

Soil Trace Gas Fluxes in Living Mulch and Conventional Agricultural Systems

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Abbreviations: Living mulch system (LMS), greenhouse gas (GHG), climate-smart agriculture (CSA), potentially mineralizable nitrogen (PMN), soil organic carbon (SOC), infrared gas analyzer (IRGA), gas chromatography (GC), permanganate oxidizable carbon (POXC), white clover (WC), crimson clover (CC), cereal rye (CR), conventional no cover crop (Tr)

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Core Ideas

White clover living mulch plots had higher overall CO_2 , N_2O , and NH_3 fluxes.

High N_2O and NH_3 fluxes in living mulch lasted through the late growing season.

Rates of CO_2 flux were partially explained by soil moisture and temperature.

Rates of N_2O flux were partially explained by soil moisture and extractable NO_3^- .

Abstract

Row crop agriculture is a significant source of two major greenhouse gases (GHGs) – carbon dioxide (CO₂), nitrous oxide (N₂O), and the air pollutant precursor, ammonia (NH₃). Fluxes of these naturally-occurring trace gases are often augmented by agricultural practices, such as fertilizer application and crop systems management. A living mulch system (LMS) maintains a live cover crop year-round and is an emerging agricultural system that can reduce pesticide and fertilizer use, while maintaining yields. Multiple trace gas fluxes of GHGs and NH₃ had never been measured together in an LMS of corn and white clover previously. This study compared soil gas fluxes in a white clover LMS with two other cover crop systems and a no cover crop system. Infrared and gas chromatography measurements were taken over two years in northern Georgia, USA. Mean soil CO₂ and N₂O fluxes (159.7 kg ha⁻¹ day⁻¹ and 0.027 kgN ha⁻¹ day⁻¹, respectively) observed in LMS plots exceeded those from other treatments. Soil temperature, moisture, potentially mineralizable nitrogen, and nitrate partially explained these differences. Mean soil NH₃ emissions were greater in LMS (0.089 kgN ha⁻¹ day⁻¹) compared to no cover crop (0.038 kgN ha⁻¹ day⁻¹). Increased N₂O and NH₃ fluxes could be from release of nitrogen (N) from decomposition of clover and release of N into the soil as the corn shades the clover. While LMS plots did not reduce trace gas emissions, labile carbon content was at least 100 mg kg⁻¹ greater than other treatments after two years, improving soil health.

Introduction

Carbon dioxide (CO₂) and nitrous oxide (N₂O) are the first and third most prevalent anthropogenic greenhouse gases (GHGs) (Myhre et al., 2013). Over 20% (10-12 Gt CO₂ eq yr⁻¹) of anthropogenic GHG emissions come from agriculture, forestry, and land use change (Smith et al., 2014) with agriculture being the largest contributor of the three since 2010 (Tubiello et al., 2015). 30-38% of agricultural GHGs and the largest anthropogenic contributions of N₂O come from soils in response to inputs such as manure and synthetic nitrogen (N) fertilizer application (Smith et al., 2014, Galloway et al., 2008, Montzka et al., 2011, Park et al., 2012). Agricultural practices that mitigate GHG emissions have the potential to reduce overall GHG emissions and increase the feasibility of meeting the 2°C increase limit outlined in the Paris Agreement within the United Nations Framework Convention on Climate Change (Paustian et al., 2016, Wollenberg et al., 2016).

N loss through N-based fertilizer application and green manure decomposition produce ammonia (NH₃) (Anderson et al., 2003, Sommer et al., 2004, Larsson et al., 1998, Ruijter et al., 2010). These emissions contribute to increased levels of secondary inorganic aerosols (SIA) which cause respiratory, cardiovascular, and other health issues (Kampa et al., 2008). Using agricultural systems that reduce soil NH₃ emissions could decrease surface SIA formation (Bauer et al., 2016) and lower indirect N₂O emissions (Bhatia et al., 2004).

Climate-Smart Agriculture (CSA) is a new paradigm for climate-risk management in agriculture that seeks to mitigate climate change, promote adaptation to climate change impacts, and enhance farm productivity and food security (Bryan et al., 2013). Using a cover crop, i.e. replacing bare fallow in the winter with crops that are suppressed and plowed as green manure in the spring, is a CSA management technique that increases soil organic carbon (SOC), offsetting GHG emissions and improving soil health (Poeplau et al., 2015).

Studies have shown reduced soil GHGs in cover crops compared to conventional tillage and no-till agricultural systems (Robertson et al., 2000, Abdalla et al., 2014, Kaye et al., 2017). However, a meta-analysis demonstrates that N₂O reductions are only present in experiments lasting less than a year, so further long-term studies are needed to determine overall effects of cover crops on N₂O (Basche et al., 2014).

A living mulch system (LMS) is a modified cover crop system where a legume cover crop grows throughout the cash crop's growing season, while contributing enough N to the soil pool to satisfy the needs of the cash crop (Zemenchik, et al., 2000). These systems have been shown to provide a variety of benefits, compared to bare soil and full cover crop suppression, including reduced erosion, nitrate leaching, and fertilizer use (as much as 75% less in some field trials), but function best in areas with ample available water (Zemenchik, et al., 2000, Nakamoto et al., 2006, Hartwig et al., 2002, Ochsner et al., 2010, Sanders et al., 2018). Due to these benefits, the LMS is being promoted as a potential sustainable alternative to conventional row crop agriculture, but the environmental impacts are relatively unknown.

Two studies have measured soil gas flux in an LMS, examining N₂O and NH₃ emissions in a kura clover (*Trifolium ambiguum*) and corn (*Zea mays*) production system (Turner et al., 2016, Alexander et al., 2019). Turner et al. (2016) found that cumulative area-scaled N₂O emissions over one growing season were higher in the LMS ($2.3 \pm 0.1 \text{ kgN ha}^{-1}$) despite lower fertilizer inputs, compared to conventional corn production ($1.3 \pm 0.1 \text{ kgN ha}^{-1}$). The majority of this increase came later in the growing season due to clover mineralization (Turner et al., 2016). Conversely, Alexander et al. (2019) found no significant differences in NH₃ or N₂O flux between management systems, indicating there is no consensus on how an LMS affects trace gas fluxes. N-rich cover crops, such as those used in LMS, can lower N loss as NH₃ from fertilizer applications (Larsson et al., 1998).

In order to better understand the broader impacts of an LMS, we aimed to analyze additional GHGs and NH₃. We also wanted to explore how a different species of clover could affect these trace gases. In white clover and corn LMS, N biomass pools in clover are mineralized later in the growing season and N uptake increases in the corn (Andrews et al., 2018). In addition, white clover grows faster than the kura clover used in previous studies (Speer et al., 1985, Zemenchik et al., 2001), and thus can have greater N decomposition rates (Tribouillois et al., 2015). Decomposition is the driving factor for N₂O production in a leguminous LMS (Turner et al., 2016), so the white clover and corn LMS may contribute to higher fluxes later in the growing season. Flux values should be analyzed with concurrently measured soil parameters to understand the soil processes contributing to GHG fluxes.

This study measured the CO₂, N₂O, and NH₃ soil fluxes and potential causal parameters in a white clover and corn LMS. We compared these fluxes to two other no-till cover crop systems (crimson clover and cereal rye) and one no cover crop system over two growing seasons. We hypothesized that in LMS plots: 1. CO₂ fluxes would be largest in LMS plots due to increased root and microbial respiration, but would be offset by increased SOC; 2. Overall N₂O fluxes in LMS would be greater than other techniques using dead mulches or bare soil. Additionally, an LMS with white clover would have higher N₂O fluxes than one with kura clover, due to greater N decomposition rate of white clover; and, 3. NH₃ fluxes would be lower than other management techniques due to reduced urea fertilizer application outweighing N loss from the green manure.

Materials and Methods

Site Description and Field Preparation

Over the 2016 and 2017 summer growing seasons, we measured the CO₂, N₂O, and NH₃ soil fluxes associated with four different management techniques for growing corn: 1) a

no-till system with crimson clover as the cover crop (CC), 2) a no-till system with cereal rye as the cover crop (CR), 3) a no-till white clover LMS (WC), and 4) a no cover crop conventional system (Tr) (Figure S1). There were three 6.1×7.3 m plots of each management technique located at the West Unit of the University of Georgia's J. Phil Campbell Sr. Resource and Education Center in Watkinsville, GA, USA, on a soil classified as a Cecil sandy loam (fine, kaolinitic, thermic type Kanhapludults) (Figure S2). Details regarding field treatment can be found in the Supplementary Material (Table S1). 15 mm irrigation was applied using a Kifco (Havana, IL) T200L portable water wheel. Each irrigation event applied 20 mm water when water-filled pore space dropped to 40% or lower to increase it to 90%.

Static Chamber Measurements of GHGs

Gaseous CO_2 and N_2O fluxes were measured weekly over each growing season in opaque PVC static chambers (Collier et al., 2014). Chamber design followed previous studies with size and material modifications explained in Figure S3 (Parkin et al., 2010, Collier et al., 2014). Three chambers were inserted in March, 2016 into each plot (Figure S2).

In WC chambers, there was living clover biomass, but biomass in CC and CR chambers had been previously killed by herbicides (Roundup for crimson clover and Dicamba for cereal rye) and was on the surface of the soil. One chamber from each of the 12 plots was sampled weekly by extracting five 10 mL samples midmorning via syringe (Collier et al., 2014). Chamber tops were placed over collars and a time 0 measurement was taken. Samples were then drawn from chambers over 30 minutes at 7.5-minute intervals for the 2016 season and over 15 minutes at 3.75-minute intervals for the 2017 season. The time interval changed in 2017 to save time and reduce potential temperature effects on the chambers after initial analysis of 2016 data showed a 15-minute measurement would provide

an accurate estimate of the flux. To account for heterogeneity in soil fluxes, each week we selected in rotation one of the three collars to sample. Gas samples were analyzed using a Shimadzu Gas Chromatograph (GC)-2014 GHG using a flame ionizing detector and methanizer for CO₂ and an electron capture detector for N₂O.

Background flux samples were taken on August 12th, November 5th, and December 28th, 2016 and February 1st, 2017 in the same 12 plots and with the same sampling techniques as in the growing season. During this time, there was no corn planted. This set of data were assumed to be minimally affected by management techniques and used as a reference to control for baseline fluxes in regression analysis.

Following GC analysis, we plotted concentrations of each gas species over time and calculated fluxes using the following formula:

$$F = m \times V/A$$

[1]

Where F is soil flux in $\mu\text{mol m}^{-2} \text{sec}^{-1}$ or $\mu\text{mol m}^{-2} \text{hr}^{-1}$ for CO₂ and N₂O respectively, m is the rate of GHG concentration change over 30 or 15 minutes in $\mu\text{mol m}^{-3} \text{sec}^{-1}$ or $\mu\text{mol m}^{-3} \text{hr}^{-1}$, V represents chamber volume in m³, and A is the chamber surface area in m². Only fluxes with a slope with an R² of 0.75 or greater were included for analysis. This followed Collier et al. (2014), which states that a linearity protocol can be determined via visual inspection when using the static chamber method. It is known that gas fluxes are sometimes non-linear (Parkin, et al., 2010), but due to the clear visual linearity of fluxes observed in this study, a simple approach was taken. Additionally, sensitivity analyses were carried out using R² values of 0.8 and 0.9 and we found minimal changes in significance of key study findings (Supplemental Tables S4-S7).

CO₂ In-Field Infrared Gas Analyzer Measurements

A Licor-6400XT Portable Photosynthesis System Infrared Gas Analyzer (IRGA) was used to measure CO₂ in 2016 due to data extending beyond acceptable limits of the GC calibration curve (Doff Sotta et al., 2004). Samples from the IRGA were not taken directly in PVC chambers, but next to them, to allow for simultaneous sampling.

Measurements from the IRGA were taken from every plot each week and recorded as an average of continuous individual measurements over a minimum change of 10 ppm CO₂. IRGA measurements were collected directly in corn rows (areas with no white clover biomass) and half way between corn rows (areas with white clover biomass) to determine if there were differences in respiration.

NH₃ Acid Trap Measurements

Ammonia fluxes were measured with a static chamber and a vacuum pump acid trap in an open system configuration. Acid trap designs came from previously tested methods (Misselbrook et al., 2005), with modifications including a Balston NH₃ filter instead of an additional tube of acid before the chamber inlet for the ambient air. Flow rate was also reduced to match different chamber volume. Sampled air came from inside the chamber and was then bubbled through a fritted Midget impinger at a flow rate of 2 L min⁻¹ for two hours. Due to only having two acid traps, only WC and Tr plots were sampled each week, rotating chambers and plots randomly to obtain a high sample size for the two systems hypothesized to be most different. Samples were analyzed colorimetrically in duplicate using EPA method 350.1 (O'Dell, 1993).

Soil and Environmental Parameters

Soil water content and temperature were measured using CS625 reflectometers (Campbell Scientific, Logan, UT) placed at two different soil depths (0-15 cm and 15-30 cm) between the corn rows. The rods were 30 cm in length and installed at an angle of 30 degrees

from the surface. Water content data and temperature were measured and recorded on 10-min intervals, stored on data loggers, and downloaded weekly. A soil moisture release curve based on the van Genuchten equation (Van Genuchten, 1980) was created using the evaporation method (Arya, 2002) with a HYPROP device (Decagon, Pullman, WA) from soil collected from the plot area.

Corn canopy light interception was measured weekly until the tasseling (VT) stage of corn development. A line quantum sensor (Model LI 191sb, Li-Cor, Lincoln, NE) measured the amount of light above the corn canopy and the amount of light reaching the clover canopy in the WC plots, the surface of the cover crop residue in the CC and CR plots, or the soil surface in the Tr plots, at four locations in each plot. The percentage of light intercepted by the corn canopy was calculated using the following formula:

$$\% \text{ light interception} = [1 - (\text{light at lower surface} / \text{light above corn canopy})] \times 100$$

[2]

Soil cores were taken from eight locations at a depth of 15 cm using a 1.5-cm inside diameter handheld soil probe within the center two rows of each plot. Two of the cores were taken from random locations within the rows and three cores were taken from random locations between the rows. These samples were taken weekly throughout the growing season (April 26th – July 20th 2016, and May 4th – July 12th 2017). The cores were combined, air-dried, and stored at 4°C. Five grams of soil from each sample was extracted at 21°C with 40 mL of 1M KCl (cold extraction) and at 100 °C with 40 mL of 2M KCl (hot extraction) for NO₃⁻ and NH₄⁺ analysis (Campbell et al., 1994). Soil extracts were analyzed for NO₃⁻ and NH₄⁺ concentration using a TL-2800 Ammonia and Nitrate Analyzer. Soil potentially mineralizable nitrogen (PMN) was calculated as the difference between cold and hot NH₄⁺ extractions. Two soil pits were dug on the periphery of the plots and 5 × 5 cm brass rings were inserted into the top 15-cm of soil. The rings and soil were dried at 105°C and the soil

bulk density was calculated. Bulk density, saturated hydraulic conductivity (K_{sat}), and total porosity were measured according to the core method, constant head method, and calculation from particle and bulk densities, respectively (Grossman et al., 2002, Klute et al., 1986, Flint, et al., 2002). Labile C was quantified by measuring permanganate oxidizable carbon (POXC) from six soil samples per system taken from the brass rings in August, 2017, following the methodology in Weil et al. (2003).

Soil N compounds were measured in paired plots directly across one irrigation lane to those where gas fluxes were measured in 2016 due to location of soil sampling equipment, and were taken within two days of flux sampling. In 2016, soil NO_3^- , NH_4^+ , and PMN data for the linear model were averaged over the three paired plots of corresponding management technique as opposed to individual data points in 2017, when soil data were taken directly from plots where flux was measured. Management system was assumed to be the limiting factor, as both sampling locations had been identically-treated experimental plots for the three years prior to the inception of this experiment. Finally, clover biomass data were measured weekly in all WC plots. Biomass measurements were used along with soil temperature to estimate the amount of respiration coming from clover versus soil in an attempt to infer soil-only respiration in following analysis.

Statistics and Missing Data

All statistics were carried out using R version 3.4.1. Observed N_2O and NH_3 fluxes were converted to N_2O-N and NH_3-N for statistical analysis. All fluxes were log transformed prior to statistical test after failing normality testing with a Shapiro-Wilks test (Das & Imon, 2016). An initial ANOVA of all individual flux observations determined that there were significant differences in CO_2 and N_2O fluxes between treatments. To specify these differences, mean GHG fluxes were compared between all four management techniques

using Tukey's pair-wise comparison. N₂O comparisons used static chamber GC data for all time points. For IRGA data, an average of three replicates of both in-row and between-row measurements from each plot every week was used for data analysis. Comparisons of CO₂ used between-row IRGA data for 2016 and GC data for 2017. A student's T-test compared mean NH₃ fluxes in WC and Tr plots. Cumulative sums for the growing seasons were also calculated by extrapolating the average from all plots for the days between sampling.

A multiple linear regression of log-transformed individual observations of flux was performed. The goals of the regression were to determine which soil and climatic parameters were related to differences in soil GHG fluxes found in the pair-wise comparisons, and which management techniques were still significantly different after controlling for these parameters. Management technique was included as a categorical variable in part, to control for differences in fertilizer application. Single variable, season specific, and management-technique specific regressions were conducted initially to develop the final model for N₂O and CO₂ as displayed below:

$$Y_s = \beta_0 + \beta_1 Tech_1 + \beta_2 Tech_2 + \beta_3 Tech_3 + \beta_4 Season_{2016} + \beta_5 Season_{2017} + \beta_6 Temp + \beta_7 Mois + \beta_8 LightInt + \beta_9 NO_3 + \beta_{10} NH_4 + \beta_{11} PMN + \varepsilon$$

[3]

Where Y_s denotes soil trace gas emissions with s for separate log transformed gas species (N₂O or CO₂). The independent variables are as follows: **Tech₁** is CC, **Tech₂** is CR, **Tech₃** is WC compared to Tr as a reference, **Season₂₀₁₆** and **Season₂₀₁₇** compared to background measurements as a reference, **Temp** is soil temperature in °C at 15 cm, **Mois** is soil moisture between corn rows at 15 cm depth in volumetric water content, **LightInt** is light interception as the inverse of the percentage of light reaching the soil, **NO₃** is soil nitrate in

$\mu\text{g/g}$, NH_4 is soil ammonium in $\mu\text{g/g}$, and **PMN** is potentially mineralizable N in $\mu\text{g/g}$.

Multicollinearity was tested for in all final models and was not found.

Percent change for each coefficient was calculated using the following formula:

$$\% \text{ Change} = 100 \times (e^{\beta} - 1)$$

[4]

For all final CO_2 and N_2O models ($N=292$ and 203 respectively), an estimate of soil temperature, moisture, and N compound were calculated for Tr plots from the average of all CR plots over the same time period. This was because instrumentation for these parameters was set up from previous experiments before Tr plots were created for this one. Plots without cover crops had similar labile C to CR plots (Table S2) and had equivalent fertilization amounts each year (Table S1). Missing values for soil temperature, moisture, and N compound data were replaced with averages of the measurements taken the week before and the week after in plots with the same corn production system. This could bias results towards the null, and underestimate the effects of these parameters in the linear regression.

Model parameters other than soil temperature and moisture were not measured during background sampling. Averages from the end of the 2016 season and the beginning of the 2017 season were paired with observed background fluxes. Light interception was considered to be zero during background periods, as no corn was present to shade the soil. Data from IRGA (2016) and GC (2017) measurements were combined for the CO_2 model.

In order to estimate CO_2 fluxes from soil without clover respiration, emissions were estimated by subtracting clover respiration rates from total CO_2 flux observed. The clover respiration rates were derived, using measured clover biomass and soil temperature and a previously developed curve of clover respiration over various temperatures (Beinhart, 1962).

The formula for the clover respiration estimation is shown below:

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$$CO_2\text{Clover} = \frac{(m \cdot T + b) \cdot BM \cdot 1,000,000 / 3600 \cdot P}{A \cdot R \cdot T_k}$$

[5]

Where $CO_2\text{Clover}$ is the estimated CO_2 flux from clover in $\mu\text{mol m}^{-2} \text{sec}^{-1}$, \mathbf{b} and \mathbf{m} are the intercept in respiration rate in $\mu\text{L O}_2 \text{mg}^{-1} \text{hour}^{-1}$ (-8.2) and slope in respiration rate of the respiration (0.48) function derived from Beinhart (1962), respectively, \mathbf{T} is soil temperature in $^\circ\text{C}$, \mathbf{BM} is the clover biomass in kg within the chamber, \mathbf{P} is the surface pressure in atm, \mathbf{A} is the area of the chamber in m^2 , \mathbf{R} is the gas constant in $\text{L atm K}^{-1} \text{mol}^{-1}$, and \mathbf{T}_k is the soil temperature in Kelvin.

Results

CO_2

Soil porosity, K_{sat} , and labile C were greater in WC plots compared to all other systems (by at least 7.4, 431, 22.9 % respectively), while bulk density was lower by at least 8.8% (Table S2). Between-row measurements from GC and IRGA correlated well ($R^2=0.73$) in 2016, with IRGA measurements greater than GC measurements in the same plot. Only GC measurements were obtained in 2017 due to IRGA instrument failure, possibly leading to an underestimation of CO_2 flux during the 2017 growing season.

Mean CO_2 fluxes for both 2016 and 2017 from between-row measurements were higher in WC plots compared to Tr plots, with a difference of $1.72 \mu\text{mol m}^{-2} \text{sec}^{-1}$ (p-value <0.001). This effect was not found in corn rows in 2016, where clover biomass was not present in the chamber (Table 1). Fluxes were greatest in WC plots in the middle of the growing season (Figure 1).

Heterotrophic estimates did not match well with corn row measurements early in the growing season (Figure S5), potentially leading to the higher fluxes observed after

subtraction. The CO₂ fluxes in corn rows and between rows in WC plots differed significantly, unlike for other treatments (Figure S5), indicating clover respiration was occurring when the clover switched from photosynthesis to respiration in the opaque chambers. Cumulative sums for CO₂ fluxes were higher in WC plots for both growing seasons (Table 1) and were greater than total SOC sequestered per year in WC plots (2.27 kg m⁻² versus 0.62 kg m⁻²).

The multiple linear regression of individual log-transformed between-row CO₂ fluxes showed greater flux in CC, CR, and WC plots compared to Tr plots (p-value<0.001), with WC plots having the greatest increase (Table 2), confirming the results of the post-hoc ANOVA analysis for between-row measurements. Soil moisture and light interception were correlated with higher CO₂ flux (p-value <0.001 and 0.04).

N₂O

Mean N₂O flux was higher in WC plots with differences of 1.15, 1.09, and 1.05 μmolN m⁻² hr⁻¹ for WC-CC, WC-CR, and WC-Tr, respectively (Table 1, p-value ≤ 0.001 for all). The N₂O fluxes were near background levels at the beginning and end of the growing season and greater, following fertilizer application. The largest fluxes were in the WC plots for both years and in Tr plots following fertilizer application in 2016. There were greater fluxes in CC, CR, and Tr plots in 2017 in the weeks following fertilizer application (Figure 2). Cumulative sums for N₂O were also higher in WC plots for both growing seasons compared to all other techniques (Table 1).

The multiple linear regression of individual log-transformed N₂O fluxes indicated that WC plots had a larger N₂O flux than Tr plots (Table 3, p-value < 0.001), confirming the results of the post-hoc ANOVA analysis. Higher N₂O fluxes compared to Tr plots were not observed in CC and CR plots (Table 3). Soil moisture and soil NO₃ were correlated at the

$\alpha=0.05$ level with higher N_2O fluxes (p-values of < 0.001 and 0.016 respectively) and soil NH_4 was correlated at the $\alpha=0.10$ level (p-value of 0.063).

NH_3

Mean NH_3 fluxes were significantly higher in the WC plots ($21.8 \text{ nmolN m}^{-2} \text{ hr}^{-1}$, 95% CI (13.8, 29.7)) compared to the Tr plots ($9.22 \text{ nmol m}^{-2} \text{ hr}^{-1}$, 95% CI (6.25, 12.2)) during the 2017 growing season. NH_3 fluxes were higher towards the middle of the growing season in both Tr and WC plots with large fluxes in the weeks following fertilizer application (maximum flux of $75 \text{ nmolN m}^{-2} \text{ hr}^{-1}$) (Figure 3).

Discussion

CO_2

Higher CO_2 fluxes in WC plots were observed in between rows, where clover biomass was present, but not within corn rows, where no clover biomass was present in 2016. This indicates a large amount of the flux between corn rows could be due to clover respiration. The discrepancy between estimated heterotrophic respiration and measurements in corn rows likely arose from an underestimation of clover respiration in the early growing season (Figure S5). Future studies should directly measure clover respiration and how it changes when the chamber is closed or open to better account for light effects on clover respiration. More in-row measurements could also avoid clover respiration masking responses in heterotrophic soil respiration. The increase in soil labile C in LMS plots indicates minimal C sequestration in comparison to increased emissions. However, labile C has rapid turnover rates, and future studies should assess total SOC at different depths to get a better measure of sequestration in an LMS (Table S2) (Martens, 2000).

Decomposition of clover could have increased CO₂ fluxes (Singh et al., 1977) and soil respiration can increase with higher C:N ratios in litter layers (Spohn, 2015). Future studies should include cover crop C:N ratios to explore these relationships further in an LMS compared to other systems.

Soil moisture was positively correlated with CO₂ flux in the linear model and has previously been shown to be an important factor altering CO₂ flux within no-till systems (Harper et al., 2005, Linn et al., 1984, Harmanjit et al., 2015).

Previous studies have shown that systems with N-rich legume biomass residue have higher soil respiration compared to systems without residue on the soil surface (Hendrix et al., 1988). This effect could be amplified in the WC plots, with clover depositing biomass throughout the entire growing season. This study showed that a WC LMS had higher soil heterotrophic respiration after subtracting estimated autotrophic clover respiration. However, this did not corroborate measurements from in corn rows, especially early in the growing season. Future studies should calculate a complete net C equivalence using inputs and outputs including irrigation, fertilizer production, and carbon sequestration.

N₂O and NH₃

Between-row fluxes of N₂O and NH₃ were significantly higher in WC plots compared to the other cover crops and no cover crop systems. Higher NH₃ fluxes in WC plots compared to Tr plots was contrary to the original hypothesis, based on the fact that a lower amount of urea-based fertilizer was applied on WC plots versus Tr plots (Table S1). Cereal grain cover crops have been shown to decrease NH₃ and N₂O emissions due to enhanced N retention (Parkin et al., 2006). The difference in LMS versus common cover crop methods could be related to the timing of N fertilization in between rows from the clover biomass. Large NH₃ fluxes tend to be restricted to short time periods after fertilizer application (Herrmann, 2001).

N₂O fluxes tend to increase exponentially following fertilizer application, then become greater than pre-fertilizer application (Hoben et al., 2011). In an LMS, more of the N is supplied from cover crop decomposition versus inorganic fertilizer, which is the main N source for the other plots (Andrews et al., 2018, Table S1), potentially causing the overall increases in N fluxes in WC plots. In-row measurements of N₂O and NH₃ fluxes should be sampled in future studies to account for potential effects of clover on flux.

Soil moisture, NO₃⁻, and NH₄⁺ have all been proven as drivers of N₂O flux (Marquina et al., 2015). The correlations presented here with N₂O and NO₃⁻/NH₄⁺ suggest both nitrification and denitrification are contributing to N₂O fluxes (Stevens et al., 1997). Plots with WC had the lowest total NH₄⁺ and NH₄⁺:NO₃⁻ ratio. This potentially indicates more NO₃⁻ is available for denitrification than NH₄⁺ is available for nitrification in these systems, and denitrification could be the greater source of N₂O formation. However, denitrification and nitrification are complex processes, so techniques such as stable isotope and acetylene inhibition (Bateman et al., 2005) should be used in future studies to quantify the amount of N₂O from both processes (Butterbach-Bahl, et al., 2013). One limitation of this study is that the 2016 soil N compound data is in paired plots, rather than in the same plots as flux measurements. Regressions with only 2017 data showed NH₄⁺ as a stronger driving force than NO₃⁻ (Table S3), so future studies should maintain consistency in measurement locations. Soils with higher SOC (as observed in WC plots) can have greater overall N₂O emissions, compared to less fertile soils (Bouwman et al., 2002).

The higher N₂O flux in WC plots compared to Tr plots supports the findings in Turner et al. (2016). Additionally, cumulative sums for LMS systems were similar between the two studies (220.2 in 2016 and 229.9 mg N m⁻² in 2017 versus 226.5 mg N m⁻² in Turner et al. (2016)), indicating that white clover and kura clover affect soil fluxes similarly despite different growth rates. Both studies observed the largest N₂O fluxes late in the growing

season, likely due to clover decomposition and mineralization. While the current study originally hypothesized that NH_3 fluxes would be decreased in WC plots due to a lower fertilizer application rate, the results suggest that the continuous release of N outweighed the reduced fertilizer amount.

Conclusion

This study found greater mean CO_2 , N_2O , and NH_3 soil between-row fluxes in WC plots compared to three conventional agricultural systems. However, for CO_2 , these increased fluxes were not observed from measurements in the corn rows, indicating further study is necessary to elucidate if the increased respiration is autotrophic, heterotrophic, or a combination. N_2O and NH_3 fluxes were higher in LMS plots despite reduced fertilization. Nitrification and denitrification rates should be explored to better understand the specific sources of N_2O fluxes. This study illustrates the complexity of developing a CSA system. A net C equivalence should be calculated from future studies, including soil flux measurements of CO_2 , N_2O and NH_3 , soil C sequestration, and agricultural inputs to assess the mitigation potential of an LMS.

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Figure Captions

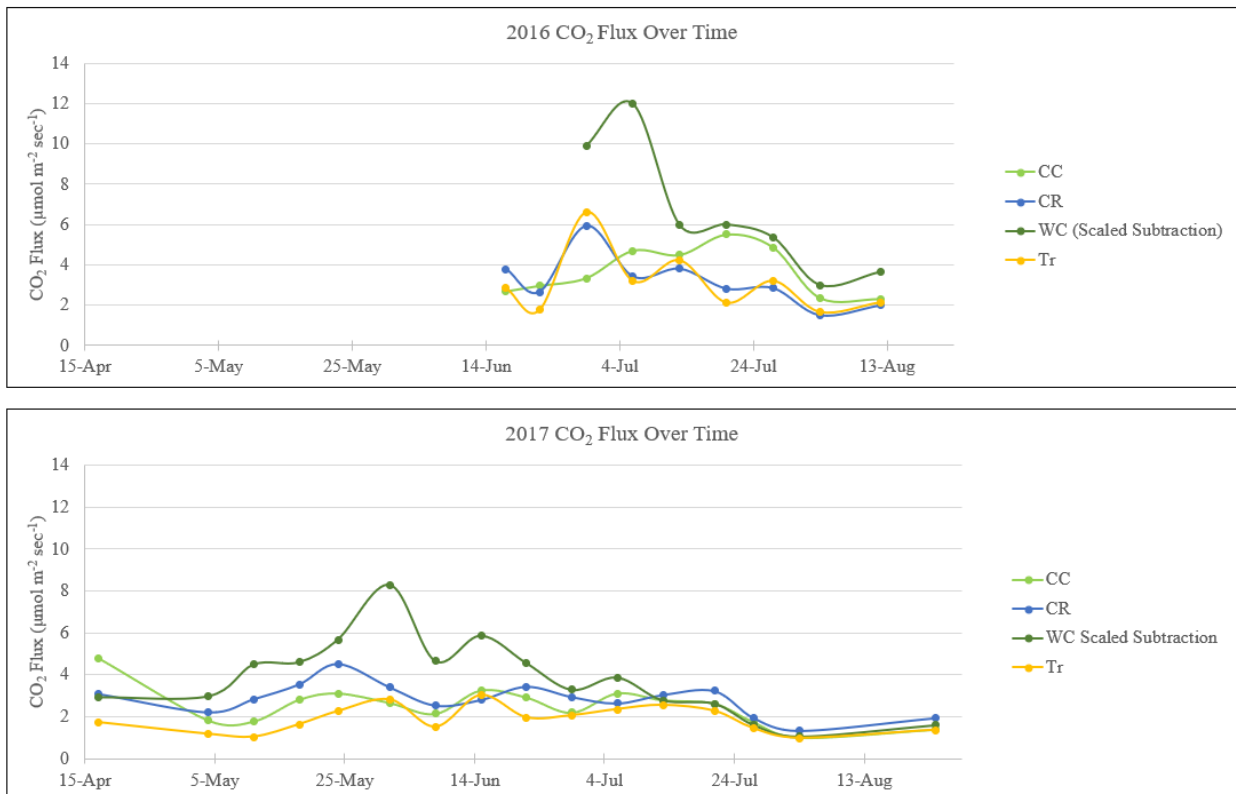


Figure 1: Time series of daily average flux measurements between the corn rows of CO₂ in $\mu\text{mol m}^{-2} \text{sec}^{-1}$ during the 2016 (A) and 2017 (B) growing seasons for crimson clover (CC), cereal rye (CR), white clover living mulch (WC), and conventional (Tr) treatments. CO₂ flux was higher in WC plots for both seasons. No error bars are included due to weekly sample sizes of three or less.

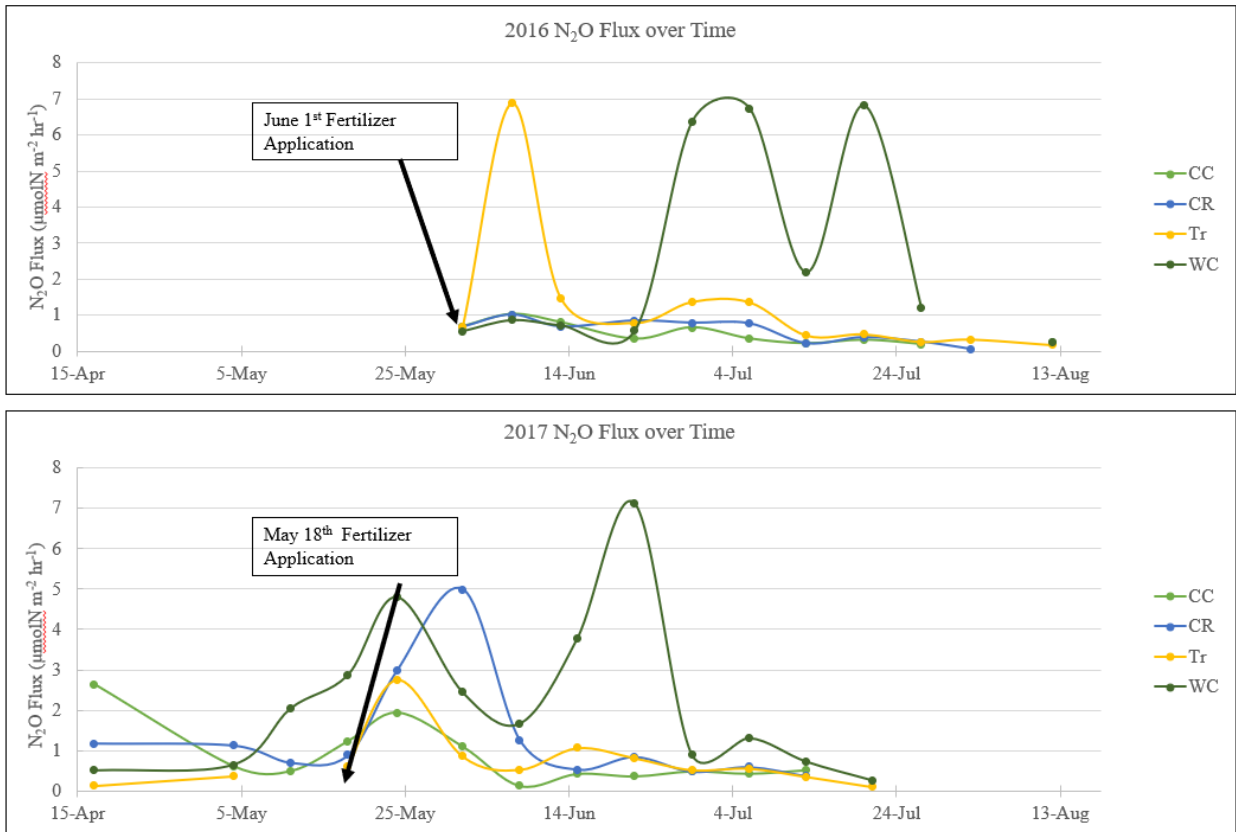


Figure 2: Time series of daily average flux measurements between the corn rows of N₂O in $\mu\text{molN m}^{-2} \text{hr}^{-1}$ during the 2016 (A) and 2017 (B) growing seasons for crimson clover (CC), cereal rye (CR), white clover living mulch (WC), and conventional (Tr) treatments.

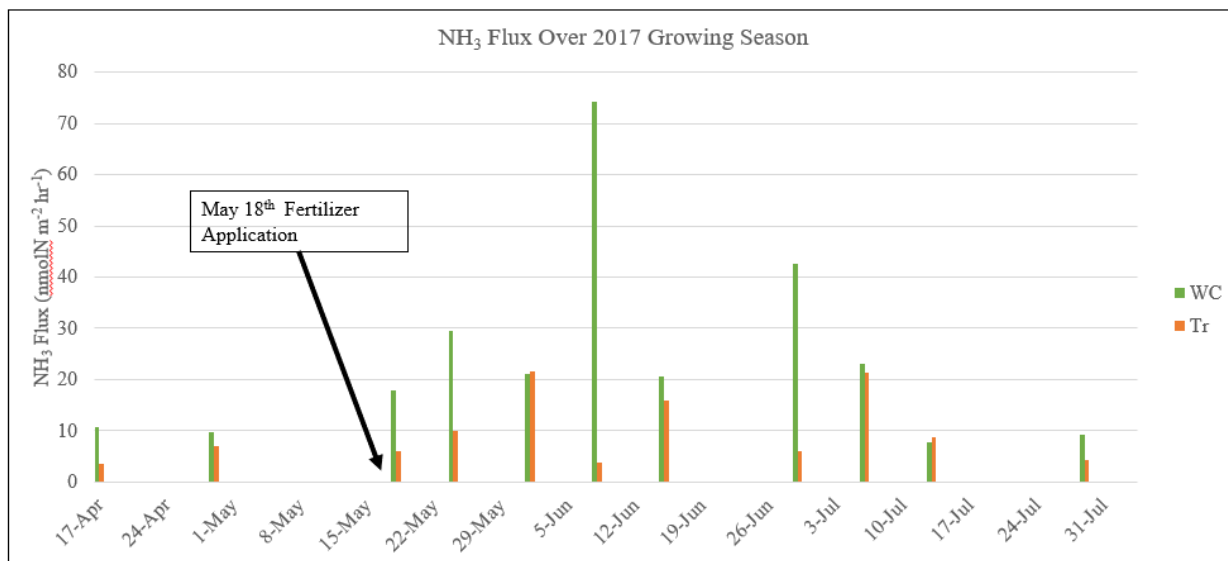


Figure 3: The NH₃ flux measurements in white clover living mulch (WC) and conventional (Tr) treatments over the 2017 growing season. Fertilizer was applied on May 18th.

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Tables

Table 1: Flux Rate Means and Comparisons for CO₂ and N₂O

Overall Means								
Mean Gas Flux		CC (95% CI)	CR (95% CI)	WC (95% CI)	Tr (95% CI)			
CO ₂ ($\mu\text{mol m}^{-2} \text{sec}^{-1}$)		2.79 (2.43, 3.15)	2.78 (2.50, 3.06)	4.20 (3.41, 4.99)	1.98 (1.73, 2.24)			
N ₂ O ($\mu\text{molN m}^{-2} \text{hr}^{-1}$)		0.50 (0.37, 0.64)	0.56 (0.40, 0.73)	1.65 (1.09, 2.21)	0.60 (0.32, 0.89)			
NH ₃ ($\text{nmolN m}^{-2} \text{hr}^{-1}$)		NA	NA	21.8 (13.8, 29.7)	9.22 (6.25, 12.2)			
Cumulative Flux Sums (in kg ha ⁻¹ yr ⁻¹)								
Gas	CC-2016	CC-2017	CR-2016	CR-2017	WC-2016	WC-2017	Tr-2016	Tr-2017
CO ₂	8,600	12,000	6,830	12,800	16,567	17,094	6,620	8,320
N ₂ O-N	0.116	0.32	0.17	0.38	0.67	0.68	0.52	0.20
Mean Log CO ₂ Flux Comparisons from Between-Row Measurements (2016 & 2017)								
Comparison	Difference in $\mu\text{mol m}^{-2} \text{sec}^{-1}$	95% CI Lower Bound		95% CI Upper Bound		P-value		
WC-Tr	0.54	0.24		0.83		<0.001*		
WC-CR	0.22	-0.06		0.49		0.19		
WC-CC	0.19	-0.09		0.47		0.28		
Tr-CR	-0.32	-0.61		-0.03		0.03*		
CR-CC	-0.02	-0.29		0.25		0.99		
Tr-CC	-0.34	-0.64		-0.05		0.02*		
Mean Log CO ₂ Flux Comparisons from In-Row Measurements (2016)								
Comparison	Difference in $\mu\text{mol m}^{-2} \text{sec}^{-1}$	95% CI Lower Bound		95% CI Upper Bound		P-value		
WC-Tr	0.18	-0.29		0.65		0.74		
WC-CR	0.18	-0.15		0.51		0.49		
WC-CC	0.16	-0.18		0.50		0.61		
Tr-CR	-0.002	-0.47		0.46		0.99		
CR-CC	-0.02	-0.35		0.32		0.99		
Tr-CC	-0.002	-0.49		0.45		0.99		
Mean Log N ₂ O Flux Comparisons from Between-Row Measurements (2016 & 2017)								
Comparison	Difference in $\mu\text{mol m}^{-2} \text{hr}^{-1}$	95% CI Lower Bound		95% CI Upper Bound		P-value		
WC-Tr	0.91	0.41		1.40		<0.001*		

WC-CR	0.86	0.41	1.30	<0.001 *
WC-CC	0.93	0.47	1.39	<0.001 *
Tr-CR	-0.05	-0.55	0.45	0.99
CR-CC	0.07	-0.39	0.55	0.97
Tr-CC	0.03	-0.49	0.54	0.99

* Significant differences at $\alpha=0.05$ level using Tukey's comparisons of log transformed fluxes.

White living mulch (WC), conventional no cover crop (Tr), cereal rye cover crop (CR), crimson clover cover crop (CC)

Table 2: Linear Regression of Weekly Log Transformed CO₂ Flux

With Autotrophic Scaled Subtraction (N=282, R ² =0.40)				
<i>Variable</i>	<i>Estimate (β) (95% CI)</i>	<i>Standard Error of Estimate</i>	<i>Percent Change (%) (95% CI)</i>	<i>P-value</i>
Intercept	-0.68	0.33	NA	<0.001***
CC	0.40 (0.21, 0.59)	0.096	49.5 (23.7, 80.6)	<0.001***
CR	0.33 (0.14, 0.52)	0.095	38.8 (15.1, 67.4)	<0.001***
WC	0.61 (0.42, 0.81)	0.099	84.8 (52.2, 124.4)	<0.001***
Season 2016	0.68 (0.42, 0.95)	0.13	98.3 (52.2, 158.4)	<0.001***
Season 2017	0.20 (-0.06, 0.45)	0.13	21.8 (-5.41, 56.9)	0.13
Temp	-0.007 (-0.03, 0.016)	0.012	-0.71 (-2.94, 1.57)	0.54
Soil Moisture	6.71 (4.97, 8.45)	0.88	6.94 (5.10, 8.82)	<0.001***
Light Interception	0.23 (0.007, 0.45)	0.11	25.7 (0.69, 56.9)	0.04*
Soil NO ₃	0.006 (-0.002, 0.01)	0.004	0.59 (-0.16, 1.34)	0.12
Soil NH ₄	-0.002 (-0.007, 0.002)	0.002	-0.25 (-0.70, 0.21)	0.29
PMN	-0.02 (-0.05, 0.004)	0.012	-1.99 (-4.35, 0.43)	0.11

***p<0.001, **p<0.01, *p<0.05, #p<0.10

White living mulch (WC), conventional no cover crop (Tr), cereal rye cover crop (CR), crimson clover cover crop (CC)

Table 3: Linear Regression of Weekly Log Transformed N₂O Flux, (N=203, R²=0.48)

<i>Variable</i>	<i>Estimate (β) (95% CI)</i>	<i>Standard Error of Estimate</i>	<i>Percent Change (%) (95% CI)</i>	<i>P-value</i>
Intercept	-4.58	0.76	NA	<0.001***
CC	0.12 (-0.21, 0.45)	0.17	12.8 (-18.9, 56.8)	0.47
CR	0.092 (-0.23, 0.41)	0.16	9.66 (-20.5, 50.7)	0.57
WC	1.19 (0.86, 1.52)	0.17	228.5 (136.3, 357.2)	<0.001***
Season 2016	0.75 (0.15, 1.36)	0.31	112.4 (16.2, 289.6)	0.015*
Season 2017	0.68 (0.079, 1.28)	0.31	97.4 (8.22, 259.7)	0.027*
Temp	0.03 (-0.029, 0.089)	0.030	3.07 (-2.86, 9.31)	0.31
Soil Moisture	11.94 (8.73, 15.2)	1.63	12.69 (9.12, 16.4)	<0.001***
Light Interception	-0.12 (-0.46, 0.23)	0.17	-10.0 (-36.9, 25.9)	0.50
Soil NO ₃	0.014 (0.0025, 0.025)	0.0056	1.38 (0.25, 2.53)	0.016*
Soil NH ₄	0.0062 (-0.00034, 0.013)	0.0033	0.62 (-0.034, 1.31)	0.063 [#]
PMN	0.0024 (-0.008, 0.13)	0.0053	0.24 (-0.80, 13.9)	0.65

***p<0.001, **p< 0.01, *p< 0.05, [#]p<0.10

White living mulch (WC), conventional no cover crop (Tr), cereal rye cover crop (CR), crimson clover cover crop (CC)

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