with the first tillage event and showed the organic systems which were tilled weekly to have increased in N_2O emissions. This further supports the argument by previous research indicating tillage promotes N_2O production by moving soil nutrients and making these more accessible to soil microbiota and increasing the rate of denitrification (Goglio et al. 2013; Bateman and Baggs 2005). Tillage also had a clear impact on nitrous oxide emissions during 2014 with systems with greater tillage emitting more as demonstrated on May 19, 2014. The CT system emitted the greatest amount with 0.16 mg N_2O m^{-2} hr^{-1} and the tilled systems of CTH, OT, and OTH all showed emissions of 0.13 mg N_2O m^{-2} hr^{-1}. The no-till systems of CNT and OMT emitted the least at levels of 0.07 and 0.05 mg N_2O m^{-2} hr^{-1}, respectively (Table 2). In 2015, those systems with the greatest levels of tillage (OT and OTH) also have the greatest emissions early season. However, 2015 was a much drier season and as the drier time period started the OMT system had the greatest N_2O emissions (Figure 3). The OMT system was likely the greatest emitter due to its ability to retain moisture which is ideal for denitrification to occur. These higher rates of denitrification in no-till systems have been noted in some studies (Grandy et al. 2006; Cavigelli et al. 2012; Benckiser and Schnell 2007). No-till systems generally have higher water content, decreased levels of diffused oxygen, and greater rates of soil organic matter decomposition which all promote denitrification (Grandy et al. 2006). With the lack of rain in 2015, it is likely that the higher water content was driving the greater N_2O emissions noted in the organic minimum till system during this year.

Seasonal and Multi-Year Fluxes

When analyzing both trapezoidal and the combination of decay and trapezoidal data by cropping season the main effect of year was significant. This was an expected result because not all three years had the same rotational crop grown. Previous studies have reported corn to produce greater N_2O than soybean due to levels of nitrogen fertilizer applied to corn systems (Parkin and Kaspar 2006). These results agree with our 2014 and 2015 results which indicate a greater seasonal flux of 1366 mg N_2O m^{-2} in 2015 with a corn crop and a flux of 533 mg N_2O m^{-2} in 2014 with a soybean crop. However, 2013 was a corn year and did not show greater emissions than our soybean crop in 2014. Lower emissions in 2013 can likely be attributed to the level of rainfall this year. Soils were saturated at times during this season. While soils with a WFPS of > 80% are suspected to primarily conduct denitrification, research indicated this situation can lead to production of N_2 rather than N_2O (Strong and Fillery 2002; Thangarajan et al. 2013; Ruser et al. 2006). Therefore, the level of moisture received in 2013 over that of 2014 and 2015 is likely the explanation for lesser seasonal N_2O.

These results align with previous studies showing differences in N_2O emissions between cropping systems with greater emissions in organic systems (Petersen et al. 2006a). The trapezoid flux both, during the 2015 season and cumulatively, indicate a cropping system difference (Figure 4 and 5). The OMT system was the greatest emitter of N_2O on both a cumulative and seasonal basis in 2015 (Figure 6 and 7). The greater seasonal and multi-year fluxes in this system could likely be attributed to the moisture retention and carbon content in this cropping system. Both moisture and greater carbon content have the potential to promote denitrification and therefore N_2O emissions (Thangarajan et al. 2013;

Strong and Fillery 2002; Ruser et al. 2006). One study indicated the conversion from a conventional to an organic system resulted in overall greater microbial respiration rates, microbial biomass carbon and nitrogen and net nitrogen mineralization of 62% during the month of June (Tu et al. 2006a). The greater carbon content in this system allows energy for soil microbes to conduct denitrification (Thangarajan et al. 2013).

Significance by system was likely noted for the trapezoid integration of seasonal and multi-year flux but not in the combined use of the trapezoid method and decay function as the trapezoid method alone tends to overestimate N_2O emissions. The connection of values collected during sampling points assumes that N_2O produced does not return to an ambient value or only does so when a sampling event resulted in this value (Figure 4). With sampling events in this study occurring following rainfall, optimal conditions for N_2O emissions are present. This type of annual flux assessment results in a clear upward bias. However, a smaller level of overestimation may occur during a dry year due to mostly baseline N_2O emissions resulting even after rainfall events (Ross 2016). Recent research measuring continuous fluxes indicated a decay function most accurately depicts real-time data for N_2O emission events above ambient values (Cavigelli et al. 2014; Ross 2016). This method shows a continuous pattern through which N_2O is emitted and then returns to the baseline value. These data indicated no seasonal or cumulative difference between systems. A lack of differences between cropping systems is likely a result of the number of contributing factors to nitrification, denitrification and the levels at which these reactions occur.

While carbon levels and tillage in organic systems have both shown evidence of contributing to N_2O emissions, a number of factors have demonstrated that organic systems have management practices healthy for soils. Previous research has determined that with the initial conversion to an organic system from a conventional results in an increase in microbial respiration rates, microbial biomass carbon and nitrogen (Tu et al. 2006). In addition to increasing these parameters of soil health, organic agriculture promotes the use of animal manures which would otherwise be left unutilized in a pasture setting.

It can be noted that during wetter years (2013 and 2014) tilled systems showed greater N_2O than that of the no-till early season (Table 2). In this case, tillage will move carbon and nitrogen to microbes in the soil for nutrients and redistribute moisture in the soil which may cause a more anaerobic environment in the soil. All of which result in denitrification (Thangarajan et al. 2013). However, looking to a drier year, those systems which are not tilled will retain moisture and have a more anaerobic environment which is a preferred environment for denitrification. The OMT system takes this effect one step further with a high level of cover crop residue that further preserves soil moisture. Fertilizer addition to the soil impacted N_2O emissions with the addition of a nitrogen source for soil microbes to use as an electron acceptor. A previous study noted that organic amendments increased N_2O production where soils were limited in organic carbon while in soils where organic carbon was not limiting denitrification, the addition of a mineral fertilizer resulted in greater emissions (Bhandral et al. 2007). The six systems took turns being the leader in emissions at specific dates but resulted in similar cumulative emissions. The availability of nitrogen, carbon, and soil moisture likewise varied temporally. The systems likely varied in which input was the limiting factor to N_2O across time suggesting future work on within system limitations is needed.

Conclusions

Within the cropping systems of CT, CTH, CNT, OT, OTH, and OMT each system provides management techniques both promoting and mitigating N_2O production. During a more humid season, tillage will promote emissions with moisture and nutrient distribution. However, a dry season will result in a no-till system emitting more N_2O due to increased water not limiting denitrification. This research also suggests that the combination of a decay function and trapezoid integration is a more accurate depiction of N_2O fluxes over time as the combination of these methods agrees with individual date analyses suggesting no single system will consistently be the greatest N_2O producer. For these reasons, it appears that each system has a factor limiting denitrification and N_2O production making annual fluxes similar across cropping systems. Management decisions to limit N_2O emissions will therefore have to vary between organic and conventional growers. Likewise, the overall impact of tillage will vary in our region depending on the amount of precipitation. The next step might be to model the range of N_2O emissions as a function of precipitation for tilled and no-till systems to determine which has the higher impact over the long-term.

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Table 1: Long Term Cropping Systems Practices

System	N Fertilizer	Spring 2014 Cover	Spring 2015 Cover
Conventional Tilled (CT)	Liquid Urea	Rye	Wheat
Conventional Crop-Hay (CTH)	Liquid Urea	Rye	Wheat
Conventional No-till (CNT)	Liquid Urea	Rye	Wheat
Organic Tilled (OT)	Legume Cover Crop/Poultry Litter	Rye	Clover + Vetch
Organic Crop-Hay (OTH)	Legume Cover Crop/Poultry Litter	Rye	Clover + Vetch
Organic Minimum Till (OMT)	Legume Cover Crop/Poultry Litter	Rye	Clover + Vetch + Rye

Table 2: Cropping System Significance within a Date

System	N$_2$O Emissions (mg N$_2$O m^{-2} hr^{-1})													
	2013													
	4-21		4-30		5-20		5-22		6-12		6-24		6-26	
CT	0.07	B	0.02	B	0.29	AB	0.54	AB	0.02	A	0.007	AB	0.07	A
CTH	0.63	A	0.09	A	0.10	B	0.34	AB	0.01	AB	0.02	AB	0.003	B
CNT	0.17	AB	0.02	B	0.28	B	0.15	BC	0.003	ABC	0.008	AB	0.01	B
OT	0.35	AB	0.06	AB	0.55	A	0.20	ABC	0.002	BC	0	AB	0.0005	B
OTH	0.09	B	0.04	AB	0.24	AB	0.56	A	0.01	AB	0.05	A	0.01	B
OMT	0.14	AB	0.06	AB	0.09	B	0.08	C	0.002	C	0.006	B	0	B

	2013													
	7-05		7-10		8-19		9-04		10-21		11-08		12-25	
CT	0.06	AB	0.08	A	0.01	AB	0.05	B	0.05	AB	0.10	AB	0.01	B
CTH	0.12	A	0.03	B	0.05	A	0.17	A	0.07	AB	0.07	AB	0.15	A
CNT	0.02	ABC	0.006	B	0.009	AB	0.04	B	0.03	AB	0.04	B	0.04	AB
OT	0.02	BC	0.03	AB	0.006	B	0.20	AB	0.11	A	0.10	AB	0.05	AB
OTH	0.03	AB	0.01	B	0.009	AB	0.14	AB	0.10	A	0.38	A	0.05	A
OMT	0	C	0.03	AB	0.009	AB	0.07	AB	0.03	B	0.42	A	0.03	AB

	2014													
	3-10		3-20		5-19		6-07		6-11		7-05		7-13	
CT	0.02	AB	0.07	A	0.16	A	0.74	A	0.59	A	0.40	A	0.09	AB
CTH	0.12	A	0.01	B	0.13	AB	0.11	B	0.15	AB	0.15	AB	0.16	AB
CNT	0.07	AB	0.12	AB	0.07	B	0.32	AB	0.07	B	0.05	B	0.03	B
OT	0.05	AB	0.04	AB	0.13	AB	0.73	A	0.36	A	0.12	AB	0.52	A
OTH	0.06	AB	0.002	B	0.13	AB	0.98	A	0.26	A	0.16	AB	0.07	AB
OMT	0.02	B	0.06	A	0.05	AB	0.32	AB	0.52	A	0.05	AB	0.06	B

	2014						
	7-17	8-06	8-11	8-14	9-05	9-16	11-30
CT	0.09 AB	0.05 B	0.08 B	0.03 BC	0.18 A	0.09 AB	0.07 BC
CTH	0.25 A	0.05 B	0.04 B	0.03 C	0.24 A	0.06 AB	0.05 C
CNT	0.10 AB	0.13 B	0.13 AB	0.08 AB	0.05 AB	0.08 AB	0.10 ABC
OT	0.05 B	0.06 B	0.07 AB	0.03 ABC	0.07 AB	0.03 B	0.21 ABC
OTH	0.04 B	0.08 AB	0.03 B	0.04 ABC	0.04 B	0.09 AB	0.30 A
OMT	0.07 AB	0.37 A	0.25 A	0.14 A	0.09 AB	0.20 A	0.24 AB

	2014
	12-27
CT	0.21 AB
CTH	0.05 C
CNT	0.27 AB
OT	0.26 AB
OTH	0.73 A
OMT	0.21 BC

	2015						
	1-02	1-06	3-08	4-17	5-04	5-13	6-29
CT	0.04 B	0.06 AB	0.19 A	0.29 AB	0.29 AB	0.99 AB	0.08 AB
CTH	0.04 B	0.06 B	0.04 B	0.07 BC	0.38 AB	0.72 B	0.08 AB
CNT	0.04 B	0.06 B	0.09 AB	0.07 C	0.30 B	0.95 AB	0.11 AB
OT	0.22 A	0.31 A	0.20 A	0.59 A	0.58 AB	2.31 AB	0.06 B
OTH	0.18 A	0.26 A	0.10 AB	0.38 A	0.68 AB	1.06 AB	0.35 A
OMT	0.10 AB	0.09 AB	0.09 AB	0.07 C	1.77 A	2.94 A	0.13 AB

	7-20
	0.12 A
	0.07 AB

0.03	B
0.08	AB
0.13	A
0.05	AB

Table 3: ANOVA Results for Seasonal and Cumulative Fluxes Using Trapezoidal and Decay + Trapezoidal Calculations

	P>F	
	Trapezoidal	
Source	Seasonal	Cumulative
Year	<0.001	--
System	0.04	0.09
Year x System	0.02	--
	Decay + Trapezoidal	
	Seasonal	Cumulative
Year	<0.001	--
System	0.73	0.13
Year x System	0.43	--

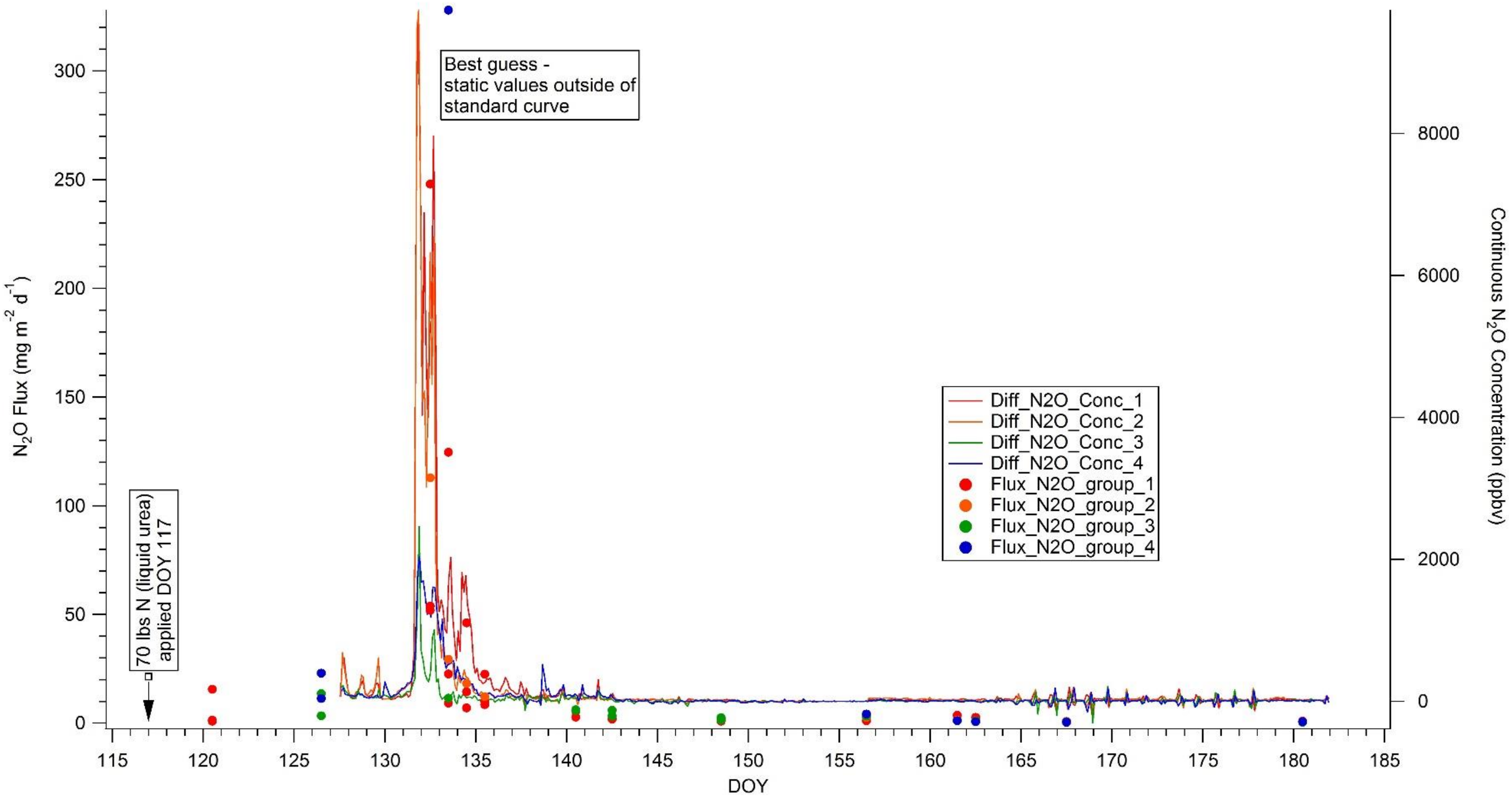
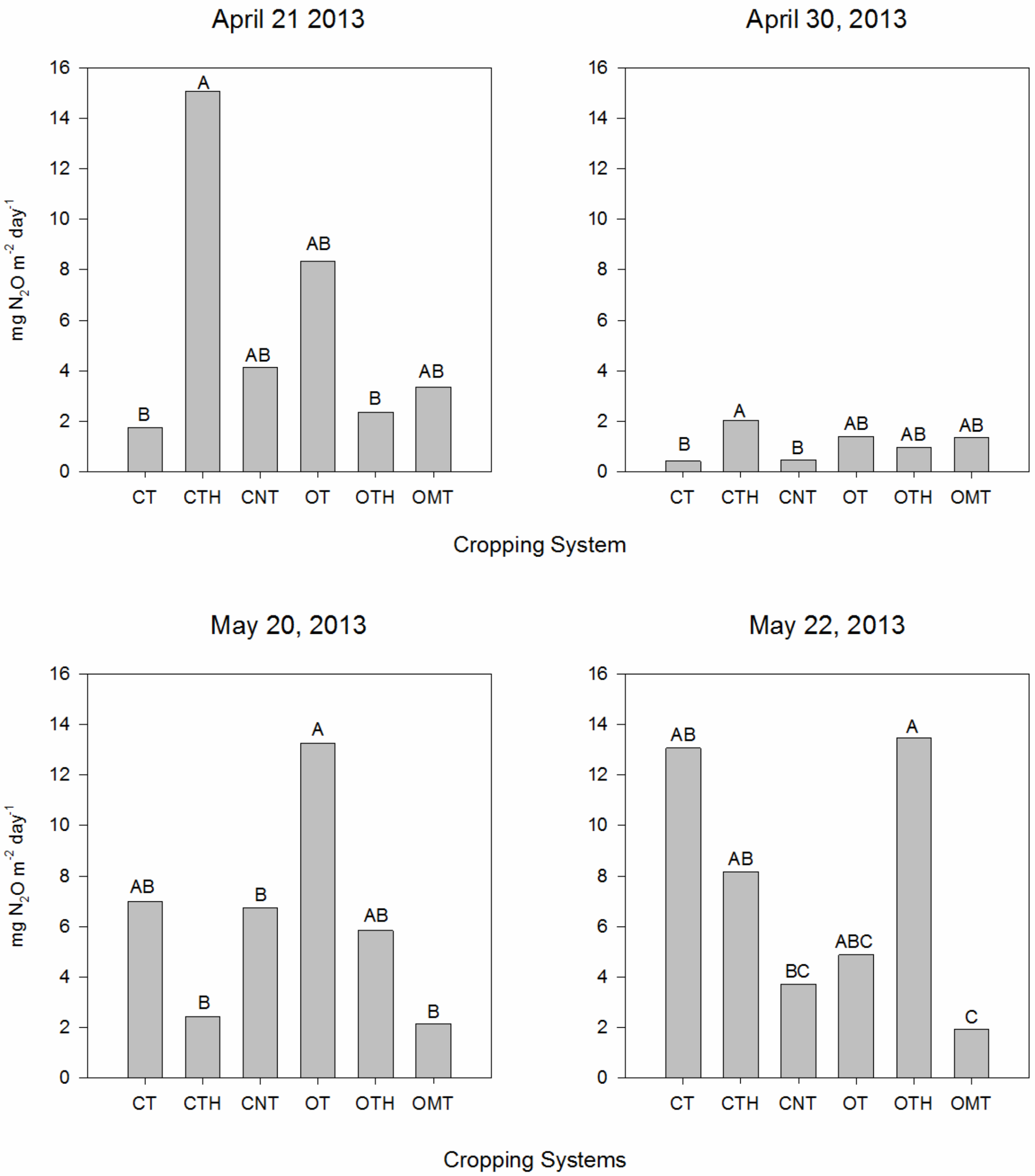


Figure 1: Nitrous Oxide (N_2O) Emissions Over Time Following a Tropical Storm in a No-Till System in Goldsboro, NC

(Ross 2016)



April 21 2013

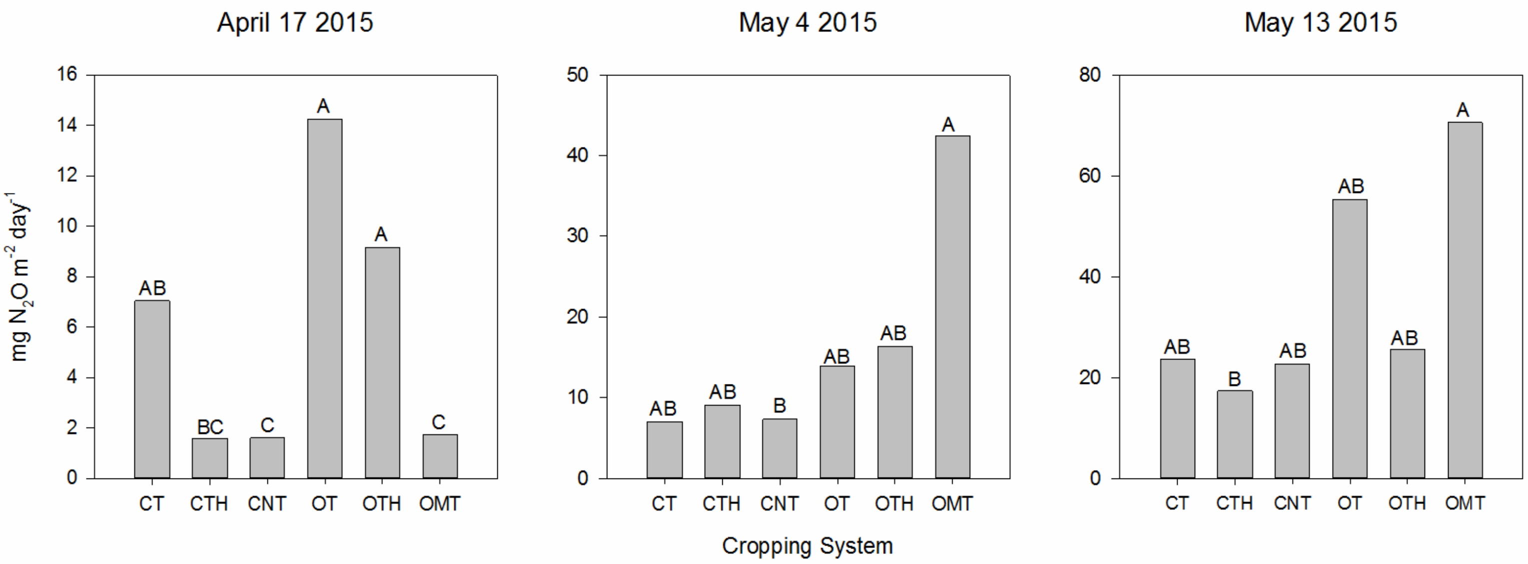
April 30, 2013

May 20, 2013

May 22, 2013

[a]Means within a date followed by the same letter are not different according to Fisher's Protected LSD at $p < 0.05$

Figure 2: 2013 Early Season N_2O Emissions with Significance Based on Tillage Levels



[a]Means within a date followed by the same letter are not different according to Fisher's Protected LSD at $p < 0.05$

Figure 3: 2015 Early Season N_2O Emissions with Significance Based on Tillage and Moisture

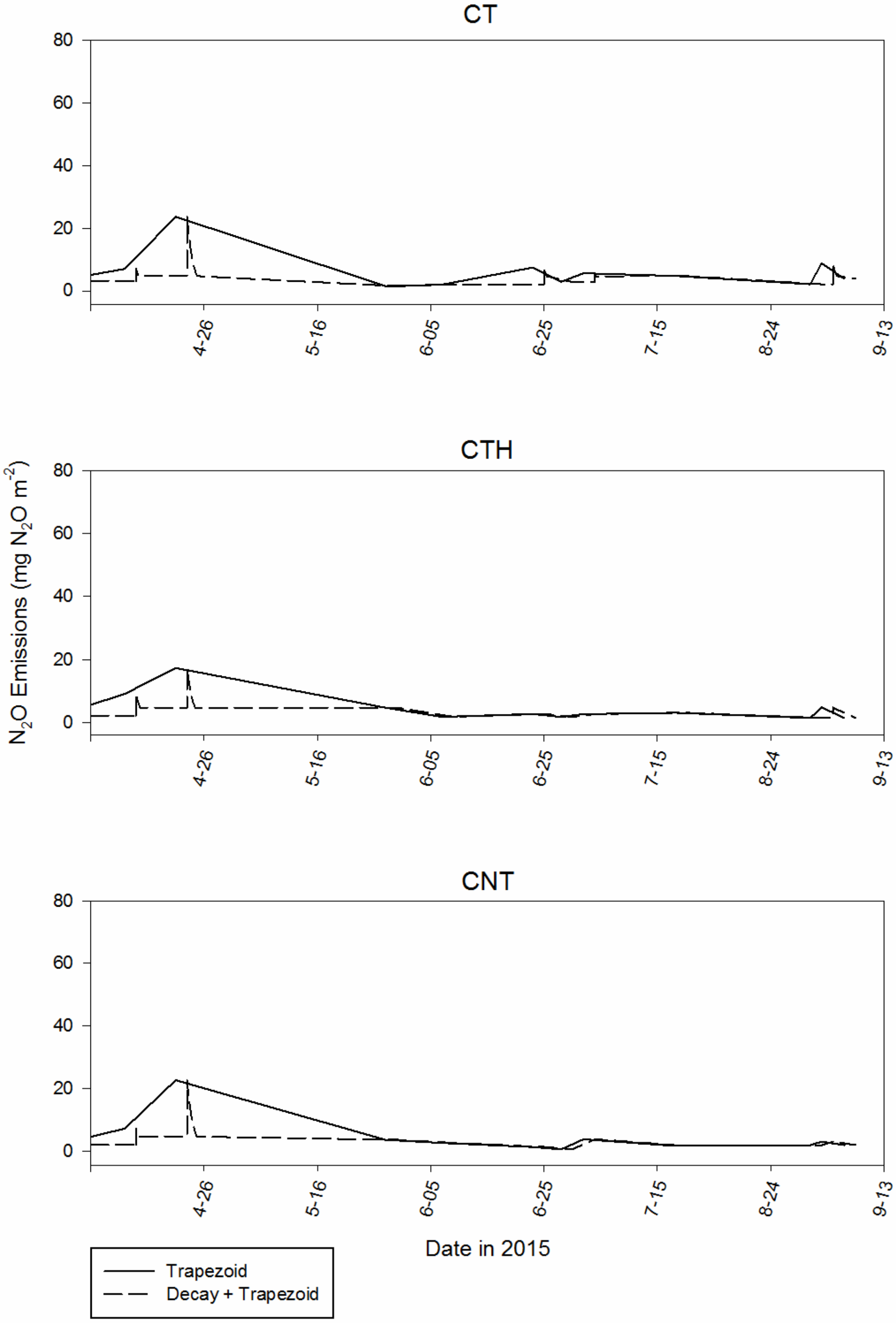


Figure 4: 2015 Decay + Trapezoidal and Trapezoidal Flux in Conventional Systems

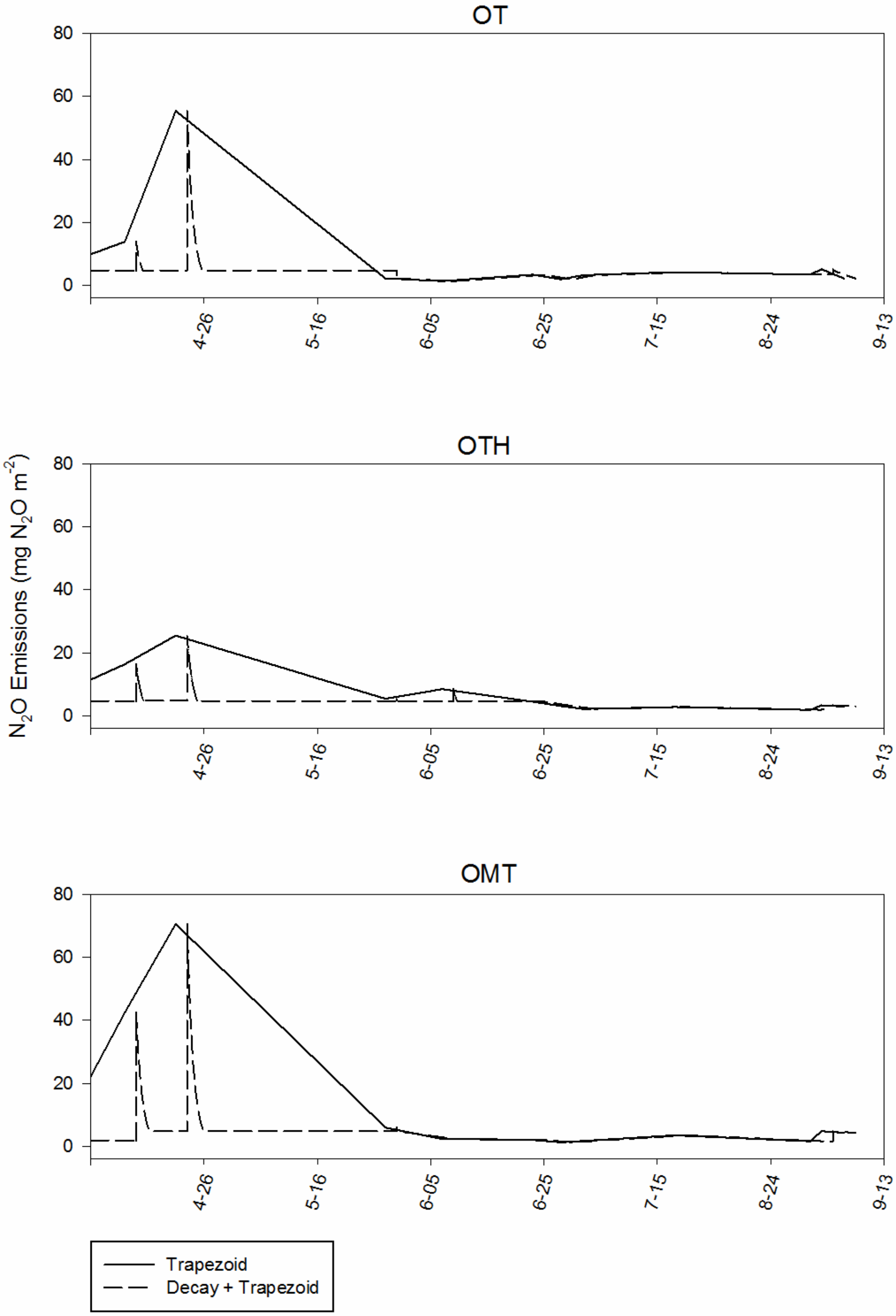
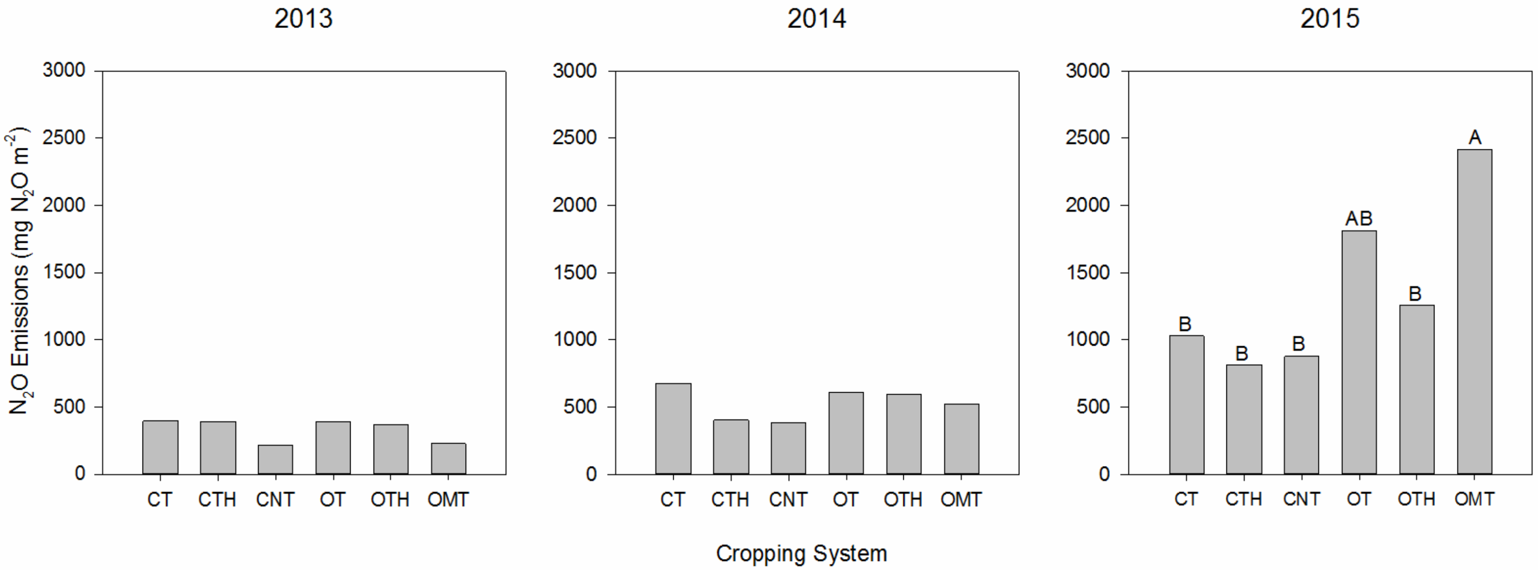
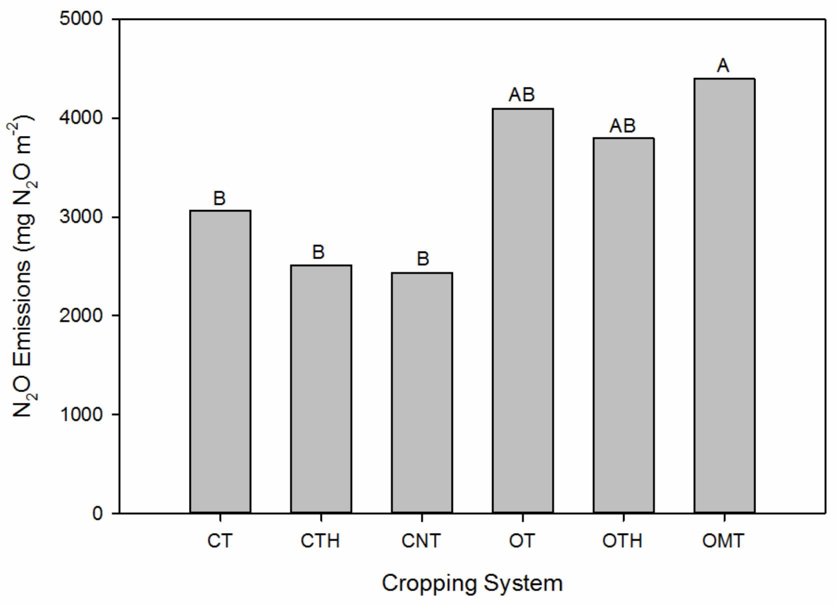


Figure 5: 2015 Decay + Trapezoidal and Trapezoidal Flux in Organic Systems



[a]Means within a year followed by the same letter are not different according to Fisher's Protected LSD at $p < 0.05$
[b]A year with no letter designation denotes no significance

Figure 6: Trapezoidal Integration Flux between April and September for 2013-2015



[a]Means followed by the same letter are not different according to Fisher's Protected LSD at $p < 0.05$.

Figure 7: Cumulative Flux Using Trapezoidal Integration Method

CHAPTER 2: NITROUS OXIDE OUTPUT BASED ON WEED MANAGEMENT IN AGRICULTURAL SYSTEMS

--------------------------Formatted for Weed Science--------------------------

Knight, Alexandra M.[1], Wesley J. Everman[1], Samuel Reberg-Horton[1], Shuijin Hu[2], David Jordan[1], and Nancy Creamer[3]

[1]Graduate Student, Assistant Professor, Associate Professor, Professor North Carolina State University Department of Crop Science. [2]Professor North Carolina State University Department of Plant Pathology. [3]North Carolina State University Department of Horticultural Science.

Abstract

Management practices in an agricultural system determine the amount of nitrogen in any given form in that cropping system. Previous studies observed differences in nitrogen and carbon content between organic and conventional agricultural systems. Organic systems have demonstrated greater microbial carbon and nitrogen levels based on organic inputs while conventional systems have shown greater nitrate (NO_3^-) levels particularly due to application of synthetic fertilizers. Research was conducted in Goldsboro, NC (35.38743 N, -78.03442 W) in a long-term cropping systems experiment from PRE herbicide application timing until 4 weeks after POST herbicide application timing during 2013, 2014, and 2015. Emissions were measured using the static chamber method in six long-term cropping systems including: conventional tilled (CT), conventional till-hay (CTH), conventional no-till (CNT), organic tilled (OT), organic till-hay (OTH), and organic minimum till (OMT). In each of these six systems a weedy and weed-free subplot were managed. In the case of organic

systems, the weedy subplot was considered to be natural and weed-free to be artificial. Conventional systems had natural subplots of weed-free and artificial subplots of weedy. In 2015, weed biomass was collected from weedy subplots and analyzed for carbon and nitrogen content. Nutrient content in weeds demonstrated the OMT system weeds have the greatest carbon and nitrogen content. When viewing the percentage of nitrogen taken up by weeds based on the amount applied, conventional system weeds take up greater nitrogen as this nitrogen is applied in plant available form.

Introduction

Management strategies in an agricultural system can determine the amount of a given form of nitrogen in a cropping system. In conventionally-managed agricultural systems, synthetic fertilizer rates are calculated based on plant available nitrogen (NO_3^- and NH_4^+) content. In organically managed cropping systems, fertilizer rates are calculated based on overall nitrogen content values which include organic nitrogen as well as NO_3^- and NH_4^+. Plant available nitrogen is more readily denitrified and, denitrification is the route in which most N_2O is produced in cropping systems (Thangarajan et al. 2013; Bateman and Baggs 2005). Differences in N content and microbial biomass in these two types of systems are known (Tu et al. 2006a; Tu et al. 2006b). Conversion of a conventional system to an organic system resulted in overall greater microbial respiration rates, microbial biomass carbon and nitrogen and net nitrogen mineralization of 62% during the month of June (Tu et al. 2006b). Based on this previous research, organic cropping systems have a steady organic source, greater microbial pool, small inorganic nitrogen pools and overall less fluctuation over time. Conventional cropping systems will have large nitrogen inputs at specific points in the season with plant available nitrogen applied in synthetic forms. Additionally, conventional systems have smaller microbial pools, greater inorganic nitrogen pools, and overall greater fluctuation in nitrogen movement over time (Figure 1).

Nitrogen fertilization increases N_2O emissions. Research demonstrated synthetic fertilizers such as ammonium nitrate result in the greatest N_2O emissions (Baggs et al. 2003) while other research demonstrated organic fertilizers to have greater N_2O emissions than synthetic fertilizers (Kaiser and Ruser 2000). The use of slow release fertilizers such as sulfur coated urea (Venterea et al. 2012; Cameron et al. 2013; Cavigelli et al. 2012) and the use of nitrification inhibitors such as dicyandiamide (DCD) have decreased N_2O emissions (Cavigelli et al. 2012). Application methods have additionally shown differences in N_2O emissions. Surface applied fertilizers have the potential of emitting two to seven times more N_2O than fertilizers which were incorporated into the soil (Baggs et al. 2003).

While incorporation of fertilizers sometimes decreases N_2O emissions, research has demonstrated tillage typically increases N_2O emissions (Omonode et al. 2011; Jin et al. 2014b; Goglio et al. 2013; Baggs et al. 2003). Tillage increases the rate of denitrification with more intense tillage by promoting decomposition and mineralization of residues (Omonode et al. 2011). However, studies have shown recent conversion to no-till treatments to emit more than the tilled treatments (Cavigelli et al. 2012; Benckiser and Schnell 2007; Grandy et al. 2006). In dry conditions, no-till systems may emit more with the capacity to maintain moisture in soil. However, in wet or humid environments, the reverse is true (Cavigelli et al. 2012).

Several studies have evaluated emissions with different treatments of tillage and nitrogen fertilizer sources in corn systems. Nitrogen fertilizer treatments tested in these studies included a polymer coated urea, conventional urea, a urea injected with an enzyme inhibitor, biochar, urea with DCD nitrification inhibitor, and chicken litter (Venterea et al. 2011; Deng et al. 2015; Smith et al. 2012). Urea and conventional tillage combined had the greatest N_2O emissions (Deng et al. 2015). The lowest N_2O emitting treatments were no-till combined with urea and DCD and no-till with biochar (Deng et al. 2015). When fertilizer treatments were applied several weeks after crop emergence, N_2O emissions were reduced due to nitrogen presence at a necessary point in growth for the corn crop. This reduced opportunity for the microbial community to transform nitrogen with nitrification and denitrification before plant uptake (Venterea et al. 2011).

The effect of promoting N_2O emissions is well documented, however differences in emissions between conventional and organic systems have been location dependent (Petersen et al. 2006b; Chirinda et al. 2010). The only location in which organic emissions were higher had greater rainfall in the organic system (Petersen et al. 2006a). This suggested moisture content was the cause of these higher emissions. Another study tied greater N_2O emissions to greater levels of soil inorganic nitrogen (Burger et al. 2005).

Research evaluating the impact of weed management on N_2O emissions indicated PRE vs PRE + POST had minimal effect on N_2O emissions in a conventional soybean [*Glycine max* (L) Merr.] cropping system (Bailey et al. 2015). Presence of some plant residues has shown to increase N_2O emissions over that of other residues (García-Ruiz et al. 2012). Presence of plant residues may be compared to weed pressure in a conventional cropping system as weeds present will be terminated at POST herbicide timing. This termination leads to carbon and nitrogen additions to the soil from the plant material of weeds just as plant residue application does. These residues were applied in combination with fertilizer which demonstrated increases in N_2O emissions. The level of increase in N_2O emissions was dependent on fertilizer applied as well as residue. However, the combination of the two varied in results based on species and other factors including lignin content in the plant matter (Garcia-Ruiz and Baggs 2007).

The objective of this study was to determine if the presence or absence of early season weeds in both organic and conventional cropping systems impacted N_2O emissions. We predicted that conventional systems would emit more N_2O due to a greater inorganic nitrogen pool. Within each cropping system, we predicted allowing weeds to remain in a cropping system would serve as a source of nitrogen storage, leading to lesser free N in a system and therefore less N_2O emissions.

Materials and Methods

Research was conducted during the summers of 2013, 2014, and 2015 in Goldsboro, NC at the Cherry Research Farm, Center for Environmental Farming Systems (35.38743, -78.03442). A total of six long-term cropping systems were evaluated. These six systems have been in place since 1996 and included a conventional no-till (CNT), conventional tilled (CT), conventional crop-hay (CTH), organic minimum till (OMT), organic full till (OT), and organic crop-hay system (OTH) (Mueller et al. 2002). These systems are further described in Table 1. The study was organized in a randomized complete block design and blocked according to soil type. Block 1 consisted of a Tarboro loamy sand (Typic Udipsamments), block 2 consisted of a Wickham sandy loam (Typic Hapludults), and block 3 consisted of a combination of State loam (Typic) and Tarboro loamy sand (Typic Udipsamments).

Each of the six systems had a split-plot design of weedy and weed-free. Due to the low weed seed bank in the conventional systems, the weedy plots were seeded with redroot pigweed (*Amaranthus retroflexus* L.) to ensure weed presence. Prevention of the weedy sub-plot from receiving PRE herbicide application was ensured through the use of covering this area with a sheet of plywood during application. Because the weedy subplots in the conventional systems were managed differently from the typical conventional practices these subplots were considered to be the "artificial" treatment. Weed-free was considered to be the "natural condition" in the conventional systems with this being the state in which weed management can typically be noted in this system type. PRE and POST herbicides were sprayed at optimal conditions as prescribed to growers. PRE application occurred immediately following planting using a pressurized CO2 backpack sprayer calibrated to apply 140 L ha^{-1} at 4.8 km hr^{-1}. POST applications were done in the same manner when weeds reached a height of 10 cm. The weed-free subplot in the organic system was managed with the combination of tillage and herbicides. Tillage occurred on a weekly basis on the main-plot scale. In addition to tillage, herbicides were sprayed between tillage events to prevent weed emergence. These herbicides were sprayed at a rate one-third that of what was applied in the conventional weed-free plots. This was done so that herbicides could be sprayed multiple times in a season as tillage broke the herbicide layer and this allowed for three applications without going over the labeled rate. The weedy subplot was considered to be the “natural” treatment in the organic plots and was managed with weekly tillage which allowed for weed emergence between tillage events. These subplot treatments are further depicted in Table 2.

In 2015, weed densities and biomass were collected in the weedy subplots. Weed fresh and dry weight was recorded. Samples were ground to particles of 2 mm or less and processed for total C and N content using a model 2400 CHN Elemental Analyzer (Perkin Elmer Corporation, Waltham, MA 02451).

Each plot was 8 rows wide for a width of 6.0 meters with static gas chambers placed between rows 6 and 7. Gas chambers were made of stainless steel and measured 1211 cm^2 with headspace of 13.48 L. Gas samples were taken within 24-48 hours following a rainfall event of $\geq$ 1.25 cm between planting and 4 weeks after POST application. These gas samples were collected at time 0, 10, 20, and 30 minutes following chamber lids being water sealed to capture gas emissions over time. These 5 mL gas samples were taken in nitrogen flushed crimp top vials between 9:00am and 1:00pm to capture the average daily temperature (Parkin and Venterea 2010). N_2O emissions will rise with increasing temperature. This is the result of greater temperatures leading to greater microbial activity and therefore greater rates of denitrification. Collecting samples during the day’s average temperatures, results in a more accurate calculation of the day’s emissions. Sampling during the daily high would result in an overestimation of daily N_2O emissions and, sampling during the daily low temperature would result in an underestimation of daily N_2O emissions (Parkin and Venterea 2010). Gas chromatography (GC) was used to measure content of N_2O for each chamber. The GC used contained both an electron capture device (ECD) and flame ionization detector (FID).

Chamber data were analyzed through the HMR program in R statistical software based on previous research (Pedersen et al. 2010). The manual operation for this program was used in which the user can view the program's selection of HMR or linear for a particular chamber. Chamber data were used based on program recommendations and data was calculated to reflect N_2O emissions per area and unit time.

Values from the HMR program were log transformed and run through a repeated measures analysis in PROC MIXED using SAS 9.4. Within the repeated measures analysis, multiple adjustments were used for the variance-covariance matrix. Models of spatial power law (SP(POW)), heterogeneous compound symmetry (CSH), unstructured (UN), and a general mixed model with date as a split-plot factor were all tested. A best fit model was selected based on Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and Akaike's Information Criterion Corrected (AICC) values. The SP(POW) indicated the best fit model in this study according to information criteria.

Weed nutrient content and gas data for 2015 were analyzed collectively using PROC CORR. A one-way ANOVA was used to measure treatment effects on nutrient content of weeds.

Results and Discussion

N_2O Emissions

The SP(POW) model in repeated measures indicated a significant main effect of date ($p \leq 0.01$). A significant interaction of system x date ($p \leq 0.01$) and system x date x weeds (p

≤ 0.05) was noted. Significant system x date interactions occurred on the following dates: May 20, 2013; May 22, 2013; June 7, 2014; June 11, 2014; and July 17, 2015. The SP(POW) model indicates a relationship between data points based on their closeness in time. Those dates within the same season were closely related as those dates contained the same crop. 2013 and 2015 were a corn [*Zea mays* (L.)] crop while 2014 was a soybean crop. Previous research has indicated that different crops will emit different levels of N_2O. One study in particular indicated corn will emit greater amounts of N_2O due to nitrogen applications in corn which are not present in soybeans (Parkin and Kaspar 2006). However, emissions in 2013 were lower than that of 2014 due to the saturation of soils in 2013 which led to complete denitrification rather than the production of N_2O (Thangarajan et al. 2013; Ruser et al. 2006; Strong and Fillery 2002).

Within a single season, early season results were predicted to consistently be greater as a result of most fertilization and tillage occurring early season. Both tillage of systems and application of nitrogen fertilizers demonstrated increases in N_2O emissions (Baggs et al. 2003; Goglio et al. 2013; Jin et al. 2014; Omonode et al. 2011; Robertson et al. 2000). Tillage and fertilization events both occurred early in the season, leading to the prediction that N_2O sampling events near these field management practices would be more closely related than those events later in the cropping season.

Early season emissions in 2013 showed those systems with greater tillage emitted the greatest amounts of N_2O. The OT and CT systems were the greatest emitters of N_2O on May 20 with 0.55 and 0.29 mg N_2O hr^{-1}, respectively (Figure 2). This agrees with a previous researcher demonstrating that continuous row crop rotations had greater NO_3^- than those with a pasture or hay rotation and therefore emitted greater N_2O (Perdomo et al. 2009). NO_3^- serves as an electron acceptor in denitrification and greater NO_3^- led to more N_2O being produced. The OTH, CT, and CTH were the top emitters on the following sampling date of May 22 (Figure 2). Because 2014 was a soybean rotation fertilizer did not contribute to N_2O emissions and tillage events began later in the season. The CT and OTH were two of the top emitters on the significant dates of June 7 and 11 in 2014. The ONT system was also one of the top emitters during significant dates in 2014. This significance suggests that the combination of greater microbial nitrogen, carbon (Tu et al. 2006a; Tu et al. 2006b) and moisture retention (Bateman and Baggs 2005; Chirinda et al. 2010) contributed to greater N_2O emissions. Tillage did not play as clear a role in 2015. 2015 was a much drier cropping season and the one significant date was following the season's tillage events. With this being the case, a given system's ability to retain moisture appeared to be the factor determining levels of N_2O emissions (Thangarajan et al. 2013).

When comparing subplots of weedy and weed-free based on emissions, system type was critical in which subplot emitted significantly greater N_2O. In the case of organic cropping systems, nitrogen is the limiting factor in denitrification. Therefore, when subplots were weed-free more nitrogen was available for denitrification as the soil microbial community was not competing with weeds for nitrogen. This was evident on May 22, 2013 and May 4, 2015 (Figure 5; Figure 7). However on April 30 and June 21 in 2013, the weedy subplot emitted greater N_2O in the OMT system than the weed-free. This was likely due to the weedy subplots retaining moisture better than that of the weed-free. Levels of water filled pore space (WFPS) are crucial in denitrification and, the absence of weeds may have allowed for greater evaporation. Conventional plots, however, tend to be limiting in carbon. Therefore, those plots with weeds had a carbon supply for denitrification. In 2014, the weedy subplot emitted greater N_2O over the weed-free on July 5. July 5 was after POST herbicide application and nitrogen from weeds was likely released back into the system at this point. This means carbon which was lacking in the conventional system was supplied by weeds. May 4, 2015 again showed greater emissions from the weed-free subplot indicating that nitrogen was retained by weeds in the weedy subplot but was available for denitrification in the weed-free subplot in the OMT system. Previous research suggests lignin content, carbon content, and lignin:nitrogen ratio in a plant material are all inversely related to N_2O emissions (Garcia-Ruiz and Baggs 2007). However, this study indicates a cropping system's management practices will determine whether plant material will mitigate or promote N_2O emissions. With weeds controlled at a size of 10 cm or less, it is possible that lignin and carbon content was very minimal when compared to a weed-free treatment thereby showing no difference in some systems. Additionally, previous studies have shown application of some plant residues led to decreases in N_2O production compared to an unamended soil (Garcia-Ruiz and Baggs 2007).

Weed Nutrients

Carbon and nitrogen content in weeds were significantly different based on cropping system from which the weeds were collected (Figure 8). Carbon content ranged between 3 and 637 kg ha^{-1} with the OTH having the least and the most in the OMT. The least amount of carbon was noted in the OTH and OT systems which both have the greatest amount of tillage and therefore, the least amount of weeds during the early portion of the field season. With decreasing levels of tillage, both the number of weeds and height to which weeds grow is increased. With an increase in weed growth, an increase in nutrient content and carbon content in particular is increased. OMT likely had such a high level of carbon within weeds due to the lack of weed control methods in this system as well as greater microbial biomass carbon and nitrogen contained in an organic system over that of the conventional (Tu et al. 2006b). Nitrogen content in weeds followed the same trend.

Carbon: nitrogen ratio also varied per system ($p \leq 0.01$). The greatest ratios were that of the no-till systems with ratios of 19.56 and 16.64 for the OMT and CNT, respectively. In those systems with tillage, organically managed systems had greater ratios than those conventionally managed systems. In the systems managed with tillage, the greatest to least C:N ratio were in the order of OTH, OT, CT, and CTH having the smallest value. These systems had ratio values of 9.42, 7.13, 6.03, and 4.67. This research agreed with previous research showing a trend of lesser N_2O emissions with a greater ratio of C:N ratio (Garcia-Ruiz and Baggs 2007).

The percent of nitrogen applied which was lost to weeds was greatest in the conventional systems (Figure 9). This is likely due to the fact that nitrogen applied in the conventional cropping systems is applied in the form of plant available nitrogen while nitrogen applied in an organic system is applied in total nitrogen content. Therefore, nitrogen in a conventional system would be readily available. In the tilled organic systems (OT and OTH), weeds were destroyed weekly by tillage events. This kept weeds from growing large and requiring greater amounts of nitrogen for survival (Blackshaw et al. 2003). The conventional systems also allowed weeds to emerge following PRE application and grow until POST applications. This allowed for a greater density of weeds to develop and take up nitrogen (Lindsey et al. 2013).

A correlation analysis between nutrient content and N_2O emissions noted that early season gas emissions had a negative correlation with weed nitrogen content (Table 3). This was additionally supported by gas emission results from the May 4, 2015 sampling date (Figure 7). This suggests weeds will be most influential in N_2O emissions shortly after fertilizer application with those systems showing fewer weeds emitting greater N_2O at this point than those systems with lesser N_2O having a greater number of weeds. Previous studies have indicated early season weed control is necessary due to the competitive ability of weeds to take up nutrients necessary for crop growth including nitrogen (Lindsey et al. 2013; Blackshaw et al. 2003; Knezevic et al. 2002; Gower et al. 2003). The focus of previous research was on the competition of weeds and crops for these nutrients. While early season weed control can be a problem from a crop competition standpoint, it appears that weeds can also act as a N_2O mitigation tool by storing nitrogen.

Weedy and weed-free subplots appear to play a role in N_2O emissions within a given cropping system. Weeds will promote N_2O production in conventional systems and mitigate these emissions in an organic system. This research agrees with previous studies suggesting that plant residue can mitigate or promote N_2O emissions (Garcia-Ruiz and Baggs 2007). While weed management plays a role within a cropping system, a system’s management practices of tillage and fertilizer use impact N_2O production to a higher degree (Baggs et al. 2003; Omonode et al. 2011c) . Both the use of fertilizer and tillage promote N_2O emissions.

Within these systems, tillage also impacts carbon and nitrogen taken up by weeds. Tillage controls weeds and thereby, leads to fewer weeds and less nutrients taken up by weeds. This research also indicates while early season tillage controlled weeds, it also promoted N_2O emissions.

Future Research

Although this research indicated the presence of early season weeds played a small role in N_2O emissions, the investigation of late season weeds on N_2O emissions could indicate greater differences in N_2O. If late-season weeds were able to mitigate N_2O and not impact yield, these weeds could serve as a vital mitigation tool. Individual system management practices have shown an impact on N_2O emissions and should further be investigated as far as stimulants and limitations of denitrification.

Source of Materials

[1] CO_2 Pressurized Backpack Sprayer, Spraying Systems Co., Wheaton, IL 60189.

[2] SAS Statistical Software, Version 9.4, Cary, NC 27513.

[3] 2400 CHN Elemental Analyzer from Perkin Elmer Corporation, Waltham, MA 02451.

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Table 1: Long Term Cropping Systems Practices

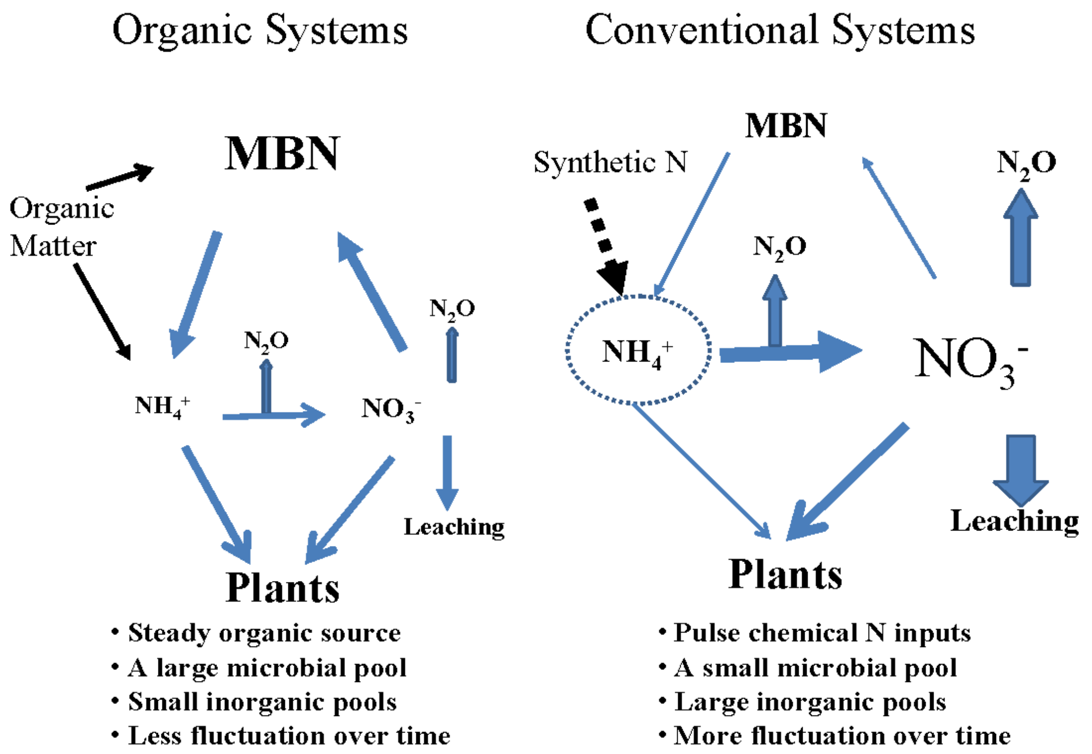
System	N Fertilizer	Spring 2014 Cover	Spring 2015 Cover
Conventional Tilled (CT)	Liquid Urea	Rye	Wheat
Conventional Crop-Hay (CTH)	Liquid Urea	Rye	Wheat
Conventional No-till (CNT)	Liquid Urea	Rye	Wheat
Organic Tilled (OT)	Legume Cover Crop/Poultry Litter	Rye	Clover + Vetch
Organic Crop-Hay (OTH)	Legume Cover Crop/Poultry Litter	Rye	Clover + Vetch
Organic Minimum Till (OMT)	Legume Cover Crop/Poultry Litter	Rye	Clover + Vetch + Rye

Table 2: Weedy and Weed-Free Subplot Practices

SYSTEM	NATURAL	ARTIFICIAL
CONVENTIONAL	**Weed-Free:** Timely PRE and POST Herbicide Applications	**Weedy:** Cover subplot during PRE application of main plot and seed weeds
CORN (2013; 2015)	PRE Atrazine 1823 g ai ha^{-1} + S-metalochlor 1412 g ai ha^{-1} POST Atrazine 1120 g ai ha^{-1} + Thiencarbazone-methyl 15 g ai ha^{-1} + Tembotrione 76 g ai ha	POST Atrazine 1120 g ai ha^{-1} + Thiencarbazone-methyl 15 g ai ha^{-1} + Tembotrione 76 g ai ha
SOYBEAN (2014)	PRE Flumioxazin 70 g ai ha^{-1} POST Glyphosate 1260 g ai ha^{-1} + Acetochlor 2521 g ai ha^{-1}	POST Glyphosate 1260 g ai ha^{-1} + Acetochlor 2521 g ai ha^{-1}
ORGANIC	**Weedy:** Weekly Tillage Events with New Flux of Weeds Following Each Event	**Weed-Free:** Weekly tillage will break up herbicide layer so PRE Herbicide applied at 1/3 rate for multiple applications without going over labeled rate
CORN (2013; 2015)	N/A	PRE Atrazine 608 g ai ha^{-1} + S-metalochlor 471 g ai ha^{-1}
SOYBEAN (2014)	N/A	PRE Flumioxazin 23 g ai ha^{-1}

Table 3: Pearson Correlations of 2015 Weed Nutrient Content and N_2O Emissions

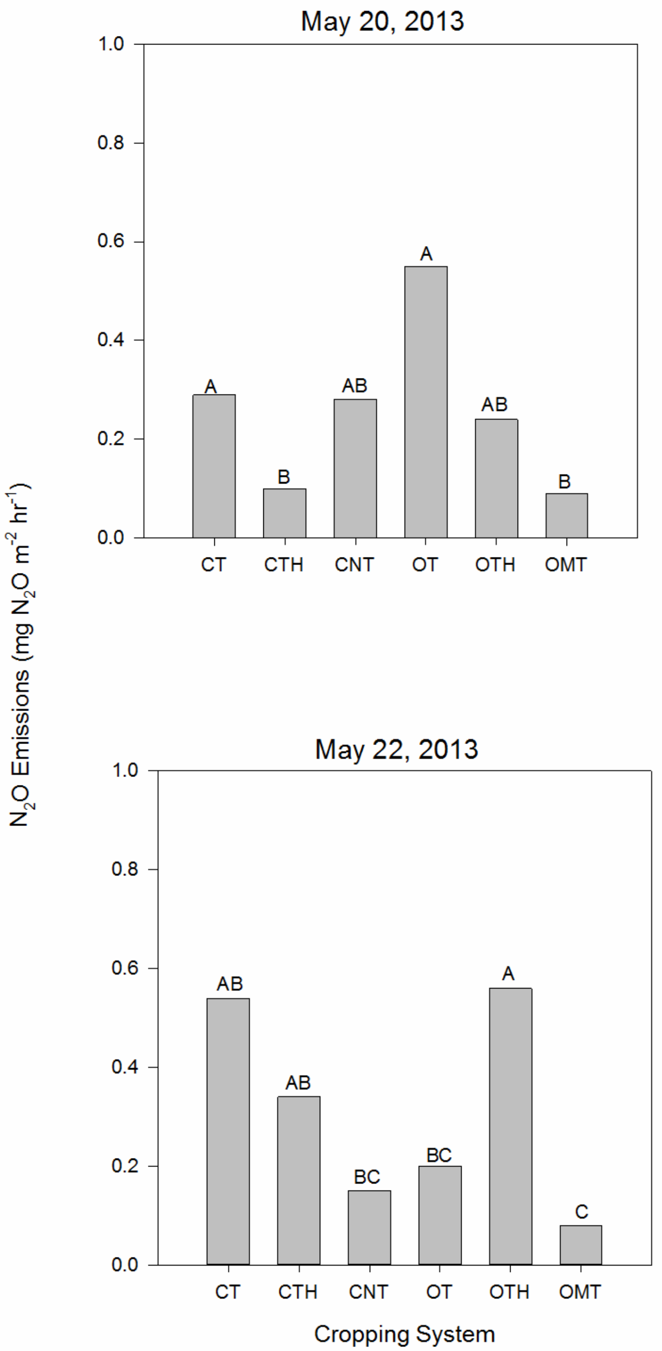
	Nutrient Content			
Gas Sampling Event	N Content		C Content	
Date	P>F	R^2	P>F	R^2
May 4	0.0324	-0.5198	0.1443	-0.3695
May 13	0.5902	0.1407	0.3991	0.2169
June 19	0.5330	-0.1626	0.8599	-0.0463
June 29	0.2321	-0.3167	0.3781	-0.2364



- Steady organic source
- A large microbial pool
- Small inorganic pools
- Less fluctuation over time

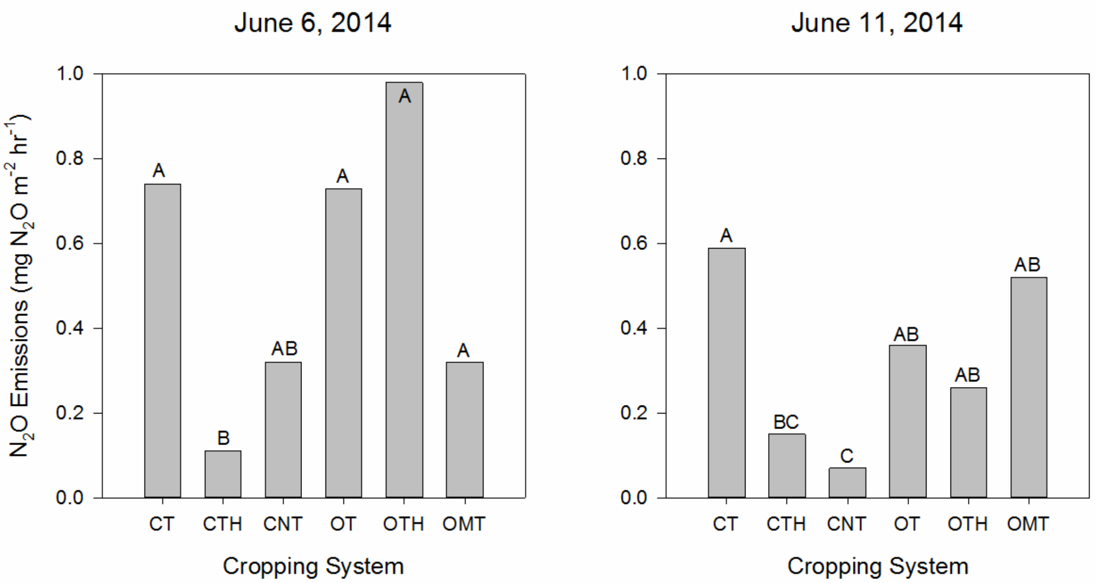
- Pulse chemical N inputs
- A small microbial pool
- Large inorganic pools
- More fluctuation over time

Figure 1: Internal N Cycle is tighter in Organic than in Conventional Systems



[a]Means within a date followed by the same letter are not different according to Fisher's Protected LSD at $p < 0.05$.

Figure 2: 2013 N_2O Emissions Based on Cropping System x Date with Significance based on Level of Tillage

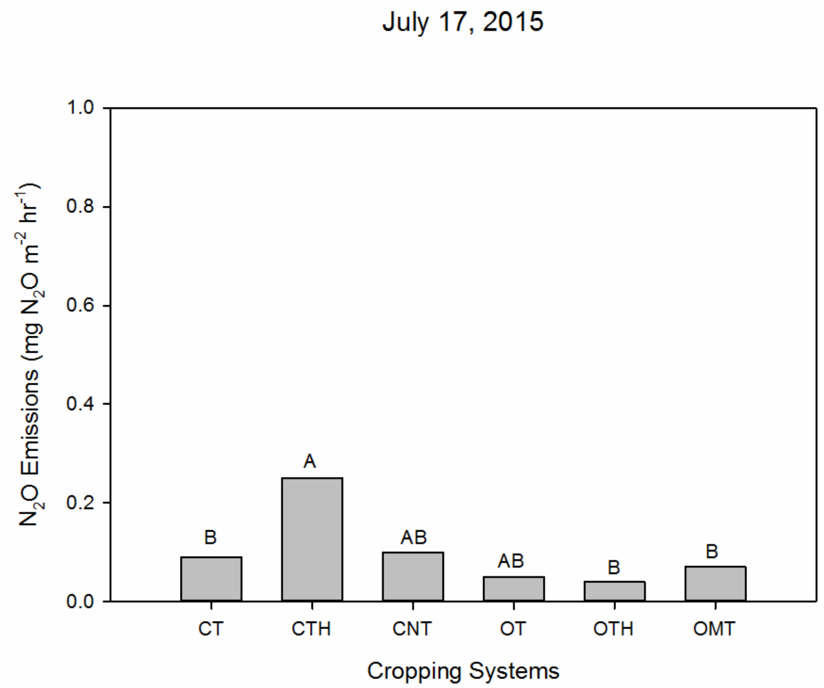


June 6, 2014

June 11, 2014

[a]Means within a date followed by the same letter are not different according to Fisher's Protected LSD at $p < 0.05$.

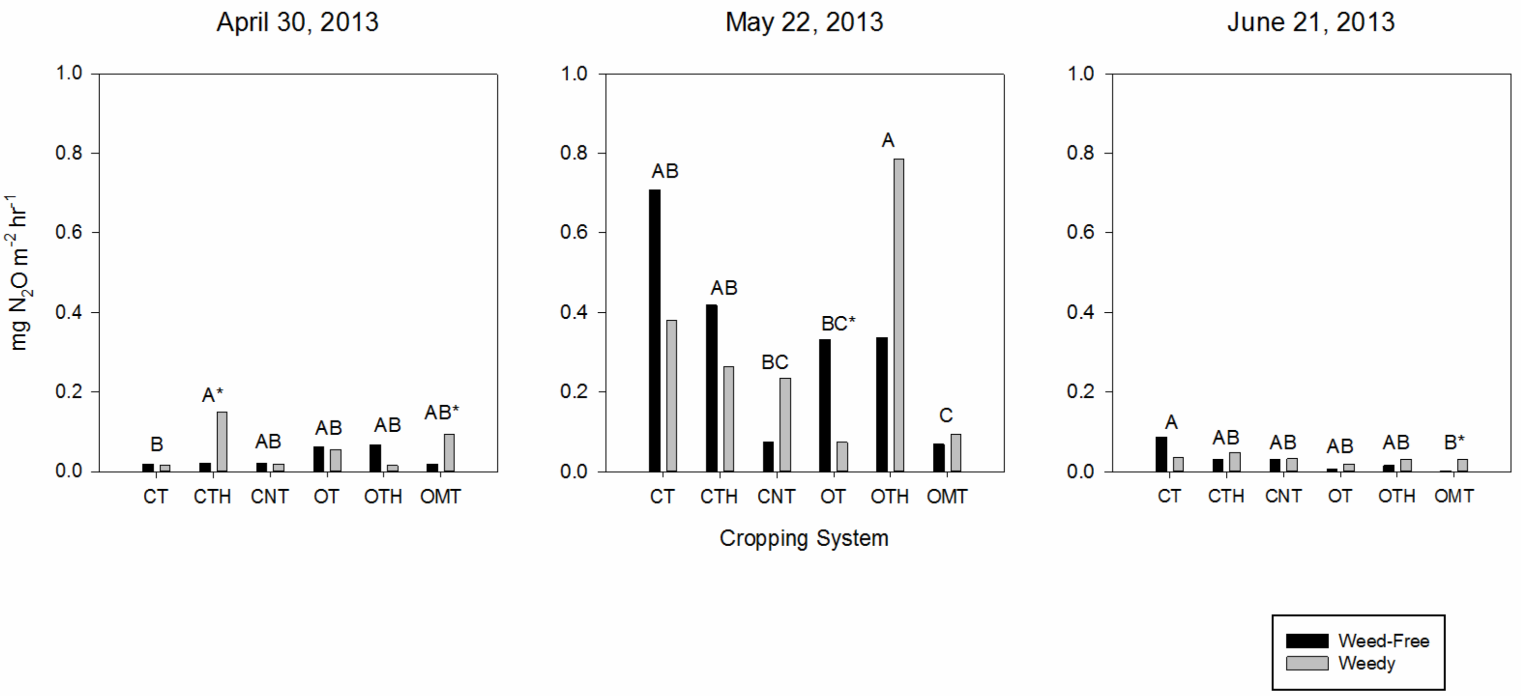
Figure 3: 2014 N_2O Emissions based on Cropping System x Date showing Significance Based on Level of Tillage



July 17, 2015

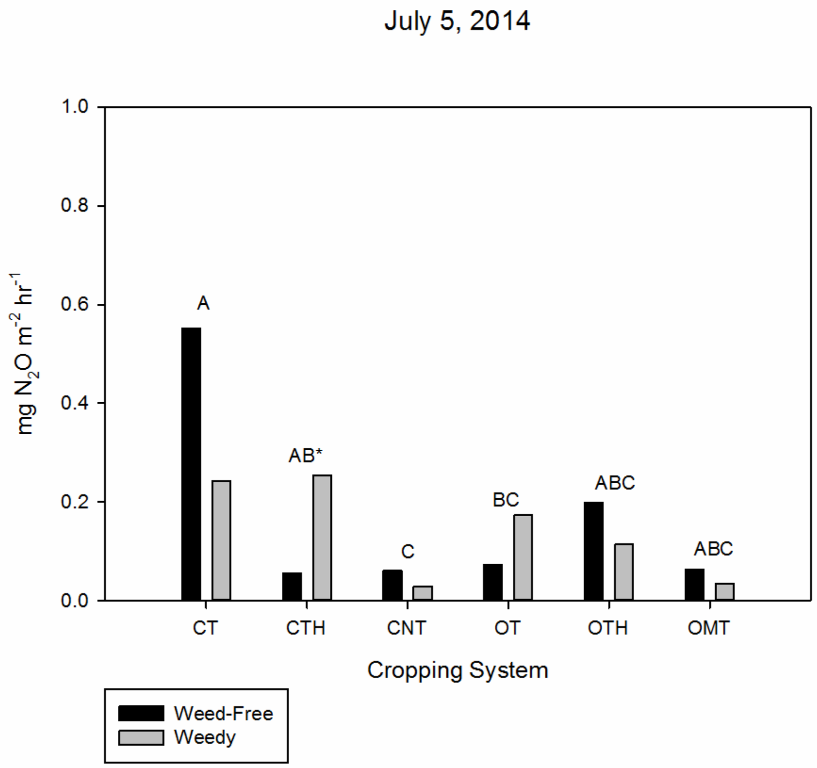
[a]Means within date followed by the same letter are not different according to Fisher's Protected LSD at $p < 0.05$.

Figure 4: 2015 Significant N_2O Emissions System x Date Late Season



[a]Means within cropping systems followed by the same letter are not different according to Fisher's Protected LSD at $p < 0.05$.
[b]Asterick(*) indicates differences between the weed-free and weedy column for a given cropping system according to Fisher's Protected LSD at $p < 0.05$.

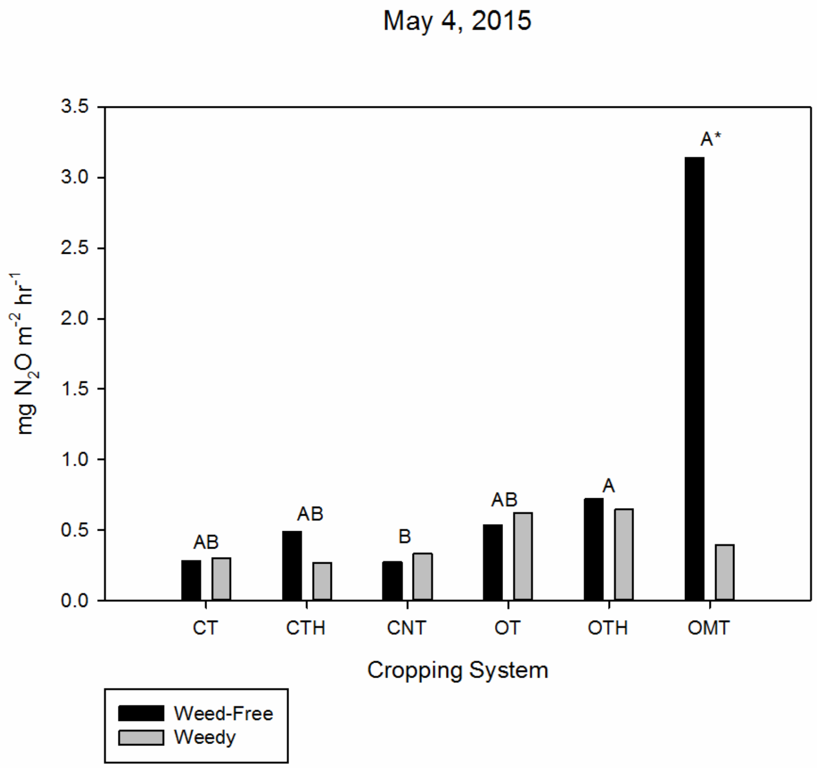
Figure 5: 2013 N_2O Emissions Based on Cropping System x Date x Weed Management



[a] Means within cropping systems followed by the same letter are not different according to Fisher's Protected LSD at $p < 0.05$.

[b] Asterick(*) indicates differences between the weed-free and weedy column for a given cropping system according to Fisher's Protected LSD at $p < 0.05$.

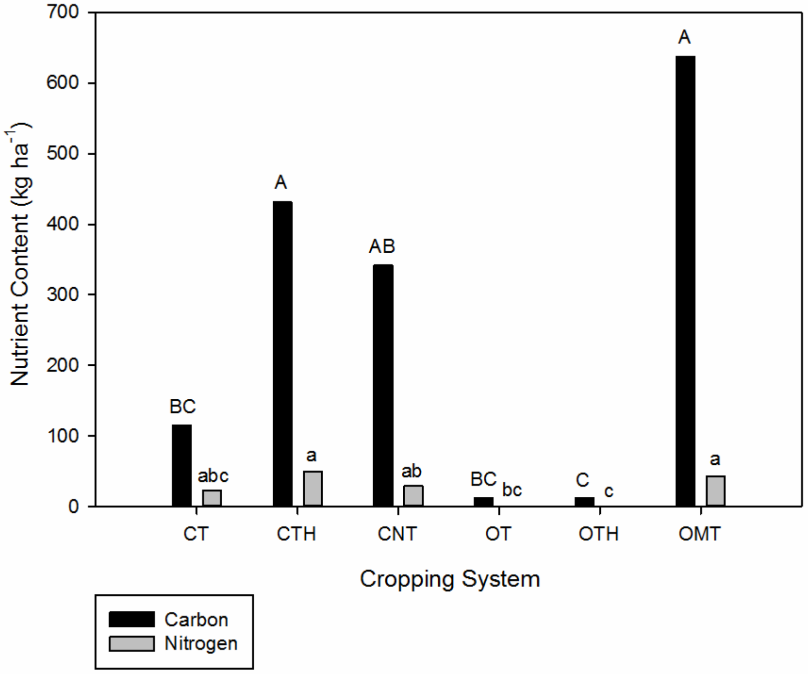
Figure 6: 2014 N_2O Emissions Based on Cropping System x Date x Weed Management



May 4, 2015

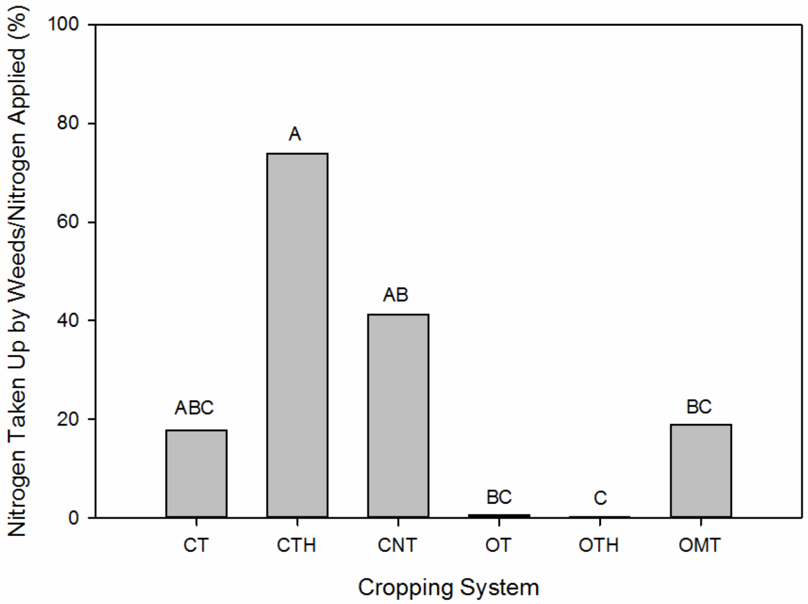
[a]Means within cropping systems followed by the same letter are not different according to Fisher's Protected LSD at $p < 0.05$.
[b]Asterick(*) indicates differences between the weed-free and weedy column for a given cropping system according to Fisher's Protected LSD at $p < 0.05$.

Figure 7: 2015 N_2O Emissions Based on Cropping System x Date x Weed Management



[a]Means within the same color column followed by the same letter are not different according to Fisher's Protected LSD $p < 0.05$.

Figure 8: Nutrient Content in Weeds Based on Cropping System



[a]Means within a column followed by the same letter are not different according to Fisher's Protected LSD $p < 0.05$.

Figure 9: Nitrogen Taken Up by Weeds

CHAPTER 3: NITROUS OXIDE EMISSIONS FOLLOWING HERBICIDE APPLICATION

--------------------------Formatted for Soil Biology and Biochemistry--------------------------

Knight, Alexandra M.[1], Wesley J. Everman[1], Samuel Reberg-Horton[1], Jude Maul[2], and Shuijin Hu[3]

[1]Graduate Student, Assistant Professor, Associate Professor North Carolina State University Department of Crop Science. [2]USDA-ARS Beltsville, Maryland [3]Professor North Carolina State University Department of Plant Pathology.

Abstract

N_2O is a potent greenhouse gas which has approximately 300 times the energy holding capacity of the baseline gas CO_2. Agriculture contributes approximately 56% of non- CO_2 emissions annually and contributes N_2O primarily through bacteria conducting denitrification. Two rates (135 kg N ha^{-1} and 22.5 kg N ha^{-1}) of urea ammonium nitrate (UAN) were combined with 13 herbicides (2,4-D, atrazine, chlorimuron, dicamba, flumioxazin, glyfosinate, glyphosate, isoxaflutole, mesotrione, nicosulfuron, paraquat, pendimethalin, *s*-metolachlor) in a lab incubation study to determine if differences in N_2O emissions could be noted between herbicides. These results indicated significantly lower N_2O emissions from nicosulfuron and chlorimuron. As a mitigator, chlorimuron + UAN was then tested in the field alongside the high emitter of *s*-metolachlor + UAN and the UAN alone treatment. Chlorimuron + UAN showed no difference from the fertilizer alone. However, *s*-metolachlor + UAN promoted N_2O emissions over that of the UAN alone. Lab soil was analyzed for the bacterial quantification gene of 16*S* and the denitrification genes *nirK*, *nirS* and *nosZ*. No differences were noted between treatments at 25 hours or 7 days

following herbicide application. The differences in field and lab results are likely a direct effect of the procedural differences in these two methods of measuring N_2O emissions. A lack of differences in denitrifying bacteria in the qPCR data indicated fungi and nitrifying bacteria may need to be considered in N_2O production.

1. Introduction

Nitrous oxide (N_2O) is an increasing concern for agricultural scientists. As a greenhouse gas, N_2O has the greatest global warming potential of the three major greenhouse gases, carbon dioxide (CO_2), methane (CH_4), and N_2O. N_2O retains energy in the atmosphere approximately 300 times greater than that of the baseline gas, CO_2 (Environmental Protection Agency 2015b). This energy retention has shown evidence of contributing to both climate change and ozone depletion (Chipperfield 2009). Agriculture is thought to contribute between 10 and 12 percent to annual, global greenhouse gas emissions and is the leading anthropogenic factor contributing to non-CO_2 greenhouse gases at a rate of 56 percent annually (Smith et al. 2014).

In a crop setting, most N_2O is contributed via bacteria conducting denitrification (Franzluebbers, 2005; Bateman and Baggs, 2005; Venterea et al., 2011; Environmental Protection Agency, 2015). Studies have shown that quantifying genes from different portions of the denitrification process could quantify which bacterial species contributed to the N_2O emissions. The genes *nirK* and *nirS* have been studied as the genes responsible for the conversion of nitrite (NO_2) to nitric oxide (NO). Additionally *nosZ* has been studied as the gene making the conversion of N_2O to N_2 possible. Species with the *nirS* gene also contained the *nosZ* gene at a rate of approximately 80% while those with the *nirK* gene lacked *nosZ* at a rate of 70%. Approximately 10% of species contained both *nirK* and *nirS* (Graf et al. 2014).

Previous research indicated that water-filled pore space (WFPS), fertilizer, and tillage are all major factors contributing to N_2O emissions in a given agricultural setting. The greatest N_2O emissions have been shown with a WFPS of 60% or greater which created anaerobic conditions in which the denitrification process was carried out (Bateman and Baggs 2005; Thangarajan et al. 2013). Additionally, research demonstrated that greater N_2O emissions occurred with greater amounts of nitrogen fertilizer applied. This held true for both organic and synthetic fertilizers. However, synthetic fertilizers showed greater emissions over that of the organic fertilizers (Baggs et al. 2003). Multiple studies have demonstrated that those systems with tillage will emit more N_2O than that of a no-till cropping system (Robertson et al., 2000; Baggs et al., 2003; Omonode et al., 2011; Goglio et al., 2013; Jin et al., 2014). While these factors demonstrated contributions to greenhouse gas emissions, little research has investigated the implications of herbicides on N_2O emissions.

As agents that control populations of pest plants in a field, herbicides are commonly applied in agricultural settings. Herbicides have the capability to have a large effect on the soil environment. Herbicides fall into two broad classifications of general and selective. A selective herbicide will be targeting a particular organism while a more general herbicide will have a range of target pests.

In the past, herbicides with the greatest non-target effects also corresponded to those which had the greatest soil mobility. In soil, the rhizosphere is impacted by the use of plant-mobile herbicides. Some soil microbes are more likely to suffer from non-target pesticides than from more specific pesticides. Overall, actinomycetes and spore-forming bacteria are less sensitive to herbicides, while fungi, nitrifying bacteria and, nematodes are more easily impacted (Killham 1994). As a general rule, herbicides will be more likely to impact bacteria in the soil than other soil microbiota (Benckiser and Schnell 2007).

Limited research exists explaining the impact of commonly used herbicides on denitrification. Sulfonylurea herbicides have demonstrated the potential to impact nitrification and denitrification through damage to the bacteria conducting these processes in a given soil (Gigliotti and Allievi 2001; Zhang et al. 2013; Xu et al. 2014). The nitrogen-fixing bacteria, *Azospirillum* spp., were the most sensitive (Forlani et al. 1995). The ability to degrade microbial activity varies with properties such as pH, organic matter, and temperature. In the case of sulfonylureas, degradation will slow as pH increases particularly when it is greater than pK_a and this will lead to persistence in the soil. An increase in temperature leads to greater hydrolysis and a faster breakdown of the sulfonylurea (Grey and McCullough 2012). A quick breakdown of sulfonylureas could in turn lead to lesser impacts on soil microbiota.

Chlorimuron showed the potential to impact microbial activity through the potential to stimulate soil activity, microbial biomass, gram-negative: gram-positive bacteria ratio, and the Shannon Index. However, higher application rates impacted microbial biomass carbon, total phospholipid fatty acid (PFLA), and overall soil microbial community structure (Xu et al. 2014). Low and high rate applications of chlorimuron demonstrated the potential to inhibit denitrifiers. As demonstrated through elevation of NO_3-N in the soil and depletion of *nirS* and *nirK*. While *nirS* recovered by the end of the seven day study, *nirK* did not (Tan et al. 2013).

This research aims to investigate common corn and soybean herbicides in the U.S. and their impact on N_2O emissions and denitrifying bacteria. Our objective of this study was to determine if corn and soybean herbicides increase or decrease N_2O emissions in the lab and field setting.

2. Materials and Methods

2.1 Lab Incubation

Research was conducted using a Tarboro loamy sand soil from Goldsboro, NC at the Cherry Research Farm, Center for Environmental Farming Systems (35.38743, -78.03442) sieved to a particle size of $\leq$ 2.00 mm. Incubations were conducted in three runs with three replications during each run using 946 mL glass jars with 300.0 g of fresh soil corrected to a water filled pore space (WFPS) of 70%. Herbicides of 2,4-D, atrazine, chlorimuron, dicamba, flumioxazin, glyfosinate, glyphosate, isoxaflutole, mesotrione, nicosulfuron, paraquat, pendimethalin, and *s*-metolachlor (Table 1) were applied in these jars in combination with a high (135 kg N ha^{-1}) and low rate (22.5 kg N ha^{-1}) of urea ammonium nitrate (UAN) formulated at 30% for a total of 28 treatments. Herbicide rates are available in Table 1. Jar lids were placed on atleast 90 minutes prior to sampling time to allow for the accumulation of gases. Gas samples of 5 mL were collected in nitrogen flushed crimp top vials and measured for N_2O content using a Shimadzu gas chromatograph containing an FID and ECD detector. Gas samples were taken 12, 25, 48, 72, 120, and 168 hours following herbicide application (Cai 2008).

2.2 Static Chamber

Research was conducted in four replications during 2015 in Goldsboro, NC at the Cherry Research Farm, Center for Environmental Farming Systems (35.38743, -78.03442). The study was organized in a randomized complete block design and blocked spatially in a Wickham sandy loam soil. Treatments of *s*-metolachlor, chlorimuron, and no herbicide were applied in combination with 30% UAN at a rate of 135 kg N ha^{-1}. Following herbicide application, 5 cm of irrigation was applied for herbicide activation and stimulation of N_2O emissions. Gas emissions were collected using static chambers at 24 time increments, following herbicide application, for a week. A total of 5 mL of gas was collected and analyzed for N_2O content using a Shimadzu gas chromatograph containing an FID and ECD detector.

Chamber data were run through the HMR program in R statistical software based on previous research (Pedersen et al. 2010). The manual operation for this program was used in which the user can view the program's selection of HMR or linear for a particular chamber. Chamber data was used based on program recommendations and data was calculated to reflect N_2O emissions per area and unit time.

2.3 qPCR

Soil samples were collected from the chlorimuron, paraquat, *s*-metolachlor, and the control treatments during separate runs of the lab incubation study at the 25 hour and 7 day mark following herbicide application. These soil samples were analyzed for copy number of the gene 16*S* for overall bacterial population and the denitrification genes *nirS*, *nirK*, and *nosZ*.

Genomic DNA was extracted from approximately 1 g of soil using the PowerSoil DNA Isolation kit (PowerSoil, MoBio Laboratories, Solana Beach, CA) according to manufacturer's instructions. DNA concentration was determined using a Nano-drop 2000 spectrophotometer at 260 nm (Thermo Fisher Scientific, Pittsburgh, PA). DNA extracts were diluted to 5 ng µl-1 and stored at -20 ºC for further analyses.

Standards for quantitative PCR (qPCR) analysis of the 16*S* gene as well as the denitrification genes *nirK*, *nirS*, and *nosZ* were prepared using the primers listed in Table 2 (Throbäck et al. 2004; Henry et al. 2006; Weisburg et al. 1991). The authors recognize that advances in understanding of microbial denitrifiers have revealed two major clades of nosZ commonly referred to as nosZ I and nosZ II (Jones et al. 2012). The primers used in this study amplify nosZ clade I only.

Amplification of qPCR products was carried out with a Stratagene Mx3000p QPCR System (Agilent Technologies, Santa Clara, CA) using SYBR green detection. Each 20 µl reaction contained 10 µl of 2X SSO Fast EvaGreen Supermix (BioRad Laboratories, Hercules, CA), 400 nM forward primer, 400 nM reverse primer and 25 ng of total template DNA (except for standards and non-template controls). Thermal cycling conditions were as follows: 1 cycle of 98 ºC for 3 min, 40 cycles of 98 ºC for 15 sec, and 62 ºC for 30 sec (16*S*, *nirK*, *nirS*, *nosZ* genes), followed by a continuous melting curve of 30 sec at 62 ºC to 95 ºC, to assess qPCR product specificity.

2.4 Statistics

Statistics were conducted using PROC GLIMMIX in SAS 9.4 for the incubation study. Data were analyzed in repeated measures using a gamma distribution and Laplace likelihood approximation. Significance differences were accepted at $p \leq 0.05$.

Field herbicide data statistics were conducted using PROC MIXED in SAS 9.4 and were log transformed as previous research deemed appropriate for gas data (Moulin et al. 2014). Repeated measures analysis was used with the heterogenous compound symmetry model (CSH). Significant differences were accepted at $p < 0.10$*, $p < 0.05$**, or $p < 0.001$***.

Statistics were conducted in PROC MIXED in SAS 9.4 for quantitative gene content of 16S, *nirS*, *nirK*, and *nosZ*. Significance differences were accepted at $p \leq 0.05$.

3. Results

3.1 Lab Incubation

Gas emissions were higher for the high nitrogen application rate (135 kg N ha^{-1}) than the low nitrogen (22.5 kg N ha^{-1}) application. The high nitrogen application had an average emission across times of 0.46 ppm while the low application had an average emission of 0.19 ppm. Gas emission data for the 28 herbicide treatments indicated significant differences in N_2O emissions with herbicide applied. Chlorimuron consistently showed the lowest level of gas emissions with an average of 0.09 ppm over time. Nicosulfuron also averaged lower than the control with 0.17 ppm while other herbicides showed comparable emissions to the non-treated control over time. *S*-metolachlor and atrazine showed the greatest emissions with an average of 0.43 and 0.44 ppm, respectively (Figure 1; Figure 2).

3.2 Field Herbicide

Analysis of week-long N_2O emissions in a field setting revealed the greatest emissions following irrigation application. Regardless of treatment, N_2O emissions decreased over time. Significant differences in herbicide applications were noted until day four (Figure 2). At 24 hours after application, the *s*-metolachlor + UAN treatment was the greatest N_2O emitter with a rate of 1.29 mg N_2O-N m^{-2} hr^{-1}. Unlike the incubation study, field data showed chlorimuron had N_2O emissions comparable to the control treatment. However, no statistical difference was present between chlorimuron + UAN and the UAN alone treatment. No herbicide difference was noted in N_2O emissions at the end of the seven day study.

3.3 qPCR for 16*S*, *nirK*, *nirS*, *nosZ*

The numbers of the 16*S* gene indicated that overall bacterial population number differences remained the same between treatments and over time. Within the denitrification genes quantified, *nirK*, *nirS*, and *nosZ* no differences were noted based on herbicide applied.

4. Discussion

The main effects of nitrogen rate and herbicide application were both found to be significant in the incubation study. N_2O emissions were consistently lower from chlorimuron and higher from S- metolachlor compared to the control (Figure 1; Figure 2). This is aligned with previous research indicating sulfonylurea herbicides such as chlorimuron have the potential to inhibit nitrifying potential in a soil (Gigliotti and Allievi 2001; Zhang et al. 2013), change the composition of soil microbiota in a soil (Gigliotti and Allievi 2001; Zhang et al. 2013; Xu et al. 2014; Jiang et al. 2015; Zhang et al. 2010; Tan et al. 2013; Joly et al. 2015), and decrease N_2O emissions (Jiang et al. 2015). Similar to evidence showing inhibition of microbiota and decreasing N_2O emissions with sulfonylurea herbicides, chloroacetamides have shown evidence of stimulating microbiota and therefore increasing N_2O emissions as shown here (Jiang et al. 2014; Min et al. 2001; Joly et al. 2015; Joly et al. 2012).

Greater N_2O emissions with greater WFPS was supported with greater N_2O emissions following field irrigation application as previous studies have indicated (Bateman and Baggs 2005; Jiang et al. 2015; Thangarajan et al. 2013). The *s*-metolachlor + UAN treatment was the greatest N_2O emitter just as other chloroacetamides increased N_2O emissions following application (Jiang et al. 2014; Min et al. 2001). While also a member of the chloroacetamide family, the herbicide butachlor was found to increase N_2O emissions via increasing NH_4^+-N and NO_3^--N content as well as through the stimulation of denitrifying bacteria and ammonia-oxidizing bacteria (Jiang et al. 2014). Another study showed an increase in denitrifying bacteria and fungi with the addition of butachlor (Min et al. 2001). Unlike the incubation study, field data showed chlorimuron had comparable N_2O emissions to that of the control treatment. Previous studies have indicated each sulfonylurea herbicide impacted soil microbiota differently (Gigliotti and Allievi 2001). No herbicide difference was noted in relation to N_2O emissions at the end of the seven day study suggesting recovery of the microbial population regardless of treatment. This recovery has been demonstrated in multiple studies where both labeled and higher rates of herbicides were applied but, soil microbiota recovered (Xu et al. 2014; Tan et al. 2013; Wardle and Parkinson 1990).

Differences between the incubation and field study results are likely reflective of the nature of the two types of sampling. In the case of the incubation study, soil samples are maintained at a 70% WFPS for the entirety of the study. Evidence from previous studies indicated 70% WFPS falls within the optimal range for denitrification to occur (Bateman and Baggs 2005; Thangarajan et al. 2013). In the field situation, 5 cm of irrigation was applied following herbicide application. This application served as a stimulant for N_2O emissions however, this moisture level was not maintained. In applying and maintaining a 70% WFPS each herbicide was well distributed through the soil. Along with differences in moisture levels, the incubation study used sieved soil which also alters the composition of soil and potentially its reactivity.

The numbers of the 16*S* gene indicated that overall bacterial population number differences remained the same between treatments and over time. Within the denitrification genes quantified, none showed a significant difference in gene number based on herbicide applied. However, changes in fungi or nitrifying bacteria could cause changes in gas emissions as suggested by previous studies regarding other chloroacetamides and sulfonylureas (Min et al. 2001; Jiang et al. 2014; Zhang et al. 2013; Tan et al. 2013; Zhang et al. 2010; Xu et al. 2014).

This research agrees with previous research suggesting that sulfonylurea herbicides such as chlorimuron mitigate N_2O emissions (Zhang et al. 2010; Xu et al. 2014; Tan et al. 2013; Zhang et al. 2013; Joly et al. 2015). Studies have suggested that sulfonylureas inhibit nitrifiers with autotrophic nitrifiers being more sensitive than heterotrophic microorganisms (Jiang et al. 2014; Gigliotti and Allievi 2001). This would explain a difference in N_2O emissions but lack of differences in denitrification genes. Previous studies also agree that chloroacetamide herbicides have the potential of stimulating soil microbiota and therefore increasing N_2O emissions (Min et al. 2001; Jiang et al. 2014).

5. Conclusions

This study indicates applications of chloroacetamides such as *s*-metolachlor increase N_2O emissions while sulfonylureas reduce N_2O emissions. The differences in field and incubation data suggest moisture levels will impact the extent to which N_2O emissions are promoted or mitigated by a herbicide. In particular, chlorimuron seems to mitigate emissions with greater WFPS and herbicide distribution. *S*-metolachlor, on the other hand, seems to promote emissions in a drier environment. Further research is needed regarding the impact of herbicides on N_2O emissions with varying environmental factors including: soil type, moisture, and temperature. The lack of differences within denitrifying bacteria suggests the quantification of fungi and nitrifying bacteria is necessary.

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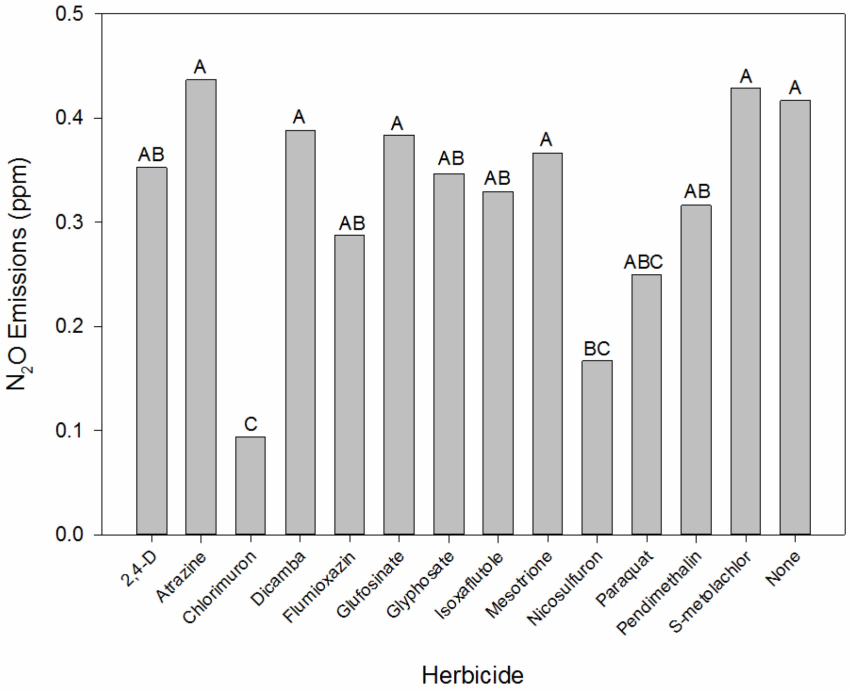
Table 1: Herbicide Applications for Lab Incubation Study

Herbicide	**Rate (g ai ha^{-1})**	**Mode of Action**	**Herbicide Family**	**WSSA Group #**
2,4-D (24D)	408	Synthetic Auxins	Phenoxy-carboxylic acids	4
Atrazine (ATZ)	1701	PS II Inhibitor	Triazine	5
Chlorimuron (CHL)	9	ALS Inhibitor	Sulfonylurea	2
Dicamba (DIC)	284	Synthetic Auxins	Benzoic Acids	4
Flumioxazin (FLU)	109	PPO Inhibitor	N-phenylthalamides	14
Glufosinate (GLU)	680	Glutamine synthetase	Phosphinic Acid	10
Glyphosate (GLY)	873	EPSP Synthase Inhibitor	Glycine	9
Isoxaflutole (ISO)	78	HPPD Inhibitor	Isoxazole	27
Mesotrione (MES)	249	HPPD Inhibitor	Triketones	27
Nicosulfuron (NIC)	35	ALS Inhibitor	Sulfonylurea	2
Paraquat (PAR)	851	PS I Inhibitor	Bipyridilium	22
Pendimethalin (PEN)	1588	Microtubule inhibitor	Dinitroaniline	3
S-metalochlor (SMT)	1588	VLC Fatty Acid Inhibitor	Chloroacetamides	15

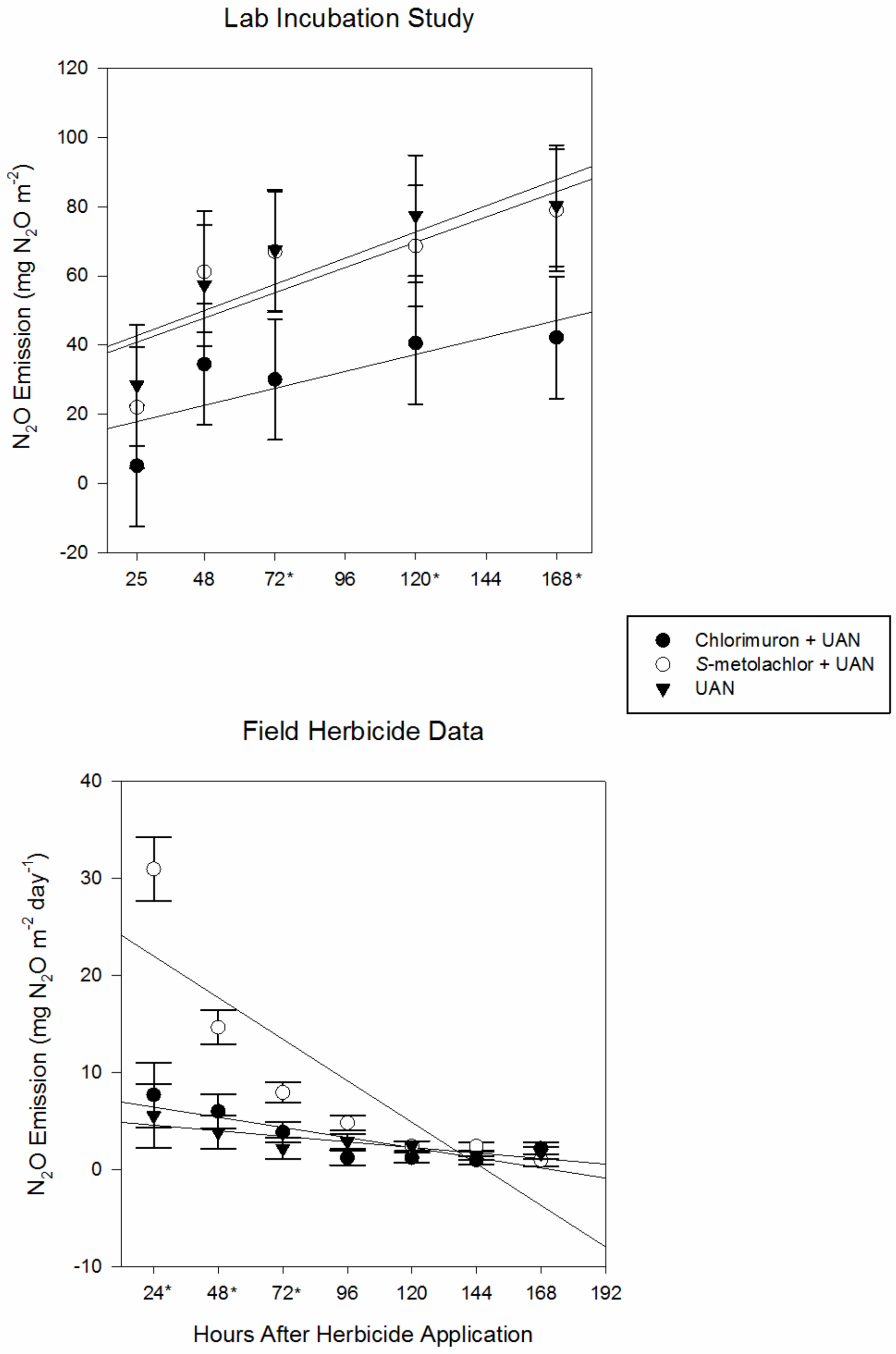
Table 2: Primer Information for Denitrification Genes

Target Gene	Sequence (5’-3’)	Reference
nirK	ATC ATG GTS CTG CCG CG (FlaCU) and GCC TCG ATC AGR TTG TGG TT (R3Cu)	(Throbäck et al. 2004)
nirS	GTS AAC GTS AAG GAR ACS GG (cd3AF) and GAS TTC GGR TGS GTC TTG A (R3cd)	(Throbäck et al. 2004)
nosZ	WCS YTG TTC MTC GAC AGC CAG (nosZ1F), and ATG TCG ATC ARC TGV KCR TTY TC (nosZ1R)	(Henry et al. 2006)
16S	ACT CCT ACG GGA GGC AGC (Eub 338F), and TTA CCG CGG CTG GCA C (533R)	(Weisburg et al. 1991)



[a]Means followed by the same letter are not different according to Fisher's Protected LSD at $p < 0.05$.

Figure 1: N_2O Emissions Averaged over Nitrogen Application and Time based on Herbicide Applied


Lab Incubation Study

Field Herbicide Data

*Treatment means differ according to Fisher's Protected LSD values at $p < 0.05$.

Figure 2: Emissions Over Time Based on Herbicide + UAN Application