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Benefits and tradeoffs of reduced tillage and manure application methods in a *Zea mays* silage system

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Abstract

A critical question is whether there are agricultural management practices that can attain the multiple management goals of increasing yields, preventing nutrient losses, and suppressing greenhouse gas (GHG) emissions. No-till and manure application methods, such as manure injection, can enhance nutrient retention, but both may also enhance emissions of nitrous oxide (N₂O), a powerful GHG. We assessed differences in soil N₂O and carbon dioxide (CO₂) emissions, nitrate and ammonium retention, and crop yield and protein content under combinations of vertical-till, no-till, manure injection, and manure broadcast without incorporation in a corn (*Zea mays* L.) silage system. During the growing seasons of 2015–2017, GHG emissions and soil mineral nitrogen (N) were measured every other week or more frequently after management events. Crop yield and protein content were measured annually at harvest. No-till reduced CO₂ emissions but had no impact on N₂O emissions relative to vertical-till. Manure injection increased N₂O and CO₂ emissions, with the magnitude of this effect being greatest for 1 mo post-application. Manure injection also increased soil ammonium and nitrate but did not increase yield or crop quality relative to broadcast application. Similarly, tillage did not affect crop yield or protein content. Despite the tradeoffs between mineral N retention and elevated GHG emissions, manure injection in no-till systems benefits farmers by reducing soil carbon losses as CO₂, retaining mineral N, and maintaining crop yields and quality.

1 | INTRODUCTION

Under traditional agricultural management, pressure to feed a growing population may degrade soils, exacerbate nutrient pollution, and enhance greenhouse gas (GHG) emissions by increasing fertilizer inputs and cultivation (Foley et al., 2011). Traditional agriculture has decreased soil organic carbon (SOC) by 40–75% (Lal, 2004), ultimately increasing carbon dioxide (CO₂) fluxes to the atmosphere (Houghton et al., 1983). Furthermore, agriculture

is responsible for >60% of nitrous oxide (N₂O) emissions; N₂O is a GHG 265–298 times more powerful than CO₂ at trapping heat (Myhre et al., 2013; Syakila & Kroeze, 2011). Given the potential for agricultural soils to be nutrient and GHG sources, many best management practices (BMPs) aim to retain nutrients and prevent transport into the atmosphere and surface or groundwater (Liu et al., 2017; Logan, 1993). However, because the primary goal of agricultural management is increasing crop productivity, a critical question is: Are there BMPs that can attain the multiple management goals of increasing yields, retaining nutrients, and suppressing GHG emissions?

Abbreviations: BMP, best management practice; GHG, greenhouse gas; PAS, photoacoustic spectroscopy; SOC, soil organic carbon.

Because GHG emissions and the fate of carbon (C) and nitrogen (N) within agricultural soils depend on soil and fertilizer management (Duncan, Dell, Kleinman, & Beegle, 2017; Flach, Barnwell, & Crossen, 1997; Lognoul et al., 2017; Plaza-Bonilla, Álvaro-Fuentes, Arrúe, & Cantero-Martínez, 2014; Wang & Dalal, 2015; Webb, Pain, Bittman, & Morgan, 2010), BMPs can be designed to retain added nutrients and reduce GHG emissions (Mangalassery et al., 2014; Ruidisch, Bartsch, Kettering, Huwe, & Frei, 2013). Best management practices affect nutrient retention and GHG emissions by altering their drivers. Nitrogen losses can be reduced by incorporating added N into soils to minimize gaseous and runoff losses (Daverede et al., 2004; Duncan et al., 2017; Kulesza, Maguire, Thomason, Hodges, & Pote, 2014) and maximize plant and microbial uptake, which are enhanced when water, temperature, and substrate (for microbes) are not limiting (Hart, Stark, Davidson, & Firestone, 1994; Sarker et al., 2017). Soil CO₂ flux, a combination of microbial and root respiration (Oertel, Matschullat, Zurba, Zimmermann, & Erasmí, 2016), increases with temperature (Lloyd & Taylor, 1994) and C availability (Hungate, Chapin, Zhong, Holland, & Field, 1997) as long as water is not limiting. Soil N₂O emissions are primarily from autotrophic nitrification or heterotrophic denitrification, which are controlled by C or N availability (ammonium [NH₄⁺] for nitrification; SOC and nitrate [NO₃⁻] for denitrification), temperature, pH, and oxygen (O₂) availability (Livesley, Kiese, & Graham, 2008; Mørkved, Dörsch, & Bakken, 2007). Thus, BMPs that alter soil microclimate, porosity, moisture or aggregation, and substrate availability (Robertson & Groffman, 2007) may affect nutrient retention and GHG emissions.

Two BMPs of interest for mitigating GHG emissions and nutrient losses while maintaining or enhancing yields are low- or no-till and manure injection. Vertical-till is a low-till method whereby only approximately 7 cm of the soil is tilled without soil profile inversion (vs. 25–33 cm for conventional-till with moldboard plow; S. Ziegler, personal communication, 2017). Another method is no-till, which can enhance soil aggregation, biological activity, and water retention (FAO, 2011; Six et al., 2002; Verhulst et al., 2010). No-till can also improve crop yields (Kassam et al., 2014), although yields may decline for 5–10 yr after converting from conventional-till (Derpsch et al., 2014; Rusinamhodzi et al., 2011). Low- and no-till can reduce SOC losses and CO₂ emissions (Alvarez, 2005; Giller et al., 2015; Paustian et al., 1997; Six et al., 2002), but recent evidence indicates C may be concurrently lost from deeper soils, resulting in no net C gain (Olson, Al-Kaisi, Lal, & Lowery, 2014; Powlson et al., 2014; Wendt & Hauser, 2013). Furthermore, no-till may increase N₂O emissions (Abdalla et al., 2013; Ball, Scott, & Parker, 1999; Burford, Dowdell, & Crees, 1981) by enhancing soil aggregation and

Core Ideas

- No-till and manure injection aim to improve crop production while reducing soil N and C losses.
- Manure injection increased soil mineral N but also increased GHG emissions relative to broadcast application.
- No-till reduced CO₂ emissions relative to vertical-till without affecting crop production or N₂O emissions.

water retention (Holland, 2003), creating the anaerobic conditions needed for denitrification.

Another BMP of interest is manure injection, which may improve nutrient retention, crop uptake, and yield relative to conventional broadcast spreading without incorporation (Duncan et al., 2017; Sutton, Nelson, Hoff, & Mayrose, 1982) but may enhance GHG emissions (Adair, Barbieri, Schiavone, & Darby, 2019; Duncan et al., 2017). Because more than 50% of manure ammonium-N can be volatilized if not immediately incorporated (Maguire et al., 2011; Powell, Jokela, & Misselbrook, 2011), incorporation is a management priority, particularly in no-till. Traditionally, manure broadcasted on the soil surface is incorporated by moldboard or chisel plow. Manure injection places manure into the subsurface using coulters or chisels. In no-till systems, manure injection can reduce volatilization and runoff losses (Duncan et al., 2017; Meisinger & Jokela, 2000); it may also enhance GHG emissions, including N₂O (Chadwick, Pain, & Brookman, 2000; Dell, Meisinger, & Beegle, 2011; Lovanh, Warren, & Sistani, 2010), by concurrently increasing C and N substrates and promoting subsurface anaerobic zones (Bremner, 1997; Duncan et al., 2017; Xue et al., 2013).

Clearly, there are tradeoffs and uncertainties when considering BMPs. Few studies have investigated the interacting effects of combining BMPs. Even fewer have quantified tradeoffs among nutrient retention, GHG emissions, and crop quality and yields. Therefore, our objective was to determine the effects of combinations of BMPs (i.e., vertical-till, no-till, broadcast manure, and manure injection) on CO₂ and N₂O emissions, soil mineral N retention, and corn (*Zea mays* L.) silage yield and protein content. We hypothesized that injecting manure in a no-till system would increase C and N substrate availability for microbes under anaerobic conditions and would therefore increase N₂O emissions yet reduce CO₂ emissions by minimally disturbing the soil profile. We also hypothesized that the same treatment combination would increase crop yield

and protein content by enhancing nutrient retention and availability. This study provides one of the first looks at how these BMPs combine to affect multiple management goals.

2 | MATERIALS AND METHODS

2.1 | Site description

The Manure Injection No Tillage (MINT) field trial in Alburgh, VT (45.005° N, 73.308° W), established in May of 2013, was continuous corn (*Zea mays* L.) silage with winter rye (*Secale cereal* L.) cover crop during the non-growing season. Prior to the trial, the field was conventional-till continuous corn with a winter rye cover crop and no manure application. The soil is a Benson rocky silt loam (loamy-skeletal, mixed, active, mesic Lithic Eutrudepts) formed in loamy till and is somewhat excessively to excessively drained with moderate permeability (Soil Survey Staff, 2017). Site measurements indicated that the soil was a sandy loam (hydrometer) with a bulk density of 1.2 g cm⁻³; pH of 6.3; 4% organic matter; and average total C and N of 2.4 and 0.2%, respectively (June–July 2015; 0–10 cm).

The trial was a randomized complete block with a split-split plot arrangement (three blocks, two plot treatments, two subplot treatments). Within each block the plot (36.6 by 7.4 m) treatments were tillage treatments: no-till and vertical-till. Vertical-till was to a depth of 7.6 cm with a blade spacing of 18.4 cm (2623VT, John Deere). Each block included a 12.2-m buffer between tillage treatments. Each tillage treatment plot had two subplot (3.7 by 12.2 m) manure application treatments: injected and broadcast without incorporation. Liquid dairy manure was broadcast or injected to a depth of 15–20 cm, but injection lines were typically filled to or just below (within 2–3 cm) the soil surface. Double-disk injection bands were approximately 10 cm wide, with 75 cm spacing between bands (NUTRIJECTOR, Jamesway Farm Equipment Inc.). Each manure × tillage treatment combination (no-till plus injection, no-till plus broadcast, vertical-till plus injection, and vertical-till plus broadcast) was replicated three times (once in each of three blocks), for a total of 12 subplots (four per block). Manure characteristics, cropping, fertilization, and harvest details are in Table 1.

2.2 | Nitrous oxide and carbon dioxide measurements

Soil N₂O and CO₂ emissions were measured every 2 wk from 6 June to 9 Nov. 2015, 11 Mar. to 18 Nov. 2016,

TABLE 1 Dates, manure characteristics/events, and cropping events for 2015–2017

| Manure characteristics/events | 2015 | 2016 | 2017 |
|---|----------|----------|----------|
| Tillage | | | |
| Date | 15 May | 17 May | 12 May |
| Liquid dairy manure application | | | |
| Date | 15 May | 17 May | 12 May |
| Rate, L ha ⁻¹ | 58,929 | 56,123 | 57,994 |
| Dry matter, % | 8 | 3.84 | 4.7 |
| Organic N, kg ha ⁻¹ | 157.4 | 75.7 | 93.8 |
| NH ₄ -N, kg ha ⁻¹ | 74.1 | 53.3 | 56.3 |
| Total N, kg ha ⁻¹ | 230.8 | 128.9 | 150 |
| Corn planting | | | |
| Date | 18 May | 19 May | 18 May |
| Rate, seed ha ⁻¹ | 83,980 | 83,980 | 83,980 |
| Starter fertilizer (10–20–20) | | | |
| Date | 18 May | 19 May | 18 May |
| Rate, kg ha ⁻¹ | 280 | 224 | 224 |
| Harvest | | | |
| Date | 30 Sept. | 21 Sept. | 21 Sept. |
| Cover crop planting | | | |
| Date | 31 Sept. | 3 Oct. | 6 Oct. |
| Rate, seed ha ⁻¹ | 112 | 112 | 112 |
| Cover crop termination^a | | | |
| Date | 15 May | 17 May | 12 May |

^aCover crops terminated with glyphosate (7 L ha⁻¹).

and 19 Apr. to 26 Nov. 2017. Measurement frequency increased to every other day for a week after manure application, then once a week for a month. We measured fluxes with static chambers (one per subplot; $n = 12$) and an infrared photoacoustic spectroscopy (PAS) gas analyzer (Model 1412i, Innova Air Tech Instruments; calibrated as in Iqbal, Castellano, & Parkin, 2013). Polyvinyl chloride chamber collars (30 cm i.d., 15 cm height) were installed to a depth of 12 cm (Parkin & Venterea, 2010). Gas concentrations were recorded every minute for 10 min in each subplot by placing a vented polyvinyl chloride lid (30 cm i.d., 9.5 cm height) on the chamber collar with an air-tight seal connected to a closed-loop system with the PAS (e.g., as Iqbal et al., 2013). The PAS measures gas concentrations nondestructively; gases pass through the detector and returned to the chamber unaltered. Chambers were removed before management events and reinstalled randomly within subplots but were large enough to be placed to capture portions of the between- and within-corn row spaces; chambers were placed 1 m from subplot edges to avoid edge effects. Residues were allowed to remain in the chambers irrespective of tillage regime, but live vegetation was clipped at ground level and removed.

Gas fluxes were calculated by fitting a linear regression of concentration against time after chamber closure. The changes in N₂O and CO₂ were calculated as:

$$F = \frac{\Delta C}{\Delta t} * \frac{V}{A} * M * \rho \alpha$$

where F is the CO₂ (mg CO₂-C m⁻² h⁻¹) or N₂O (mg N₂O-N m⁻² h⁻¹) production rate, $\Delta C/\Delta t$ is the change in chamber gas concentration (10⁶ mol⁻¹ h⁻¹), V is chamber volume (0.00954 m³), A is chamber surface area (0.0707 m²), M is the molecular weight of CO₂ or N₂O (mg mol⁻¹), ρ is the density of gas at 20 °C and 0.101 MPa (1 mole per 24.04 m³), and α is a conversion coefficient (28/44 for N₂O and 12/44 for CO₂).

2.3 | Soil sampling and analysis

Soil samples (0–15 cm) were collected during each gas sampling within one meter of the chamber. Samples were placed on ice for transportation to the laboratory. Within 24 h, we extracted 5-g field-wet soil subsamples with 2 M potassium chloride to determine available NO₃⁻ and NH₄⁺ via colometric analysis (BioTek Synergy HTX, BioTek Instruments, Inc.). We also determined gravimetric soil moisture by drying a 5-g field-wet subsample at 60 °C to constant weight. At each gas sampling, soil temperature was recorded adjacent to each chamber.

2.4 | Crop analysis

To determine corn yield, the entire plot was harvested with a John Deere 3950 two-row chopper into a wagon equipped with an Avery Weigh-Tronix weighing system at a target cutting height of 15–20 cm. A 500-g sample from each subplot was collected and used to determine dry matter content (gravimetric) and crude protein concentration. A 500-g fresh whole-plant sample from each subplot was collected, weighed, dried at 60 °C for 7 d, and reweighed for dry matter. Dried samples were ground with a Wiley mill (Arthur H. Thomas Co.) to pass a 2-mm screen and with a cyclone forage mill (UDY Corporation) to pass a 1-mm screen. Ground samples were scanned on a Foss DS2500F near-infrared reflectance spectrophotometer system, and near-infrared reflectance calibrations obtained through the Dairy One Forage Laboratory were used to determine crude protein. Spectra were scanned between 400 and 2,500 nm every 2 nm using ISIScan software v.4.6.1 (Infrasoft Intl., LLC). All samples were scanned in duplicate to maximize spectral input and to minimize the impact of a poor scan.

2.5 | Statistical analysis

Daily CO₂ and N₂O emissions and soil NH₄⁺ and NO₃⁻ were analyzed using linear mixed models that included (a) *subplot* as a random effect to account for nonindependent subplot measurements over time, (b) *date* as a factor to examine how treatment effects changed among days, (c) a constant variance function to account for heterogeneous errors among tillage (N₂O) or tillage and manure application treatments (NH₄⁺ and NO₃⁻; none for CO₂), and (d) two- and three-way interactions among *tillage*, *manure*, and *date*. Data were cube root (N₂O), Box-Cox (CO₂), or log (NH₄⁺ and NO₃⁻) transformed to meet normality assumptions. Daily CO₂ fluxes showed evidence of temporal correlation, so the model included an auto-regressive correlation structure. Cumulative growing season CO₂ and N₂O and crop yield and protein content (all untransformed) were analyzed with *subplot* as a random effect, a constant variance function for manure application and tillage treatments, and two- and three-way interactions among *tillage*, *manure*, and *year* (continuous).

To examine direct and indirect effects of management practices and hypothesized drivers of soil N and daily GHG emissions, we developed and compared structural equation models. Classical statistical techniques do not permit the investigation of causal relationships; however, the use of structural equation models develops causal understanding from data by testing networks of causal relationships (Figure 1) (Eisenhauer, Bowker, Grace, & Powell, 2015; Grace & Irvine, 2020; Grace et al., 2012). While relying on some correlative information, structural equation model approaches causal understanding (as in Shipley, 2002) by fitting data to models that represent alternative causal hypotheses (e.g., Supplemental Figure S1) and by testing and comparing model fit (based on model-implied vs. observed covariance matrices; Eisenhauer et al., 2015; Grace et al., 2006, 2012). Structural equation models may contain unidirectional causal relationships among variables (Figure 1; single-headed arrows) and correlations (Figure 1; double-headed arrows). Direct effects are indicated by one arrow linking two variables (e.g., direct effect of CO₂ on N₂O; Figure 1); indirect effects are where two variables are linked through one or more other variable(s) (e.g., indirect effect of Temp on N₂O via CO₂; Figure 1). Structural equation models also allow researchers to compare the strength of various pathways by using either standardized (values near 1 are strong; values near 0 are weak) or unstandardized path coefficients. Unstandardized path coefficients estimate the response of the variable of interest in the original units (e.g., g N₂O-N ha⁻¹ d⁻¹ for N₂O) to a one-unit increase in a driving variable (e.g., a 1 °C increase in soil

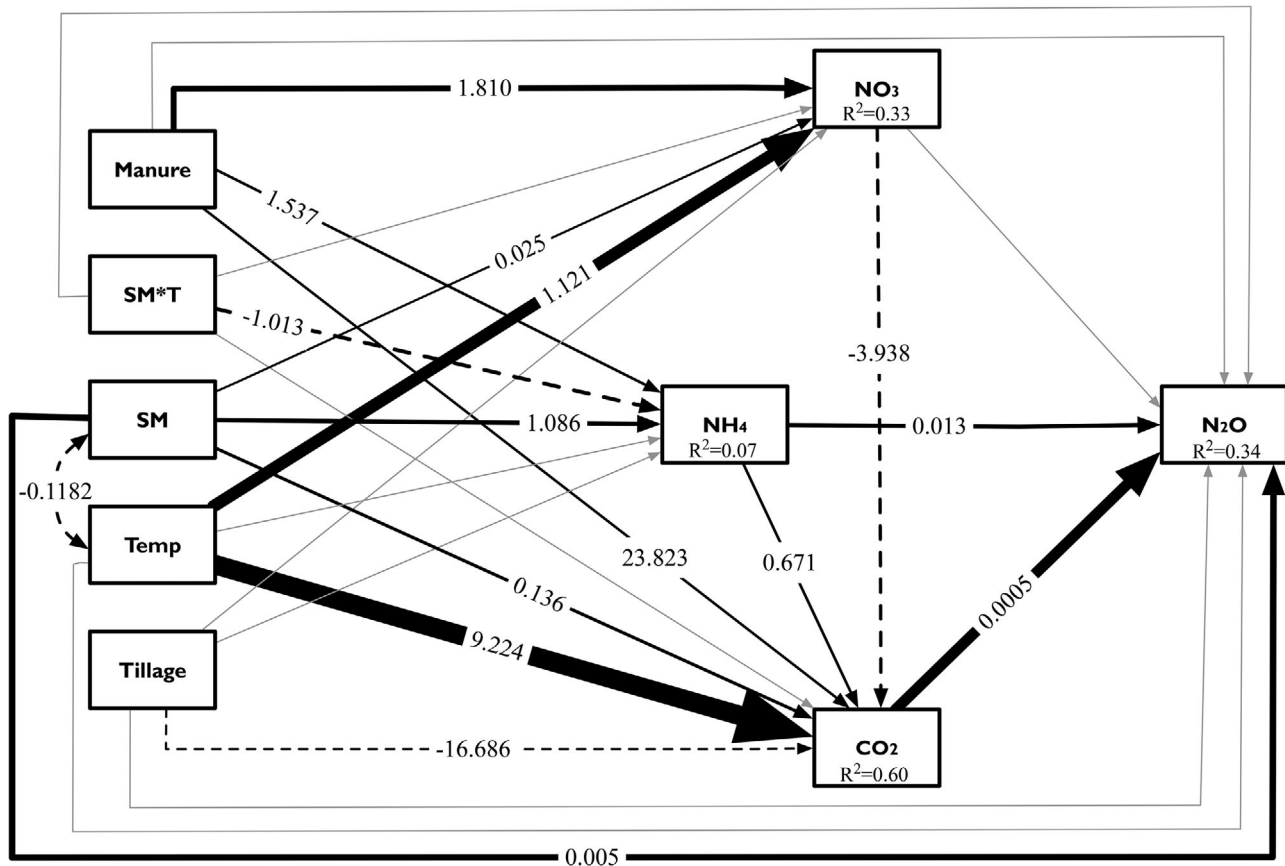


FIGURE 1 Best fit full structural equation model for daily greenhouse gas flux data and covariates. Single-headed arrows represent causal relationships; double-headed arrow denotes correlation between soil moisture (SM) and soil temperature (Temp). Arrows are scaled by standardized path coefficient values. Untransformed unstandardized path coefficients are shown. Dashed lines indicate negative and solid lines indicate positive path coefficients. Significant pathways are black arrows with path coefficients ($P < .05$). Gray arrows are nonsignificant pathways. SM*T, soil moisture \times temperature interaction; Manure, manure treatment; Tillage, tillage treatment. Positive estimates for Tillage indicate that vertical-till increased the response variable relative to no-till. Positive estimates for Manure indicate that manure injection increased the response variable relative to broadcast

temperature) while holding all other variables constant at their average values.

Our three structural equation models had (a) the structure in Figure 1 or (b) the structure in Figure 1 plus an arrow from NH_4^+ to NO_3^- or (c) as (b) plus an arrow from manure and tillage treatments to soil moisture and temperature to test whether treatments altered soil microclimate (Supplemental Figure S1). In the structural equation models, data were transformed, and model structures were as in the ANOVAs. Because C availability, as it influences denitrification and N_2O fluxes, can be quantified as concurrent CO_2 fluxes (Farquharson & Baldock, 2008; Xu, Tian, & Hui, 2008), we included it as a driver of N_2O . Akaike's Information Criterion modified for small sample sizes (AIC_c) was used to select the best structural equation model. To choose the best model(s) we considered models with $\text{dAIC}_c \leq 2$ to have substantial support, where dAIC_c is the difference between the model under consideration and

the model with the lowest AIC_c value (Burnham & Anderson, 2002). When no single model was best, we chose the simplest model with a $\text{dAIC}_c < 2$ (i.e., the model with the fewest independent variables). Furthermore, we examined model fit using Fisher's C ($P > .05$ indicates good fit). Using the best structural equation model, we calculated total effects as the sum of statistically significant direct and indirect effects. To better understand each variable's impact on daily N_2O and CO_2 emissions, we multiplied the unstandardized total effect (transformed into original units; e.g., $\text{g N}_2\text{O-N ha}^{-1} \text{d}^{-1}$) by the maximum daily range of each variable. This provided an estimate of the maximum change in N_2O or CO_2 emissions (in $\text{g N}_2\text{O-N ha}^{-1} \text{d}^{-1}$ or $\text{kg CO}_2\text{-C ha}^{-1} \text{d}^{-1}$) due to a model driver (e.g., temperature) within one day.

For cumulative GHG emissions, corn yield, and corn protein content, we did not have sufficient sample sizes to construct structural equation models ($n = 36$), so we

constructed analysis of covariance (ANCOVA) models using the above-described ANOVA model structures and measured covariates: soil mineral-N, soil temperature, soil moisture, and, for N₂O, CO₂ flux. To characterize covariate availability or conditions throughout the growing season, we calculated the area under the curve (*trapz* function in *pracma* package; Borchers, 2019) during each growing season (15 May to 1 Sept.). The ANCOVAs did not include interactions among covariates or treatments and covariates, except for a soil temperature × moisture interaction.

All linear mixed effects models were fit using the *nlme* package in R Studio (Pinheiro, Bates, DebRoy, & Sarkar, 2018; R Development Core Team, 2008; RStudio Team, 2015). Treatment significance ($P < .05$) was assessed using F-tests. We fit structural equation models and calculated marginal and conditional R^2 values using *piecewiseSEM* (Lefcheck, 2016). Marginal R^2 describes the proportion of variance explained by fixed factors (e.g., manure, tillage, date). Conditional R^2 describes the proportion of variance explained by fixed and random factors (Nakagawa & Schielzeth, 2013).

3 | RESULTS AND DISCUSSION

3.1 | Manure injection increased nitrous oxide and carbon dioxide emissions

3.1.1 | Nitrous oxide emissions

Consistent with other research (Duncan et al., 2017; Flessa & Beese, 2000; Rubaek, Henriksen, Petersen, Rasmussen, & Sommer, 1996; Vallejo, García-Torres, Díez, Arce, & López-Fernández, 2005; Velthof, Kuikman, & Oenema, 2003; Wulf, Maeting, & Clemens, 2002), manure injection increased N₂O emissions but not on all days (significant ANOVA manure and manure by date effects; Supplemental Table S1). On average, daily N₂O emissions resulting from manure injection (107.9 ± 12.1 g N₂O–N ha⁻¹ d⁻¹) were 2.4 times higher than those resulting from broadcast (45 ± 5.7 g N₂O–N ha⁻¹ d⁻¹), but the impact of injection was greatest post-application, with N₂O emissions 4 times greater than broadcast (Figure 2b).

In the best structural equation model for the full growing season, manure application method only increased N₂O emissions indirectly by increasing NH₄⁺ and CO₂ as an index of C availability (Farquharson & Baldock, 2008; Xu et al., 2008) (Figure 1). In the best structural equation model, manure and tillage treatments did not affect soil moisture and temperature (ANOVAs also indicated soil moisture and temperature were unaffected by treatments [Supplemental Tables S2–S4]). Two structural equa-

tion models had $dAIC_c < 2$. One included a nonsignificant pathway from NH₄⁺ to NO₃⁻ ($dAIC_c = 1.9$), and the other did not ($dAIC_c = 0$; Supplemental Table S2). Of these, we selected the simpler model without a pathway from NH₄⁺ to NO₃⁻ as the best model (Fisher's $C P = .817$; Supplemental Table S2). In this structural equation model, the total effect of injection across the growing season was small, increasing N₂O emissions by 0.03 g N₂O–N ha⁻¹ d⁻¹ relative to broadcast application (direct plus indirect effects; Supplemental Table S5). Soil moisture, CO₂ emissions, and available NH₄⁺ had the largest impacts on N₂O emissions via direct and/or indirect effects (Figure 1). Across the maximum range of values for each variable measured within a day, increasing CO₂, soil moisture, and available NH₄⁺ increased N₂O emissions by 109.2, 118.7, and 12.3 g N₂O–N ha⁻¹ d⁻¹, respectively (Supplemental Table S5).

Manure injection had the greatest impact during pulse events (i.e., 1 mo after application), when manure application method had direct and indirect effects on N₂O emissions (Figure 3a; Supplemental Table S4). On average, daily N₂O emissions from injection soils were 9.4 g N₂O–N ha⁻¹ d⁻¹ greater than from broadcast during pulse events (Supplemental Table S4). Because manure is rich in C and N substrates for nitrification and denitrification (Table 1) (Firestone & Davidson, 1989), elevated post-injection N₂O emissions may be related to the addition of these substrates paired with a lack of soil aeration, which increases denitrification activity (Vallejo et al., 2005; Webb et al., 2010). The positive indirect impact of injection on N₂O emissions via CO₂ (Figure 3a) supports these ideas and suggests that injection may increase N₂O emissions by increasing C availability (Farquharson & Baldock, 2008; Xu et al., 2008) and by increasing microbial activity (e.g., the large post-application CO₂ fluxes; Figure 2c–d) and O₂ consumption to create anaerobic conditions (Van Groenigen et al., 2006). Furthermore, the direct positive effect of NH₄⁺ (single-headed arrow from NH₄⁺ to N₂O; Figure 3a) after application suggests that there may also be a role for nitrification in promoting N₂O emissions, particularly because NO₃⁻ had no direct effect on N₂O emissions despite NO₃⁻ being a precursor to N₂O via denitrification. However, nitrification is an autotrophic process, and the positive direct effect of CO₂ mineralization on N₂O (Figure 3a) suggests that denitrification, a heterotrophic process, likely also plays a role in N₂O production. Indeed, injection may promote coupled nitrification–denitrification by creating conditions favorable for nitrification on the outer edges of the manure slot, with denitrification taking place in anaerobic microsites within the manure (Comfort, Kelling, Keeney, & Converse, 1990). Apart from manure application, soil moisture and temperature interacted to directly affect N₂O (Figure 3a): At low soil moisture, increasing soil temperature had little or no effect on

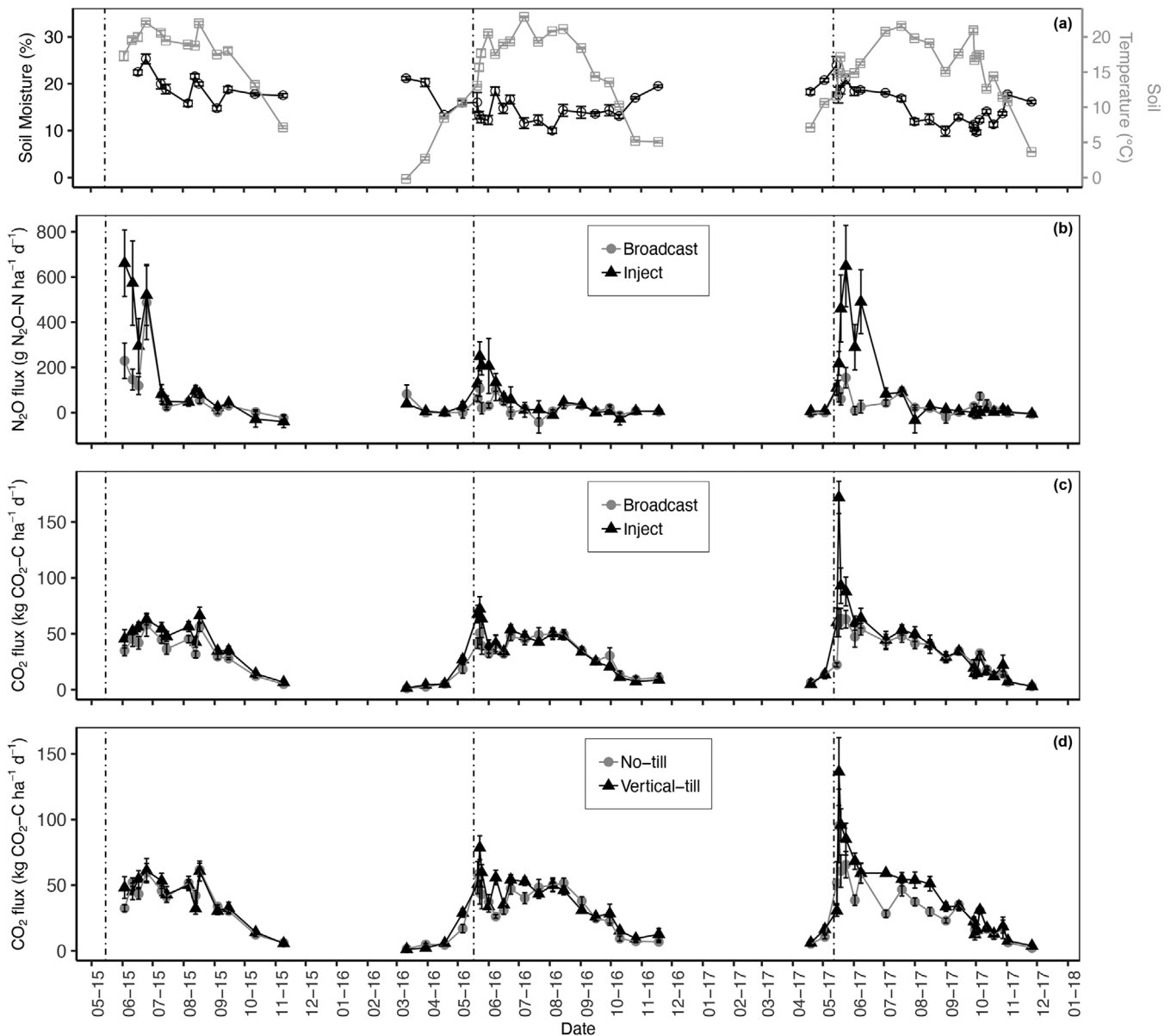


FIGURE 2 Daily (a) soil moisture (black line) and soil temperature (grey line) averaged across manure and tillage treatments, (b) N₂O fluxes and (c) CO₂ fluxes by manure application treatment, and (d) CO₂ fluxes by tillage treatment. Error bars are ± 1 SE. Vertical dashed lines show manure application and tillage events

N₂O emissions, but at high soil moisture, N₂O emissions increased with temperature (Supplemental Figure S2). Carbon dioxide emissions and soil NH₄⁺ also directly increased N₂O emissions by 22.5 and 8 g N₂O-N ha⁻¹ d⁻¹, respectively, across the maximum daily range of values (Supplemental Table S5).

Later in the growing season, N₂O fluxes were low, and between-treatment differences became indistinguishable, as in Duncan et al. (2017). In the non-pulse structural equation model, manure application method had only small indirect effects on N₂O emissions (Figure 3b). During this time, N₂O emissions were directly increased only by soil moisture and available C (CO₂ flux), which

increased N₂O emissions by 37.9 and 49.8 g N₂O-N ha⁻¹ d⁻¹, respectively, across the maximum daily range (Supplemental Table S5). This suggests that these N₂O fluxes may have been associated with periods of high soil moisture and O₂ depletion that triggered small bursts of denitrification (Dobbie, McTaggart, & Smith, 1999; Robertson & Groffman, 2007; Sierra, Malghani, & Loesch, 2017; Smith et al., 2003).

On average, cumulative growing season N₂O emissions from manure injection ($15,900 \pm 300$ g N₂O-N ha⁻¹) were two times greater than broadcast ($7,350 \pm 490$ g N₂O-N ha⁻¹; Supplemental Figure S3) and increased with cumulative CO₂ fluxes, soil moisture, and temperature (Table 2).

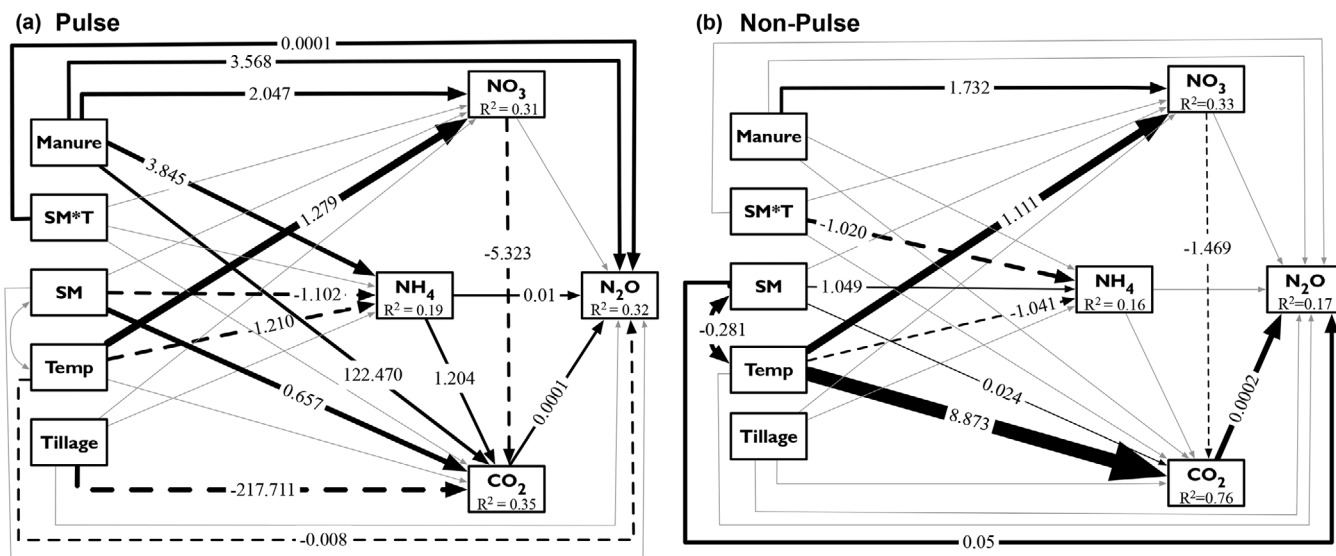


FIGURE 3 Pulse (a) and non-pulse (b) structural equation models for daily greenhouse gas flux data and covariates. Pulse relates to measurements 1 mo after manure application and tillage events; non-pulse are all other measurements. Single-headed arrows represent causal relationships; double-headed arrow denotes correlation between soil moisture (SM) and soil temperature (Temp). Arrows are scaled by standardized path coefficient values. Untransformed unstandardized path coefficients are shown. Dashed lines indicate negative and solid lines indicate positive path coefficients. Significant pathways are black arrows with path coefficients ($P < .05$). Gray arrows are nonsignificant pathways. SM*T, soil moisture \times temperature interaction; Manure, manure treatment; Tillage, tillage treatment. Positive estimates for Tillage indicate that vertical-till increased the response variable relative to no-till. Positive estimates for Manure indicate that manure injection increased the response variable relative to broadcast

Cumulative N_2O flux was smaller in 2016 ($\sim 6,300 \text{ g N ha}^{-1}$) than in 2015 ($\sim 15,000 \text{ g N ha}^{-1}$) and 2017 ($\sim 13,500 \text{ g N ha}^{-1}$), as was peak flux (Figure 2b); however, soil mineral N concentrations were greater during 2016 than during 2015 and 2017 (Figure 4). Mean precipitation during 2016 was lower than during 2015 and 2017 (2.65 mm in 2016 vs. 3.57 and 3.20 mm in 2015 and 2017, *daymetr* package) (Hufkens, Basler, Milliman, Melaas, & Richardson, 2018), and soil moisture directly before and after application (through 2 June) averaged 14.0 and 20.1%, respectively (Figure 2a). The role of soil moisture in promoting N_2O fluxes in the cumulative flux ANOVA and structural equation models suggests that the low 2016 N_2O fluxes may be related to relatively low precipitation and post-application soil moisture impeding denitrification and resulting in an accumulation of NO_3^- . This highlights the importance of abiotic parameters, such as sufficiently high soil moisture, for N transformations and N_2O fluxes from agricultural soils via their impacts on microbial processes (Xu et al., 2012). Low soil moisture in 2016, which likely reduced denitrification and plant uptake rates and subsequently drove low N_2O emissions and high soil NO_3^- accumulation, may explain why we did not observe an impact of soil NO_3^- on daily or cumulative N_2O emissions. Denitrification may have been the primary mechanism for N_2O production, but, given the relatively high levels of NO_3^- in each year (Figure 4a), NO_3^- was likely not limiting. Rather, low soil moisture and

elevated soil O_2 likely impeded denitrification and N_2O production.

3.1.2 | Carbon dioxide emissions

Emissions of CO_2 were generally highest from manure injection plots, consistent with Dosch and Gutser (1996) and Phan et al. (2012). Cumulative CO_2 emissions from manure injection ($7,200 \pm 70 \text{ kg CO}_2\text{-C ha}^{-1}$) was, on average, slightly greater than from broadcast ($6,300 \pm 80 \text{ kg CO}_2\text{-C ha}^{-1}$) (Table 2; Supplemental Figure S3). However, for daily emissions, the size of the difference varied by day (significant ANOVA manure and manure by date effects) and was relatively small on average (Figure 2c; injection averaged $39 \pm 4.7 \text{ kg CO}_2\text{-C ha}^{-1} \text{ d}^{-1}$; broadcast averaged $31.7 \pm 4.1 \text{ kg CO}_2\text{-C ha}^{-1} \text{ d}^{-1}$).

In the full structural equation model, manure injection increased CO_2 emissions via direct and indirect effects by $13.8 \text{ kg CO}_2\text{-C ha}^{-1} \text{ d}^{-1}$; the positive direct impact of manure injection was reduced by the negative impact of increased NO_3^- availability in manure-injected soils on CO_2 emissions (Figure 1). Soil temperature had the greatest total effect on CO_2 emissions: across the maximum daily range of values, increasing soil temperature increased CO_2 emissions by $408.4 \text{ kg CO}_2\text{-C ha}^{-1} \text{ d}^{-1}$ via direct and indirect effects. Soil moisture, soil NH_4^+ , and soil NO_3^-

TABLE 2 Analysis of variance (ANOVA) and analysis of covariance (ANCOVA) results for cumulative N₂O, cumulative CO₂, corn yield, and protein content

| Treatment | Cumulative N ₂ O | | | Cumulative CO ₂ | | | Yield | | | Protein | | | | | | | | | |
|--|-----------------------------|-------|-------|----------------------------|-------|-------|--------|-------|-------|---------|-------|------|-----|------|-----|-------|---------|-------|---------|
| | ANOVA | F | P | ANOVA | F | P | ANOVA | F | P | ANOVA | F | P | | | | | | | |
| Manure | 14.16 | .006* | .001 | 29.39 | .001 | .04 | 5.76 | .04 | .1 | 2.78 | .1 | 0.34 | .57 | 0.25 | .63 | 0.01 | .94 | 0.04 | .85 |
| Till | 0.05 | .83 | .74 | 0.12 | .74 | .02 | 8.13 | .02 | 8.86 | .04 | .04 | 0.73 | .42 | 0.93 | .36 | 0.12 | .73 | 0.26 | .62 |
| Year (Yr) | 2.71 | .12 | .11 | 2.91 | .11 | .001* | 13.88 | .001* | 52.18 | .003* | .003* | 0.16 | .69 | 0.02 | .88 | 39.64 | <.0001* | 176 | <.0001* |
| Manure/Till | 0.01 | .91 | .77 | 0.09 | .77 | .8 | 0.07 | .8 | 0.27 | .62 | .62 | 1.59 | .24 | 1.15 | .32 | 0.5 | .5 | 0.78 | .4 |
| Manure/Yr | 0.95 | .34 | .09 | 3.26 | .09 | .99 | 0.0003 | .99 | 0.16 | .7 | .7 | 1.01 | .33 | 0.68 | .42 | 0.15 | .7 | 0.54 | .48 |
| Till/Yr | 0.1 | .76 | .43 | 0.65 | .43 | .04 | 4.79 | .04 | 1.65 | .22 | .22 | 0.23 | .64 | 0.75 | .40 | 0 | .96 | 0.23 | .64 |
| Manure/Till/Yr | 0.76 | .39 | .22 | 1.61 | .22 | .89 | 0.02 | .89 | 0 | .99 | .99 | 0.91 | .35 | 0.70 | .42 | 0.12 | .74 | 0.08 | .78 |
| NO ₃ ⁻ , mg-N kg ⁻¹ | - | - | .57 | 0.32 | .57 | - | - | - | 0.25 | .62 | .62 | - | - | 0.48 | .50 | - | - | 0.06 | .81 |
| NH ₄ ⁺ , mg-N kg ⁻¹ | - | - | .9 | 0.02 | .9 | - | - | - | 2.76 | .12 | .12 | - | - | 1.49 | .24 | - | - | 2.13 | .17 |
| SM, % | - | - | .003* | 12.52 | .003* | - | - | - | 0.07 | .8 | .8 | - | - | 0.07 | .78 | - | - | 38.24 | <.0001* |
| ST | - | - | .01 | 8.47 | .01 | - | - | - | 3.6 | .08 | .08 | - | - | 0.00 | .97 | - | - | 33.61 | .0001* |
| SM/ST | - | - | .55 | 8.18 | .55 | - | - | - | 0.88 | .36 | .36 | - | - | 0.06 | .82 | - | - | 3.88 | .07 |
| Cum. CO ₂ flux | - | - | .001* | 16.29 | .001* | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Marginal R ² | .16 | | | .57 | | | .48 | | .36 | | | .35 | | .26 | | .43 | | .83 | |
| Conditional R ² | .16 | | | .57 | | | .48 | | .36 | | | .35 | | .38 | | .43 | | .83 | |
| n _{observations} | 36 | | | 36 | | | 36 | | 36 | | | 36 | | 36 | | 34 | | 34 | |
| n _{groups} | 12 | | | 12 | | | 12 | | 12 | | | 12 | | 12 | | 12 | | 12 | |

Note. SM, soil moisture (%); ST, soil temperature (°C); SO₄²⁻, and NH₄⁺ values for the ANCOVA were calculated as the area under the curve during each year.

*Significant at the .05 probability level.

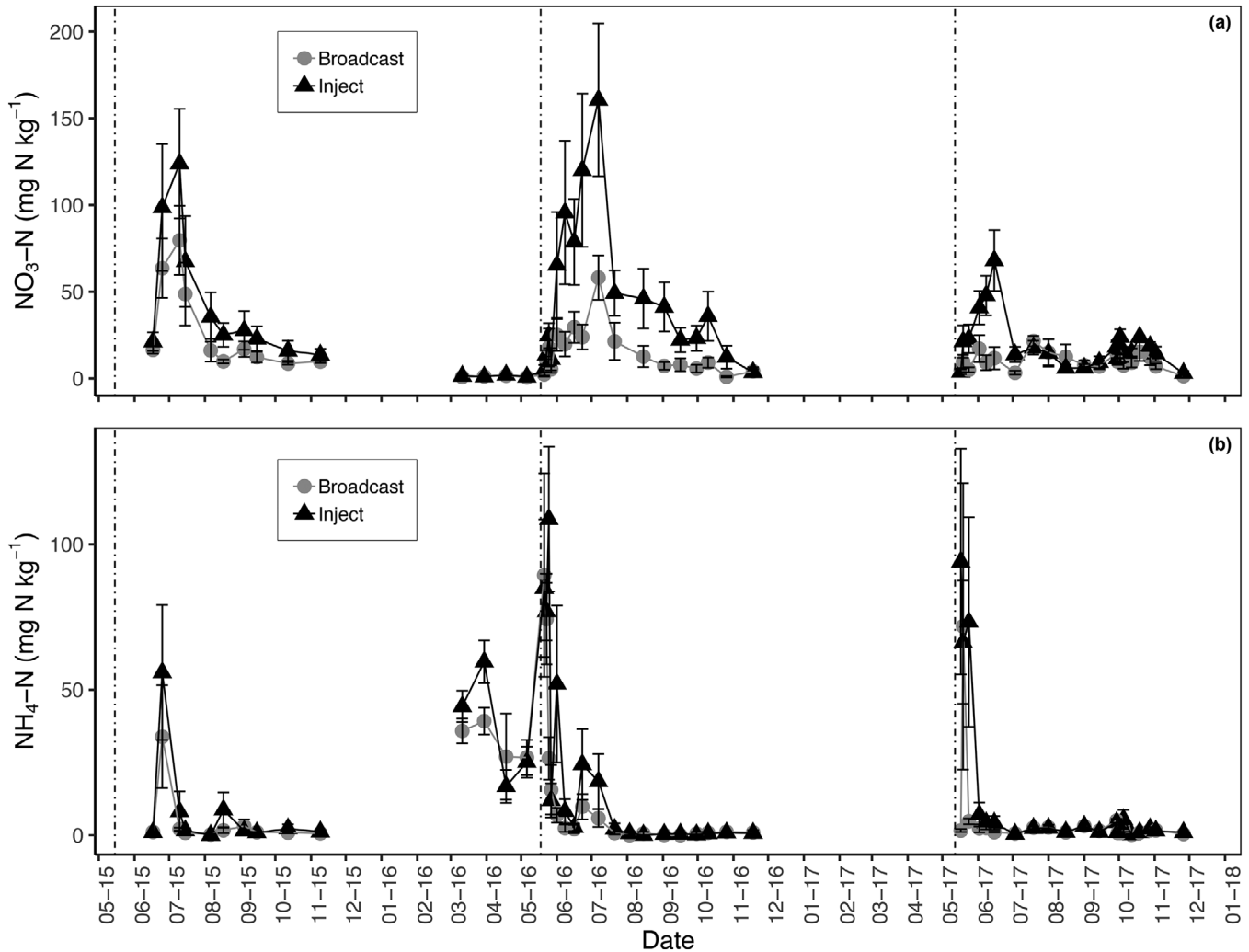


FIGURE 4 Soil (a) NO_3^- and (b) NH_4^+ concentrations by manure application method for 2015 to 2017. Error bars are ± 1 SE. Vertical dashed lines show manure application and tillage events

had somewhat smaller total effects on CO_2 emissions: Across the maximum daily range of values, increasing soil moisture and soil NH_4^+ enhanced CO_2 emissions by 87.2 and 133.8 $\text{kg CO}_2\text{-C ha}^{-1} \text{d}^{-1}$, respectively; increasing soil NO_3^- decreased CO_2 emissions by 332.8 $\text{kg CO}_2\text{-C ha}^{-1} \text{d}^{-1}$ (Figure 1; Supplemental Table S5).

Despite the minimal impact of manure application in these results, we observed large pulses of CO_2 post-injection, indicating that injection had a relatively large, but short-lived, effect on emissions (Figure 2c). Post-application, manure injection emissions were 1.5 times broadcast emissions. Unlike N_2O emissions, CO_2 emissions peaked quickly at about 74 h after manure application and tillage and were likely related to the addition of readily oxidizable manure C entering the soil (Comfort, Kelling, Keeney, & Converse, 1988, 1990; Farquharson & Baldock, 2008; Xu et al., 2008). In the post-application pulse structural equation model, manure application method had direct and indirect effects on CO_2 emissions

(Figure 3a; Supplemental Table S4). Compared with broadcast, manure injection increased emissions by 126.8 $\text{kg CO}_2\text{-C ha}^{-1} \text{d}^{-1}$ via direct and indirect effects (Supplemental Table S5). Soil moisture had the largest total effect on CO_2 emissions, increasing emissions by 1,706.3 $\text{kg CO}_2\text{-C ha}^{-1} \text{d}^{-1}$ across the maximum daily range (Supplemental Table S5). Soil NO_3^- and NH_4^+ also had large impacts on CO_2 emissions: soil NO_3^- decreased CO_2 emissions by 449.8 $\text{kg CO}_2\text{-C ha}^{-1} \text{d}^{-1}$, whereas soil NH_4^+ increased emissions by 240 $\text{kg CO}_2\text{-C ha}^{-1} \text{d}^{-1}$ across the maximum daily range (Supplemental Table S5).

In the non-pulse structural equation model, manure application had no direct impact on CO_2 flux. The largest impacts were from soil temperature and moisture (Figure 3b). Across the maximum daily range of values, soil temperature increased CO_2 emissions by 422.7 $\text{g CO}_2\text{-C ha}^{-1} \text{d}^{-1}$, whereas soil moisture decreased emissions by 87.2 $\text{g CO}_2\text{-C ha}^{-1} \text{d}^{-1}$, likely due to the negative correlation with soil temperature (Figure 3b). Soil NO_3^-

also decreased CO₂ emissions by 124 kg CO₂-C ha⁻¹ d⁻¹ across the maximum daily range of values (Supplemental Table S5). In all three structural equation models, manure injection increased soil NO₃⁻ concentrations, which led to a decrease in daily CO₂ emissions (Figures 1 and 3). The relationship between soil NO₃⁻ and CO₂ emissions has only recently been re-evaluated as previous modeling studies (e.g., Li & Harriss, 1994), generally predicted higher soil CO₂ emissions with N fertilization. However, Gagnon et al. (2016) found that N-fertilization reduced heterotrophic respiration, particularly when NO₃⁻ was added. Combined with our results, this suggests that NO₃⁻ may reduce microbial oxidation of SOC. Alternatively, the co-occurrence of high NO₃⁻ and low soil moisture (which reduced CO₂ emissions) in 2016 could be driving this negative relationship.

3.2 | No-till reduced carbon dioxide emissions without enhancing nitrous oxide emissions

Overall, no-till reduced CO₂ emissions without affecting N₂O emissions. This demonstrates that even reduced-tillage practices, such as vertical-till, have the potential to increase SOC losses relative to no-till. Although there was a significant tillage × date interaction for N₂O emissions, the effect of tillage was small and inconsistent ($P < .05$ in ANOVA) (Supplemental Figure S4; Supplemental Table S1). In the full structural equation model, no-till had only a small negative indirect impact on N₂O emissions by decreasing CO₂, which decreased N₂O emissions 0.01 g N₂O-N ha⁻¹ d⁻¹ relative to vertical-till (Figure 1). In the post-application pulse structural equation model, no-till slightly decreased N₂O emissions by <1 g N₂O-N ha⁻¹ d⁻¹, again via a negative indirect impact through CO₂ emissions (Figure 3a). In the non-pulse SEM, tillage method had no direct or indirect effects on any variable. Our results are consistent with Chen, Kolb, Cavigelli, Well, and Hooks (2018), who found no-till emissions to be less than reduced- and conventional-till emissions in coarse-textured soils. The soil at our site is well-drained, and soil moisture was not affected by tillage or manure application treatments, further suggesting that no-till practices on well-drained soils may not promote N₂O losses.

Tillage did affect CO₂ emissions, with the greatest emissions from vertical-till plots, although the size of the difference varied by date (significant tillage by date effect in ANOVA), with the largest fluxes occurring post-application and tillage (Figure 2d). This temporal pattern was also evident in the structural equation models, where no-till had a small negative impact on CO₂ emissions in the full structural equation model, a much larger impact in the

post-application/tillage pulse structural equation model (decreasing emissions by 217.7 kg CO₂-C ha⁻¹ d⁻¹ via direct and indirect effects), and no impact in the non-pulse structural equation model (Figures 1 and 3). Although soil temperature and moisture at times increased CO₂ fluxes, the largest CO₂ fluxes occurred directly after manure application and tillage events and did not coincide with the highest soil temperatures. Thus, our results suggest that nutrient addition and soil disturbance can, at times, overwhelm abiotic drivers of CO₂ emissions.

Annually, vertical-till (7,230 ± 90 kg CO₂-C ha⁻¹) slightly increased emissions relative to no-till (6,270 ± 50 kg CO₂-C ha⁻¹), but the size of this difference increased over time, with very little between-treatment difference in 2015 and average differences of 720 and 2,020 kg CO₂-C ha⁻¹ in 2016 and 2017, respectively (Table 2; Supplemental Figure S3). Cumulative CO₂ emissions were not significantly affected by covariates (Table 2). Our results suggest that the benefits of no-till for reducing CO₂ emissions may increase over time.

3.3 | Soil mineral nitrogen

Manure injection roughly doubled soil NO₃⁻ and NH₄⁺ on average, suggesting that it enhances soil N retention. However, manure application only enhanced soil NH₄⁺ during the post-application pulse, whereas soil NO₃⁻ was elevated throughout the sampling period, likely as a result of post-application nitrification (Figures 3 and 4). Manure injection increased average soil NH₄⁺ concentrations by 1.7 times versus broadcast application, but this difference varied with date (in ANOVA; Supplemental Table S6) and was largest post-application and negligible later in the growing season (Figure 4b). In the structural equation models, soil moisture increased NH₄⁺ during non-pulse times but decreased soil NH₄⁺ immediately after manure application (Figure 3). Soil temperature in the structural equation models consistently decreased NH₄⁺ (Figure 3). On average, soil NO₃⁻ was 2.2 times higher in manure injection than in broadcast soils but also varied by date (in ANOVA) (Supplemental Table S6; Figure 4a). Soil NO₃⁻ increased with soil moisture and temperature in the full structural equation model but only with temperature in the pulse and non-pulse structural equation models (Figures 1 and 3).

3.4 | Corn yield and protein content

In contrast to our expectations and despite higher soil mineral N, manure injection did not enhance corn yield or protein content. Yield was relatively consistent across years

with no treatment effects (Table 2) but was most variable in no-till broadcast plots: Average yield was $43,500 \pm 4,400$ kg ha⁻¹ in no-till broadcast; $51,000 \pm 2,900$ kg ha⁻¹ in no-till injection; $51,000 \pm 2,200$ kg ha⁻¹ in vertical-till broadcast; and $50,800 \pm 1,900$ kg ha⁻¹ in vertical-till injection (Supplemental Figure S5). Yield did not change significantly with any covariates (Table 2). Similarly, corn protein content was not affected by treatments but did increase with soil moisture and temperature (Supplemental Figures S5b and S6). Overall, our results indicate that no-till did not reduce crop yields or quality, as in other studies (Derpsch et al., 2014; Rusinamhodzi et al., 2011), although using no-till with broadcast application resulted in more variable yields. Thus, no-till with manure injection may be viable BMPs for reducing CO₂ fluxes without risking crop production or quality.

4 | CONCLUSIONS

Our findings highlight the tradeoff between mineral N retention and elevated N₂O emissions with manure injection. Manure injection more than doubled N₂O emissions and mineral N concentrations relative to broadcast, but average growing season N₂O losses were only equivalent to 9.5% of the annual N applied. Yet, there is still concern with choosing management practices that exacerbate N₂O losses due to its potent global warming potential. Alternatively, practices that do not immediately incorporate manure, such as broadcast application in no-till systems, pose a concern for N loss via ammonia volatilization (Duncan et al., 2017; Gordon, Jamieson, Rodd, Patterson, & Harz, 2001) or surface runoff (Diaz, Sawyer, Barker, & Malarino, 2010; Kleinman & Sharpley, 2003). For these reasons, manure injection stands as a BMP for reducing nutrient losses (Webb et al., 2010).

We also found that no-till mitigated SOC losses via CO₂ emissions without enhancing N₂O emissions relative to vertical-till. Because no-till and manure injection did not reduce crop yields or quality, our results suggest that these BMPs are viable options to reduce SOC losses and nutrient pollution while providing stable crop production. Efforts to mitigate N₂O production from manure injection may focus on applying manure when climatic conditions suppress denitrification or on co-management strategies, such as reducing N inputs to match crop requirements (Kim & Giltrap, 2017).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

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